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Relationship between *Brucella* species distribution and some environmental factors in Babylon province, Iraq

A Thesis

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By

Wasan Mohammed Abdulzahra Hussein Murshedi

B.Sc. Biology //Environment/ University of Babylon
M.Sc. Biology/Environment/ University of Babylon

Supervised by

Prof.
Dr. Ayad M. j. Al-Mamoori

Prof.
Dr. Jason Rao

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بِسْمِ اللهِ الرَّحْمٰنِ الرَّحِيْمِ

{يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ

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﴿صَدقَ اللهُ العليُ العظيمُ﴾

سورة المجادلة

الآية (١١)

Dedication

To **Soul of My grandmother and my father** who lived in my life

To **The source of my strength, patience and all** love…

My mother

To my brothers , sisters and their children

To my relatives and friends.

To anyone who help me

I dedicate this work

Wasan

2023

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Summary

This investigation aimed to knowledge of the presence of *Brucella* spp .in the samples from Babylon province and the relationship with environmental factors. They are made up of two sections of the gathered samples: one from the animal's soil and the other from sheep's blood.

The first part of the seasonal collection of sixty-three animal soil samples for various villages from February to November,2021 in the middle, south, and north of Babylon province, Hilla City, Iraq, with physicochemical parameters (air and soil temperature, pH, EC, TDS, salinity, TOC), as well as heavy metals (Fe, Cu, Cd, and Pb). The second portion of the collection from twenty sheep's blood samples (eighteen females and two males) from Al-Mihnawiya village in Babylon province, Middle of Hilla, Iraq at the end of May, 2021. The blood samples firstly were assessed by an automatic (count blood cell) CBC test for diagnosis of the infection in sheep's blood and then initially identified *Brucella* spp by real-time PCR .

The results showed the presence of *Brucella* species in some of soil samples and its percentage varies according to the four seasons, where the highest percentage of *Brucella* spp. was in summer season and the lowest in winter season and these percentages were related to seasonal environmental indicators. The mean percentage of *Brucella* spp. were (60%, 78.13%, 100%, 91.67%), the mean values of physical and chemical properties were air temperature (27.40, 30.69, 42.11, 32.83) ^{0}C, soil temperature (21.85, 26.17, 37.11, 23.01) ^{0}C, pH (7.62, 7.56, 7.81, 7.51), electrical conductivity (3957.0, 3768.34, 4958.89, 5123.33µs/cm),total dissolved solids (2812.0, 2645.94, 3548.89, 3593.33 mg/L), salinity (2.53, 2.47, 3.17, 3.28‰) and total organic carbon(42.44, 35.68, 34.57, 27.93%) in winter; spring; summer and autumn seasons respectively

,2021 while the mean values of heavy metals were cupper(Cu) (12.51, 5.36, 4.11, 5.95 mg\kg), iron(Fe)(412.29, 304.08, 314.95,392.44 mg\kg) , cadmium (1.88, 0.52, 1.17, 1.77 mg\kg) and lead (Pb) (647.99, 726.97, 731.77, 722.32 mg\kg) in winter, spring, summer, autumn seasons respectively,2021. It was also noted that this low percentage of *Brucella* spp.in the winter season had the highest values of heavy metals (cupper(Cu) , iron(Fe), and cadmium(Cd)) except for lead (Pb), which had the lowest value, while *Brucella* spp. percentage in the summer season had the lowest values of heavy metals (cupper(Cu) , iron(Fe), and cadmium(Cd)) except for lead (Pb), which was higher. The parameters of sheep's blood samples were their mean [8.69(10_3\ µl), 92.72 (%), 3.24 (%)] in white blood cells count, lymphocyte, granulocyte respectively. Statistical analysis of blood samples showed that all blood samples had infection due to the presence of significant differences ($P<0.05\%$) in lymphocytes but no differences ($P>0.05$) in WBC count and granulocytes.

After sending the positive bacterial samples isolated from the soil and blood for sequencing analysis, it was found that the bacterial samples isolated from the soil have eleven *Brucella* species for (twenty eight) new strains recorded in Genbank as following: six strains for *Brucella melitensis*, five strains for *Brucella intermedia*, four strains for *Brucella pseudogrignonensis*, two strains for *Brucella anthropi*, two strains for *Brucella ovis*, two strains for *Brucella inopinata*, two strains for *Brucella oryzae*, two strains for *Brucella lupini*, one strain for *Brucella rhizosphaerae*, one strain for *Brucella pituitosa* and one strain for *Brucella thiophenivorans*. Whereas deposited the four new strains in Genbank for two strains of *Brucella melitensis* and two strains of *Brucella abortus*. Also, phylogenetic analysis was for the drawing of

phylogenetic tree and the determination of phylogenetic relationship for Knowledge the degree of convergence between species and their source.

Through our research, we concluded that ed that proportion of *Brucella* spp. presence in summer was high and also environmental factors which included (Air and Soil temperature, pH, EC, TDS and Salinity) were high, except TOC was lower in summer season whereas heavy metals for Cu, Fe, Cd were lower in summer except Pb was high. This indicated that differences of seasons affect to *Brucella* ratio in soil. The findings of sequencing for the bacterial samples from soil demonstrated that *B. melitensis* was found in both the summer and autumn seasons, but *B. pseudogrignonensis* was found in the winter, spring and autumn seasons. *B. intermedia* in both the spring and fall seasons, *B. oryzae*, *B. anthropi*, *B. ovis* in the spring season while *B. thiophenivorans* and *B. pituitosa* in the autumn season. These types of *Brucella* had genetic variations. while results of the bacterial samples from blood showed presence of two types of *Brucella* spp. (*B. Melitensis and B. abortus*) also had genetic variations. Also concluded that during phylogenetic analysis of the 16S rRNA sequences indicated that *B. abortus* was nearest to Ukraine. While *B. melitensis* was nearest to with Greece in blood samples whereas *the two strains* of *B. pseudogrignonensis* one was nearest to India and the other was nearest to India and France, the two strains of *B.melitensis* one was nearest to India and China and the other was nearest to Mexico and USA, *B.intermedia* was nearest to Pakistan, *B.anthropi* was nearest to India: Pudungi and South Korea, *B.oryzae* was nearest to India: Maharashtra, *B.ovis* was nearest to India and USA , *B.inopinata* was nearest to Oman, *B.lupini* was nearest to Nigeria. *B.intermedia* was nearest to Russia, *B.pituitosa* was nearest to China and USA, *B.thiophenivorans* was nearest to India and Poland, *B.rhizosphaerae* was nearest to Ukraine and Pakistan.

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List of Abbreviation

Symbol	Description
GPS	Geographical positioning system
EC	Electrical Conductivity
TDS	Total Dissolved Solid
TOC	Total organic carbon
TBE	Tris-E Borate-EDTA Buffer
EPA	Environmental protection agency
APHA	American public health organization
DNA	Deoxyribonucleic acid
PCR	Polymerase chain –reaction
CDC	centers for disease control and prevention
OIE	World Organization for Animal Health
NCBI	National Center for Biotechnology Information

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CHAPTER ONE

INTRODUCTION

&

LITERATURES REVIEW

Chapter one

1.Introduction and literatures Review

1.1. Introduction

Brucella belongs to the Brucellaceae family, the orders Rhizobiales, Daeguia, Crabtreella, Mycoplana, Pseudochrobactrum, and Paenochrobactrum are all members of the Alpha proteobacteria class (Leclercq *et al*. 2020). It is heat sensitive and can survive for several weeks in water. The survival of *Brucella* abortus in the environment is influenced by sunlight and temperature (Jones *et al*., 2010).

Microorganisms have a diverse habitat in the soil, but their numbers are particularly high near macropores on the surface(Bundt *et al*., 2001). Earthworms, plant roots, and other soil biota build soil channels that are coated with organic materials in top soil to form macropores (Fierer *et al*., 2007). Bacterial development and diversity are linked to organic matter; thus, microbial numbers are greatest at the soil's surface (10 cm) and decrease with depth (Eilers *et al*., 2012). Identify different ecotypes and the potential for new strains, giving them insight into the environmental maintenance and transmission of emerging and re-emerging disease risks in Iraq. The presence of *Brucella* species in soil is one of the risk factors for the disease's spread to animals and humans (Seleem *et al*., 2010). Brucellosis is a zoonotic infection that can affect both animals and humans and it is still prevalent in the United States (Fouskis *et al*., 2018). *Brucella* species are pathogenic bacteria that adapt to new hosts and are naturally transmitted to their primary hosts by direct or indirect contact, as well as to other vulnerable hosts unwittingly (Moreno, 2014). The use of cows, buffaloes, sheep, and goats in mixed farming has increased the risk of brucellosis with small ruminants acting as primary hosts and cattle acting as an overflow host for *B. melitensis* (Abd El-Wahab *et al*., 2019). The current study investigates the association between soil risk variables

(physical and chemical) and the occurrence of *Brucella* species. the epidemiological relevance of brucellosis in terms of risk of transmission to humans and cattle (Motsi *et al*., 2013). It is a bacterial illness with a global distribution that is related to the emergence of agricultural society, in which animal husbandry plays an important role. The Food and Agriculture Organization and the World Health Organization rank it as one of the most common zoonotic diseases (WHO, 2005; WHO, 2012; Corbel, 2006).

The World Organization for Animal Health (OIE) classifies brucellosis as a disease, infection, and infestation that affects various species (OIE, 2018). According to Khan and Zahoor (2018), this is one of the most common infectious and communicable zoonotic infections with high morbidity and lifetime sterility rates. Livestock, as well as the owners' can have a significant economic influence. Brucellosis in cattle is typically caused by *B. abortus*. In some locations, particularly in southern Europe and western Asia, where cattle are maintained in close quarters with sheep or goats, *B. melitensis* infection can occur, although it has never been linked to abortions or transmission to other animals (Pal *et al*., 2017). Pasteurization renders *B.melitensis* inactive and its survival outside the host is primarily dependent on external factors. The pathogen can live for up to eight months in an aborted fetus in the shade, two to three months in wet soil, one to two months in dry soil, three to four months in the face, and eight months in liquid manure tanks (Ward *et al*., 2012).

Developing diagnostic assays for molecular epidemiological investigations is critical to understanding the population structure, evolutionary history and host connection in a bacterial community (O'Callaghan and Whatmore, 2011). Bacteria that live in isolation in stable environments, such as the intracellular milieu often have clonal populations with smaller and degraded genomes than their have larger and more diverse genomes (Toft and Andersson,2010). A certain degree of adaptation is required to meet environmental challenges. It has

been proposed that mutation and internal genomic rearrangements are the only mechanisms driving genetic drift and speciation in live clonal bacteria (Moreno, 1997). A variety of techniques for maintaining genetic diversity have been documented for mammalian bacterial pathogens with small genomes (Bolotin and Hershberg, 2015).

Aim of Study:

This study is design to identify the differences between diversity of *Brucella* species according to the environmental factors.

Study Objectives :

1- Survey for all areas for Brucellosis in different villages in Babylon province

2- Isolate *Brucella* spp. from different soil samples and sheep's' blood samples by cultured on the culture media.

3-Studing effect the environmental factors that included air temperature, soil temperature ,pH, salinity , EC ,TDS ,TOC on presence *Brucella* spp. in soil and also estimating heavy metals (Cu, Fe, Cd, Pb) in soil to determine degree of poisoning it to *Brucella* species.

4- Identify patterns in their spatial and temporal distribution and predicted pathogen dispersal patterns and their relation with *Brucella* spp. distribution.

5- Document the genetic subtypes of high-risk pathogens present in the diverse samples types with emphasize on difference and comparison of regional pathogen soil and blood samples in animals as well as population-level disease risk by detection with real time PCR and *16S rRNA* gene with achieve the sequences for isolates and study the phylogeny between different species .

1.2. literatures Review

1.2.1 The Relationship between *Brucella*, The Environmental Factors , Heavy Metals and its Phylogenetic Relations

1.2.1.1 Geographical Distribution of Brucellosis

Brucellosis is an endemic disease in Iraq where studied acute Brucellosis (Al-Shok,1997).The high prevalence of brucellosis in large herds will be determined by the increased likelihood of identifying. the increased potential for disease transmission through contact among them, Farming many species in the same herd has been described as a risk of infection due to duplicate sources of infection. By two mechanical carriers or by the spread of the organisms into the environment through urine, vaginal secretions, and aborted fetuses or feces. Dairy animals have a much larger chance of brucellosis but also the disease's distribution is faster than beef animals. Because animals that live in smaller areas come into close contact when they are suitable for milking (Kazi *et al*., 2005). The distribution of brucellosis in different geographies is highly dynamic with the emergence of new areas of infection and the re-emergence of infection in areas where infection existed earlier. New areas of prevalence of brucellosis have emerged in Central Asia and Middle East countries where prevalence is continuously increasing (Pappas *et al*. 2006).

Borregaard and Rahbek, (2010) showed that the positive relationship between a species' geographic distribution and its abundance is one of ecology's most well-documented patterns. An ecosystem is a geographical area or a specific place in which certain living organisms interact with each other. This includes the interactions of species with their environment. The ecosystem has served as the central organizing concept of ecology and the study of ecosystems has become an important applied science for the analysis of global change and human environmental impacts (Currie,2011).Brucellosis is a major public health concern in developing countries. This disease is found worldwide but is more

common in the Mediterranean countries, the Indian subcontinent, the Arabian Peninsula, and in parts of Mexico and Central and South America (Peeridogaheh *et al*., 2013).

The geographical distribution of animal brucellosis is constantly changing. New cases of animal (and consequently human) brucellosis may emerge or re-emerge as new cases of infected areas or re-emerge in previously free areas. Therefore, a knowledge of the epidemiology of the disease in animals, particularly with respect to the geographical characterization of *Brucella*, is importance to establish and reliable and efficient control measures against brucellosis in a "One Health" perspective. Sasan *et al*., (2012) noted that Iran is the second country in the world for the prevalence of brucellosis. Al-Shabaa, (2016) studied brucellosis that Undulant fever, Mediterranean fever, or Malta fever is different names for one disease, which is an infectious disease caused by a type of bacteria called *Brucella* that is almost invariably transmitted by direct or indirect contact with infected animals or their products and affects people of all age groups and of both sexes. The eradication poses major difficulties because this disease is largely under diagnosed and underreported. A study was conducted in the period from March, 2013 to March, 2014 to estimate the seroprevalence of brucellosis and identify risk factors associated with *Brucella* infections in Al-Najaf province. Furthermore, the study recorded relatively low *Brucella* seroprevalence in rural areas, recording 70 (38.9%), compared to the urban areas, recording 110(61%),that suggestion may be due to persistence involving multiple social and ecological factors like varying geographic regions. Corbel,(2020) demonstrated that as the number of *Brucella* isolates accumulated from different host species and different geographical locations accumulated, it was realized that the original criteria used for defining these species did not always lead to consistent results. Brucellosis was an

endemic of specific geographic areas and represents the most common zoonotic infection (Bonaventura *et al*., 2021).

1.2.1.2 Pathogenicity of *Brucella* spp.

Brucella spp. are Gram-negative, non-motile, non-spore-forming, slow-growing, facultative intracellular bacteria causing brucellosis (Bonaventura *et al*., 2021). *Brucella* pathogenesis was divided into four stages: adhesion, invasion, establishment, and dissemination inside host tissue (Lopez *et al*.,2002; Christopher *et al*., 2010). *Brucella ovis* can also cause abortions and placentitis in ewes, but this appears to be uncommon. Infected ewes may give birth to weak lambs that die soon after birth. Systemic signs are rare in adult ewes and rams (Xavier *et al*., 2010). Features of pathology in infected hosts is divided into three distinct phases: the incubation phase before clinical symptoms are evident; the acute phase during which time the pathogen invades and disseminates in host tissue; and the chronic phase that can eventually result in severe organ damage and death of the host organism (Baud and Greub, 2011).

Mechanisms of intracellular survival and immune evasion contribute to systemic persistence by the pathogenic *Brucella* species that enter into the bodies of animals through inhalation, ingestion, and through mucous membranes or broken skin. *Brucella* induces acute or chronic infection of the reproductive tract that leads to abortion or severe reproductive tract diseases (He,2012). Poester *et al*., (2013) noted that all *Brucella* species establish persistent infection in the reticuloendothelial system of the natural hosts of *Brucella*. The mechanisms of placenta localization, trophoblast tropism and abortion are poorly understood. the molecular determinants and mechanisms of the cell internalization process began to emerge only recently where cyclic β-1,2-glucan is a molecule secreted into the periplasm of *Brucella* and is required for intracellular *Brucella* to avoid fusion of the phagosome with lysosomes. Secretion system translocate *Brucella* effector proteins into host cells and is

useful for both survival and replication of *Brucella* in infected host cells. as in shape in (1-1). In infected rams, clinically detectable lesions become apparent from 3 weeks to 8 weeks after inoculation. Rams can develop epididymitis, orchitis, and impaired fertility due to *Brucella ovis*. Some rams shed *B. ovis* for long periods without clinically apparent lesions (Poester *et al*., 2013).

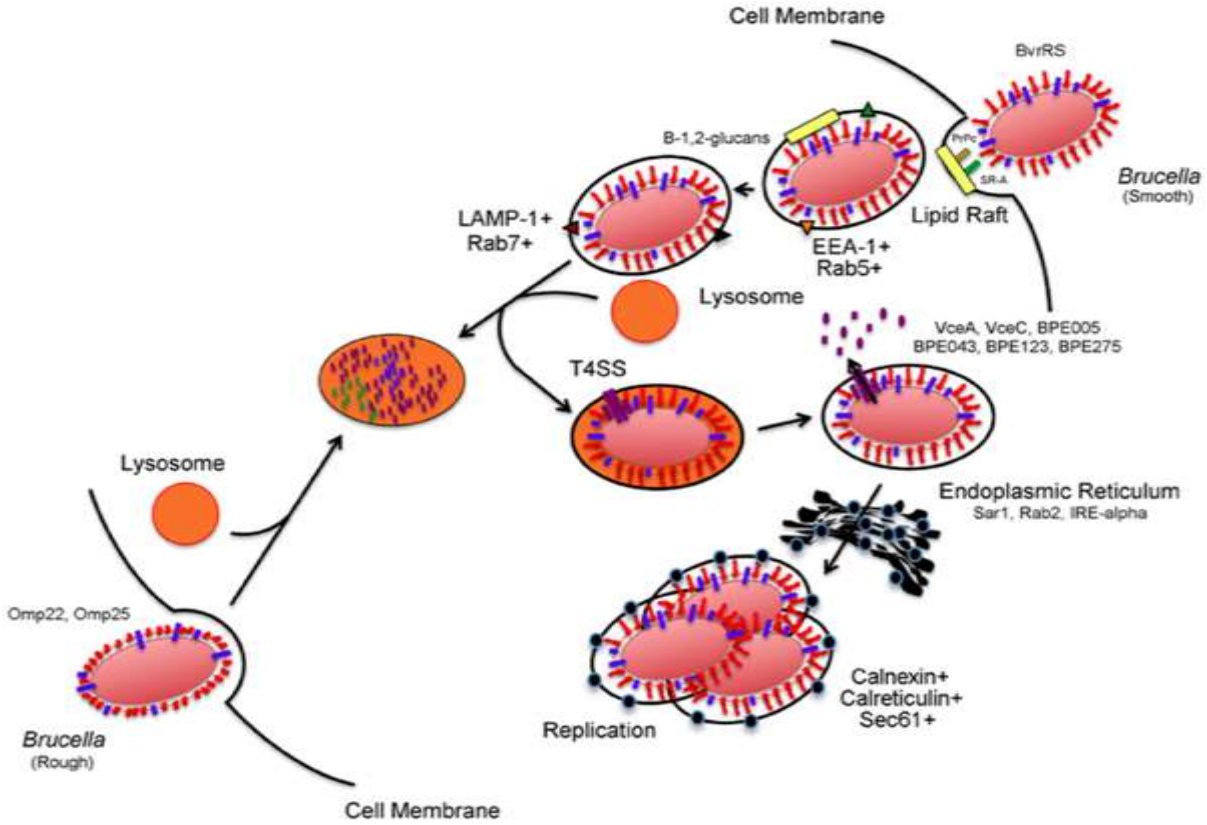


Figure (1-1): *Brucella* invasion to the host immune system(Gomez *et al*., 2013).

Brucella could enter a host through the lymph nodes and then translocate to the reproductive tract's preferred tissues (Kim, 2015). Coelho *et al*., (2015) showed that *Brucella* spp. is the aetiological agent of brucellosis, a highly infectious disease that causes reproductive failure and has widespread public health implications due to its zoonotic properties. This disease also had a significant economic impacts. Identification of brucellosis risk factors that maintain infection in animals and the environment is critical to achieving brucellosis control and eradication. Brucellosis is characterized by abortion,

infertility, placentitis in females and epididymitis in males(Jafer, 2018). *B. melitensis, B. abortus, B. suis, B. ovis, B. canis, and B. neotomaes* are among the six species of *Brucellae* (Deng *et al*., 2019).The initiation of *Brucella* infection depends on the exposure dose, virulence of the *Brucella* species, and natural resistance of the animal to the organisms (Bedore and Mustefa, 2019).

Amjadi *et al*., (2019) showed that brucellosis was one of the major zoonotic infections. Considering the economic burden, its prevalence in endemic regions. *Brucella* is able to survive and replicate within host cells by expressing different virulence factors and using various strategies to avoid the host's immune response. This led to progression of the disease from an acute phase to chronic brucellosis. Exploration of genetic variations has confirmed the expected influence of gene polymorphisms on susceptibility and resistance to brucellosis.

While Saavedra *et al*., (2019) noted that *B. melitensis* and *B. ovis* are the aetiological agents of small ruminant brucellosis. The term "reservoir" refers to an ecological species that maintains live circulating organisms throughout the ecosystem over time within the niche of a host reservoir, *Brucella* can remain at a low replication rate for a long time and under favorable conditions, regress to infect other cells and start new replicative cycles (Godfroid *et al*., 2013; O'Callaghan, 2020**).** Contagious abortion or Bang's disease is the name for bovine brucellosis (Hayoun *et al*., 2020). The species of brucella may infect people was *B. melitensis* being the most virulent and dominant species associated with human brucellosis in China (Ye *et al*., 2020).Stranahan and Gamboa,(2021)studied facultative intracellular bacterial pathogen and the cause of worldwide zoonotic infections for its ability to evade the immune system and persist chronically within host cells, a rough lipopolysaccharide phenotype is retained by *Brucella canis* and *Brucella ovis*, which remain virulent in their natural canine and ovine hosts, respectively. While these natural rough strains lack the O-polysaccharide ,they like their smooth counterparts that are able to

evade the host immune system by exhibiting low endotoxic activity resisting destruction by complement and antimicrobial peptides entering and trafficking within host cells.

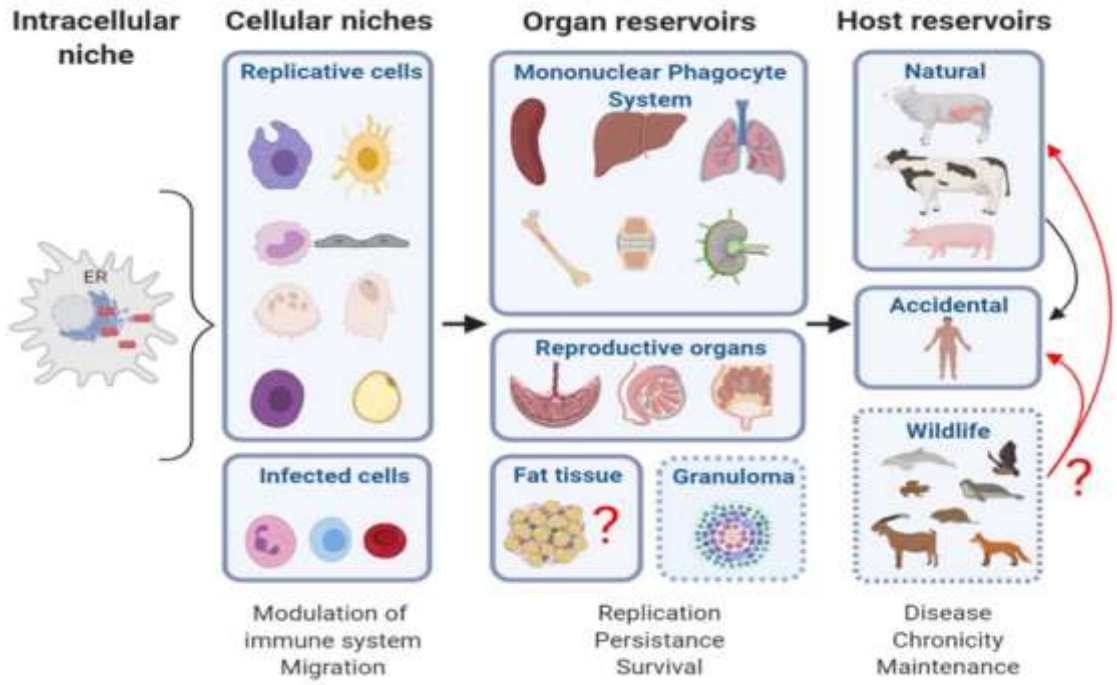
1.2.1.3 Survival *Brucella* in The Environment

The ability of *Brucellae* to persist outside mammalian hosts is relatively high compared with most other non-sporing pathogenic bacteria under suitable conditions. Numerous studies have assessed the persistence of *Brucella* spp. under various environmental conditions. When pH, temperature, and light conditions are favorable, including high humidity, low temperature, and absence of direct sunlight, *Brucellae* may retain infectivity for several months in water, aborted fetuses and fetal membranes, feces and liquid manure, wool, hay, and on buildings, equipment, and clothes. This bacteria is able to withstand drying, particularly in the presence of extraneous organic material, and remain viable in dust and soil. Survival can be prolonged at low temperatures, especially below 0^0C (European Commission, 2001). The soil provides a complex habitat for the microorganisms, but their numbers are very high in surface soil around macropores (Bundt *et al*., 2001). The environment was a medium for replication and aids bacterial expansion and subsequent transmission to new host cells, for example, through extensively infected tissues such as the aborted fetus (Gorvel and Moreno, 2002). *Brucellae* are sensitive to exposure to heat and most disinfectants but can survive in the environment for up to two years under specific conditions, becoming a continuous threat to both humans and animals (Boosi *et al*., 2004). Sobsey *et al*., (2006) mentioned that fecal wastes and other wastes (such as respiratory secretions, urine or skin) of various agricultural livestock and feral animals often contain high concentrations of human and animal pathogens (disease-causing microorganisms). The macropores were the channels in the soil that are formed by the activities of earthworms, roots of plants, and other soil biota that are lined with organic matter, mostly in top soil

(Fierer *et al*., 2007). *Brucella* was a robust pathogen that persist outside and inside the mammalian hosts for a long time despite the unfriendly conditions; it remains in food for up to 15 months given adverse conditions such as acidity and temperature between 11°C and 14°C or for two to three days under 37°C, When *Brucella* is exposed directly to sunlight, it may survive for few hours while its survival in contaminated manure and aborted feti is more than 2 months during the winter season. Furthermore, in an ideal environment, the survival of *Brucella* spp. was reported to last up to 135 days (Aune *et al*.,2012).

Seiler & Berendonk, (2012) showed that the risk of metal resistance in the environment was assessed based on heavy metal concentrations, Analyses of the data indicate that agricultural and aquacultural practices represent major sources of soil and water contamination with moderately to highly toxic metals such as mercury (Hg), cadmium (Cd), copper (Cu), and zinc (Zn). If those metals reach the environment and accumulate to critical concentrations, they can trigger co-selection of antibiotic resistance. Li *et al*.,(2013) found that incidence of brucellosis were strongly associated with lower temperatures and less sunshine in the winter and spring, climatic factors likely influence the ecology of brucellosis both directly and indirectly by affecting several parameters, including the growth and reproduction dynamics of domestic animals, interactions between sheep, goats and humans, pathogen replication, and population immunity(Morales,2013). Hellberg&Chu,(2016) showed the effects of climatic factors, such as temperature, rainfall, drought, and wind, on the environmental dispersal and persistence of bacterial foodborne pathogens, called *Bacillus cereus, Brucella*, *Campylobacter*, *Clostridium, Escherichia coli, Listeria monocytogenes, Salmonella, Staphylococcus aureus, Vibrio,* and *Yersinia enterocolitica.* While Reshma *et al*., (2016) investigated the relationship between soil characteristics and micronutrient availability in the Salem area of Tamil Nadu. In total, 1691 soil samples were gathered from 385

villages of Salem district, with four samples per village. Samples were analyzed to determine the soil characteristics and the status of accessible micronutrients. analysis was used to determine the association between available micronutrients and soil characteristics. Electrical conductivity was strongly and positively associated with Fe, while Zn, Mn, and Cu were negatively related. Organic carbon had a positive relationship with Mn and Cu, but it was not significantly positive with Fe and Zn. According to Estrada *et al*., (2016), Brucellosis was a zoonotic disease that affects many animal species globally. It is possible to extrapolate the notions of niche and reservoir to the infectious, as in figure (1-2). The effects of climatic factors on this important zoonotic disease. The growing burden of brucellosis, understanding the quantitative relationships between climatic factors and the seasonality of brucellosis and providing early warning of disease epidemics through climate forecasting. Previous studies showed that the seasonality of brucellosis might be linked to human activities and ecological factors, especially climatic variability (Zhu *et al*., 2017).



Figure(1-2): Summary of *Brucella's* cellular niches and reservoirs (Espinoza *et al*., 2021).

While Al-Kaisi *et al*., (2017) established that a complex and dynamic natural system is the soil that includes minerals, organic matter, countless organisms, liquids, and gases that support life on earth through many services. The soil physical environment includes components of soil structure and aggregation. The soil biological environment includes all soil organisms (macro-and microorganisms) and soil-plant relationships (plant root-soil interactions). Seasonal climatic conditions may affect multiple aspects of climate-sensitive infectious diseases (Xiang *et al*., 2018). High transmission rates between domestic and wild animals are expected during the dry season due to sharing of pastures and water points, while the within-herd transmission is expected during the wet season due to a high birth rate and abortion (Kimaro *et al*., 2018). According to the WHO,(2018) in countries with cold or temperate climates, there are notable seasonal variations in brucellosis incidences with most occurring cases in the summer and spring. Consequently, climatic factors can be used to forecast the occurrence of disease outbreaks within periods of weeks to months (Morin *et al*., 2018).

Liu *et al*., (2020) noted that brucellosis is a serious public health problem primarily affecting livestock workers. The strong seasonality of the disease indicates that climatic factors may play an important role in the transmission of the disease. However, the associations between climatic variability and brucellosis are still poorly understood. they assessed the quantitative relationships and response effects between monthly climatic factors and brucellosis. Temperature, sunshine duration, and evaporation were significantly affected by seasonal fluctuations in the transmission of brucellosis. Nyerere *et al*., (2020) concluded that variations in seasonal weather have a great impact on the transmission dynamics of brucellosis in humans, livestock, and wild animals.

1.2.1.4 The Relationship between Environmental Factors and *Brucella* Diversity

Since 1937, one of Iraq's most prevalent illnesses, brucellosis, has been regarded as an endemic problem (AlZahawi, 1938). The organism's capacity for survival in the environment affects the epidemiology of the disease for (4-8^{0}C) in tap water, for months at (0^{0}C), for 2.5 years in frozen tissues and for years in media. In wet soil, *Brucella* can survive for 60 days and at (20^{0}C) and 40% relative humidity, it can last 144 days. Additionally, it persists in the body for more than 200 days in uterine exudate, 75 days in aborted fetuses and 30 days in urine (King,1957). According to research conducted by Bercovich,(1998) on a few epidemiological features, *Brucella* was able to live, propagate, and sustain itself in the environment. *Brucella*-free herds are also at risk from infected cattle with murky results from undetectable cases. Deqiu *et al*., (2002) reported cases of inhaling contaminated aerosolized particles such as dust and soil. Alphaproteobacteria species can associate extra-and intracellular with unicellular and multicellular eukaryotes in a variety of systems, including soil and water (Batut *et al*., 2004).Mantur and Amarnath, (2008) showed that intimate contact with domestic or wild animal populations occurred in hundreds of small cities and 575,000 villages throughout India. In recent decades, a number of studies on the of brucellosis in Iraq have been conducted. The northern provinces of Iraq are bordered by Iran, Turkey, and Syria. Kuwait, Jordan, and Saudi Arabia also share borders with other Iraqi regions. This disease could spread through the close animal-human contact, it is a particular problem in Iraq (John *et al*., 2008). Rasul and Mansoor, (2012) noted that the prevalence rate in 2012 was 10.7% in Erbil city and 6.36 in Dohuk (Omar *et al*., 2011).

A dynamic array, such as dietary behaviors, milk product processing techniques, husbandry procedures, and environmental cleanliness, have an

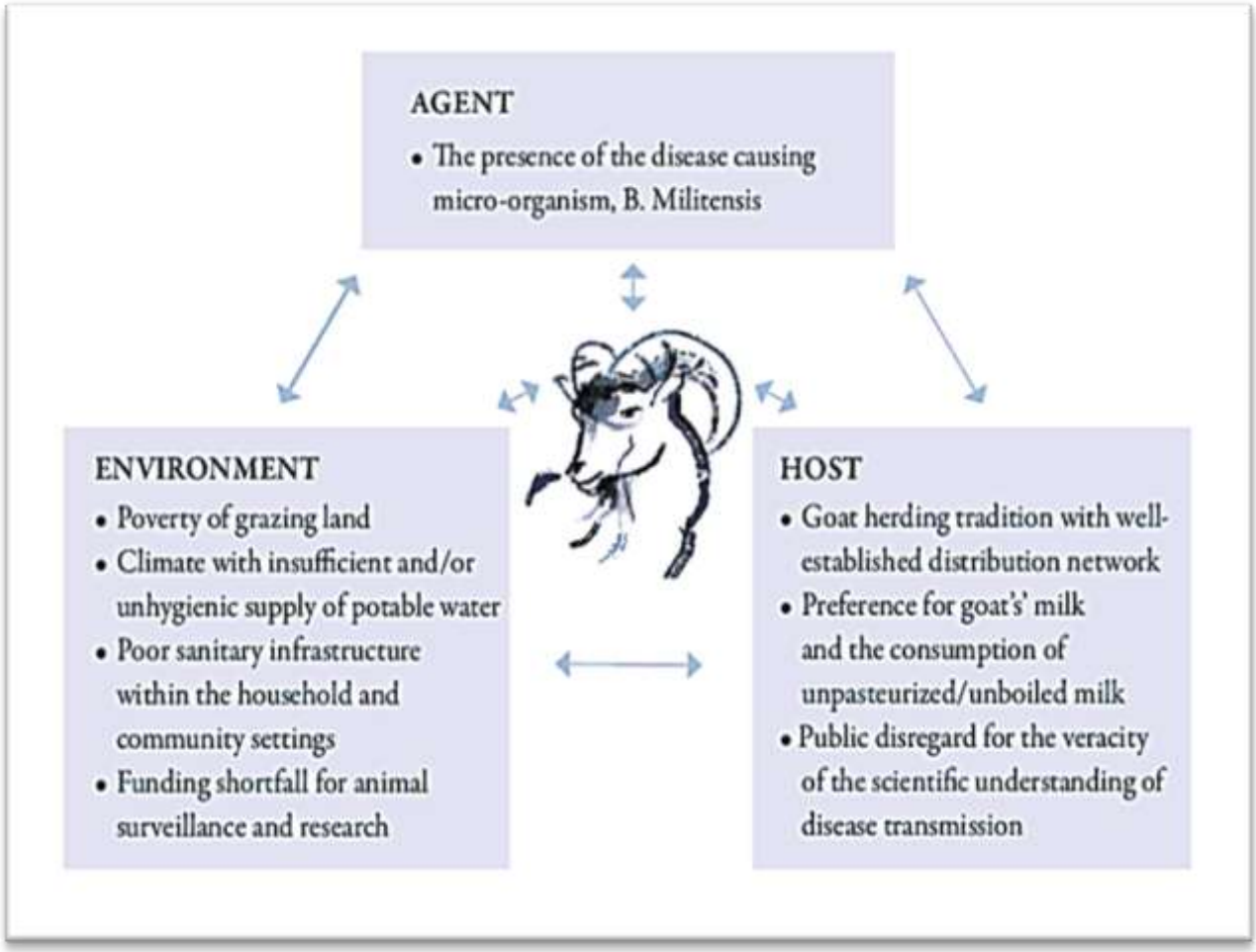
impact on the occurrence of brucellosis in people. This explains why the typical route of infection was either direct ingestion or via mucous membranes, broken skin and in rare cases, intact skin. In addition, the geographic position of Iran is seen to be a key risk factor for the transmission of infectious illnesses, notably from Eastern and Western surrounding nations like Iraq, Pakistan, and Afghanistan. Due to the endemic nature of the disease and the absence of efficient animal disease management systems in these places, there is a risk that Iran might get brucellosis from these countries (Esmaeili, 2014). Between October 2012 and April 2013, Salman *et al*., (2014) researched on certain agricultural animals, farmers, and veterinarians in Baghdad. The high incidence of brucellosis in humans and animals suggests that the illness is endemic in this region. The primary risk factors for brucellosis in Iran include consumption of unpasteurized dairy products (particularly raw milk and fresh cheese), close contact with animals, animal husbandry and laboratory and veterinary professions (Sofian *et al.,* 2008; Bahador *et al*., 2012; Alavi *et al*., 2014). According to Gül *et al*., (2014), the prevalence of brucellosis in Turkey ranges from (2% to 6%), *B. melitensis* was most common in the Mediterranean region. A few Central American countries are affected by it, along with the Middle East, Central Asia, the Arabian Gulf region, and other areas. It appears that this organism is not native to northern Europe, north America (with the exception of Mexico), southeast Asia, Australia, or New Zealand, despite accounts of it coming from Africa and India (CFSPH, 2007).

The incidence of brucellosis increases during the spring and summer because of factors including dairy product intake and direct contact between ranchers and aborted fetus. The seasonal pattern of brucellosis revealed that the disease was most prevalent in the spring and summer and least prevalent in the winter and fall. There were still endemic areas of brucellosis in the Middle East (Iran),Africa, Latin America, Central Asia, and the Mediterranean Basin, to

name a few. According to Ministry of Health and Medical Education research, the disease is endemic throughout Iran, particularly in areas where humans live near cattle, in a report by Esmaeili, (2014). Rostami *et al*., (2015) showed that spring and fall seasons had the greatest and lowest rates of brucellosis, respectively. In endemic areas, the incidence rate varies from 0.03 to 200 per 100,000 individuals (Yang *et al*., 2013 ; Rostami *et al*., 2016). Additionally, rare incidences of transmission by tissue transplantation, person-to-person transmission, blood transfusions, bone marrow transplants, and aerosols from an infected patient have all been reported (Tuon *et al*., 2017).It infects human hosts by contact with mucosa or inhalation, puncture wounds such as needles, and ingestion after proliferating within macrophages and escaping host defense mechanisms (Hull and Schumaker, 2018). It is an occupational zoonosis for farmers and veterinarians that will affect people is correlated with the likelihood that it will affect the area's cattle population (Musallam *et al*., 2016 ; Proch *et al*., 2018). Espinoza *et al*., (2021) showed that *Brucella* is an intracellular bacterium that causes abortion, reproduction failure in livestock and leads to a debilitating flu-like illness with serious chronic complications. *Brucella* developed strategies to avoid recognition by the immune system of the host and promote its survival and replication. In vivo, *Brucellae* reside mostly within phagocytes and other cells, including trophoblasts throughout the body, where they established a preferred replicative niche inside the endoplasmic reticulum. This activity is crucial because it allows *Brucella* to maintain replicating-surviving cycles in its cellular niches for long periods of time, even when bacterial populations are low. *Brucella* takes advantage of the environment supplied by the cellular niches which in it lives to form reservoirs and spread to other organs. In addition, *Brucella* infection favors cellular niches in the host, resulting in anatomical reservoirs that can lead to chronic infections or persistence in asymptomatic people, also posing a risk of future contamination.

1.2.1.5 Epidemiology of Brucellosis

The epidemiological triangle, which included agent, host, and environment, was a conventional model of infectious disease etiology. The three components of the triangle are an agent (pathogen), a vulnerable host, and the physical, social, behavioral, cultural, political, and economic conditions that bring the agent and host together and lead to infection and disease in the host. According to the epidemiological triangle (Snieszko,1974), as in figure(1-3).



Figure(1-3): The epidemiology triad model of *Brucella* (Tripp & Sawchuk, 2011).

Brucellosis is a zoonotic disease that may spread everywhere in the world, although it is most prevalent in the Mediterranean, North and East Africa, the Middle East, South and Central Asia, and Central and South America (Corbel, 2006).The disease's global epidemiology had not significantly change in recent

years. Existing strains may adapt to new social and agricultural practices while other strains may develop , due to a number of hygienic, social, and political factors, brucellosis is becoming increasingly prevalent in many developing countries despite advances in detection and control (Pappas *et al*., 2006). The disease may have significant socio-economic effects on individuals, especially in low-income countries (Benkirane *et al*.,2006). Atluri *et al*.,(2011) found that a zoonotic infection caused primarily by the bacterial pathogens *B. melitensis* and *B. abortus.* Globally, it is one of the most widespread zoonotic diseases, with 500,000 new cases reported each year. In endemic areas, *Brucella* infections represent a serious public health problem that results in significant morbidity and economic losses (He,2012). The highest incidence of brucellosis in Kurdistan of Iraq in 2013 was 976 cases in the province of Sulaimani (Mohammed, 2015). While Chitupila *et al*., (2015) showed that epidemiological study of bovine brucellosis was carried out in the Western Tanzanian Kigoma region's Kibondo and Kakonko Districts. Every year, more than 500,000 people become infected with Brucellosis. The illness continues to be a severe public health concern in the Mediterranean region, western Asia, parts of Africa, and Latin America despite its limited geographic distribution. Due to miscarriage, early birth, decreased milk supply and a lower reproduction rate, animals with Brucellosis have severe economic losses. Beauvais *et al*., (2016) used a switching model based on season to examine the transmission of brucellosis on a sheep and cattle farm. Furthermore, *Brucella* spp. infection is mediated by direct contact with the placenta, fetus and fetal fluids or byproducts(e.g., milk, meat, and cheese)from infected animals (Tadesse, 2016). Fadihl and Khalil, (2016) examined the presence of *Brucella* spp. in the unpasteurized milk-based cheese " local cheese manufactured locally in Baquba city. Fifty cheese samples were gathered from street vendors in Baquba's old town between May and September 2015; they were detected into two species: *B.melitensis* and

B.abortus . Samples of "local cheese" fresh cheese include *B.abortus* and *B.melitensis* infections.

Seventer and Hochberg (2017) showed that understanding of the mechanisms affecting transmission is essential for infectious disease control and prevention. the fundamental concepts of infectious illness diagnosis, management, and prevention. There are many different ways that infectious pathogens might migrate from a natural reservoir to a vulnerable host, categorization systems employed to categorizes transmission into two categories: direct transmission the infectious form of the agent is conveyed directly reservoir to an infected host and indirect transmission (the agent is passed via a living or inanimate intermediate). AL-Busultan *et al*., (2018) showed that both animal and human brucellosis are still prevalent in a number of Middle Eastern nations, including Iraq. Studies include many transmission routes and an incidence ratio that is used to describe indirect transmission from the environment to individuals (Zhang *et al*., 2019). Dahl ,(2020) noted that Brucellosis, an illness that affects animals that are raised for food. This study employed a systematic review technique to identify and evaluate the evidence and knowledge gaps in published studies that investigated about brucellosis in various food-producing animals in Mosul, Iraq. Local professional phagocytes, such as macrophages, dendritic cells, and neutrophils, once inside, internalize the bacteria and travel to the nearest draining lymph nodes as part of the immune system's routine sampling process. This causes spread to the reticuloendothelial system's various organs, including the lungs, spleen, liver, and bone marrow (Moreno and Calvo, 2020).According to Holt *et al*., (2021), Brucellosis was a zoonotic disease that significantly affects both public health and animal productivity globally. Tulu, (2022)studied the globally contagious zoonotic illness brucellosis poses a significant threat to Ethiopia and other developing nations. The most common cause of brucellosis in cattle is *Brucella abortus*, but rarely, *Brucella melitensis*

and *Brucella suis* can also infect calves with the brucellosis. Normally, brucellosis spreads to non-infected cattle by direct or indirect contact with infected animals or their excretions. In pastoral and mixed cattle management systems in Ethiopia, where people live near to cattle and have a higher risk of the *Brucella* organism, the prevalence of brucellosis is high. There are more than 10 cases of brucellosis per 100,000 people in some areas. Kumar *et al*., (2021)demonstrated that the emergence of brucellosis in new areas also transmission of brucellosis from wild to domestic animals are of utmost significance in terms of new epidemiological aspects.

1.2.2 Genotypic Methods for Identification of *Brucella* Species.

The arbitrarily primed polymerase chain reaction was used by Fekete *et al*., (1992) to establish the DNA heterogeneity among species of BruceUla (AP-PCR). With the use of five randomly selected primers, both individually and in pairs, the PCR was used to create straightforward, reproducible genomic fingerprints from the DNA of 25 distinct BruceUla strains. With every primer, a number of DNA stretches were amplified in every sample. For each primer, reaction circumstances were perfected and polymorphism was visible. Polymorphic markers are PCR products that are distinct from those produced by all strains. According to Michaux *et al*., (1993), *Brucella* organisms have two circular chromosomes with average genomic sizes of 2.1 Mb and 1.2 Mb each. The BruceUla strains can be identified by changes in the agarose gel banding patterns of their amplified DNA, which can serve as a diagnostic marker for particular strains. Similarity coefficients were evaluated to determine the genetic relatedness of the *Brucella* strain. Using primers complementary to two enterobacterial short repetitive sequences-repetitive extragenic palindromic and enterobacterial repetitive intergenic consensus sequences-thirty-four *Brucella* reference or field strains representing all the species and biovars were studied by repetitive element sequence-based PCR, as demonstrated by Mercier *et al*.,

(1996). All of the strains displayed positive amplifications, indicating that these sequences may be present in the *Brucella* genome. A combination of the two techniques was able to distinguish all the isolates, with the exception of some strains that belonged to *B.abortus* biovars3and9.Repeated extragenic palindromic PCR was less discriminating than enterobacterial repetitive intergenic consensus PCR in terms of differentiating strains. A straightforward and practical tool for brucellosis epidemiology research seems to be repetitive element sequence-based PCR. While Bilak *et al*., (1998) showed that certain *Brucella* species have a single 3.3 Mb megareplicon as a result of the fusion of the two chromosomes. There are intracellular-extracellular facultative organisms, such as the zoonotic *Brucella* and *Bartonella* species, with intermedium genome sizes ranging from 3.3 to 1.6 Mb (Moreno, 1998).The identification of *Brucella* by Probert *et al*., (2004) revealed that the technique can be labor-and time-intensive and expose people to sickness through work in a lab. *Brucella* isolation confirmation using real-time PCR that includes *B.abortus* and *B.melitensis* can all be easily identified.

In the study by Dahouk *et al*., (2005), *Brucella* spp. were shown to be a relatively homogeneous collection of bacteria. However, RFLPs of particular genes showed enough variation to discriminate between *Brucella* species and biovars. The results of the PCR-RFLP analysis demonstrate good type ability, repeatability, stability, and epidemiological concordance. As a result, PCR-RFLP assays of certain gene loci can be used as instruments for studies in taxonomic, evolutionary, diagnostic, and peri-epidemiological research. Mutations were found in some virulence-related genes (Moreno and Moriyón 2006; Martin *et al*., 2009). Despite their close genetic relatedness and lack of lysogenic phages or detectable plasmids, there is a high link between genotypes, virulence, and host preference (Moreno and Moriyón 2006). To improve resolution, the sequences used as the outgroup were removed from the tree

(Williams *et al*., 2007). *B. melitensis* (derived from sheep and goats), *B.suis* (hogs), *B.abortus* (cattle), *B.ovis* (sheep),*B.canis* (dogs), and *B. neotomae* (wood rats) are the six species that have been identified based on genetic analysis. *B. pinnipedialis* (seals), *B. ceti* (dolphins, porpoises), *B. microti* (voles, foxes), and *B. inopinata* (unknown) are among the newly identified species (Mantur and Amarnath, 2008). Mukherjee *et al*., (2007) employed *Brucella*-specific nucleotide sequences encoding the BCSP 31 kDa protein, Omp2, and the 16S rRNA in three distinct diagnostic PCR methods. The three PCR assays performed on six *Brucella* reference strains. The results of PCR testing on bcsp and omp2 from 19 Indian field isolates (human, bovine, and murine tissues) were also completely similar. When the *16S rRNA* gene was employed as the diagnostic target in the PCR, only 14 of the 19 isolates and 2 of the 7 isolates from bovine milk were recognized as *Brucella*. In cow blood samples, the 16S rRNA PCR proved unsuccessful. When a consensus result of omp2 and bcsp blood PCR was incorporated for comparison with ELISA, the correlation between ELISA and blood PCR improved (at P=0.05). The use of numerous marker-based PCRs resulted in increased sensitivity and specificity. Due to these characteristics, phage integration or traditional recombination cannot acquire external genetic material. *Brucella* is classified strictly molecularly based on its genetic characteristics(Whatmore, 2009).

The real-time PCR tests were compared to conventional PCR experiments that targeted the same genes previously disclosed. The genus-specificity of 26 *Brucella* strains, comprising all species and biovars, was assessed. Using decreasing amounts of DNA from *B. ovis, B. melitensis and B. Abortus* Finally, repeatability and reproducibility within and between assays were assessed. In all three assays, the DNA of all *Brucella* species was amplified. However, the real-time PCR detected the earliest signal. And it was always more sensitive than conventional PCR experiments. The real-time PCR assay had the same or

higher sensitivity than the other two tests. Variability was extremely minimal in all cases. To summarize, real-time PCR tests are simple to use, give data faster than traditional PCR methods, and reduce the possibility of DNA contamination. The real-time PCR assay is specific and highly sensitive and it looks to be an efficient and reproducible approach for detecting the *Brucella* spp.in a timely and safe manner (Bounaadja *et al*., 2009).The automated Sanger method is described as a "first-generation" technology, while more recent methods are known as Next Generation Sequencing NGS (Metzker,2010).

Environmental and plant-related taxa with sizable genomes, such as Caulobacter and Brady rhizobium, have been found to exist (Kaneko *et al*., 2011). According to Kiran *et al*., (2011), computational genomics is one of the most crucial techniques for comprehending the distribution of closely related genomes in an organism, including simple sequence repeats (SSRs), which offer important knowledge about genetic variants. Two ultrafast DNA sequencing methods and platforms currently under development are Sanger and Next Generation Sequencing (NGS), which combines a number of unique DNA polymerase-dependent technologies (Guzvic, 2013). Statistical investigations reveal an imbalance between tri-nucleotide SSRs and tetra nucleotide SSRs in *Brucella* genomes. Tri-nucleotide SSRs that have been overexpressed in the genomic and coding regions may be accountable for the functional diversity in expressed proteins that could result in variable pathogenicity. A sensitive quantitative-LAMP (Q-LAMP) assay for quantifying brucellosis was created by Soleimani *et al*., (2013) and a LAMP technique was built for speedy *Brucella* identification. The researchers used specially designed primers to target *Brucella* spp. in their study. By analyzing LAMP products in successive dilutions with the real-time turbid meter system and then graphing time threshold values against log of copy number to construct a standard curve, the assay was developed as a quantitative test. *Brucella* genomic DNA were used to

test the assay's specificity. Furthermore, it is evident from a biological, biochemical, genetic, and medicinal standpoint that the traditional *Brucella* organisms meet the concept of ecotypes. the diverse *Brucella* species and strains demonstrate distinct host preferences (Tsolis, 2002), zoonotic potential (Atluri *et al*., 2011) and virulence (Moreno, 2014), despite having a genetic link that is relatively close (97%) similarity. The phylogeny of a few typical alphaproteobacteria as inferred from a *16S rRNA* gene-based maximum likelihood reconstruction. These characteristics make *Brucella* an ideal model for studying bacterial host adaptability. Surprisingly, pseudogene accumulation in prokaryotes has been shown to be a sign of recent host adaptation (Chain *et al*., 2005; Wattam *et al*., 2009; Goodhead and Darby, 2015).

On the other hand, any departure from the regular DNA sequence is referred to as a mutation. This indicates that the mutation turns an allele that is typically abundant in the population into a rare and aberrant variant (Karki *et al*., 2015). A recent study found little genetic diversity among 124 *B. abortus* strains recovered from animals in two locations in Kazakhstan (Shevtsov *et al*., 2015). Mathew *et al*. (2015) showed that Brucellosis is a disease with global public health and economic implications, effective control requires knowledge of epidemiology and strains found in a given place. This study aimed to determine *Brucella* spp. within-herd seroprevalence, identify and characterize *Brucella* strains using Multiple Loci Variable Number of Tandem Repeats Analysis (MLVA-VNTR) and investigate probable spillover to other species in a dairy herd undergoing abortions. As indicated by high within-herd incidence, pathogen isolation and abortion, *B. abortus* is circulating in this herd, with cattle serving as reservoir hosts. The low seroprevalence in sheep and goats indicates that *B. abortus* has transferred from cattle to small ruminants in the herd. This was the first time *B. abortus* biovar 3 identified from an aborted dairy cow in Tanzania. The Tanzanian genotypes appear to be related to genotypes found in

Europe, Turkey, and China, but not to the *B.abortus* biovar3 reference strain or Kenyan genotypes. These genetic variables have an impact on phenotypic characteristics. Individual genetic susceptibility is correlated with specific allele variants (polymorphisms) in a number of genes. They have the power to change how exposure to the environment and interactions between genes and the environment work (Rosero *et al*., 2016). Gene sequencing, however, continues to be the gold standard diagnostic strategy for the detection of mutations in polymerase chain reaction (PCR) amplicons with significant allelic heterogeneity (Kiehl *et al*., 2016). The arrangement of nucleic acids in polynucleotide chains ultimately serves as the code for the genetic and metabolic characteristics of terrestrial life. As a result, the ability to measure or infer such sequences is required for biological and agricultural research (Heather and Chain, 2016). Sanger sequencing is time-and money-consuming, which has led to the development of a number of sequencing techniques over the past ten years to reduce these drawbacks in large-scale operations (Kulski, 2016). The close phylogenetic relationship of *Brucella* organisms with soil-arthropod-plant-associate bacteria suggests that the common ancestor of *Brucella* evolved from opportunistic organisms close to 500 million years ago that adapted to an intracellular life probably in a cold-blooded vertebrate (Eisenberg *et al*.,2017),later on adapting to mammals (Calvo *et al*., 2009).These are soil bacteria associated with plants that may behave as opportunistic bacteria causing nosocomial infections in immunocompromised hosts (Kettaneh *et al*., 2003). Ahmed *et al*., (2017) discovered DNA of *Brucella* species in soil samples (n=1280) from nine districts in Punjab. Using a typical multiplex polymerase chain reaction, only two *Brucella* species (*B. abortus and B. melitensis*) were found (mPCR). *B.abortus* was found in three districts (33.33 %) while *B. melitensis* was found in two districts (22.22 %).The *IS711* gene was amplified from soil DNA samples using mPCR and the PCR products were

sequenced to obtain IS711 nucleotide data, *IS711* gene fragment nucleotide sequencing from soil-borne *Brucella* isolates: homology level and phylogenetic relationship, The nucleotide sequence of soil-borne *Brucella* isolates' *IS711* gene fragment was compared to isolates from other countries found in GenBank for homology and phylogenetic relationships. The neighbor-joining method was employed to create a phylogenetic tree from various sequence alignments and the findings revealed a considerable level of homology (up to 99 %). Soil-borne *Brucella* isolates from Pakistan were shown to be closely related to isolates from the United States, Iran, India, Korea, and China. Other differences between the so-called classical and non-classical *Brucella* groups, which are clustered in two phylogenetic groups, include genes coding for the O chain of lipopolysaccharide (LPS), functional flagella, phage truncated sequences, pili-like structures, and some broader metabolic alternatives (most of them non-essential for survival) (Al Dahouk *et al*., 2017). Common polymorphisms are common differences in DNA sequence in a population, alterations to DNA that occur more frequently than 1% of the time in a population, such as mutations, offer two or more equally viable possibilities (Teama, 2018). Stand-alone ONP sequencing for genomic epidemiology investigations may require further progress in order to be useful in endemic areas. According to outbreaks and regional clustering of *B. melitensis*, a gene-by-gene phylogenetic analysis of up to six allelic differences would be judged an appropriate threshold to designate clustered isolates as epidemiologically related (Janowicz *et al*., 2018). While the Office International des Epizooties (OIE) classifies Brucellosis as a multiple species disease infection and infestation (OIE, 2018). Fuks *et al*., (2018) demonstrated that sequencing the 16S rRNA gene provides the majority of knowledge regarding Earth's extraordinary microbial diversity. The use of NGS methods has increased the number of samples and the depth of sequencing, but the read length of today's most widely used sequencing platforms is quite short,

requiring the researcher to select a subset of the gene to sequence (typically 16-33% of the total length). As a result, multiple bacteria may share the same amplified area, and profiling resolution is intrinsically reduced. Platforms with ultra-long read lengths, whole-genome shotgun sequencing techniques, and computational frameworks previously proposed that all provide for alternative strategies to avoid this problem, but each has its own set of drawbacks. There is a need for a simple and low-cost *16S rRNA* gene-based profiling approach that takes advantage of the short read length to offer significantly greater coverage of the gene, allowing for high resolution even under difficult conditions of low bacterial biomass and fragmented DNA. At the genomic level, *Brucella* species share 97-99% identity. Peker *et al*., (2019) stated that for improving antimicrobial therapy, rapid and reliable detection of bacterial infections directly from patient samples is essential. Although Sanger sequencing of the 16S ribosomal RNA (rRNA) gene is used as a molecular approach, due to significant sequence homology in their 16S rRNA genes, species identification and discrimination for bacteria is not always possible. Unfortunately, sequencing every PCR amplicon reaction, especially in large-scale applications, is expensive, time-consuming and cumbersome (Hashim and Al-Shuhaib, 2019). Esmaeel, (2019) accomplished the goal by collecting 35 blood samples and 50 cow milk samples from Al-Diwaniyah Teaching Hospital and local herd areas in Al-Diwaniyah governorate, Iraq. A PCR technique was used to identify the bacteria and determine the presence of its VGs in the samples. A partial gene sequencing (PGS) technique targeting the 16S rRNA gene was also used to authenticate *B.abortus* infection in the samples. Abdel-Hamid *et al*., (2020) in his investigation, 18 *Brucella* isolates that were bacteriologically and molecularly identified as *Brucella abortus* (n=6) and *Brucella melitensis* (n=12) by AMOS-PCR were subjected to Multiple Locus Variable Number Tandem Repeat Analysis (MLVA-16). In addition to the global genotypes database, in

this study attempted to examine the genetic relationships among a few Egyptian *Brucella* genotypes identified between the years of 2002 and 2013. *B.melitensis* strains showed more marker diversity than *B. abortus*, which was primarily explained by the divergence in panel 2B markers.

Ntirandekura *et al*.,(2020) illustrated the importance of brucellosis to both public health and the livestock sector, this illness is endemic in Tanzania, and little is known about the molecular characterization of *Brucella* species in pastoral contexts. This study tried to define *Brucella* species (targeting genus *Brucella*) infecting humans, cattle, and goats in the Kagera region using real-time PCR, PCR amplification of 16S rRNA genes, and Sanger sequencing (Ngara and Karagwe districts). *Brucella* spp. was discovered using real-time PCR in 47 samples out of 125 samples. Out of 47 real-time PCR positive samples, 20 produced the predicted 16S rRNA gene PCR result. Following sequencing analysis and blasting, *Brucella* spp. were detected in pastoral areas of the Kagera region. *Brucella* spp. from Kagera were classified into two clades and three branches, and were all related to *Brucella melitensis*, *B. abortus*, and *B. suis* from the United States, Sudan, and Iran. They were distinct from other species found in the US, New Zealand, Germany, and Egypt. The distance between the geographical regions from where the phylogeny reconstruction data (nucleotide sequences from 16S gene sequencing) was acquired determined this. Esquivel *et al*., (2020) noted that *Brucellae's* 97% genomic similarity between species is one of their most remarkable characteristics. Nevertheless, the various *Brucella* species exhibit varied host preferences, zoonotic risk, and virulence. Numerous facets of the biology of *Brucella*, such as host adaptation and virulence mechanisms, remain poorly known despite 133 years of study, focusing on the connection between the genetic diversity and host preference of the various *Brucella* species is one way to approach understanding these traits.

Local *B.melitensis* genotypes studied by Abdel-Hamid *et al*., (2020) revealed that geographic proximity is not the main cause for the Egyptian *Brucella* genotypes' remarkable genetic similarity. These little alterations could have been induced by a stepwise mutational event at the most variable loci from a very limited number of ancestors, particularly during non-preference host transmission. According to Akoko *et al*., (2021), the number of *Brucella* species circulating in Kenya's various hosts was mostly unknown, restricting the use of focused management techniques. As a result, tests were carried out on Kenyan livestock populations with several hosts to detect circulating *Brucella* species and analyze evidence of host–pathogen relationships. For *Brucella* detection, all samples were examined utilizing genus-level real-time PCR assays using primers specific for IS711 and bcsp31 targets. DNA from both *B. abortus and B. melitensis* is found in humans and several animal host species, implying that these species can be transmitted from one host to another. Craddock *et al*., (2022) showed that *Brucella melitensis* is a prominent etiological agent of brucellosis and it is described utilizing sequencing techniques. The phylogenetic analysis using long-read assemblies revealed a number of inconsistencies in cluster assignment. Ashford and Whatmore, (2022) studied brucellosis, significant advances in molecular typing applied to the genus over the last few decades and molecular methods are now an important component for diagnosis , surveillance and understanding the disease's epidemiology on a local and global scale. The shift from gel-based procedures to multi-locus methodologies based on tandem repeats (MLVA) or gene sequences (MLSA) and finally, the increasing use of whole genome sequencing(WGS) approaches to assess the group's diversity in the face of future pandemics.

CHAPTER two

Materials & Methods

Chapter Two

2. Materials and Methods

2.1. Materials:

2.1.1 Equipments:

The laboratory instruments and equipment that were used in this study and their origin were listed in (**table 2-1).**

Table (2-1): List of Equipment and their Origin

Equipment & instruments	Company / Country
GPS	Garmin
Air Thermometer	Gallen Kamp/England
Soil Thermometer	Weksler/USA
pH Meter	Oakton/USA
Fume Hood	Cryste/Korea
Flame Atomic absorption Spectrophotometer	shimadzu /Japan
microwave	Panasonic\Japan
water bath	Jonalab\Korea
Autoclave	Hirayama / Japan
Ultrasonic	China
CBC device	Dymind\China
Real TM PCR (Rotor-Gene)	QiA Gene
Centrifuge	DLAB /Ghain
Sensitive balance	Sartorius /Germany
Electrophoreses	Clarivate /UK
Water Distillatory	GFL/ Germany
Plate Magnetic Stirrer, Vortex	Fisher Scientific/ USA
Thermostatic Incubator	Zxinstrument/Chain

Power Supply	Biorad/ USA
Applied Biosystems™ ProFlex™ PCR System	Fisher Scientific/ USA
UV-trans illuminator	Vilber Lourmat Sté /Farance
Nano drop	Optizen Pop – Korea

2.1.2 The Biological and Chemicals Materials

The biological and chemicals materials which used in present study together with their producing companies were listed in (**table 2 -2**).

Table (2-2): The Biological & Chemicals Materials

Chemical	**Company/Origin**
Sulphuric acid (H_2SO_4)	Chem -Lab\Belgium
Orthophosphoric acid(H_3pO_4)	BHD\England
Nitric Acid (HNO_3)	Central Drug House Ltd (CDH) \India
Perichloric acid($Hclo_4$)	Himedia\U.S.A
Horse serum	Himedia\India
Normal saline	Karada Pharma\ Iraq
Glycerol ($C_3H_8O_3$)	Merck-England
Polymxin B sulphate	Italy
Nystatin	Asia-Syria
Nalidixic acid	Flamingo pharma-(UK)
Vancomycin	Turkey
Bacitracin	Taro\U.S.A
Diphenyl amine	BDH\England
Ammonium Ferrous Sulphate	BDH\England

$[(NH_4)_2\ So_4.FeSo_4]$	
Di potassium Chromate($K_2Cr_2O_7$)	Panreac\ Spain
Agarose	Carl Roth/Germany
Primers	Macrogen \ Korea
Red safe staining solution (Simplysafe)	Intron / Korea
Loading dye	Intron / Korea
Premix master mix	Intron /Korea
100bp DNA Ladder (Markers)	Kapa /USA
TBE buffer	Condalab/Spain

2.1.3 The Commercial Kits and Culture Media

The commercial kits and culture media used in this study and their origin were listed in (**table 2-3**).

Table (2-3): list of Commercial Kits and Culture Media

Types	Company
Brucella Agar Base	Himedia\India
Brain Heart Infusion (BHI) Broth	Himedia\India
Brucella Real –TM Kit	Sacace\Italy
FavorPrep Blood/ Cultured Cells Genomic DNA Extraction Mini Kit	Intron \ Korea

2.1.4 Study Area

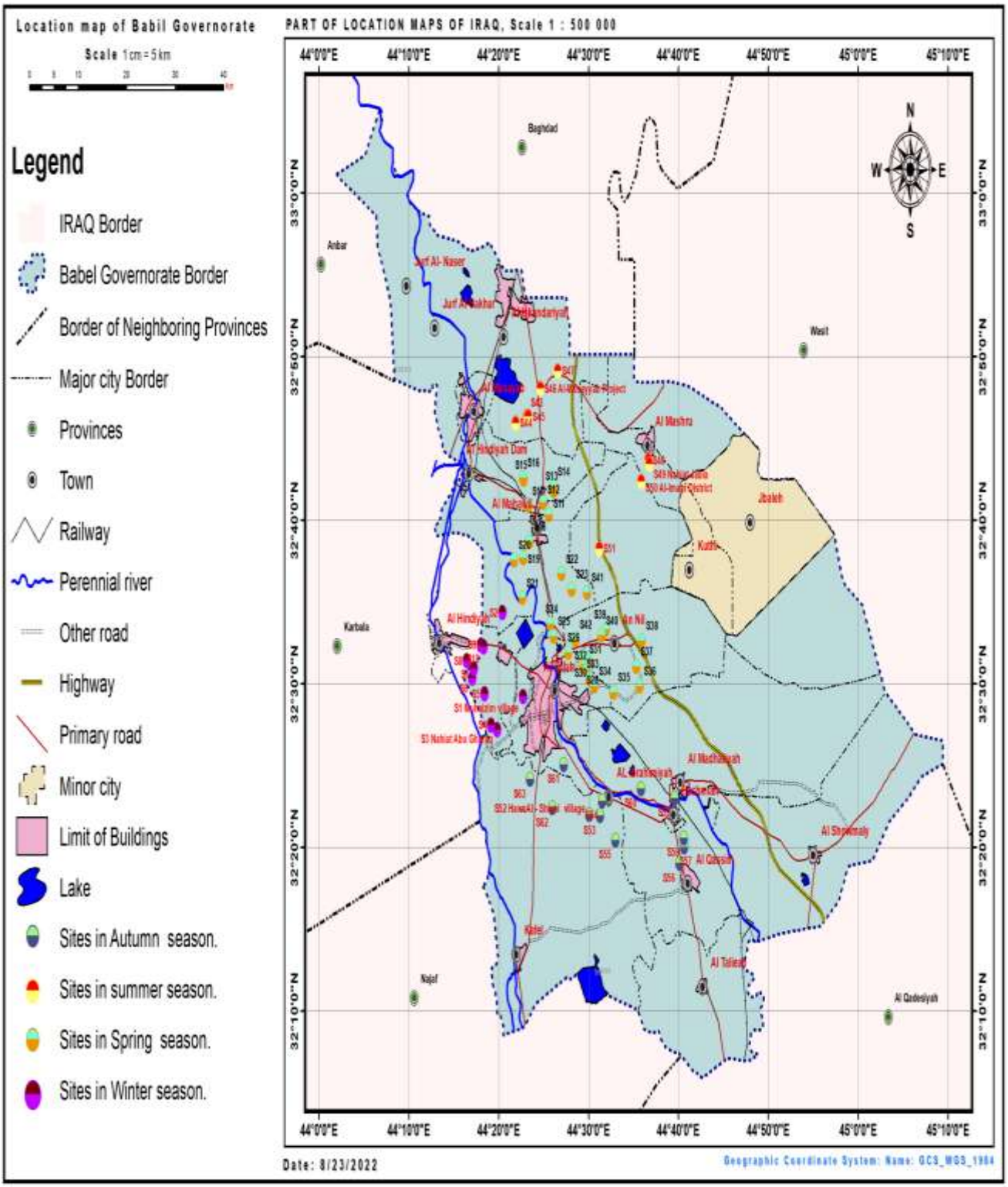
The study area included sites of different villages for the four seasons from animals soils and determination their locations by GPS in Babylon province , Hilla City, Iraq. These samples included 63 sites from north, middle and south Babylon province as following , site number ,village name and their coordinate mentioned in table(2-4) and figure(2-1) while sheep's blood samples were collected from Al-Mihnawiya village.

Table (2-4): Study Area for Different Villages and their Coordinates

Site Number	The Village Name	The Coordinates
S1	Muhaizim village	N=32^0 29.211, E=044^0 22.702
S2	Abu Gharaq Al-Awsat village ,Nahiat Abu Gharaq	N=32^0 34.333, E=044^0 20.414
S3	Bani Sala village , Nahiat Abu Gharaq	N=32^0 27.186 , E=044^0 19.841
S4	Bani Sala village, Nahiat Abu Gharaq	N=32^0 27.462 , E=044^0 19.156
S5	The end of Abu Garq Al-Awsat , Nahiat Abu Gharaq	N=32^0 29.367 , E=044^0 18.410
S6	Albuhamdan village	N=32^0 30.339, E=044^0 17.065
S7	Zughaib village , Nahiat Abu Gharaq	N=32^0 30.908, E=044^0 17.218
S8	Al-rghila village , Nahiat Abu Gharaq	N=32^0 31.397, E=044^0 16.447
S9	Al-rghila village , Nahiat Abu Gharaq	N=32^0 32.373, E=044^0 18.002
S10	Youssoufia village , Nahiat Abu Gharaq	N=32^0 32.275 , E=044^0 18.317
S11	Saidia village , Mahaweel district	N=32^0 40.355, E=044^0 25.596
S12	Bida Almahaweel village , Mahaweel district	N=32^0 41.096, E=044^0 24.944
S13	Bida Almahaweel village , Mahaweel district	N=32^0 41.819, E=044^0 26.147
S14	Bida Al-Kabeer village , Mahaweel district	N=32^0 42.086, E=044^0 26.077
S15	Tawaleb village , Nahiat Sudat Al-Hindia	N=32^0 42.493, E=044^0 22.768
S16	Tawaleb village , Nahiat Sudat Al-Hindia	N=32^0 42.622, E=044^0 22.715

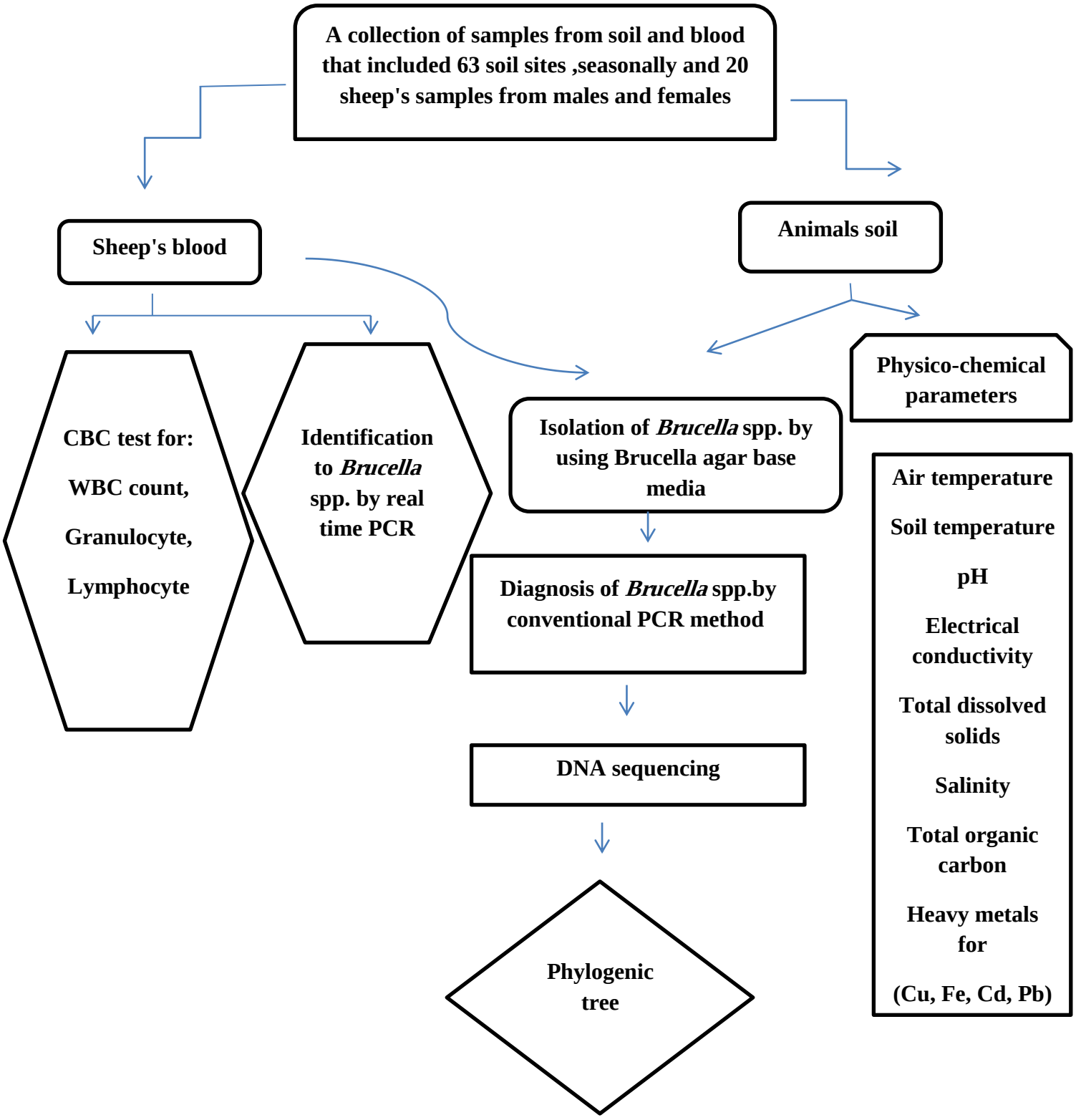
S17	Jafar Fort village , Mahaweel district	N=32^0 40.888, E=044^0 23.251
S18	Khanfara village , Mahaweel district	N=32^0 38.705 , E=044^0 23.431
S19	Albualwan village, Mahaweel district	N=32^0 37.723, E=044^0 22.738
S20	Khafaga village , Mahaweel district	N=32^0 37.585 , E=044^0 21.709
S21	Albualwan village, Mahaweel district	N=32^0 35.263 , E=044^0 22.630
S22	Al-Khatounia village ,Mahaweel district	N=32^0 36.732 , E=044^0 26.995
S23	Al-Fendia , Haji Kazem , village , Mahaweel district	N=32^0 35.798 , E=044^0 28.128
S24	Queresh area ,the ruins of Babylon	N=32^0 33.676 , E=044^0 25.709
S25	The ruins of Babylon	N=32^0 32.920 , E=044^0 26.098
S26	Al-Bujaj village, Hilla center	N=32^0 31.931 , E=044^0 27.739
S27	Nahiat Alwardi	N=32^0 31.488 , E=044^0 29.207
S28	Al-Busltan village , Alwardia Kharji , Nahiat Alwardi	N=32^0 31.089, E=044^0 29.364
S29	Al-Zariga village , Nahiat Alwardi	N=32^0 31.360 , E=044^0 29.614
S30	Al-Zardi village , Nahiat Alwardi	N=32^0 31.564 , E=044^0 29.998
S31	S31 Alwardia Kharji , Nahiat Alwardi	N=32^0 31.202 , E=044^0 29.607
S32	Al-Zariga village , Alwardia Kharji , Nahiat Alwardi	N=32^0 30.839 , E=044^0 29.846
S33	Alwardia Kharji Nahiat Alwardi	N=32^0 30.313 , E=044^0 30.119
S34	Zalli village , Alwardia Kharji , Nahiat Alwardi	N=32^0 29.868 , E=044^0 30.669
S35	Albutif village, Alwardia Kharji Nahiat Alwardi	N=32^0 29.489 , E=044^0 32.799
S36	Albutif village , Alwardia Kharji Nahiat Alwardi	N=32^0 29.865 , E=044^0 35.685
S37	Albutif village , Alwardia Kharji Nahiat Alwardi	N=32^0 31.060 , E=044^0 35.317
S38	Kish village , Nahiat Alniyl	N=32^0 32.644 , E=044^0 35.913
S39	Nahiat Alniyl Center	N=32^0 33.326 , E=044^0 31.915
S40	Al -Maamra , Ishakah Al-Fayhan village , Nahiat Alniyl	N=32^0 32.975, E=044^0 31.461

S41	Shehib Hamad village , Nahiat Alniyl	N=32^0 35.588 , E=044^0 29.836
S42	Al-Kuseirat village , Nahiat Alniyl	N=32^0 32.717 , E=044^0 28.533
S43	Ibn Al-Kaddim village , Al-Musayyab	N=32^0 46.311 , E=044^0 23.106
S44	Albu jassim village , Al-Musayyab	N=32^0 45.933 , E=044^0 21.896
S45	Albu Jassim village , Al-Musayyab	N=32^0 46.331 , E=044^0 23.307
S46	Tunisia village , Al-Musayyab Project	N=32^0 48.044 , E=044^0 24.620
S47	Muwailiha village , Al-Musayyab Project	N=32^0 49.124 , E=044^0 26.597
S48	Khirbana village, Nahiat Jabla	N=32^0 43.661 , E=044^0 36.657
S49	Khirbana village, Nahiat Jabla	N=32^0 43.428 , E=044^0 36.755
S50	Bani Zaid village , Al-Imam District	N=$32^0$42.364, E=044^0 35.884
S51	Al-Imam District	N=32^0 38.266 , E=044^0 31.197
S52	Hawa Al-Shami village, Al-Hilla Center	N=32^0 22.004 , E=044^0 30.068
S53	AbuDabaa village, Nahiat aldabla	N=32^0 21.934 , E=044^0 31.285
S54	AbuDabaa village, Nahiat aldabla	N=32^0 22.839 , E=044^0 31.515
S55	Al-Nazim village, Nahiat Al-Qasim	N=32^0 20.430 , E=$044^0$32.998
S56	Doro'village , Nahiat Al-Qasim	N=32^0 19.018 , E=044^0 40.129
S57	Al-Agilat village, Nahiat Al-Qasim	N=32^0 20.031 , E=044^0 40.634
S58	Abu Lahima village, Nahiat Al-Qasim	N=32^0 20.582 , E=044^0 40.595
S59	Umhairija village , Al-Hashmiyah district	N=32^0 23.005 , E=044^0 39.568
S60	Imashmish village , Nahiat Al-Hamza Al gharbiu	N=32^0 23.595 , E=044^0 35.841
S61	Iglis village , Al-Hilla Center	N=32^0 25.063 , E=044^0 27.243
S62	Ibn Al-Kadhim village, Nahiat Al-Kifil	N=32^0 22.405 , E=044^0 25.974
S63	Karim Al-Rawi village , Al-Hilla Center	N=32^0 24.159 , E=044^0 23.485



Figure(2-1): Location Map of Babylon Province, Hilla City .

2.1.5 Study Design



2.1.6 The Samples Collection

The samples collection consist of two parts :

2.1.6.1 Soil Samples

The animals soil samples collected seasonally from February to November, 2021 were taken the samples from the upper layer for bacteriological study and from the deepest layer (25-30 cm in depth) for environmental study, dried by air and sieved (to ~ 2 mm particle size) during the collecting the samples measured air temperature and soil temperature and determination the sites by GPS, Figure(2-2).

Figure(2-2): Studied Collected Sites of the Animals Soil in Babylon Province

2.1.6.2 Blood Samples

The whole blood samples were collected from jugular vein of the sheep and putting into polyethylene EDTA tubes and then transferred to the laboratory using special container with ice directly for CBC test and then were kept at -20 C^0 until using for bacteriological and molecular analysis .

2.2. Methods:

2.2.1 Environmental Measurement

2.2.1.1 Physical and Chemical Parameters

Air temperature was measured by a simple thermometer (0^0C-100^0C)and soil temperature by soil thermometer in the field directly. While pH, Electrical Conductivity(EC), Total Dissolved Solid(TDS), Salinity were measured by multi-parameters.

2.2.1.2 TOC (Total Organic Carbon)

The wet oxidation technique was utilized exothermic heating and oxidation of organic carbon of the sample with potassium dichromate and concentrated H2SO4 and the titration of excess dichromate with 0.5N ferrous ammonium sulphate solution to a sharp one drop end point. In this procedure was taken 10 ml of 1 N K2Cr2O7 solution and added to 0.2 to 0.5 g dried sample in a 500 ml Erlenmeyer flask and are then mixed by swirling. After that, Twenty (20) ml of concentrated H2SO4 was added and mixed gently. The mixture was allowed for 30 minutes after is diluted to 200 ml volume of distilled water and 10 ml of 85% H3PO4, and then was added 15 drops of diphenylamine indicator. The solution was back titrated with 0.5 N ferrous solution (Gaudette *et al*., 1974).

2.2.1.3 Heavy Metals

Soils samples for heavy metals determination were digested according to the procedure described Sharidah (1999). One gram of dried soil samples were digested with (10ml) di-acid mixture (9ml HNO_3 : 4ml $HCLO_4$) , the mixture was boiling gently in sandy bath on hot plate until release fumes, after cooling and filtering through whatman No.42 filter paper and <0.45µm Millipore filter paper and transferred quantitatively to 25 volumetric flask by adding distilled water and the concentration of (Cu, Fe, Cd, Pb) were then determined with by Flame Atomic absorption - spectrophotometer (Type Aa 7000),Shimdzu /Japan was used to determine the concentrations (mg/L) of the elements (APHA, 1985).

2.2.2 Microbiological Study:

2.2.2.1 Preparation of Culture Media:

Culture media used in this study were prepared according to the manufacturer's instructions:

2.2.2.1.1 Brucella Agar Base

It was prepared according to Himedia manufacturing company (India) to Isolation and detection of *Brucella* spp:

1- Suspend(21.55gm) in (500ml) D.W , heating to boiling to dissolve the medium completely . Sterilize by autoclaving at (15^0C) Ibs pressure (121^0C) for 15 minutes .

2- Cool to (45-50^0C) and aseptically add sterile (5%) v\v inactivated horse serum (RM 1239, inactivated by heating at (56^0C) for (30 minutes) and then add the antibiotics (Polymxin B sulphate ,Vancomycin, Bacitracin, Nystatin, Nalidixic acid, Cycloheximide) as supplement .

2.2.2.1.2 Brain Heart Infusion Broth (BHI)

This media were prepared according to the manufacturer's instruction. It was used for preservation of bacterial isolates as stock for long time (Forbes *et al*., 2007) .

2.2.2.2 Bacterial isolation from soil and blood samples:

2.2.2.2.1 *Brucella* isolation from soil samples

After samples collection in sterile bags from sites taken (1gm) from soil's sample and put in plain tube completed the size to (5ml) by normal saline then mixed by vortex and leave to settle after that incubation for (15) minute in Incubator. Two hundred (200 µl) were taken from mixing by micropipette and published in petridishs where all samples subcultured in selective Brucella agar base media with Polymyxin B (2,500IU), Vancomycin(10.0mg), Bacitracin (12,500IU), Cycloheximide(50.0mg), Nystatin(50,000 IU) and Nalidixic acid (2.5mg) and then5% of inactivated horse serum. Bacterial cultures were incubated

for 14 days at (37°C) and 10% carbon dioxide until appearance of growth(Alton *et al*., 1988;Dadar *et al*., 2019; Dadar and Alamian , 2021).

2.2.2.2.2 *Brucella* isolation from blood samples

1- Blood samples was mixed by vortex

2- These samples were taken from it 1ml and put in screw cap bottles contain 4ml of brain heart infusion and then added horse serum to mixing.

3-The samples were incubated for (21 days) for appearance turbidity

4- After that ,the samples were subcultured on brucella agar base medium by taking (100µl) from cultured broth by micropipette and published in petridishs and leaved in incubator for (14 days)until appearance of growth (Forbes *et al*., 2007).

2.2.3 Molecular and Blood Study:

Blood samples transferred to laboratory directly for identification of infection and then saved in freezer to bacteriological and molecular tests .

2.2.3.1 Blood tests

All samples measured by automatic (Count blood cell) CBC device to diagnosis of the infection in sheep 's blood and after that was identified *Brucella* species by real time PCR.

2.2.3.2 Molecular Study

Molecular diagnostic methods are also currently being used for the detection of *Brucella* spp. in various samples (Şahin *et al*., 2008).

2.2.3.2.1 Genomic DNA Extraction:

2.2.3.2.1.1 Bacterial DNA Extraction:

Genomic DNA extracted from bacterial isolates cultured from the soil and blood according to the manufacturer's protocol FavorPrep^{TM} Blood/ Cultured Cells Genomic DNA Extraction Mini Kit as following :

Protocol

Step 1. Cell lysis

1- the appropriate number of cell (up to 5 x 10^9) was transferred to a 1.5 ml microcentrifuge tube contained 200µl normal saline and then centrifuge at

14000 rpm for1min and remove the supernatant carefully and completely.

2- If RNA-free genomic DNA was required, 5µl of 100 mg/ml RNase would be added to the sample and was incubated for 2 minutes at room temperature .
3- FATG Buffer was added from it 200 µl to the sample and mix by vortexing. or pipetting Incubate in room temperature for 5 min to lyse the sample.
4- FABG Buffer was added from it 200 µl to the sample and mix thoroughly by vortexing by 5 sec.
5- The samples were Incubated at 70 ºC for 10 minutes to Lysate.
6- Preheat required Elution Buffer in a 70 ºC water bath for DNA Elution.

Step 2. DNA Binding

7- Ethanol (96~100%) was added from it 200 µl to the sample and mixed by vortex for 10 sec. the sample to mix well by if there is any precipitate formed.
8- FABG Column was placed to a collection tube and was transferred the sample mixture carefully to FABG Column. Centrifuge at speed 14,000 rpm for 1 min. the collection tube was discarded and then the FABG Column was placed to a new collection tube.

Step 3. Column Washing

9. W1 Buffer was added 400 µl to the FABG Column and centrifuged for 30 sec at speed 14,000 rpm and then discarded the flow-through and placed the FABG Column back to the collection tube.

10. Wash Buffer was added 600 µl to the FABG Column and centrifuged for 30 sec at speed 14,000 rpm. And then discarded the flow-through and placed the FABG column back to the collection tube. (Ethanol was added to Wash Buffer when first open) .

11. The samples was centrifuged for an additional 3 min at speed 14,000 rpm to dry the column. (This step will avoid the residual liquid to inhibit subsequent enzymatic reactions).

Step 4. DNA Elution

12. The dry FABG Column was placed to a new 1.5 ml microcentrifuge tube.

13. Preheated Elution Buffer was added 100 µl to the membrane center of FABG Column. (For effective elution, make sure that the elution solution is dispensed onto the membrane center and absorbed completely).

14. The FAGB Column was incubated at 37 ºC for 10 min in an incubator.

(Standard volume for elution is 200 µl , and if less sample volume (sample has low number of cells), reduce the elution volume (100µl) to increase DNA concentration, then If higher DNA yield was required, DNA elution step was repeated to increase DNA recovery and the total elution volume to approximately 200 µl).

15. The samples were centrifuged for 1min at full speed 14,000 rpm to elute the DNA and store the DNA fragment at (-20ºC).

2.2.3.2.1.2 Extraction DNA from Blood for Real-TM PCR

Genomic DNA from sheep's blood samples were extracted using FavorPrep^{TM} Blood/ Cultured Cells Genomic DNA Extraction Mini Kit according to manufacturer's protocol as following :

Step 1. RBC lysis

1- Blood sample (frozen) 200µl was transferred into a clean 1.5 ml microcentrifuge tube. If the sample volume is less than 200 µl , the appropriate volume of PBS was added to complete the volume.

2- A Proteinase K (10 mg/ml) about 20 µl and FABG Buffer about 200 µl were added to the sample of blood and mixed thoroughly by pulse-vortexing (Proteinase K do not add directly to FABG Buffer) then the mixture was incubated at 60ºC for 15 min to lyse the sample (During incubation vortex the sample every 3 minutes).

2- Briefly spin the tube to remove drops from the inside of the lid.

The rest of steps include DNA Binding, Washing, DNA Elution was same the steps of DNA extraction.

2.2.3.2.2 Measurement of Concentration and Purity of Extracted DNA.

The extracted DNA was checked by using Nanodrop, which measured DNA concentration (ng/µl) and check the DNA purity by reading the absorbance at (260 /280 nm) as following steps:

1. After opening up the Nanodrop software, choose the appropriate application (Nucleic acid, DNA).
2. A dry paper-wipe was taken and cleaned the measurement pedestals several times. Then carefully pipet 2µl of ddH2O onto the surface of the lower measurement pedestals for blank the system.
3. The sampling arm was lowered and clicking OK to initialized the Nanodrop, then cleaning off the pedestals and 1µl of extracted DNA carefully pipet onto the surface of the lower measurement pedestals, then check the concentration and purity of extracted DNA (Krebs *et al* ., 2009) .

2.2.3.2.3 Gel Electrophoresis to Analyze DNA Quality:-

1- An agarose solution was prepared by dissolve (1g) of agarose powder in (100) ml of (1x TBE) in the flask, agarose was melted in hot plate stirrer until the solution became clear.

2- The agarose solution was made cool to about (50^0C), swirling the flask occasionally to cool evenly.

3- Red stain (3µl) was added to the warm gel then sealed the ends of the casting tray.

4- The combs were placed in the gel-casting tray.

5- Melted agarose solution was poured into the casting tray.

6- The agarose was allowed to solidify at room temperature, the comb pulled out carefully. The gel was placed onto the electrophoresis chamber that was filled with TBE (1x) buffer.

7- DNA samples (5µl) were mixed with (3µl) DNA loading buffer and loaded in agarose gel wells.

8- The agarose gel electrophoresis was completed at 70V, 65Amp for 1hour ,

DNA moves from the negative side (cathode) to the positive side (anode).The DNA was observed by viewed under UV transilluminator. The stained bands in gel were visualized using Gel imaging (Sambrook and Russel , 2006) .

2.2.3.2.4 Primer Solution Preparation :

A primer working solution was prepared from the lyophilized primers after dissolving in nuclease free water(n.f.w), according to the manufactured company mentioned in table (2-2) to make a stock solution with a concentration of (100 Pmol/µl) for each primers and stored at (-20°C). A working solution from concentration of (100 Pmol/µl) was prepared by diluting (10µL) of primers stock solution in (90 µL) of nuclease free water to reach a final volume (100 µl) and stored at (-20°C) as mentioned in table (2-5) (Sambrook *et al*., 2012).

Table(2-5):Reaction Components and Volume for PCR from Bacteria Isolated from the Soil and Blood

Component	Final volume (25 µl)
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	1.5 µl
Nuclease-free Water	16.5 µl

2.2.3.2.5 Real time PCR for Blood Samples :

This method used for primary detection of *Brucella* species in the blood samples by Real Time PCR for qualitative detection of *Brucella* species, (Brucella Real -TM). Reaction components and the optimum condition of detection run according to leaflet's instrument as mentioned in (table 2-6)and (table 2-7).

Table (2-6):Reaction Components and Volume for Real Time PCR

Component	**Volume reaction (20 µl)**
2×Rotor .Gene SYBR Green PCR Master Mix	12.5 µl
Primer A	1 µl
Primer B	1 µl
Nuclease-free Water	Variable (2.5 µl)
DNA	Variable (3 µl)

Table (2-7):The Optimum Condition of Detection

PCR Program			
	Temperature ^{0}C	Time	Cycle
PCR initial activation step	95 ^{0}C	5 min	1 Cycle
Two step cycling			40 Cycles
Denaturation	95 ^{0}C	5 sec	
Combined annealing\Extension	60 ^{0}C	10 sec	

2.2.3.2.6 Conventional PCR for Bacteria Extracted from Soil and Blood

Conventional PCR were used to amplify the target bacterial DNA using specific primer pairs. It includes three consecutive steps that repeated for specific number of cycles to get PCR product which can be finally visualized after agarose gel electrophoresis. The primer sequence, PCR product size and thermal cycling conditions mentioned in (table 2-8) and (table 2-9).

Table (2-8): The Sequence of Primers that Used this Study

Primer	Sequence	Primer sequence	Tm (°C)	GC %	Size of Product (bp)
***16s rRNA* bacterial primers**	**27F**	**5'- AGAGTTTGATCCTGGCTCAG- 3'**	**54.3**	**50.0**	**1250** Srinivasan *et al.*, (2015)
	1392R	**5'- GGTTACCTTGTTACGACTT- 3'**	**49.4**	**42.1**	

Table (2-9) The Optimum Conditions for Detection the Bacterial Isolates from Soil and Blood (Stages and Temperature of PCR for *16s rRNA* gene)

		Temperature ^{0}C	Time	cycle
Stage 1	Initial Denaturation	95ºC	5 min	1
Stage 2	Denaturation	95ºC	45 sec	35
	annealing	56ºC	45 sec	
	Extension	72ºC	1 min	
Stage 3	Final Extension	72ºC	5 min	1

2.2.3.2.7 16S rRNA Sequence Analysis and Phylogenetic Tree

Sequencing method was performed for study of genetic changes and phylogenetic tree draw of *16SrRNA* gene in some local *Brucella* isolates by comparing with NCBI-GenBank *Brucella* isolates.

The sequencing of the *16SrRNA* gene were done after assurance in presence amplification of PCR products for required volume; These products were sent to company (Macrogen) in Korea for performing Sanger sequence , after getting on nitrogenous bases sequence for *16S rRNA* gene amplified products of *Brucella* isolates. This sequence analyzed by NCBI-blast programme for purpose compared

homology or diversity degree to local *Brucella* isolates with the world isolates recorded in NCBI-GenBank.

Also phylogenetic analysis for draw of phylogenetic tree and determination phylogenetic relationship used (MEGA 6) programme to compare local one strain with strains all of the world states.

2.2.3.2.8 Recording of Iraqi *Brucella* isolates in gene bank –NCBI

Sequences of *Brucella* isolates were isolated from animals soils and sheep's blood sources in Al-Hilla city\Iraq and each sequences have variations.

2.2.4 Biosafety and Hazard Material Disposing:

Biosafety aspects followed during the work include disposing of all swabs, petri dishes and all contaminated supplies by autoclaving and then incineration. All benches cleaned with alcohol before and after the work. Eco Safe dye was used alternately ethidium bromide**.**

2.2.5 Statistical Analysis:

Data was analyzed using SPSS(version 23, SPSS Inc. Chicago, Illinois, USA). Descriptive statistics (mean, standard Error, standard deviation), and differences were compared by One-way ANOVA at $p \leq 0.05$ using Duncan's test. While statistical analysis was carried out using t-test student for comparing between two groups. Whereas data analysis for *Brucella* species(%) and environmental factors by multivariate analysis of ecological data using Canonical Correspondence Analysis (CCA).

CHAPTER THREE

Results

&

Discussion

Chapter Three

3. Results and Discussion:

3.1. Bacterial and Molecular Study:

3.1.1 Presence of *Brucella* spp. in Soil and Blood Samples

3.1.1.1 Soil Samples

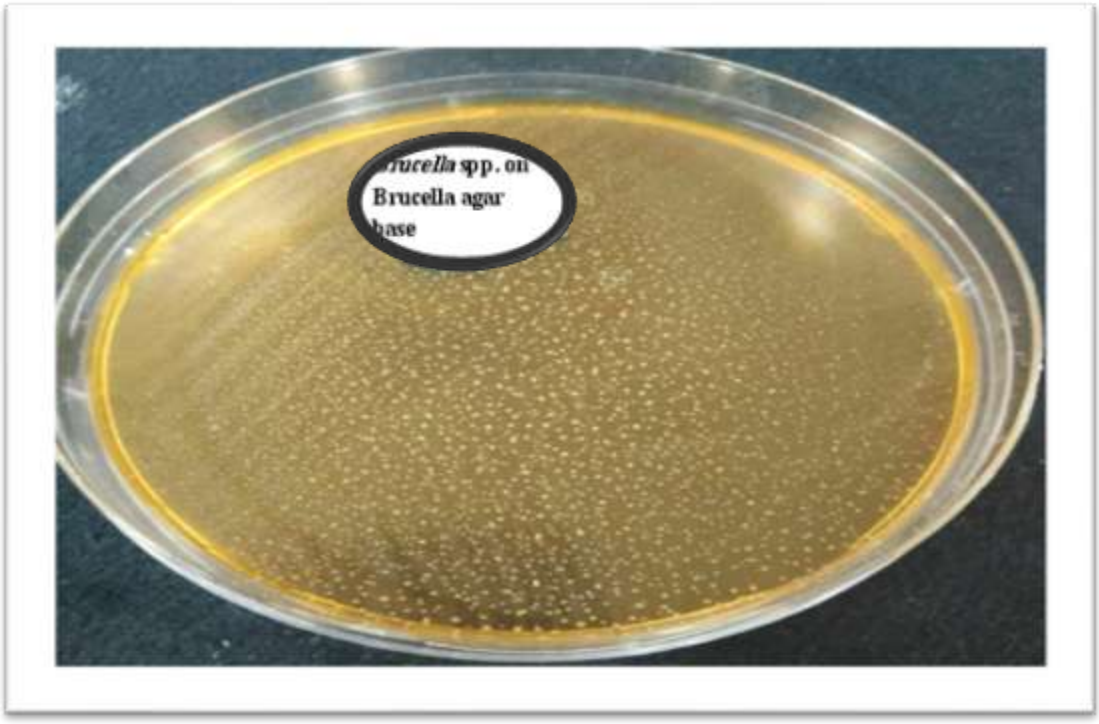
The current study results showed that *Brucella* spp. was found in all seasons, with 6 positive samples out of 10 samples in the winter season; 25 positive samples out of 32 samples in the spring season; 9 positive samples out of 9 samples in the summer season; and 11 positive samples out of 12 samples in the autumn season as mentioned in table (3-1).The samples were cultured on *Brucella* Agar Base in figure (3-1) .

Table (3-1):The *Brucella* Percentage for the Four Seasons

Samples number / Seasons	The total number of samples	The positive number of samples	*Brucella* percentage (%)
Winter	**10**	**6**	**60**
Spring	**32**	**25**	**78.13**
Summer	**9**	**9**	**100**
Autumn	**12**	**11**	**91**

The most important confirmatory method for *Brucella* infection is bacteriological diagnosis, which is recognized as the gold standard diagnostic method because of its high specificity when compared to other diagnostic procedures. It is critical to understand that there are many *Brucella* biotypes within the *Brucella* spp. in order to confirm an infection and pinpoint the source of the infection (Leyla *et al*., 2003). *Brucella* spp. is also diagnosed using molecular, bacteriological, allergic reaction, and serological approaches (Simsek *et al*., 2004). *Brucella* isolation and identification of the etiological agent's

biotypes are required steps in designing epidemiology and eradication strategies (Zinstag *et al*., 2005).



Figure(3-1): Colonies of *Brucella* on Brucella agar base media incubated at 37^0C for 14 days appeared round with smooth margins, round edges, translucent and golden color .

A microbiological study is necessary for detecting cases of *Brucella* spp. because the symptoms are varied and non-specific, as shown by Bonaventura *et al*.,(2021). The foundation of laboratory diagnosis is comprised of three separate microbiological techniques:1- direct diagnosis by culture, 2- indirect diagnosis by serological testing, and 3- direct rapid diagnosis utilizing PCR-based techniques. A culture is still regarded as the "gold standard" in the laboratory diagnosis of brucellosis due to its clinical and epidemiological importance, despite the established experience with serological tests and extremely sensitive nucleic acid amplification tests (NAATs). Bacteriological and molecular diagnostics should always be used in addition to *Brucella* species diagnosis in herds at risk (Marianelli *et al*., 2008). AL-tememy *et al*., (2013) showed that these isolates were *Brucella* based on the shape of the bacterial colonies, gram stain, and

biochemical testing. High levels of bacterial isolates were found in the ALmanathera (44%), Alshabaka (30%), and Kuzweenah (26%) areas of AL-Najaf, which indicate that *Brucella melitensis* is widely distributed in these areas of AL-Najaf. *B.melitensis* coincidenced with PCR results, all collected samples recorded from aborted ewes that showed positive test expressed positive *Brucella* isolates. This evidence corroborated the findings of Şahin,(2008). Brucellosis must be correctly and swiftly recognized for both health and financial reasons. Isolation of *Brucellae* from tissues and milk samples is the only way to definitively diagnose bovine brucellosis. However, making a diagnosis based solely on culture is not always practicable, especially in the outdoors or in slaughterhouses. Several serological tests were developed and used as an alternative. Each test has distinct advantages and disadvantages that influence how commonly it is utilized (Poester *et al*., 2010). *Brucella* isolation and identification remains the gold standard for diagnosis and it is recognized as a critical tool for ensuring the flock's health (OIE, 2012). Each isolate had colonies that were convex, transparent, and smooth. The luster of the surface revealed a honey color in transmitted light. Previously, the standard biotyping approach was employed to characterize *Brucella* isolates (Dadar *et al*., 2019).

3.1.1.2 Blood samples

3.1.1.2.1 Blood detection:

The results from blood samples revealed infection all samples by CBC device.

Table (3-2):The Relationship between the Blood Parameters with the Normal Value in Sheep's Blood (Mean±S.E) . p≤0.05

Parameters	Mean±S.E	Normal value	p-value
WBCs count (10_3\ µl)	8.69±1.01	10	0.122
Lymphocytes (%)	92.72±0.68	4	≤0.0001
Granulocytes (%)	3.24±0.51	27	0.067

This table (3-2), sig<0.05 in lymphocytes refers to the occurrence of infection while no (sig >0.05)in both WBC and neutrophils. This indicates the presence of infection in all samples of blood.

The current study's findings (3-2) corroborated Jiao *et al*., (2015) findings that WBC, neutrophils (NE), eosinophils (EO) and neutrophils percent were lowered, but lymphocytes (LY) and lymphocytes percent were elevated. This difference exhibits statistical significance (P=0.05). In 90.73% (137/151) of Brucellosis cases, the white blood cell count is normal or lower. When an animal is exposed to toxins, blood can be used as a pathological indicator of its health and other issues (Olafedehan *et al*., 2010). An opportunity to examine the presence of many metabolites and other elements in an animal's body is offered by blood analysis. The physiological, dietary, and pathological status of an organism are all critically impacted by it as well (Doyle, 2006). An increase in lymphocytes and a lack of white blood cells count and granulocytes indicate the presence of the causative factor in the animal (sheep)and the causative factor is cause of the infection. This causative agent is either a virus or a bacterium. In this study, the causative agent was *Brucella* bacteria, which attacked lymphocytes when they entered the body . In real time PCR, *Brucella* spp. was found in all twenty samples, two male and eighteen female. According to Olafedehan *et al*., (2010), dissecting the blood's constituent parts can reveal important information for the diagnosis and prognosis of illnesses in animals. Physiological health problems and blood composition change (Togun *et al*., 2007). The response of animals to various physiological situations can be predicted using these modifications (Khan and Zafar, 2005). The study of the quantities and morphology of the cellular constituents of the blood-the red cells (erythrocytes), white cells (leucocytes), and platelets (thrombocytes)-and the use of these data in the diagnosis and monitoring of disease is referred to as haematology (Merck, 2012). Hematological studies can help with the identification of numerous disorders as well as the assessment of the extent of

blood damage (Togun *et al*., 2007). Afolabi *et al*., (2011) indicated that the variations in haematological markers are widely used to distinguish between the many states of the body and to pinpoint pressures brought on by environmental, nutritional, and pathological variables. It has been discovered that both genetic and non-genetic factors influence the hemological traits of farm animals (Xie *et al*., 2013). Several factors, including physiological (Alodan and Mashaly, 1999), environmental (Graczyk *et al*., 2003), dietary (Kurtoğlu *et al*., 2005), age (Alodan & Mashaly, 1999) and circumstances (Seiser *et al*., 2000). According to Addass *et al*., (2012), a number of factors, including age, nutrition, health, amount of physical activity, sex, and environmental effects, affect animal blood values. Daramola *et al*.,(2005) claim that the sex and age of farm animals affect many haematological factors. In situations of *Brucella* infection, the lymphocyte/granulocyte ratios in the blood may range from 80/15% to 90/9% depending on the kind and breed of the animal (Grillo *et al*., 2012 ; Singh *et al*., 2013). An increase in acute-phase reactants is associated with the brucellosis-related inflammatory process (Bozkurt *et al*., 2014). Neutrophils and lymphocytes were crucial for the inflammation process, Physiological immune responses were affected by changes in neutrophils and lymphocytes. According to studies, compared to control groups. Numerous studies have shown that infections, especially bacterial infections that caused an inflammatory responses (Skendros and Boura, 2013). When the intracellular cycle of the organism ends within the vacuoles of the autophagocytic apparatus, a competent *Brucella* bacterium that is prepared to infect leukocytes and other cells is liberated (Starr *et al*., 2012). White blood cells, neutrophils, and eosinophils are significantly reduced in Brucellosis, whereas lymphocytes are significantly increased. When compared to the healthy control group, both an increase in lymphocyte percentage and a decrease in eosinophils show a significant difference. It demonstrates that brucellosis in blood could be distinguished in this infection from other bacterial infections.

Following a more thorough examination of the blood cell classification for brucellosis, it was discovered that the majority of cases had normal or decreased white blood cell proportions, normal or decreased neutrophil proportions, and increased lymphocyte percentages (Solmaz *et al*., 2014; Köse *et al*., 2014). In pastoral areas, the infectious disease brucellosis was common and can be transmitted from animals to humans. However, sporadic brucellosis cases have recently grown in non-endemic areas. The large-scale epidemic was the new trend in occurrence, which was formerly multiple and irregular (Cui,2014). A range of hematological and inflammatory markers have garnered significant attention as indicators of bacterial infections in recent years, and there is a wealth of evidence to support their utility in the early diagnosis of infections (Markanday,2015; Akya *et al*., 2019). *Brucella* may thrive in phagocytic cells such as neutrophils and macrophages because it is an intracellular bacterium (Santiago *et al*., 2019). Brucellosis was commonly accompanied by inflammatory signs. After infection, *Brucella* spreads to the lymph nodes and then to the bloodstream, causing systemic disease (Bozdemir *et al*., 2017). The WBC count in brucellosis may act as a marker for leukocytosis (Togan *et al*., 2015). Neutrophils and lymphocytes have a substantial impact on inflammatory processes (Skendros and Boura, 2013). The interaction between the pathogen and the host immune system was critical in combating bacteria or establishing parasitism inside an erythrocyte and remaining there without reproducing. This will shield the pathogen from detection by the immune system and medications (Vitry *et al*., 2014). The primary functions of white blood cells and their variants were to combat infections, guard the body from invasion by foreign organisms via phagocytosis and to generate or at the very least, transport and distribute antibodies during immune response. Animals with low white blood cell counts are more susceptible to disease infection, whereas those with high counts can produce antibodies during the phagocytosis process and have a high level of disease resistance (Soetan *et al*., 2013), which

improves adaptability to the environment and the prevalence of diseases in a given area (Iwuji and Herbert, 2012; Isaac *et al*., 2013).

Afolabi *et al*. (2011) claim that age, sex, breed of animals, geographic location ,climate, season, day length, time of day, nutritional state, species' life behaviors and present health were all factors that could influence an animal's hematological values. in addition to physiological and environmental factors such as age of the animal, method of breeding of animal, pregnancy and parturition, genetics, housing, feeding, extreme climatic conditions, stress, transport and diseases. According to Chineke *et al*., (2006), differences in hematological indices can be linked to dietary, environmental, and hormonal factors. These variables can also include genotype, age, and gender. *B. melitensis* affects both animals and humans, causing significant economic losses mostly due to miscarriages, stillbirths, and reproductive abnormalities in animals, as well as being potentially dangerous to animal handlers. granulocytes rose immediately, while lymphocytes increased significantly afterwards. Although the cells primarily involved in *Brucella* clearance from infected cells that rose immediately upon exposure, the overall cell percentage remained nearly unchanged. Only the total number of lymphocytes and granulocytes could be utilized as haematological indicators to determine the status of infection in animals (Kumar *et al*., 2017). *Brucella* infection raised intracellular calcium levels, which was essential for *Brucella* intracellular survival in macrophages (Cui *et al*., 2014). It may be advantageous to undertake haematological investigations, which were of ecological and physiological interest in understanding the relationship between blood properties and the environment, in order to choose animals that were genetically resistant to environmental conditions and specific diseases (Ovuru & Ekweozor, 2004 ; Akpan & Ekaette 2013). *Brucella* Invasion mechanisms necessitate the presence of virulence factors (Verri *et al*., 2001). The impact of hematological parameters and hemoglobin types on sheep adaption and reproduction that hematological

markers can be used to detect adaptability and physiological features in these animals. The ability of each type and breed of sheep to adapt to diverse environmental conditions has been critical to their reproduction. Furthermore, hemoglobin types have been linked to sheep's environmental adaptation and physiological features (Al-Thuwaini, 2021).

After internalization, *Brucella* were exposed to harsh and diverse environmental conditions. For its adaptability, the pathogen developed various methods, such as evasion and resistance to intracellular host defense mechanisms, which were predicted by specific structural components or the presence of virulence factors. Survival and reproduction of brucellosis were mostly dependent on macrophages, dendritic cells, and placental trophoblasts (Copin *et al*., 2012). *Brucella* is stopped at the G1 stage of growth and replication resumes once it enters the intracellular compartments (De Bolle *et al*., 2015). It is similar to Bruce Lee in the film Game of Death in that winning means survival while losing means death (Yu *et al*., 2022).The virulence of the bacteria, which results in chronic disease, is connected with its capacity to enter and multiply within cells. In order to prevent lymphocytes from presenting the antigen, *Brucella* uses a lipopolysaccharide defense mechanism, which results in an accumulation of bacteria within phagocytes (Smith, 2018). When Raj *et al*., (2017) studied infected cattle in India, neutrophil levels dropped by 30%. In their study found an increasing in lymphocytes reaching 51% in infected cattle in India. Brucellosis in small ruminants is caused by *B. melitensis*, which is a frequent zoonotic disease in many sheep and goat-producing countries (WAHIS, 2018).

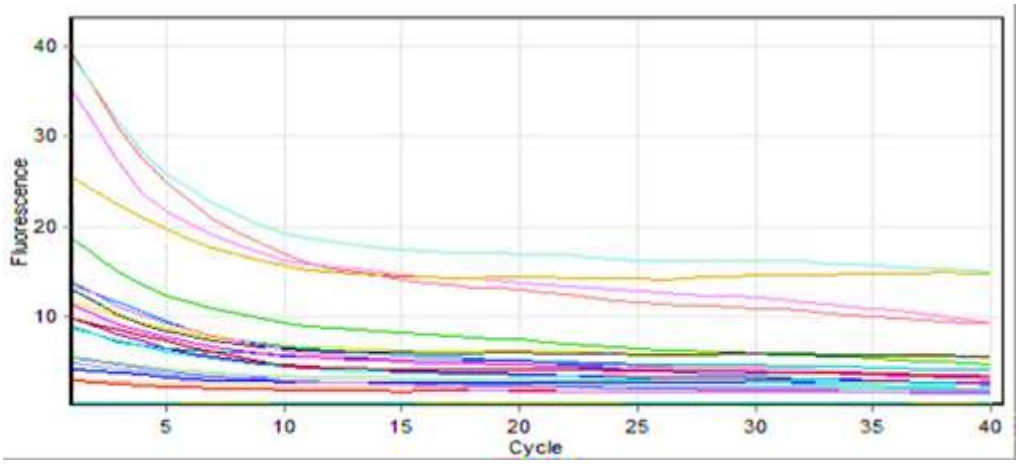
The epidemiological area, the animal reservoir, and the occupationally exposed people all influence the bacteria's mechanism of transmission (Geresu *et al*., 2016). Aborted fetuses, the fetal membranes after birth, vaginal discharges, and milk from infected animals were sources of infection for the transmission of bovine brucellosis (Tolosa *et al*., 2010; Geresu *et al*., 2016).

Although severe cases of brucellosis require an incubation period of up to 7 days, the automatic systems' typical incubation duration is up to 5 days (Raj *et al*., 2014). Yagupsky *et al*., (2019) claim that *Brucella* infections can be identified using nucleic acid amplification assays, serological testing, and culture. Modern automated blood culture techniques enable the identification of acute cases of brucellosis within the customary 5 to 7days incubation protocol used in microbiology laboratories, even though longer incubations and the performance of subcultures may be necessary for protracted cases. A definitive diagnosis of brucellosis can be made through the isolation and identification of the organism, although this approach has some drawbacks, including *Brucella's* sluggish growth and variable sensitivity depending on the disease's stage. The PCR experiments employed oligonucleotide primers made from 16S rRNA sequences that are genus-or species-specific. Field samples from sheep and cattle were examined using enhanced PCR tests and bacteriological methods. Overall, it was shown that PCR assays were more sensitive than conventional bacterial isolation. *Brucella* DNA was discovered using PCR assays in samples from aborted fetuses, blood, milk, semen, and serum. In conclusion, it was found that, when carried out under perfect circumstances, the PCR assay is useful for the sensitive and exact detection of *Brucella* infections in animals. The two main techniques for brucellosis laboratory confirmation are serological tests and blood cultures (Lai *et al*., 2017). Currently, the blood cultures was the most reliable method for diagnosing brucellosis (Araj, 2010).

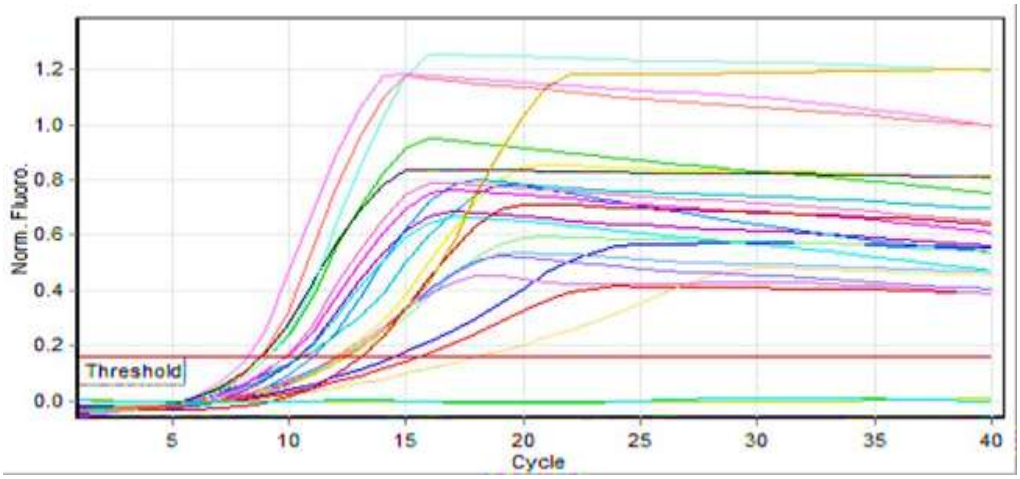
3.1.1.2.2 Genetic Detection

3.1.1.2.2.1 By real time PCR

The blood samples identified by real time PCR for knowledge of *Brucella* genus and all samples revealed that its contain *Brucella* spp., as in figure (**3-2**).



Quantitation data for Cycling A.Green



Standard Curve

Figure (3-2): Real time PCR reaction for sheep's blood samples and all samples showed positive detection signals by diagnostic kit (*Brucella* Real -TM) , (Real Time PCR Kit for qualitative detection of *Brucella* species).

In many laboratories, real-time PCR is the technique of choice for diagnostic purposes. This technology combined the chemistry of the polymerase chain reaction with the use of fluorescent reporter molecules to monitor the amplification products produced during each cycle of the PCR reaction. It also provided a rigorous description of fluorescent-based methods for real-time PCR nucleic acid analysis. There were two primary categories for real-time PCR techniques; the first included double-stranded DNA intercalating molecules like SYBR Green I and EvaGreen, while the second category includes fluorophore-

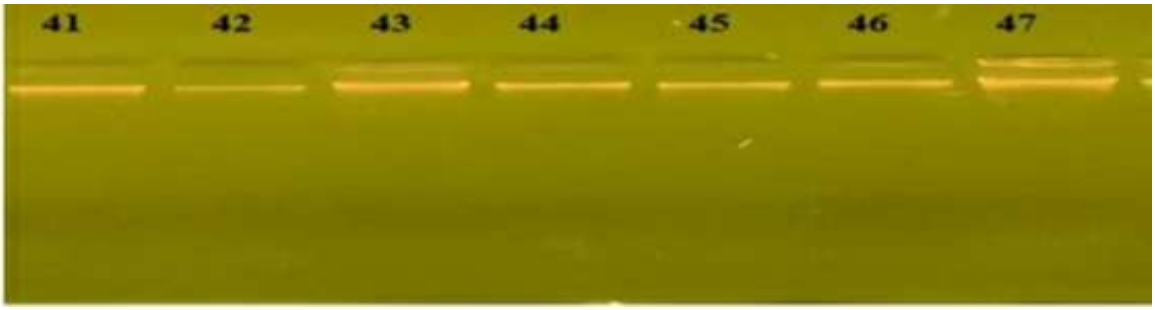
labeled oligonucleotides (Navarro *et al*., 2015). Due to the serious adverse effects of antibiotic treatment, the rapid and precise detection of *B.abortus* and *B.melitensis* from the samples was crucial. Real-time PCR and high-resolution melt (HRM) curve analysis was used in the study to provide a novel technique for brucellosis detection in samples (Piranfar *et al*., 2015).

O'Leary *et al*., (2006) claimed that serological and microbiological methods can be used to diagnose the extremely contagious disease brucellosis. They assessed the potential diagnostic usefulness of both real-time and conventional PCR techniques for the detection of *B. abortus* in naturally infected cows. Using real-time PCR, it was determined whether the test's predicted detection was Blood, milk, and lymph tissue samples were obtained from naturally infected animals. Real-time or traditional PCR can be used to analyze *Brucella* blood samples taken from cows that have the infection on their own and did not contain any abortion. In his study used a specific kit to diagnose *Brucella* spp. Molecular methods like real-time PCR can now be used to identify *Brucella* isolates (Gopaul *et al*., 2008).The melting temperature profile, which has a special sensitivity to create a successful species diagnosis was swiftly and accurately adjusted in order to achieve this (Zeinzinger *et al*., 2012). Members of the genus *Brucella* were well-known as diseases of animals and livestock, as well as the most common organisms of zoonotic infection in humans. In general, brucellae demonstrate a wide variety of host specificity in animals leading to the discovery at least seven *Brucella* species. Because the genomes of the many *Brucella* species were extremely conserved, real-time PCR tests based on these SNPs might be developed. Six of the seven clade-specific assays detected DNA quantities of less than 10 fg, showing a high level of sensitivity. These PCR assays offer a quick and extremely sensitive way to distinguish between the main *Brucella* groups (Foster *et al*., 2008). *Brucella* species were discovered and identified in bovine and buffalo samples using both conventional and real-time PCR tests. *B.abortus*

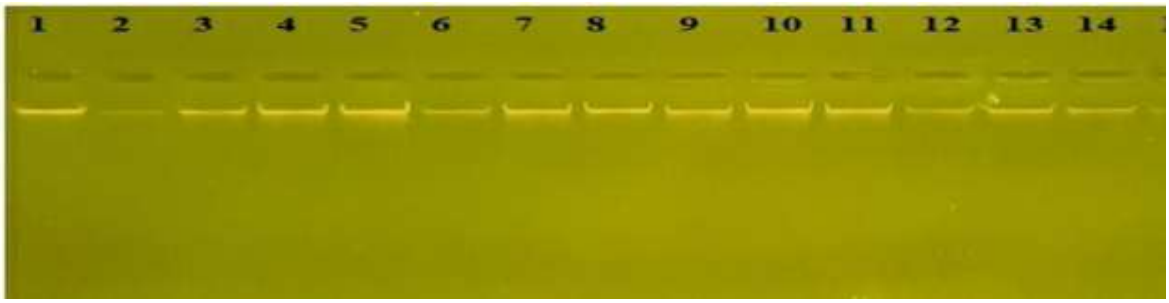
and *B.melitensis* were positive results. The highest and lowest incidences of *Brucella* species were found in the examined locations. The highest infection rate was seen in samples that were taken in the spring (Dehkordi *et al*., 2014).

3.1.1.2.2.2 Conventional PCR for Bacterial Isolates and 16SrRNA Sequencing

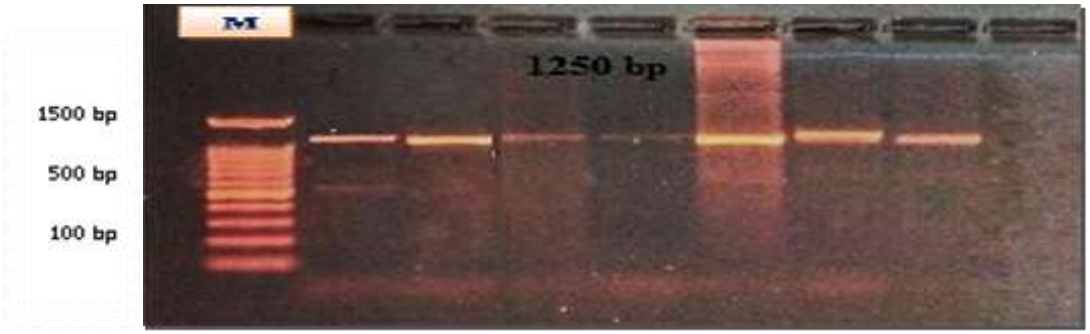
After the culture for both samples from the soil and blood on Brucella agar base media and DNA extraction from bacterial isolates and electrophoresis of DNA as in figure(3-3),(3-4) after that, identified the positive samples by conventional PCR to knowledge *Brucella* sp., as in figure (3-5) ,figure (3-6).



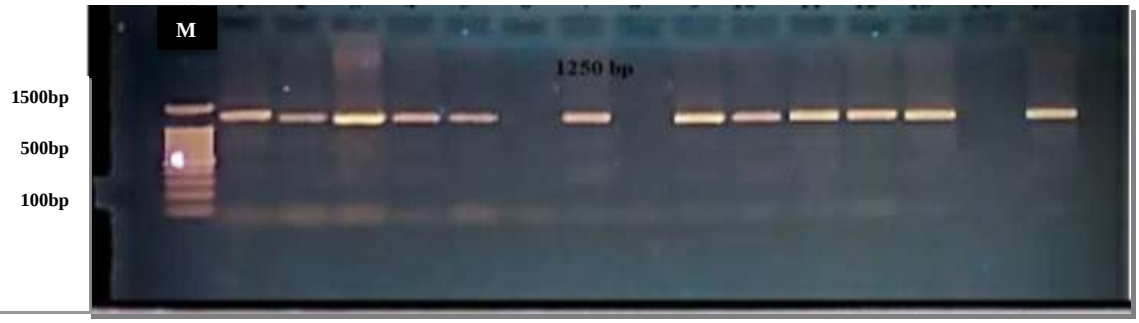
Figure(3-3): Gel electrophoresis for Extracted DNA from soil bacterial isolates, (Agarose 1%, at 70 volts, 60 min). Visualized after staining with ethidium bromide stain.



Figure(3-4): Gel electrophoresis for Extracted DNA from blood bacterial isolates, (Agarose 1%, at 70 volts, 60 min). Visualized after staining with ethidium bromide stain.



Figure(3-5): Agarose gel electrophoresis (1%) for 16s-rRNA bacterial primer-bacteria isolated from the soil samples (1250 bp), Primer Ta at (56^0C), at (65Amp ,70 volts, 60min). It was visualized under U.V light after staining with Eco Safe dye , Lane M 100 bp DNA Ladder**.**



Figure(3-6): Agarose gel electrophoresis(1%) for16s-rRNA bacterial primer-bacteria isolated from the blood samples (1250 bp), Primer Ta at (56^0C), at (65Amp ,70 volts, 60min). It was visualized under U.V light after staining with ethidium bromide stain, Lane M 100 bp DNA Ladder.

Animal samples from sheep were cultured for identifying *Brucella* spp. (Rahman *et al*., 2017). The type of *Brucella* involved, the medium for culture and the procedure. Serological tests are essential tools for the early detection of the disease (Ali *et al*., 2011). For each of the aforementioned reasons, it would be extremely beneficial to have the methods that allow for the quick and precise identification of *Brucella* both from agar plates and straight from the blood culture bottles, once this is identified as positive by the continuous-monitoring blood culture systems. Direct detection methods based on PCR have been established (Navarro *et al*.,2004). In certain situations, the American Society for Microbiology and the WHO recommend extending the incubation of bottles for

up to 4 weeks in addition to subcultures, but this protocol has a number of disadvantages, including expensive and intensive work, organizational problems, and a delaying in the automatic systems' automatic diagnosis(Yagupsky *et al*.,2019). According to estimates from the World Health Organization of 2011 showed that infectious diseases continue to be one of the top five global causes of death, microbial identification continues to be dominated by time-consuming culture-based techniques. Members of this domain often contain the *16S rRNA* gene, a molecular marker for bacterial species identification that is a useful tool for bacterial identification owing to continually growing databases of sequence data. All of them were initially discovered by conventional culture-based or non-16S molecular techniques. 16S rRNA's capacity for species-level identification was thoroughly assessed. There have been numerous cases of possible clinical misidentification of gram-negative rods, gram-positive cocci, and common gram-negative cocci using traditional culture-based identification mentioned by Srinivasan *et al*., (2015) who utilized 10 ng of genomic DNA extracted from each strain to amplify this gene. Sanger sequences have been created. Isolated 16S rRNA sequences with a genus and species name (Isolated named- strains 16S aligned. fasta) were obtained from the Greengenes database (DeSantis *et al*., 2006). PCR was used to diagnose the risk of brucellosis in people who were exposed to animals at work. It is a more accurate for finding *Brucella* spp. To tackle this potentially hazardous zoonotic disease in Pakistan, especially where brucellosis is common in animals, there is an urgent need for more accurate and focused diagnostic tools like PCR (Asif *et al*., 2014). A popular method for nucleic acid amplification used in vitro to detect infectious illnesses is the polymerase chain reaction (PCR). The use of PCR for pathogen identification, genotyping, and quantification has several benefits, including high sensitivity, high specificity, reproducibility, and technical simplicity. Typical zoonotic illnesses *Brucella* spp. was the source of the disease brucellosis, which continues

to pose a serious health risk in many underdeveloped nations worldwide (Wang *et al*., 2014). The *16S rRNA* gene was the target of the PCR technique which was used to identify the bacteria in the samples (Hamdy and Zaki, 2018). A final diagnosis of the condition is made using either serological or cultural methods, or both (Etman *et al*., 2014). As a result, PCR-based approaches have improved in accuracy, sensitivity, speed, and ability to work with DNA rather than highly contagious live cultures as a result of their increased using for detecting and identifying *Brucella* species (Moussa *et al*., 2011). In particular, for slow-growing bacteria like *Brucella*, Rahman *et al*., (2014) found that the PCR assays were efficient for speedy and precise diagnoses. It was shown by Manivannan *et al*., (2021) to be a sensitive and specific approach for identifying *Brucella* spp. PCR-based techniques were gaining popularity because they could identify organisms with greater accuracy, sensitivity and speed and also they used DNA rather than live cultures. Differentiating between various *Brucella* species can be done using a variety of genetic polymorphisms, such as amplified fragment length polymorphisms (Whatmore *et al*., 2005).

After Sequencing analysis for both bacteria isolated from soil and blood samples found that *Brucella* isolates from blood samples was four strains for two species (*Brucella melitensis and Brucella abortus*) as mentioned in table(3-3). Whereas *Brucella* isolated from soil for the four seasons from the positive fifty one samples out of sixty three and sented for sequencing was thirty seven revealed (11) *Brucella* species included (28) new strains recorded in GenBank mentioned in table (3-4). These samples divided to(3) new strains for one *Brucella* species (*Brucella pseudogrignonensis*) in winter,(16) new strains for seven *Brucella* species (*Brucella pseudogrignonensis, Brucella rhizosphaerae, Brucella oryzae, Brucella intermedia , Brucella anthropi, Brucella ovis , Brucella inopinata*) in Spring , (7) new strains for three *Brucella* species (*Brucella inopinata , Brucella melitensis, Brucella lupini*) in Summer and (11) new strains

for five *Brucella* species (*Brucella pseudogrignonensis, Brucella melitensis, Brucella pituitosa , Brucella intermedia , Brucella thiophenivorans*) in Autumn.

Table(3-3): *Brucella* isolates recorded in GenBank for *Brucella* spp. isolated from blood.

Isolate name	Accession number	*Brucella* sp.
AyJaWa-17	OM246525	*B.abortus*
AyJaWa-18	OM246526	*B.abortus*
AyJaWa-19	OM246527	*B.melitensis*
AyJaWa-20	OM246528	*B.melitensis*

Table(3-4): *Brucella* isolates recorded in GenBank for *Brucella* spp. isolated from soil

Isolate name	Accession number	*Brucella sp.*
AyJaWa-1	OM246509	*B.pseudogrignonensis*
AyJaWa-2	OM246510	*B.pseudogrignonensis*
AyJaWa-3	OM246511	*B.oryzae*
AyJaWa-4	OM246512	*B.oryzae*
AyJaWa-5	OM246513	*B.intermedia*
AyJaWa-6	OM246514	*B. intermedia*
AyJaWa-7	OM246515	*B.anthropi*
AyJaWa-8	OM246516	*B.anthropi*
AyJaWa-9	OM246517	*B.ovis*

AyJaWa-10	**OM246518**	***B.ovis***
AyJaWa-11	**OM246519**	***B.inopinata***
AyJaWa-12	**OM246520**	***B.inopinata***
AyJaWa-13	**OM246521**	***B.melitensis***
AyJaWa-14	**OM246522**	***B.melitensis***
AyJaWa-15	**OM246523**	***B.lupini***
AyJaWa-16	**OM246524**	***B.lupini***
AyJaWa-31	**ON158069**	***B.melitensis***
AyJaWa-32	**ON158070**	***B.pituitosa***
AyJaWa-33	**ON158071**	***B.pseudogrignonensis***
AyJaWa-34	**ON158072**	***B. pseudogrignonensis***
AyJaWa-35	**ON158073**	***B. melitensis***
AyJaWa-36	**ON158074**	***B. intermedia***
AyJaWa-37	**ON158075**	***B. melitensis***
AyJaWa-38	**ON158076**	***B.intermedia***
AyJaWa-39	**ON158077**	***B.melitensis***
AyJaWa-40	**ON158078**	***B.thiophenivorans***
AyJaWa-41	**ON158079**	***B.intermedia***
AyJaWa-42	**ON158080**	***B.rhizosphaerae***

The results of 16SrRNA sequencing revealed the presence variation for (17 isolates) from sheep's blood samples established (4new strains) for two species of *Brucella*, also *Brucella* isolates from soil established (28 new strains) for eleven species, indicating the presence of genetic diversity among the isolates , this genetic variation mentioned in tables (3-3),(3-4) and in (appendix1) & (appendix2).

Table (3-5): The relationship between *B. melitensis* and *B. abortus* blood parameters of infected sheep's, Mean ±S.E. P≤0.05

Blood parameters	***B. melitensis***	**P= value**	***B. abortus***	**P= value**
Lymphocyte %	93.37±1.77	0.72	92.76 ±1.30	0.49
Granulocytes %	2.37±0.79	0.66	4.98 ±1.08	0.02*
WBC count (10_3\ µl)	8.03±1.14	0.46	7.03±0.80	0.002*

This table (3-5) showed that there was no significant differences (p>0.05) between genotype and sheep's blood in *B.melitensis*, indicating that genetic variation existed but has no influence on bacteria because *B.melitensis* is the sheep a reservoir host to it, this genetic polymorphism may help it to be more resistant to bacteria. While in *B.abortus*, the variation occurred and significant differences (p<0.05) between genotype and sheep's blood were observed, indicating the presence of genetic variation in *B.abortus*. This is due to the reservoir host of *B.abortus* being cattle, and extending to other hosts (sheep) that live in the same place (animal barn) and have contact with each other therefore the sheep was susceptible to infection by *B.abortus* from infected livestock. Khamesipour *et al*., (2015) claimed that Brucellosis was a commercially relevant zoonotic disease that reduces productivity in cattle operations by resulting in miscarriage in affected animals. The current findings in table (3-5) are consistent with previous research conducted in Iraq, which revealed that *B.melitensis* and *B.abortus* had the highest isolation rates compared to other species (Seleem *et al*,

2008). *B.abortus* has been described as a cause of abortion in sheep in Nigeria, which corresponded with study results showing that *B.abortus* easily infected cattle and the spreading among pregnant animals causing abortion. This means that plans for controlling and eradicating bovine brucellosis in areas where infected sheep and goats were present and they must include eradication of the disease from these species as well (Okoh, 1980). *Brucella* generates lipid A, which played an important role in the immunological evasion process. The pathogen had a longer fatty acid residue than other enterobacterial LPS and this alteration in LPS structure decreased its endotoxic characteristics (Lapaque *et al*., 2009). Failure to add lipid A to the acyl chain results in a more severe inflammatory condition in *B. abortus* mutants than in the wild-type parent strain, contributing to lower infectivity and in macrophages as well (Parent *et al*., 2007). In addition to evasion, *Brucella* produces flagellin, which aids in evasion detection (Nissen *et al*., 2005). Ocholi *et al*., (2005) also reported on a sporadic, naturally acquired *B.abortus* infection of sheep on a privately owned farm in Toro, near Bauchi, Nigeria. Five ewes in a flock of 28 Yankassa sheep had abortions during the third month of pregnancy. All isolates were recognized as *B.abortus* biovar1 and biotyped. This biovar was also isolated from the cattle kept on the farm alongside the sheep. The infection was traced back to the farm's animal husbandry techniques. All isolates were identified as *Brucella*. The natural reservoir for (Sheep and goats) are *B. melitensis*. It is the most common *Brucella* species in the Middle East, accounting for the vast majority of cases (Mustafa *et al*., 2017; Al-Adsani *et al*., 2018). As a result, the epidemiologic situation makes a chronically diseased sheep population continuously spreads to consumers. *B. melitensis* in sheep is a major public health concern with a high risk of human infection. All *Brucella* species are closely related and can be thought of as pathovaiors of a single species (Martirosyan *et al*., 2011), making them a serious threat to the cattle population, livestock owners, abattoir employees, meat dealers,

and professional animal health workers (Seyman *et al*., 2015). Thus, it was not unexpected that *Brucella spp*. is not "absolute" but "relative," although ruminants which in general are vulnerable to infection with *B.abortus* in tiny ruminants is uncommon (Aparicio, 2013). Infected pregnant ewes with *B.abortus* result in late-term abortions. The terminated ovine embryos had lesions as a result of systemic infections, which were comparable to those observed in bovine fetuses following natural and artificial infections(Gorham *et al*.,1986). *B.abortus* infections have been documented in sheep in the United States (Kreeger *et al*., 2004), Nigeria (Ocholi *et al*., 2005) and Iran (Behroozikhah *et al*., 2012) and the current findings are consistent with these reports. The presence of DNA from both *B.abortus* and *B.melitensis* in one animal in this investigation indicated that a single host can be infected with two different species of *Brucella* at the same time. *Brucellae's* potential host range may also be affected by breeding conditions (Martirosyan *et al*., 2011). *Brucella* species are pathogenic bacteria that adapted to new hosts and naturally transmitted to their primary hosts by direct or indirect contact, as well as to other vulnerable hosts unwittingly (Moreno, 2014).This study compared brucellosis to Egypt, where it is still endemic and infects a wide range of animal species, inflicting enormous economic damage (Holt *et al*., 2011). *B. melitensis* has previously been isolated from cattle, buffalo, sheep, goats, and Nile catfish (El-Tras *et al*., 2010). The presence of cows, buffaloes, sheep, and goats in mixed farming has increased the risk of brucellosis, with small ruminants functioning as major hosts and cattles serve as an overflow host for *B.melitensis* (Abd El-Wahab *et al*., 2019). In contrast, *B.abortus* was isolated from cattle, buffalo and camels (Hamdy and Amin, 2002), but not from small ruminants (Wareth *et al*., 2014), indicating that *Brucella* species could infect additional hosts in addition to natural hosts through contact. The most significant consequence was the apparent drop in brucellosis incidence, which fell to nearly17cases per100,000 people in the middle and south of Iraq in 2009,

compared with 27.23 cases per 100,000 in 2002 and 88.55cases per100,000in1995 (Mugabi, 2012). Farm animals' haematological parameters were affected by genetic and non-genetic causes (Xie *et al*., 2013). Several elements, including physiological (Alodan and Mashaly,1999) and environmental conditions (Graczyk *et al*., 2003). Dietary composition affects the blood profile of healthy animals (Kurtoğlu *et al*., 2005).They provide diverse feed products to their animals without regard to the health and physiological ramifications. Although an animal's nutritional state is independent of dietary intake and metabolic process effectiveness, feed quality is a critical determinant in determining production success or failure. Diets have been shown to have detectable impacts on blood components, which are therefore frequently employed in nutritional evaluation and animal surveys. Hematological data could be used as a baseline for comparison in terms of nutrient deficiency, physiology, and farm animal health status, Haematological parameters and the factors that influence their values. Blood serves as a pathological indicator of the status of animals exposed to toxicants and other situations. The analysis of blood allows for the clinical investigation of the existence of metabolites and other elements in the bodies of animals, and it plays an important part in an animal's physiological, nutritional, and pathological health. Blood constituents alter in response to an animal's physiological state. These alterations are significant in analyzing farm animals' responses to varied physiological circumstances and frequently induced by a combination of genetic and non-genetic influences. Among the factors influencing blood-based parameters in farm animals include age, sex, breed, and management practices, these parameters was used to assess the health and physiological status of farm animals. The effects of these parameters on cattle, sheep, and goats have been researched. (Etim *et al*., 2014). *Brucella* spp. were facultative intracellular bacteria that colonize macrophages. After completing replication in these cells, they travel to the lymphoid tissues of the reproductive

system as a result of primary bacteremia. The agent produced secondary bacteremia in these tissues, which led to a widespread infection and finally abortion. As a result, these chronic bacteremia phases, which nearly invariably involved germs in the bloodstream, and repeated in the following gestational period (Pappas and Papadimitriou, 2007). Modern PCR methods may identify both active bacteria and bacteria that have been phagocyted or destroyed by macrophages in various compartments of the blood during bacteremia episodes (Khan and Zahoor, 2018). PCR techniques could also be used to determine the progression of an infection. Blood, which is constantly circulating with bacteria-laden macrophages, is a relevant clinical resource that can be used for diagnostics as a source of infectious agent DNA (Zerva *et al*., 2001). Molecular methods was used to look for DNA polymorphisms in *Brucella spp*. Because of the degenerate nature of the genetic code, mutational changes in the sequence are completely reflected, which does not occur in proteins; this type of study was conducted in 2015, in which the authors sequenced the complete genome of many *B.melitensis* strains and they distinguished between vaccine and endemic species, undertaking a phylogenetic reconstruction of the species' history and proposing a possible global distribution of the bacterium (Tan *et al*., 2015). The *rRNA* gene, specifically the *16S rRNA* gene employed in this study, is a common genetic target for strain identification and phylogeny. These genes are extremely conserved and diverge at a very slow rate. Only a few percent of DNA sequences from different species within the same genus will differ. Sequence identity among 16S rRNA sequences is commonly interpreted as showing the presence of a single species (Bricker *et al*., 2000). The current study concurred with Kaltungo *et al*.,(2014) who used two distinct diagnostic approaches (serological test and PCR analysis). the positive PCR samples were negative on serological testing, demonstrating that, in some situations, PCR can discover *Brucella* genes in

seronegative animals due to its ability to detect accurate levels of the pathogen in infected animals' body fluids.

Table(3-6): The relationship between *B.intermedia* and environmental factors, Mean ±S.E. p≤0.05

Parameters	*B. intermedia*	P=value
Air Temperature (^{0}C)	30.238±1.282	0.38
Soil Temperature (^{0}C)	24.53±1.394	0.65
pH	7.622±0.278	0.46
EC (µs/cm)	5134.73±1301.224	0.47
TDS (mg/L)	4037.7965±902.598	0.30
Salinity (‰)	3.296±0.844	0.45
TOC (%)	30.294±4.142	**0.05***
Cu (mg\kg)	11.54±1.926	**<0.001***
Fe (mg\kg)	513.178±57.904	**0.001***
Cd (mg\kg)	1.326±0.166	**0.004***
Pb (mg\kg)	746.268±16.602	0.14

Table(3-7): The relationship between *B.oryza*e and environmental factors, Mean ±S.E. p≤0.05

Parameters	*B. oryzae*	P=value
Air Temperature(^{0}C)	32.875±1.8675	0.35
Soil Temperature(^{0}C)	24.1±0.5975	0.53
pH	7.53±0.0675	0.37
EC (µs/cm)	3559±58.145	0.05*
TDS (mg/L)	2527.415±22.84	0.003*
Salinity (‰)	2.1025±0.315	0.17
TOC (%)	38.805±2.6025	0.05*
Cu (mg\kg)	6.4025±0.61	0.024*

Fe (mg\kg)	385.24±17.23	0.008*
Cd (mg\kg)	0.25±0.054	0.28
Pb (mg\kg)	740.0825±11.525	0.011*

Table(3-8): The relationship between *B. pseudogrignonensis* and environmental factors, Mean ±S.E. p≤0.05

Parameters	*B. pseudogrignonensis*	P= value
Air Temperature(^{0}C)	29.69±2.817	0.52
Soil Temperature(^{0}C)	23.085±0.944	0.06
pH	7.545±0.103	0.83
EC (µs/cm)	5585.998±401.263	0.34
TDS (mg/L)	3615.237±33.1	0.58
Salinity (‰)	3.45±0.467	0.41
TOC (%)	42.02±3.648	0.15
Cu (mg\kg)	7.24±1.547	0.05*
Fe (mg\kg)	486.188±24.208	0.79
Cd (mg\kg)	1.222±0.182	0.028*
Pb (mg\kg)	681.838±10.531	0.019*

Table(3-9): The relationship between *B. thiophenivorans* and environmental Factors, Mean ±S.E. p≤0.05

Parameters	*B. thiophenivorans*	P =value
Air Temperature(^{0}C)	34.11± 0.53	0.001*
Soil Temperature(^{0}C)	26.00± 0.09	0.0001*
pH	7.80± 0.06	0.01*
EC(µs/cm)	6624.00± 0.95	0.001*
TDS(mg/L)	4401.00± 0.82	0.001*

Salinity(‰)	3.98± 0.08	0.0001*
TOC(%)	37.00± 0.57	0.007*
Cu(mg\kg)	6.50± 0.64	0.49
Fe(mg\kg)	280.00± 0.72	0.10
Cd(mg\kg)	1.75± 0.06	0.001*
Pb(mg\kg)	672.00± 0.81	0.0001*

Table(3-10): The relationship between *B. pituitosa* and environmental factors, Mean ±S.E. p≤0.05

Parameters	***B. pituitosa***	**P =value**
Air Temperature(^{0}C)	32.55± 0.44	0.001*
Soil Temperature(^{0}C)	23.77± 0.25	0.0001*
pH	7.46± 0.09	0.019*
EC(µs/cm)	6742.22± 6.18	0.001*
TDS(mg/L)	4241.00± 52.00	0.93
Salinity(‰)	4.36± 0.06	0.001*
TOC(%)	34.00± 0.81	0.003*
Cu(mg\kg)	8.45± 0.08	0.001*
Fe(mg\kg)	643.00± 0.52	0.0001*
Cd(mg\kg)	1.50± 0.04	0.001*
Pb(mg\kg)	717.00± 5.00	0.0001*

Table(3-11): The relationship between *B.melitensis* and environmental factors, Mean ±S.E. p≤0.05

Parameters	*B. melitensis*	P=value
Air Temperature (^{0}C)	34.193±0.71	0.003*
Soil Temperature (^{0}C)	27.426±0.901	0.19
pH	7.613±0.1016	0.004*
EC (µs/cm)	4520±19.083	<0.001*
TDS (mg/L)	3420.166±11.75	0.001*
Salinity (‰)	3.111±0.14	<0.001*
TOC (%)	36.648±1.28	<0.001*
Cu (mg\kg)	3.075±0.058	0.30
Fe (mg\kg)	271.971±2.418	0.47
Cd (mg\kg)	1.513±0.075	0.035*
Pb (mg\kg)	704.441±2.261	<0.001*

Table(3-12): The relationship between *B. anthropic* and environmental factors, Mean ±S.E. p≤0.05**.**

Parameters	*B. anthropic*	P=value
Air Temperature (^{0}C)	32.5±0.57	0.14
Soil Temperature (^{0}C)	24.13±0.595	0.004*
pH	7.48±0.195	0.036*
EC(µs/cm)	3460±32.17	<0.001*
TDS(mg/L)	2400±20.845	<0.001*
Salinity(‰)	2.2±0.05	0.0001*
TOC(%)	28.5±0.66	0.001*
Cu(mg\kg)	4.79±0.029	0.001*
Fe(mg\kg)	238.325±1.085	0.001*
Cd(mg\kg)	0.72±0.0275	0.008*
Pb(mg\kg)	778.83±1.165	0.001*

Table(3-13): The relationship between *B. ovis* and environmental factors, Mean ±S.E. p≤0.05**.**

Parameters	*B.ovis*	P=value
Air Temperature(^{0}C)	32.833±0.503	0.022*
Soil Temperature (^{0}C)	25.066±0.466	0.023*
pH	7.4±0.266	0.47
EC (µs/cm)	1680.333±2	<0.001*
TDS (mg/L)	1187.833±0.5	0.001*
Salinity (‰)	1.026±0.036	0.002*
TOC (%)	12.13±0.035	<0.001*
Cu (mg\kg)	5.416±0.05	0.004*
Fe (mg\kg)	308.833±0.35	0.001*
Cd (mg\kg)	0.863±0.005	0.001*
Pb (mg\kg)	795.5±0.5	<0.001*

These tables (3-6),(3-7),(3-8),(3-9),(3-10),(3-11),(3-12),(3-13) showed that all isolates have significant differences (p<0.05) between genotype and environmental factors that included physical and chemical parameters (air and soil temperature, pH, EC, TDS, salinity, TOC) and heavy metals (Cu, Fe, Cd ,Pb) according to statistical analysis.

This indicates presence of genetic variation in them. This polymorphism of these bacteria was due to environmental indicators and heavy elements that caused change in the bacteria. This alteration may be in the structure of DNA or in the nucleotide bases so that it adapted to the external environmental conditions and could resist and survive. Also, its resistance to the poisonous heavy elements presented in the soil has been transformed into new strains in this soil to be able to survive. This genetic change in bacteria caused the moral differences between them. *B. melitensis* was founded two in the summer season and four in autumn season, while *B. pseudogrignonensis* existed three in spring season, two in

autumn season and three in winter season. *B. intermedia* three in both the spring and autumn seasons , *B.oryzae* was three, *B.anthropi* and *B.ovis* were two in the spring season, whereas *B. thiophenivorans* and *B. pituitosa* were one in the autumn season as mentioned in (appendix 2). This implies the presence of genetic variation in them and suggests that these bacteria changed as a result of polymorphism caused by environmental indicators and heavy metals. This caused differences between them, allowing them to adapt to and live in harsh environments. In addition, to survive in this soil, its resistance to the poisonous heavy metals may be changed into new strains. The psychological differences amongst microorganisms are caused by genetic changes.

There was many ways to become infected by the dangerous bacteria known as *Brucella*. Long-lasting resistance was possible in both inside and outside of mammalian hosts, even in harsh circumstances. It can linger in food for up to 15 months under adverse conditions such as acidity and temperatures ranging from 11 to 14 °C or for a few days if kept below 37 °C for up to two months in the winter and for only a few hours if exposed to direct sunshine, *Brucella* can also survive in contaminated manure and aborted infected feti (Lucero *et al*., 2010). Most publications classified it as a facultative intracellular pathogen but due to its shared evolutionary history with other alpha-proteobacteria, they were reclassified as facultative extracellular intracellular pathogens (Lamontagne *et al*.,2009). The different *Brucella* species were renamed in the 1980s because it was thought that *Brucella* was a monospecific genus (*Brucella melitensis*) with six biovars distinguished by their host prevalence (Whatmore, 2009). It had long been assumed that the disease originated in animals and had a high zoonotic potential with infected humans serving as the disease's final host. On the other hand, man-to-man infection has recently been demonstrated. This might be as a result of development in epidemiological and diagnostic techniques as well as the organism's continual capacity to adapt to its hosts (Lucero *et al*., 2010). Newly

discovered *Brucella* species exhibit high levels of genetic flexibility. In addition to having a great capacity for adaptability to novel non-mammal hosts like frogs, many of these isolates are mobile have rapid development and can persist in soil. Additionally, they have a high metabolic rate and may quickly adjust to their environment in order to increase the number. The genetic diversity of newly discovered *Brucella* species was far higher than the thousands of isolates of the traditional *Brucella* species discovered over the twentieth century. These unusual *Brucella* species share a close genetic relationship with soil microbes. As a result, they are unable to acquire new genetic traits from their surroundings (Al Dahouk *et al*., 2017). *Ochrobactrum* has been identified as an emerging pathogen in immunocompromised people. The genus *Ochrobactrum* was recently reclassified within *Brucella* but the clinical characteristics of the two groups differ. Human infections have been documented by *Ochrobactrum anthropi (B.anthropi*), *Ochrobactrum intermedium* (*B. intermedium*) and *Ochrobactrum pseudogrignonense* (*B.pseudogrignonensis*) (Ryan and Pembroke, 2020)**.** *Brucella pseudogrignonense* is a gram-negative, rod-shaped, oxidase-positive, anaerobic, non-motile, and non-spore-forming bacterium that has been discovered in clinical specimens rather occasionally. It was first detected in Scandinavia in 1992 from male blood and from a baby's ear in 2000 in Sweden. Nonetheless, this was Taiwan's first instance of *B. pseudogrignonense* infection and full genome sequencing yet, its pathogenicity in humans has not been thoroughly examined. They reported the first case of *B. pseudogrignonense* in Korea and only the third in the globe. Whole genome sequencing of *B. pseudogrignonense* from Malaysian tropical soil was reported in 2016 (Kampfer *et al*., 2007). O. pseudogrignonense was isolated from environmental sources and it was discovered to be a fungal pathogen (Wu *et al*., 2016). It was a sort of environmental organism that can be found in both water and soil (Kettaneh *et al*., 2003). In Germany, sewage water was used to isolate *Brucella thiophenivorans*, a gram-negative, oxidase-positive,

non-spore-forming, non-motile *Brucella* bacteria (Kampfer *et al*.,2008). *Brucella pituitosa*, a gram-negative, oxidase-and catalase-positive, non-spore-forming, non-motile member of the *Brucella* genus, was discovered in a Swedish industrial environment (Huber *et al*., 2010). *Brucella intermedia* is a bacterium in the genus *Brucella* (Liu, 2011). Velasco *et al*., (1998) described it initially . Only one case of cholangitis following liver transplantation has been reported in humans (Möller *et al*., 1999).

A member of the Brucellaceae family, *Brucella* ovis is a gram-negative coccobacillus. It was the cause of ovine brucellosis, a disease that must be reported along with *Brucella melitensis*. The stable fly is capable of spreading *B. ovis*. The epididymis, especially the tail becomes severely inflamed as a result of infection. *Brucella melitensis* is a brucellaceae-related gram-negative coccobacillus bacterium. It typically affects sheep and goats, although cases have been reported in cattle, yaks, water buffalo, camels, alpacas, dogs, horses, and pigs, among others. Infection can occur in humans if they come into contact with an infected animal or its leftovers. Animals acquire *B. melitensis* by venereal transmission and contact with infected animals' placentas, fetuses, fetal fluids, and vaginal discharges. Blood, urine, milk, and semen contain the bacterium, it is zoonotic cause malta fever or localized brucellosis in humans (CDC, 2019). *Brucella oryzae* , the type strain is MTCC 4195T (also known as DSM 17471T) that it is gram-negative, motile, and non-pigmented and is associated with endophytic bacterial species (Tripathi *et al*.,, 2006). According to Holmes *et al*., (1988), *Brucella anthropi or O. anthropi* strains have a rod shape are aerobic, gram-negative, non-pigmented and move by means of peritrichous flagella, The type strain is CIP 82.115 (= CIP 14970 = NCTC 12168 = LMG 3331) (Kettaneh *et al*., 2003).They are becoming significant opportunistic pathogens (Ryan *et al*., 2020).

The metals are materials that are malleable, lustrous that have excellent electrical conductivity. They voluntarily lose their electrons to produce cations. Natural metal deposits can be found in the earth's crust and because the compositions of these deposits vary locally, so the concentrations in the immediate vicinity. The characteristics of the provided metal, along with a number of environmental parameters serve to monitor the distribution of metals in the atmosphere (Khlifi & Hamza-Chaffai, 2010). When present at extremely low concentrations, these metals maintain a variety of biochemical and physiological processes in living organisms, but when their concentrations rise above a certain threshold, they become toxic. Exposure to Heavy metal persists and is rising in many regions of the world the fact that heavy metals were known to have several negative health impacts and that these effects could endure for a very long time. For ecological, evolutionary, nutritional and environmental reasons, heavy metals were significant environmental contaminants and their toxicity is a problem that is becoming more and more important (Jaishankar *et al*., 2014;Nagajyoti *et al*.,2010). The oxidative degradation of biological macromolecules were mostly caused by heavy metal binding to DNA where it displaced original metals from their native binding sites and caused malfunction to cells and finally became toxic (Flora *et al*., 2008). Ionic and oxidative stress-related mechanisms were how lead metal toxicology affects live cells (Wadhwa *et al*., 2012). At very high concentrations, ROS may damage cells, proteins, nucleic acids, membranes, and lipids structurally, which could lead to a stressed state at the cellular level (Mathew *et al*., 2011). Cadmium in the environment can be found in soils and sediments for decades. Plants gradually absorb the metals that accumulate in them and concentrate them along the food chain (Bernard, 2008; Mutlu *et al*., 2012). It was primarily detected in vegetables due to its rapid transfer from soil to plant (Satarug *et al*., 2011). And it is a very hazardous non-essential heavy metal that is well known for its negative effects on cell enzymatic

systems, oxidative stress, and producing nutritional shortages in plants (Irfan *et al*., 2013). The transition metal iron is the most plentiful in the earth's crust, it is the most crucial nutrient for most living species. Most aerobic organisms benefit from iron-mediated processes in their respiration mechanisms. If not appropriately insulated, it can catalyze reactions that result in the creation of radicals, which can harm biomolecules, cells, tissues, and the entire organism. Iron poisoning has always curiosity (Albretsen, 2006).

Cadmium was emitted into the atmosphere as a result of natural or man-made activity and it could have a wide range of effects on both animals and people (Hayat *et al*., 2019). Lead was a non-biodegradable metal that occurred at trace levels in nature. Atmospheric lead levels are steadily rising as a result of human activities such as manufacturing, mining, and the use of fossil fuels. Lead is dangerous to the human body when exposed to amounts higher than ideal. Children are particularly vulnerable to lead poisoning and the severity of poisoning increases when they come into contact with lead-contaminated dust (Loh *et al*., 2016). Vegetable bioaccumulation of heavy metals was a health risk due to the possibility for heavy metals to enter the food chain via contaminated soil and water (Khan *et al*., 2015). Significant environmental and health problems were caused by heavy metal contamination, absorption, and bioaccumulation in food crops, especially in developing countries. Heavy metal concentrations were influenced by soil type, plant genotype, and their interactions (Ding *et al*., 2013). Because inorganic fertilizers have larger concentrations of heavy metals than organic manures, using them causes soil to become more contaminated with heavy metals (Hu *et al*., 2013). Fertilizer application increased the amounts of Cd and Pb in agricultural soils. Despite statistical evidence that these heavy metals increased significantly ($P = 0.05$), lead concentrations increased dramatically in comparison to Cd concentrations and a decrease in pH, increasing heavy metal availability and exacerbating the problem of deteriorating food quality, metal

leaching and impacts on soil organisms (De Vries *et al*., 2002). The geological features of the soil determine heavy metal concentrations in the soil. In contrast, normal farming operations tend to collect these components. These metals were built in the soil as a result of the application of liquid and soil manure (or their derivatives, compost, or sludge) or inorganic fertilizers. These considered significant heavy metal sources (Martin *et al*., 2006).The majority of heavy metals are harmful in low quantities and can enter the food chain, where they accumulate and harm living species. At higher concentrations, all metals have the potential to cause harm, and the toxicity of each metal is determined by the amount available to organisms, the absorbed dose, the route, and the length of exposure (Gall *et al*.,,2015). There were significant environmental and public health concerns due to the harmful effects of these metals (Fashola *et al*., 2016). The physiological and poisonous effects of a metal on living creatures were determined by the metal's speciation and bioavailability (Olaniran *et al*., 2013). According to D'amore *et al*., (2005), the geochemical cycle of heavy metals caused an accumulation of heavy metals in the environment, when they were above permissible levels, they could pose a risk to all life forms. The weathering of parent materials, man-made changes to the geochemical cycle, soil ingestion (the main way that humans are exposed to soil-borne metals), transportation from mines to other locations and industry discharge of high concentrations of metal waste are some of the common ways that metals enter the environment. Although some heavy metals were crucial to the physiological, biochemical and metabolic processes of living things, the majority of them were toxic when produced in excess and have no known biological functions in living things. Metal toxicity is the capacity of a metal to have adverse effects on organisms. This depends on the absorbed dose and heavy metal bioavailability (Rasmussen *et al*., 2000). The threat was posed by heavy metals to the health of living creatures that exacerbated by their prolonged presence in the environment. When the medium is acidic and

nutrient-deficient, as well as when the soil structure is weak, toxicity increases (Mukhopadhyay and Maiti, 2010). Heavy metals tend to generate free ionic species at acidic pH levels available to saturate metal binding sites. This means that when the concentration of hydrogen ions increases. As a result, heavy metals become more accessible, increasing their toxicity to microorganisms and plants. A modest variation in pH levels could affect the solubility and bioavailability of heavy metals. Variations in soil composition, such as organic matter concentration, also influence heavy metal toxicity. Heavy metal pollution was commonly detected in soil with a low organic matter level. Metals in organic soils contaminated with a mixture of heavy metals were less mobile and bioavailable to microorganisms and plants than metals in mineral soils (Olaniran *et al*., 2013).
The stability of metal ion species was affected by temperature fluctuations and the stability of the microorganism-metal complex was dependent on biosorption sites, microbial cell wall structure, and ionization of chemical moieties on the cell wall. When the temperature was elevated from 25 to 40 ^{0}C, the sorption capacity of pb increased (Arjoon *et al*., 2013). Due to their toxicity, heavy metal-contaminated soils restricted the range of plants that may grow and caused trouble with nutrition, evolution, and ecology (Mani and Kumar, 2014). Heavy metal toxicity varied according to the species affected, the concentration of the metal, the chemical form of the metal, the soil composition and pH (Nagajyoti *et al*., 2010).

Microbial population size, diversity, and activity are impacted by heavy metal toxicity. Inhibiting enzyme activity and oxidative phosphorylation, altering the nucleic acid structure, disrupting cell membranes, causing functional disturbance are just a few of the ways that affects the morphology, metabolism, and growth of microorganisms (Xie *et al*., 2016). A microorganism's cellular structure could capture heavy metal ions and then sorbed them onto cell wall binding sites (Malik, 2004). The amount of metal sorbed was determined by the kinetic

equilibrium and metal composition at the cellular surface. Electrostatic, ion exchange, precipitation, the redox process and surface complexation are all parts of the mechanism (Yang *et al*., 2015). As passive uptake via surface complexation onto the cell wall and other outer layers was carried out by pieces of dead biomass, living cells, or fragments of cells and tissues (Fomina and Gadd, 2014). The organism would accumulate heavy metals should have a tolerance to one or more metals at higher concentrations and must have improve transformational abilities, converting toxic chemicals into harmless forms that allow the organism to reduce the metal's toxic effects while keeping the metal containe (Mosa *et al*., 2016). The cellular surface of microorganisms, as well as the exchange of metal ions and complex forms with the metal ions on the reactive chemical sites of the cell surface, influence the metal uptake mechanisms by different biosorbents. The impact of many elements was known, including pH and organism biomass. The extra metal ions were precipitated at the cell surface. Due to the existence of an ionic structures that allowed them to attach to metal cations, all microorganisms have a negative charge on the surface of their cells (Gavrilescu, 2004). Different microorganisms take up metal in different ways, so it is helpful to analyze the cell wall components that vary among them. Enzymes, glycoproteins, lipopolysaccharides, lipoproteins and phospholipids are present in the peptidoglycan layer of gram-negative bacteria, which serves as the active site for activities involving metal binding (Gupta *et al*., 2015). On the surface of the cell, metals and metalloids were linked to these ligands, which displace necessary metals from their typical binding locations. Once the metal and metalloid were bonded, microbial cells are able to change them from one oxidation state to another, hence lowering their toxicity (Chaturvedi *et al*., 2015). Depending on the composition of the polysaccharide and related components, microorganisms could also secrete a variety of metal-binding metabolites form extracellular polymeric structures consisting of polysaccharides, sheaths, and biofilms (Fomina

and Gadd, 2014). The environment was regarded as a massive reservoir for bacterial species as well as a source of human infections. Some environmental bacteria were capable of a wide range of actions, including the stimulation of disease, the breakdown of contaminants, the synthesis of unique biomolecules, as well as resistance and pathogenicity. Bacterial lifestyle flexibility includes adaptation to numerous niches. Adaptation to both open environments and human-specific niches is a significant difficulty that includes intermediate creatures that allow for pre-adaptation to humans. Pseudomonas, Aeromonas, and Ochrobactrum were excellent examples of opportunistic behavior coupled with specific genetic structure and evolution. Original genomic comparisons between aeromonads and between the mild opportunistic pathogens *Ochrobactrum* spp. and strictly intracellular pathogens *Brucella* spp. led to the conclusion that the adaptation to humans may have occurred concurrently with a speciation in action that was revealed by changes in both genomic and population structures. In addition to the acquisition of specific virulence components, this adaptation-driven speciation may be a significant process for the development of real diseases (Aujoulat *et al*., 2012). The pathogen's endemic persistence in Austria and the Czech Republic over a decade demonstrated the pathogen's endemic persistence within a limited geographical zone. It was probable that enzootic transmission cycles involving rodents, predators and the natural environment contribute to *Brucella's* long-term survival in this region. Although soil may be the principal reservoir of infection, other vectors cannot be ruled out. Although the pathogenicity of *Brucella* for cattle and humans has not yet been shown, new foci of *Brucella* infections that may posed a public health risk must be monitored (Morris and Southwick, 2010). *Brucella intermedia*/*Ochrobactrum intermedium* strain DF13 can degrade 2,4-dichlorophenoxyacetic acid in Brazil (2,4-D). It has a 4,570,268 bp genomic sequence with 57.8 percent G1C (Silva *et al*., 2022). In light of *B.ovis*, the taxonomy of *Brucella* has been contested. The DNA

homologies were astonishingly close, supporting the genus's categorization as a single species with subspecies. According to phylogenetic analysis, the genus has four clades, which may have descended from a common soil ancestor that resembled *Brucella* and *Ochrobactrum*. *B. ovis*, along with *B. melitensis* and *B. abortus*, formed the tree's root lineage. According to 16S-rRNA sequencing and other molecular investigations (Banai and Corbel, 2010), the newly found isolate of *B. inopinata* differed from standard *Brucella* in some ways. *B. ovis* was first discovered as a bacterial agent connected to sheep epididymitis and abortion in New Zealand and Australia in the 1950s (Buddle, 1956). It was one of the two classical *Brucella* species with no zoonotic potential was stably rough. The bacterium infected sheep and could cause clinical or sub-clinical chronic infections characterized by epididymitis, male sterility and sporadic abortions in pregnant ewes (Ficapal *et al*., 1998). It was a non-zoonotic species that caused epididymitis and other genital lesions in rams, as well as greater perinatal mortality in lambs, and placentitis, miscarriages and infertility in ewes (OIE, 2017b). Lipopolysaccharide (LPS) from *B. ovis* is distinguished by the absence of O-polysaccharide (O-PS) chains (R-LPS). Only one species in the *Brucella* genus, *B. ovis* was fully composed of R strains that are toxic to their natural hosts. Their independence from O-PS for full virulence distinguishes them from smooth (S) *brucellae* (e.g., *B.melitensis* and *B.abortus*). *B.melitensis* infection was removed because, among other things, it produces antibodies that hinder the serological identification of S *brucellae* infections (Nicoletti, 2010). This bacteria was found in sheep-raising regions all over the world and there have never been any successful cases of eradication (Blasco, 2010). In contrast to other *Brucella* species, *B. ovis* has a specific environmental persistence. Some *Brucella* species, such as *B.ovis*, may be able to survive in hostile environments for extended periods of time. *Brucella* had an extremely limited chance of surviving in situations such as dryness, severe heat, and direct sunlight. Under favorable

conditions such as pH > 4, cool temperature, high humidity and a lack of direct sunlight, *Brucella* spp. can live in aborted fetuses for extended periods of time and cause ovine epididymitis(*Brucella ovis*). The most important mode of infection transmission was assumed to be passive ram-to-ram venereal transmission via the ewe. Ram-to-ram transmission could also occur through mucosal contact between infected and healthy rams, as well as urogenital transmission induced by licking the preputial area of infected rams (Ridler *et al*., 2000). Understanding the relationships between *Brucella* species was essential for understanding the ecology, evolutionary history, host interactions and the development of precise genotyping procedures. The evaluation of single nucleotide polymorphisms (SNPs) and other differences in genes, known as multilocus sequence typing, revealed that there was a significant amount of taxonomically valuable variability among *Brucella* isolates (Whatmore *et al*., 2007). Single SNPs can be used to identify *Brucella* species due to their evolutionary stability and ability to be used in genotyping procedures (Foster *et al*.,2008). Ability to build very accurate, high-resolution phylogenies is fortunately being quickly improved by recent developments in innovative sequencing technologies (Hall, 2007). According to earlier whole-genome investigations (Halling *et al*., 2005), *B. ovis* was the most basal species in the phylogeny of the genus, *B. abortus* and *B. melitensis* share a close relationship. The rooted phylogeny suggested that brucellosis first spread to pigs, goats and cattle through contact with infected sheep. Furthermore, this contact was recent, occurring within the last 86,000 to 296,000 years. Instead of emerging as a result of domestication, this disease was widespread within wildlife populations in the Middle East for at least 10,000 years prior to the domestication of livestock (Zeder, 2008). The coevolution of *brucellae* with their individual hosts is inconsistent with the whole-genome phylogeny based on topology and the projected pace of mutational change(Chain *et al*., 2005). All well-studied

Brucella organisms resided naturally in the intracellular environment of animal cells. However, despite their capacity to be momentarily isolated from contaminated materials, they have never been shown to multiply in open environments, even in organic substrates and they were infrequently in contact with other bacteria when they were metabolically active (Espinoza *et al*., 2021). *Brucella* organisms were selectively impacted by relatively consistent host conditions which included antagonistic natural host defenses, adaptive immune effectors and the availability of nutrients within cells (Martirosyan *et al*., 2011). Because of the potential for brucellosis transmission, yellow beans were known as a source of pathogenic infectious agents. To avoid misunderstandings, some authors used both the names *Brucella* and Ochrobactrum for the same bacteria in the publication's title (Li *et al*., 2021). Ochrobactrum anthropos cells were typically rod-shaped with dimensions of 0.5 mm in width and 1-3 mm. However, in nitrogen-deficient conditions, cells grow more rounded and are less prevalent, which reduce their length and area, The amplification of the gene also supports *Ochrobactrum anthropi's* capability to fix nitrogen through an as-yet unexplained mechanism, bypassing N2-limiting conditions (Villagrasa *et al*., 2019). *O. anthropi* is now recognized as a nosocomial, opportunistic pathogen that may be hazardous. This opportunistic pathogen was a widespread soil alphaproteobacterium that colonizes a wide range of organisms. Recorded *O. anthropi* infections included potentially lethal conditions, including endocarditis. Due to the reports, the sparse number of investigations and the organism's strong phylogenetic link to the extremely pathogenic *brucellae*, *O. anthropi* was a desirable example of bacterial pathogenicity (Chain *et al*., 2011). Bacteria belonging to the *Ochrobactrum* genus were regularly discovered in soil and adjacent to plant roots. The O-polysaccharide and glucan were generated by the *O. rhizosphaerae* PR17T strain. Purified polysaccharides were analyzed chemically. Sugar and absolute configuration assignment were used to determine

the chemical makeup of the repeating unit of the O-polysaccharides (Szpakowska *et al*., 2020). Two isolates of bacteria, one from the potato rhizosphere and the other from an industrial setting, were tested for their taxonomic classification. *O. pseudogrignonense* was discovered to be the strain's most distant relative after these strains' (*16S rRNA*) gene identities as members of the Alphaproteobacteria were established. The isolates from each of the previously described *Ochrobactrum* species may be separated based on genotype and phenotypic characteristics according to the outcomes of physiological, biochemical and DNA-DNA hybridization tests. In view of this, the names *O. rhizosphaerae* sp. nov. and Ochrobactrum thiophenivorans sp. nov. were proposed for these two species of the genus Ochrobactrum (Kämpfer *et al*., 2008). The strains were *O.grignonense*OgA9aT, *O.thiophenivorans* DSM 7216T, *O. pseudogrignonense* CCUG 30717T, *O. pituitosum* CCUG 50899T. The sequencing of the genomes of all strains has substantially expanded the possibility of genome-based investigations in *Ochrobactrum* spp. (Krzyanowska *et al*., 2019).

Table(3-14): The relationship between *B.inopinata* and environmental factors, Mean ±S.E. p≤0.05**.**

Parameters	*B. inopinata*	P=value
Air Temperature (C^0)	38±1.333	0.89
Soil Temperature (C^0)	34.693±5.07	0.62
pH	7.666±0.133	0.80
EC (µs/cm)	5777.57±9.2	0.64
TDS (Mg\L)	4137.5±19.2	0.50
Salinity ‰	3.746±0.566	0.56
TOC %	48.663±4.276	0.64
Cu (mg\kg)	1.976±0.24	0.60
Fe (mg\kg)	210.686±8.623	0.63

Cd (mg\kg)	0.846±0.081	0.65
Pb (mg\kg)	777.913±8.586	0.61

Table(3-15): The relationship between *B.lupini* and environmental factors, Mean ±S.E. p≤0.05**.**

Parameters	*B.lupini*	P=Value
Air Temperature(^{0}C)	44.553±0.653	0.49
Soil Temperature(^{0}C)	39.01±1.626	0.28
pH	7.713±0.253	0.79
EC (µs/cm)	5707.333±15.03	0.89
TDS (Mg\L)	4056±40	0.88
Salinity ‰	3.56±0.696	0.9
TOC %	20.773±6.043	0.52
Cu (mg\kg)	2.676±0.17	0.67
Fe (mg\kg)	118.51±7.018	0.55
Cd (mg\kg)	1.346±0.0606	0.32
Pb (mg\kg)	734.423±10.873	0.32

Table(3-16): The relationship between *B.rhizosphaerae* and environmental factors, Mean ±S.E. p≤0.05**.**

Parameters	*B.rhizosphaerae*	P=value
Air Temperature (^{0}C)	21.5±0.57	0.14
Soil Temperature (^{0}C)	24.38±0.385	0.23
pH	7.15±0.05	0.14
EC (µs/cm)	3665±5.735	0.143
TDS (Mg\L)	2559.165±3.73	0.39
Salinity ‰	2.355±0.04	0.22

TOC %	35.63±0.065	0.13
Cu (mg\kg)	5.45±0.05	0.14
Fe (mg\kg)	584.5±0.685	0.14
Cd (mg\kg)	0.1415±0.0065	0.38
Pb (mg\kg)	596.5±0.725	0.4

While the three *Brucella* species mentioned in tables (3-14),(3-15),(3-16) including *Brucella rhizosphaerae*, *Brucella lupini* and *Brucella. inopinata*, do not exhibit significant differences ($p>0.05$) between genotype and environmental parameters, this suggests that genetic diversity is present but has no impact on the makeup of the bacterium. *B.rhizosphaerae* founded one in the spring season and *B. lupine* was four in the summer season while *B. inopinata* existed one in both spring and the summer seasons as mentioned in (appendix 2). This may be because *B. lupine* and *B. rhizosphaerae* are related to roots, whereas *B. inopinata* can be transported from water to soil by frogs, because *B. inopinata* is present in toads. *B. inopinata*, only the type strain BO1 has been classified. Other *Brucella* strains, genetically distinct from the traditional species, have been described, including isolates from wild rats in Australia and frogs from Africa (Fischer *et al*., 2012). *B. rhizosphaerae* is a gram-negative, oxidase-positive bacterium discovered in the rhizosphere of a potato in Austria (Kampfer *et al*., 2008). *B. lupini* is a bacteria that grows on non-rhizobial roots. It gets its name from the fact that it nodulates Lupinus albus. LUP21T (LMG 20667T) is the reference strain and are a group of microbes known as rhizobia and belonging to the proteobacteria were solely responsible for the nodulation of legumes (Trujillo *et al*., 2005). *B.inopinata* is a gram-negative, nonmotile, non-spore-forming coccoid bacterium discovered at a breast implant infection site. BO1T (=BCCN 09-01T =CPAM 6436T) is the type strain, Scholz *et al*., (2009) identified it as a possible cause of brucellosis.

Sequences from isolates 3278 and 5043 have been added to GenBank (accession numbers MT471347, MT471348, and MT482342). Although it was genetically more distant from the traditional *Brucella* species, which comprise a homogenous ancestor to the classic *Brucella* species, molecular analysis indicated that these strains belong to the genus *Brucella*. On the other hand, *B. inopinata* was more distant from classic *Brucella* species than *Ochrobactrum* species (Audic *et al*., 2011). As *B. inopinata*, strain BO2 and strains from wild Australian rats were among the genetically distant strains from the traditional *Brucella* species that have recently been discovered (Tiller *et al*., 2010). Grilló *et al*. (2012) conclude that BO1 and *Brucella* species are the most vulnerable to *Brucella* infection with mice being the least susceptible. Amphibian isolates show a degree of horizontal gene transfer with the insertion of genomic areas with sequence identity to soil-dwelling or facultative pathogenic Alphaproteobacteria, most notably from the genus Ochrobactrum (Al Dahouk *et al*., 2017). Any trace elements, necessary or non-essential, presented in excess of safe amounts can cause physiological or morphological abnormalities, as well as genetic alterations, such as slowing or stopping growth or producing mutations (Luo *et al*., 2011). Based on *16S rRNA* sequences, one group's sequence was identical to the *B. inopinata* consensus sequence, whereas the other group's sequence has an Ochrobactrum-derived 44-nucleotide insertion (Al Dahouk *et al*., 2017).Thacker *et al*., (2007) discovered that a locally isolated gram-negative *Brucella* sp. strain, identified using biochemical techniques and *16SrRNA* analysis, was found to be virulent. Multiple heavy metal (Ni, Zn, Hg, Pb, Co) tolerances and antibiotic resistance were found in the strain. When heavy metals impacted soil microbial communities, autochthonous lead-and cadmium-resistant isolates were discovered, which had a negative influence on biomass, metabolic activity, and diversity. The microbial community in several metal-stressed soils is comprised of two groups, one resistant to lead and the other vulnerable to it. A lead-resistant

isolate was also isolated from a control soil that had never been exposed to lead, implying widespread lead resistance. These findings demonstrate that heavy metals have an impact on the bacterial types (Wu *et al*., 2020). Wild animals served as reservoirs for causative agents that can stay dormant for lengthy periods of time until they are activated. The presence of agents in uncommon hosts can occasionally raise the risk of the replication mistakes, which could result in mutations. This can result in the emergence of novel strains or species that are more virulent and/or antibiotic-resistant. Increased transmission rates in sensitive groups could be one of the consequences (Cupertino *et al*., 2020). Disease reservoirs in the environment are still being recognized as important factors in disease outbreaks, pathogen persistence, and ongoing transmission in humans (Zhao *et al*., 2012). Animal manures include a variety of zoonotic infections according to Dungan, (2012) which are suspected of being carried off-site as aerosols from confined feeding operations. For bacterial identification and phylogenetic classification, DNA extracted from aerosol samples which were produced and a portion of the *16SrRNA* gene was sequenced. Although the findings imply that aerosolized bacteria are diverse, since 2007, novel *Brucella* strains have been identified as having phenotypic traits that are more similar to those of strains belonging to the genus *Ochrobactrum* than to those of classic *Brucella* species (Al Dahouk *et al*., 2012). Since its discovery on the island of Malta, brucellosis has garnered recognition as the second-most significant zoonotic disease after rabies (Seleem *et al*., 2010; Abubakar *et al*., 2012).

3.1.2 Phylogenetic Tree Draw

Sanger sequencing was successful for 17 out of the 20 samples for blood and 37 out of the 63 samples for soil with an expected PCR product size (1250 bp) by 16S rRNA bacterial primers. The sequences were identified as belonging to *Brucella* spp. following similarity searched by blast (sequence identity of 99%). 16S rRNA sequencing method was performed for study of genetic changes

and phylogenetic tree draw of *16SrRNA* gene in some local *Brucella* species isolates by compared with NCBI-GenBank *Brucella* species. The sequences were deposited in GenBank with accession numbers as mentioned in tables (3-17),(3-18). In addition, each sequence on blast search showed similarity and diversity with different *Brucella* spp. (*B. abortus, B. melitensis*) for blood and (*Brucella pseudogrignonensis, Brucella rhizosphaerae, Brucella oryzae, Brucella intermedia, Brucella anthropi , Brucella ovis, Brucella inopinata, Brucella melitensis, Brucella lupini, Brucella pituitosa, Brucella thiophenivorans)* for soil deposited in GenBank considering the percent identity, where (17) strains in blood and (37) strains in soil were used for partial sequence alignment (except one with complete sequence) and phylogeny construction. Occurrence types of substitutions mutations (Transversion and Transition) for both isolates in blood (the sheep's blood *Brucella* samples) for two species of *Brucella* spp. and also in soil (different villages of Babylon province middle , north and south) for 11 species of *Brucella* by compared local one strain with strains to one state of the world states . These sequences recorded and published in the Gen Bank database taken as a reference to identify the polymorphisms. Also phylogenetic analysis for draw of phylogenetic tree and determination phylogenetic relationship used (MEGA 6) programme to compare local one strain with strains all of the world states , Compared (4 strains) from the sheep's blood *Brucella* with 16 strains of all the world states were different strains and (28 strains) from the soil *Brucella* with 38 strains of all the world states were different strains.

Phylogenetic analysis of the *16S rRNA* gene sequences indicated that *B. abortus* was nearest to Ukraine. While *B. melitensis* was nearest to with Greece as mentioned in table (3-17) and figure (3-7). While (11 strains) of *Brucella* spp. isolated from the soil compared many states as mentioned in table (3-18) and figures(3-8) ,(3-9).

Table(3-17): The Phylogenetic Tree for the Sheep's Blood Samples.

Accession	Country	Source	Compatibility
ID: KF780870.1	**China**	***B. abortus***	%99
ID: CP046721.1	**USA**	***B. abortus***	%99
ID: CP034696.1	**India**	***B. abortus***	%99
ID: CP023309.1	**Italy**	***B. abortus***	%99
ID: CP009098.1	**South Africa**	***B. abortus***	%99
ID: CP077765.1	**Russia**	***B. abortus***	%99
ID: MZ020787.1	**Nigeria**	***B. abortus***	%99
ID: CP003177.1	**South Korea**	***B. abortus***	%99
ID: NR_042460.1	**Germany**	***B. abortus***	%99
ID: CP066176.1	**Ukraine**	***B. abortus***	%99
ID: MK684240.1	**Greece**	***B. melitensis***	%99
ID: MT611105.1	**China**	***B. melitensis***	%99
ID: CP044986.1	**India**	***B. melitensis***	%99
ID: CP024716.1	**USA**	***B. melitensis***	%99
ID: LT963351.1	**Norway**	***B. melitensis***	%99
ID: CP058600.1	**Croatia**	***B. melitensis***	%99

Table (3-18): The Phylogenetic Tree for the Animals Soil's Samples

Accession	Country	Source	Compatibility
ID: OK178876.1	**India:Maunath**	***B. pseudogrignonensis***	%99
ID: MZ960147.1	**India: West Bengal**	***B. pseudogrignonensis***	%99
ID: MZ468615.1	**India**	***B. pseudogrignonensis***	%99

ID: MZ026423.1	**China**	***B. pseudogrignonensis***	%99
ID: MW990011.1	**China**	***B. pseudogrignonensis***	%99
ID: OL662885.1	**India :Maharashtra**	***B. oryzae***	%99
ID: MZ169425.1	**Malaysia**	***B. oryzae***	%99
ID: OK655705.1	**Nigeria**	***B. oryzae***	**98%**
ID: OM125096.1	**India**	***B. oryzae***	%99
ID: LC667796.1	**Pakistan**	***B. intermedia***	%99
ID: OL687412.1	**Viet Nam**	***B. intermedia***	%99
ID: OK356788.1	**Bangladesh**	***B. intermedia***	%99
ID: MZ676069.1	**China**	***B. intermedia***	%99
ID: MZ642684.1	**Austria**	***B. intermedia***	%99
ID: MZ621132.1	**Germany**	***B. intermedia***	%99
ID: MZ311845.1	**Svalbard**	***B. intermedia***	%99
ID: OK217265.1	**India: Pudungi**	***B. anthropi***	%99
ID: CP044971.1	**South Korea**	***B. anthropi***	%99
ID: CP088965.1	**China**	***B. anthropi***	%99
ID: OL614485.1	**India**	***B. anthropi***	%99
ID: OL469515.1	**Egypt**	***B. anthropi***	%99
ID: CP066053.1	**USA**	***B. anthropi***	%99
ID: MN990902.1	**India**	***B. ovis***	%99
ID: NR_074146.1	**USA**	***B. ovis***	%99
ID: KU587137.1	**Oman**	***B. inopinata***	%99
ID: NR_116161.1	**USA**	***B. inopinata***	%99
ID: KJ372229.1	**India**	***B. melitensis***	%99

ID: CP034104.1	**China: Ulanqab**	***B. melitensis***	%99
ID: CP024716.1	**USA**	***B.melitensis***	%99
ID: LT963351.1	**Norway**	***B. melitensis***	%99
ID: OK342148.1	**Greece**	***B. melitensis***	%99
ID: OK655702.1	**Nigeria**	***B. lupini***	%99
ID: OK583830.1	**India**	***B. lupini***	%99
ID: OK254133.1	**Iran**	***B. lupini***	%99
ID: OK655724.1	**Nigeria**	***B. lupini***	%99
ID: MT275734.1	**India**	***B. melitensis***	**99%**
ID: MT611105.1	**China**	***B. melitensis***	**99%**
ID: MK684236.1	**Greece**	***B. melitensis***	**99%**
ID: CP024716.1	**USA**	***B. melitensis***	**99%**
ID: LT963351.1	**Norway**	***B. melitensis***	**99%**
ID: MT912851.1	**Mexico**	***B. melitensis***	**99%**
ID: CP058600.1	**Croatia**	***B. melitensis***	**99%**
ID: CP018782.1	**USA**	***B. pituitosa***	**99%**
ID: MF928319.1	**China**	***B. pituitosa***	**99%**
ID: MN017830.1	**France**	***B. pituitosa***	**99%**
ID: MK382471.1	**New Zealand**	***B. pituitosa***	**99%**
ID: NR_115043.1	**Germany**	***B. pituitosa***	**99%**
ID: MK177585.1	**India**	***B. pituitosa***	**99%**
ID: KU712002.1	**South Korea**	***B. pituitosa***	**99%**
ID: ON111441.1	**Uruguay**	***B. pituitosa***	**99%**
ID: KY880942.1	**France**	***B. pseudogrignonensis***	**99%**

ID: KX896756.1	**Iran**	***B. pseudogrignonensis***	**99%**
ID: KY038213.1	**India**	***B. pseudogrignonensis***	**99%**
ID: CP015776.1	**Malaysia**	***B. pseudogrignonensis***	**99%**
ID: KU597487.1	**Dominican Republic**	***B. pseudogrignonensis***	**99%**
ID: KT005456.1	**China**	***B. pseudogrignonensis***	**99%**
ID: KT726993.1	**Thailand**	***B. pseudogrignonensis***	**99%**
ID: CP091785.1	**Hong Kong**	***B. pseudogrignonensis***	**99%**
ID: OL360758.1	**Russia**	***B. intermedia***	**99%**
ID: MT649859.1	**Japan**	***B. intermedia***	**99%**
ID: MT605439.1	**China**	***B. intermedia***	**99%**
ID: MT383651.1	**India**	***B. intermedia***	**99%**
ID: MT114490.1	**Egypt**	***B. intermedia***	**99%**
ID: MT113104.1	**Sri Lanka**	***B. intermedia***	**99%**
ID: KY194764.1	**Saudi Arabia**	***B. intermedia***	**99%**
ID: LT601262.2	**Spain**	***B. intermedia***	**99%**
ID: MG214517.1	**Brazil**	***B. intermedia***	**99%**
ID: OM729685.1	**India**	***B. thiophenivorans***	**99%**
ID: MN094106.1	**Russia**	***B.thiophenivorans***	**99%**
ID: MK883203.1	**Chile**	***B. thiophenivorans***	**99%**
ID: KY819002.1	**China**	***B. thiophenivorans***	**99%**
ID: KY178282.1	**Germany**	***B.thiophenivorans***	**99%**
ID: KT282999.1	**Iran**	***B. thiophenivorans***	**99%**
ID: MW763245.1	**Poland**	***B. thiophenivorans***	**99%**
ID: MW414495.1	**Portugal**	***B. thiophenivorans***	**99%**

ID: MT373404.1	**Ireland**	***B. thiophenivorans***	**99%**
ID: MZ165353.1	**Ukraine**	***B. rhizosphaerae***	**99%**
ID: NR_042600.1	**Germany**	***B. rhizosphaerae***	**99%**
ID: MZ026405.1	**China**	***B. rhizosphaerae***	**99%**
ID: MT505117.1	**Brazil**	***B. rhizosphaerae***	**99%**
ID: KU711995.1	**South Korea**	***B. rhizosphaerae***	**98%**
ID: MN985315.1	**Pakistan**	***B. rhizosphaerae***	**99%**
ID: MZ424578.1	**India**	***B. rhizosphaerae***	**99%**

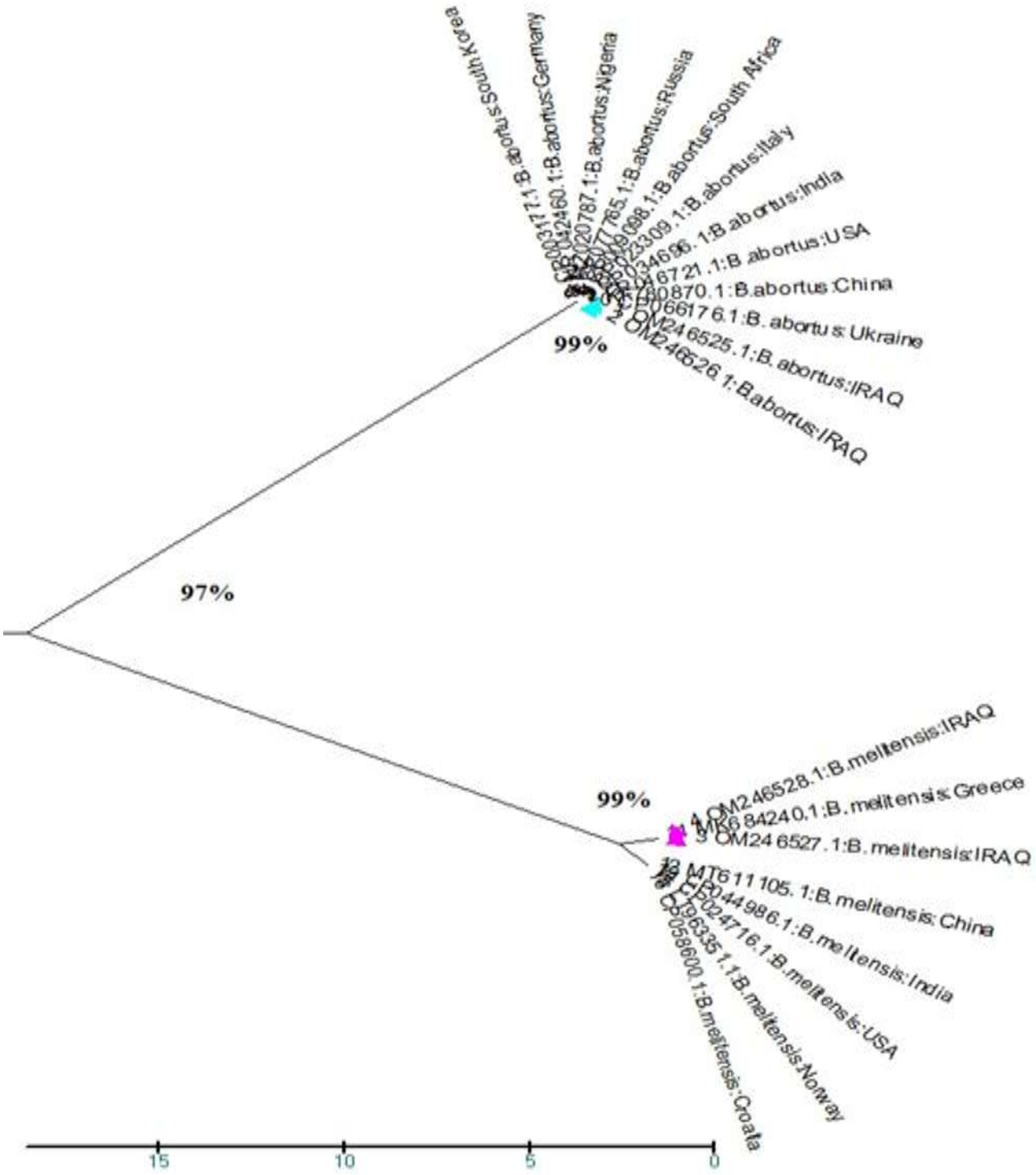


Figure (3-7): Evolutionary analysis (phylogenetic tree of *Brucella* isolates isolated from blood) 16S *rRNA* gene sequences of *Brucella* at compared the two strains (*B.abortus*, *B.melitensis*) with different states.

This figure(3-7) found that *B.abortus* was nearest to Ukraine while *B.melitensis* was nearest to Greece.

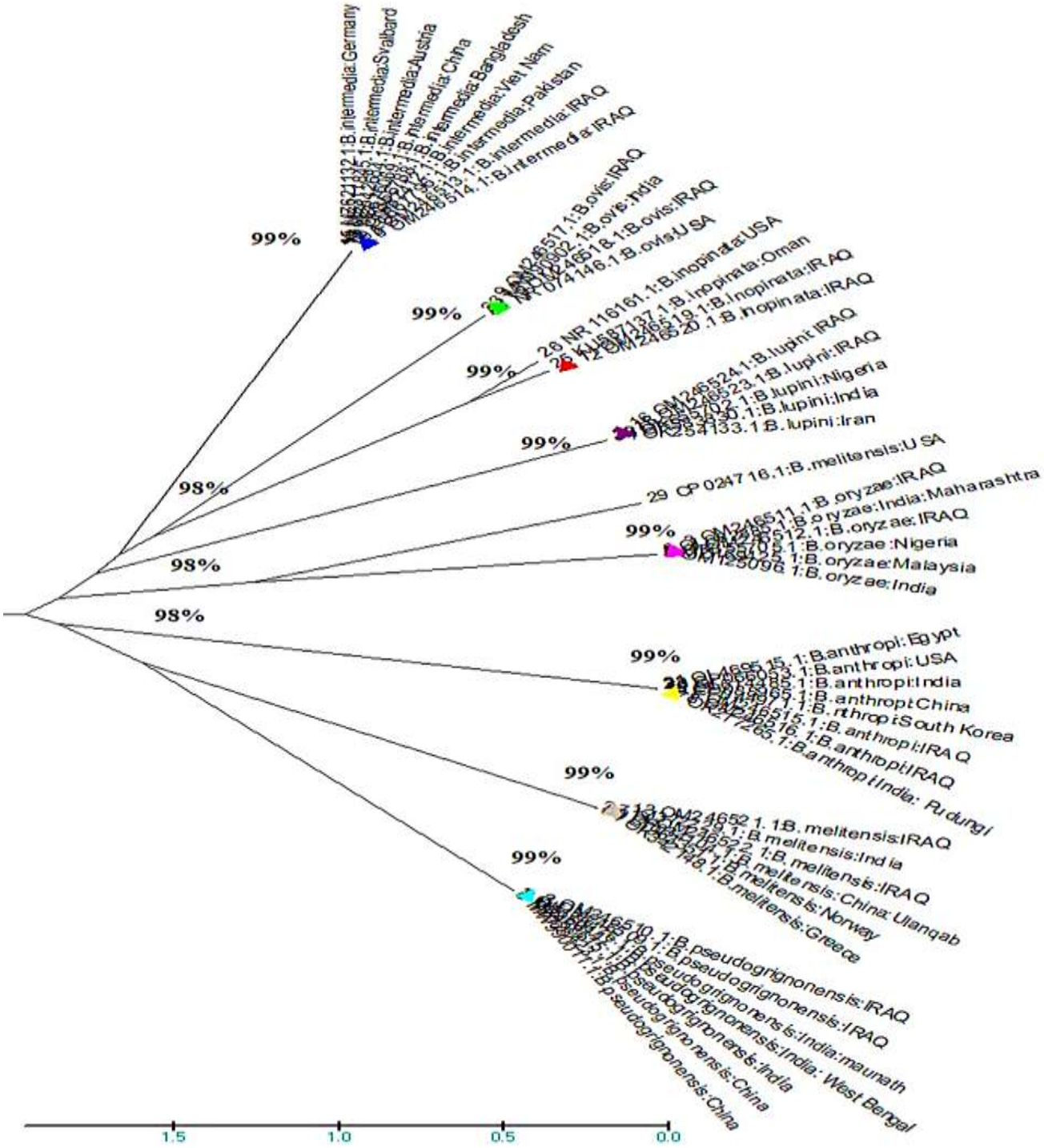


Figure (3-8): Evolutionary analysis (phylogenetic tree of *Brucella* isolates isolated from soil)) of *16S rRNA* gene sequences of *Brucella* at compared the 8 strains (*B. pseudogrignonensis, B.melitensis , B.intermedia, B.anthropi, B.oryzae , B.ovis, B.inopinata , B.lupini*) with different states.

This figure(3-8) found that *B. pseudogrignonensis* was nearest to India

,*B.melitensis* was nearest to India and china, *B.intermedia* was nearest to Pakistan, *B.anthropi* was nearest to India: Pudungi and South Korea, *B.oryzae* was nearest to India :Maharashtra, *B.ovis* was nearest to India and USA, *B.inopinata* was nearest to Oman , *B.lupini* was nearest to Nigeria.

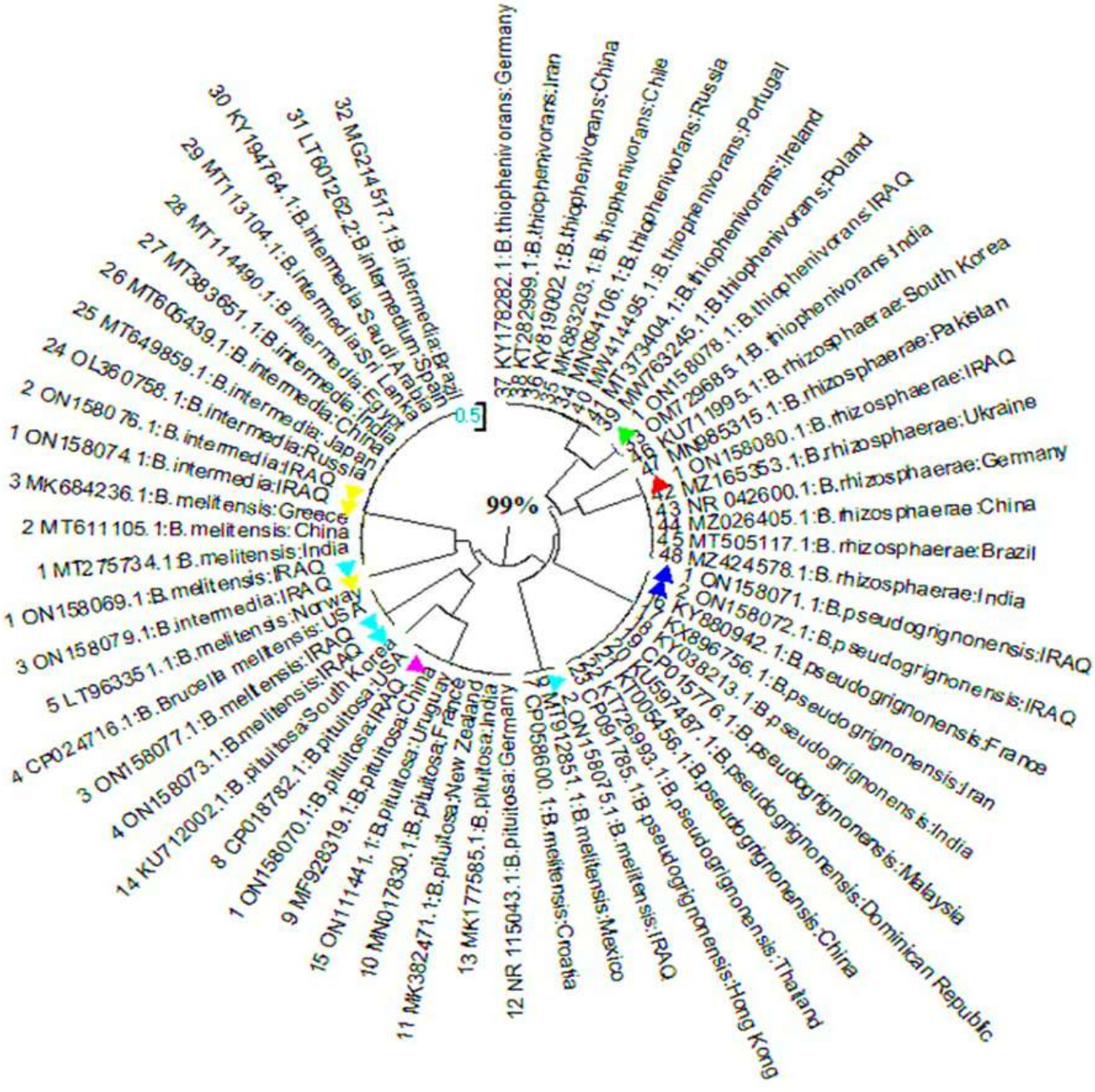


Figure (3-9): Evolutionary analysis (phylogenetic tree of *Brucella* isolates isolated from soil)) of *16S rRNA* gene sequences of *Brucella* at compared the 6 strains (*B.melitensis*, *B.pseudogrignonensis*, *B.intermedia*, *B.pituitosa*, *B.thiophenivorans*, *B.rhizosphaerae*) with different states .

This figure(3-9) found that *B.melitensis* was nearest to Mexico and USA, *B.pseudogrignonensis* was nearest to India and France, *B.intermedia* was nearest to Russia, *B.pituitosa* was nearest to China and USA , *B.thiophenivorans* was nearest to India *and* Poland, *B.rhizosphaerae* was nearest to Ukraine and Pakistan.

The phylogenetic tree of *Ochrobactrum* spp. already shows the presence of the A44T-related strains in a separate branch (Kampfer *et al*., 2007). They employed Multilocus Sequence Analysis (MLSA) (Ramette *et al*., 2011) based on the concatenated sequences of 16S rRNA and genes to acquire higher phylogenetic resolution within this group, which reveals that *O*. *rhizosphaerae* PR17T is the closest relative of A44T. The strain *Ochrobactrum* sp. was identified in the rhizosphere of a field-grown potato in Gelderland, the Netherlands. According to phylogenetic analysis based solely on the *16S rRNA* gene and concatenation of the *16S rRNA* gene and *MLSA* genes. The recA sequence had only 64% coverage and 98.71% sequence similarity to *B. inopinata* strain BO1 (GenBank accession no. FM177719) and they also submitted the DNA sequences from isolates 3278 and 5043 to GenBank (accession nos. MT471347, MT471348 and MT482342). They isolated an atypical *Brucella inopinata*-like sp. from two adult marine toads, one of these was an asymptomatic carrier, a classic lesion seen in mammalian *Brucella* infections. The results suggested that marine toads were another amphibian species susceptible to atypical *Brucella* bacteria and that infection can result in long-term asymptomatic carriers as well as more typical reproductive lesions. RecA strain BO1 had 98.71% sequence similarity with only 64% coverage in the recA sequence (GenBank accession no. FM177719).

3.2. Environmental Study:

3.2.1 The Relationship between the Environmental Factors and *Brucella* species .

Our results proved existence relation between the presence of Brucella and the physical, chemical properties. Environmental parameters was recorded during this study for four seasons, *Brucella* percentage (%) have rates between (60%-100%) where the lowest rate in Winter season while the higher rate in Summer season. Air temperature (At) has values ranged between (27.40-42.11^{0}C), while values between (21.85-37.11^{0}C) for soil temperature (St) where lowest value in winter season while highest value in summer season for both. pH has ranged between (7.51-7.81) where highest value in Summer season. Electrical conductivity (E.C) has ranged between (3768.34-5123.33µs/cm),Total dissolved solids (TDS) have ranged between (2645.94-3593.33 mg/L)and Salinity(S) has ranged between (2.47- 3.28‰) where the lowest values in Spring and Winter seasons while highest values in autumn and summer seasons for both. Whereas Total Organic Carbon (TOC) has ranged between (27.63-42.44) which have lowest value in autumn and summer seasons while higher value in winter season.

Statistical Analysis showed that *Brucella*(%) have significance differences between all seasons. *B.melitensis*(%), *B.pseudogrignonensis*(%) do not have significance differences between winter and spring seasons but have significance differences between these seasons with summer and autumn seasons, *B.thiopheniphorans*(%) and *B.pituitosa*(%) do not have significance differences between winter, spring and summer seasons but have significance differences between these seasons with autumn, *B.anthropi*(%), *B.ovis*(%), *B.oryzae*(%), *B.rhizosphaerae*(%) do not have significance differences between winter, summer and autumn seasons but have significance differences between these seasons with spring season, *B.intermedia*(%) do not have significance differences between winter and summer seasons but have significance differences between these

seasons with spring and autumn seasons, *B.inopinata*(%) and *B.lupini*(%)have significance differences between all seasons. Air temperature, soil temperature, electrical conductivity, total dissolved solids & total organic carbon have significance differences between all seasons while pH do not have significance differences between winter, spring & autumn seasons but these seasons have significance differences with summer season. Whereas salinity does not have significance differences between winter and spring seasons but these seasons have significance differences between summer and autumn seasons. mentioned in table (3-19) according to anova analysis and figure (3-10) according to canonical correspondence analysis CCA (CANOCO analysis).

Table (3-19): The Relationship between the Four Seasons and the Physical , Chemical Parameters and the Percentage of *Brucella* species (Mean ± S.D). p≤0.05

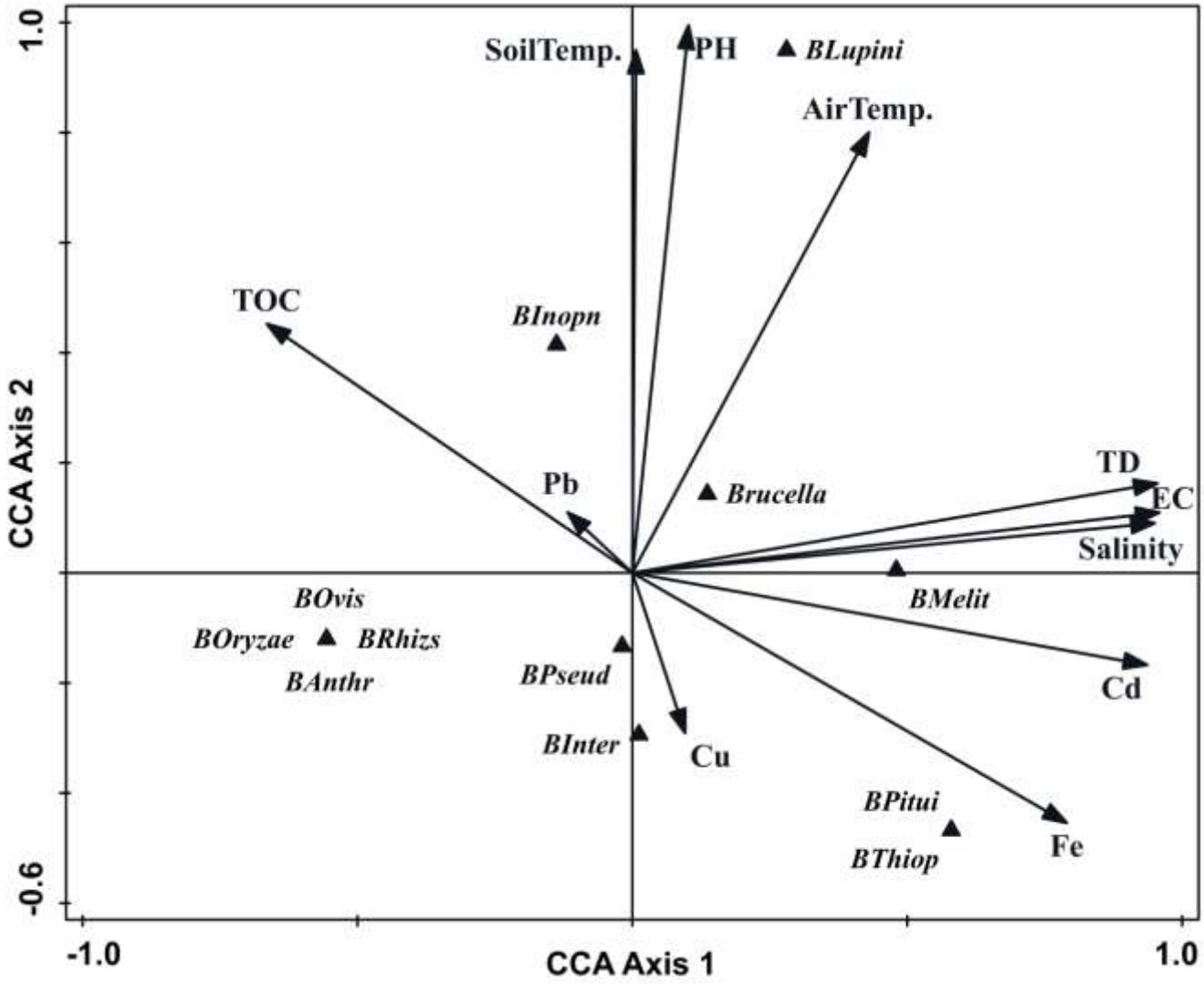
Seasons / *Brucella* species (%) and Environmental Parameters	Winter, 2021	Spring, 2021	Summer , 2021	Autumn, 2021
***Brucella* per.(%)**	60±0.5^{a}	78.13±0.87^{b}	100±11.4 ^{c}	91.67±6.7 ^{d}
***B.melitensis* per. (%)**	0.00 ^{a}	0.00 ^{a}	33.33±1.85^{b}	66.67±2.83^{c}
***B.pseudogrignonensis* per. (%)**	37.5±4.95 ^{a}	37.17±1.65^{a}	0.00 ^{b}	25.00±4.08^{c}
***B.thiopheniphorans* per. (%)**	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	100±1.63^{b}
***B.pituitosa* percentage (%)**	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	100±4.50^{b}
***B.anthropi* per. (%)**	0.00 ^{a}	100.0±4.97^{b}	0.00 ^{a}	0.00 ^{a}
***B.ovis* per. (%)**	0.00 ^{a}	100.0±5.44^{b}	0.00 ^{a}	0.00 ^{a}
***B.oryzae* per.(%)**	0.00 ^{a}	100±2.45^{b}	0.00 ^{a}	0.00 ^{a}
***B.intermedia* per. (%)**	0.00 ^{a}	50.00±3.68^{b}	0.00 ^{a}	50.00±4.08^{b}
***B.inopinata* per. (%)**	0.00 ^{a}	50.00±2.62^{b}	50.00±2.16^{c}	0.00 ^{a}

***B.lupini* per. (%)**	0.00 [a]	0.00 [a]	100.0±4.92[b]	0.00 [a]
***B.rhizosphaerae* per.(%)**	0.00 [a]	100.0±10.14[b]	0.00 [a]	0.00 [a]
Air Temp. (^{0}C)	27.40 [a]	30.69±4.8 [b]	42.11±2.4 [c]	32.83±1.8[d]
Soil temp. (^{0}C)	21.85 [a]	26.17±3.4[b]	37.11±3.1 [c]	23.01±7.3 [d]
pH	7.62±0.3 [a]	7.56±0.4 [a]	7.81±0.2 [b]	7.51±03. [a]
EC (µs/cm)	3957±14.5[a]	3768±22.6 [b]	4959±72.4 [c]	5123±18.1 [d]
TDS (mg/L)	2812±10.5[a]	2646±15.8 [b]	3549±21.6 [c]	3593±12.7 [d]
Salinity(‰)	2.53± 0.9[a]	2.47±0.3 [a]	3.17±0.7 [b]	3.28±1.1 [b]
TOC(%)	42.44±3.4 [a]	35.68± 2.3[b]	34.57±3.1 [c]	27.63±2.03[d]

This table(3-19) noted that proportion of *Brucella* presence in summer is higher that it is in relation with environmental parameters which include (Air and Soil temperature, pH, EC, TDS and Salinity) that are high , except TOC is low while proportion of *Brucella* presence in winter is lower, also relate with environmental parameters include (Air and Soil temperature, pH, EC, TDS and Salinity), these the parameters (the physical and chemical) is low except TOC which is high**.** This indicated that variation of seasons affect to *Brucella* ratio in soil .

The explanation for this is that when temperatures rise, the speed of chemical and biological reactions in the soil increases, which increases the growth and activity of bacteria, so their presence in the soil increases. These microorganisms live in a large range of heat and adapt to the temperature changes that occur in the soil. In the soil, these bacteria decompose the organic matter greatly, the accumulation of organic matter decreases, and thus the percentage of organic matter in the soil decreases. Also, the increase in salt affects the activity of microorganisms because it causes an increasing in plant residues, which are a source of energy for microorganisms. Temperature determines the organic matter in the soil, when the temperature decreases, it helps to accumulate organic matter in the soil, as the organic matter increases with the amount of rain, and the low temperature also reduces the chemical and biological reactions in the soil, so the percentage of bacteria's presence also affects the decomposition of the

accumulated matter a little, and thus the percentage of organic matter increases in the soil. Bacteria prefer to live in a neutral medium between 6 and 8, whereby *Brucella* prefers a neutral medium with a value of 6.8. *Brucella* strains are resistant to drying and can live for lengthy periods of time in biological material, especially at low temperatures (Quinn *et al*., 2004). Bacterial cells are able to survive for a prolonged time in water, aborted fetuses, soil, dairy products, meat, dung, and dust(Gwida *et al*., 2010).



Figure(3-10): The relationship between *Brucella* species (%) and the environmental factors

This figure(3-10) shows a strong positive relation between *B.lupini*(%) with pH , air temperature, soil temperature and a weak relation with TD,EC, salinity ,Cd, Fe, Cu but has a negative relation with TOC, Pb. *Brucella* species(%) has a strong positive relation between TD, EC, salinity, Cd, Fe ,Cu and a weak relation with air temperature, pH, soil temperature while has a negative relation with TOC and Pb. *B.melitensis*(%) has a strong positive relation with Cd, salinity, EC, TDS, Fe, Cu and a weak relation with air temperature ,pH, soil temperature. *B.pituitosa* (%) has a strong positive relation with Fe, Cd, Cu ,salinity, EC, TDS and a weak relation with air temperature, pH, soil temperature while a negative relation TOC and pb. *B.inopinata*(%), *B.ovis*(%), *B.oryzae*(%), *B.rhizosphaerae*(%), *B.anthropi*(%) have strong positive relation with TOC and pb while negative relation with pH, air temperature ,soil temperature, TDS, EC, salinity, Cd, Fe, Cu. While *B.pseudogrignonensis*(%) and *B.intermedia*(%) have influenced by other environmental variables.

3.2.2 Relationship of the Heavy Metals with *Brucella* Species.

Our results confirmed existence relation between the presence of *Brucella* spp. and the heavy metals **,** as mentioned in table **(3-20).** *Brucella* spp. percentage (%) have rates between (60%-100%) where the lowest rate in Winter season while the higher rate in Summer season. Heavy Metals concentrations in study sites showed copper (Cu) ranged (4.11- 12.51mg\kg) has the lowest concentration in summer season but the highest in winter season. While Iron (Fe) ranged (304.08-412.29 mg\kg) and Cd (0.52- 1.88mg\kg) have the lowest concentrations in summer & spring seasons but the highest concentration in winter season for both. Whereas Lead (Pb) recorded (647.99-722.32mg\kg) the lowest concentration in winter season and the highest in summer season. Some significant differences according to statistical analysis and all previously mentioned concentrations of heavy metals.

Statistical Analysis showed that *Brucella*(%) have significance differences between all seasons. Copper(Cu) do not have significance differences between spring ,summer & autumn seasons but these seasons have significance differences with winter season while (Iron)Fe & lead(Pb) have significance differences between all seasons whereas cadmium (Cd) do not have significance differences between winter & autumn seasons but these seasons have significance differences between spring & summer seasons. All measured metals followed this trend (Pb >Fe >Cu >Cd).

Table (3-20): The Relationship between the Four Seasons with *Brucella* species (%) and the Concentrations of Heavy Metals (Mean±S.D). p≤0.05

Seasons / *Brucella* species (%) and Heavy Metals	Winter, 2021	Spring, 2021	Summer, 2021	Autumn, 2021
***Brucella* per.(%)**	60±0.5^{a}	78.13±0.87^{b}	100±11.4 ^{c}	91.67±6.7 ^{d}
***B.melitensis* per. (%)**	0.00 ^{a}	0.00 ^{a}	33.33±1.85^{b}	66.67±2.83^{c}
***B.pseudogrignonensis* per. (%)**	37.5±4.95 ^{a}	37.17±1.65^{a}	0.00 ^{b}	25.00±4.08^{c}
***B.thiopheniphorans* per. (%)**	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	100±1.63^{b}
***B.pituitosa* per. (%)**	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}	100±4.50^{b}
***B.anthropi* per. (%)**	0.00 ^{a}	100.0±4.97^{b}	0.00 ^{a}	0.00 ^{a}
***B.ovis* per. (%)**	0.00 ^{a}	100.0±5.44^{b}	0.00 ^{a}	0.00 ^{a}
***B.oryzae* per.(%)**	0.00 ^{a}	100±2.45^{b}	0.00 ^{a}	0.00 ^{a}
***B.intermedia* per. (%)**	0.00 ^{a}	50.00±3.68^{b}	0.00 ^{a}	50.00±4.08^{b}
***B.inopinata* per. (%)**	0.00 ^{a}	50.00±2.62^{b}	50.00±2.16^{c}	0.00 ^{a}
***B.lupini* per. (%)**	0.00 ^{a}	0.00 ^{a}	100.0±4.92^{b}	0.00 ^{a}
***B.rhizosphaerae* per.(%)**	0.00 ^{a}	100.0±10.14^{b}	0.00 ^{a}	0.00 ^{a}
Cu (mg\kg)	12.5±0.41^{a}	5.12±0.83 ^{b}	4.04±0.82 ^{b}	5.32±0.93 ^{b}
Fe(mg\kg)	412±0.83 ^{a}	304±0.82 ^{b}	315±0.46 ^{c}	396±2.89 ^{d}
Cd(mg\kg)	1.83±0.09^{a}	0.52±0.02 ^{b}	1.18±0.01 ^{c}	1.72±0.09 ^{a}
Pb(mg\kg)	647±0.94 ^{a}	726±0.94 ^{b}	732±0.53 ^{c}	722±0.83 ^{d}

In the current study, it was observed that heavy metals affect the percentage of *Brucella* presence in soil, The high amounts of Cu ,Fe, Cd are in winter , where the percentage of *Brucella* is low, except for lead (Pb), which is low in the presence of little *Brucella*. while low amounts of copper ,iron, cadmium be in the proportion of the presence of high *Brucella* except for lead (Pb), which is high in the presence high *Brucella* .

To explain this, when heavy metals increase, the percentage of bacterial killing increases and this is what was observed in the above-mentioned results, except for lead, which was less. While in summer was high may be due to the frequent appearance of insect pests during the summer and moderate seasons lead to frequent using of lead-containing pesticides. This pesticides contain lead that transmitted to soil by spraying the nearby plants to or as aerosols in air deposited to soil or transmit to water that use in drinking and cleaning the floors of the barns, which causes an increase in the accumulation of lead in these soils, Also may be due to using veterinarian drugs to treat animals, Pb also comes from tillage machines that use gasoline and from fuel combustion, which accumulates in the bones of animals and is excreted with urine. It is also one of the causes of abortion for pregnant females and males sterility. According to Wu *et al*., (2004), Chemical fertilizers alone do not appear to be as effective at preserved organic matter in soil as organic matter inputs in the form of manure or straw, either alone or in combination with them . The effects of organic manure plus inorganic fertilizer on topsoil carbon storage and crop productivity were larger than those of chemical fertilizer alone (Ding *et al*., 2012). A high concentration of soil organic matter in soils aided in the different interactions of micronutrients, resulting in the production of more stable micronutrient complexes, because Fe and Mn were less sensitive to redox changes therefore soil organic matter binds more Cu than Fe. Organic matter accretion at the soil surface promotes transformations of Fe

while potentially decreasing in availability of Cu by their redistribution among other complex fractions (Dhaliwal *et al*., 2019).

The results mentioned in the tables(3-19),(3-20) and figure(3-10) showed that there is relationship between the *Brucella* (%) with environmental factors and heavy metals according to seasons. These results agreed with Ahmed *et al*., (2017) that animals were infected by *Brucella* species, which also have a zoonotic effect. Risk variables associated with the presence of *Brucella* species in soil were investigated 256 soil samples (n = 1280) were collected. The rate of *Brucella* was higher in the summer in agreement with Salari *et al*., (2003) which showed that the frequency of *Brucella* sp. was highest in the summer (39.5%). The majority of infections (98%) had a history of consuming infectious cheese and milk products.

Our findings were also consistent with those of Liu *et al* ., (2019). This incidence was the highest in the summer and fall, with a peak in May. Six virulence genes were discovered in two separate strains, indicating that the *Brucella melitensis* strains were highly virulent. Because soil has the greatest carbon reservoir in the terrestrial ecosystem, tiny changes in soil carbon can have a large impact on the carbon balance of the ecosystem (Schmidt *et al*., 2011). According to the recent studies, the carbon stored in the subsurface has a longer turnover time than the carbon contained in the topsoil. it is necessary to identify the depth at which high and low amounts of soil organic carbon is stored in order to implement sustainable soil management (Meersmans *et al*., 2009). There were many reports demonstrating strong correlations between environmental factors and soil organic carbon content, stocks, or turnover in addition to vegetation and soil microbes (Meersmans *et al*., 2008).

However, relatively few studies have concentrated on the relationships between these abiotic factors and SOM composition, air temperatures, and land uses. However, numerous studies demonstrate that a variety of factors interact with soil organic matter. After conducting soil organic matter analyses across a variety of

grasslands in New Zealand, Wang *et al*., (2016) reported that temperature, rainfall, soil order, landscape, and land-use might all contribute to the explanation of the heterogeneity of SOM composition. Vancampenhout *et al*., (2010) also looked at a number of soil factors that affect SOM composition, such as dominating vegetation. In order to comprehend the multidimensional character of SOM composition, it is necessary to take soil environmental elements into account. The interrelationship of a species and its position in a particular ecosystem, including the relationship of the species with the components of the ecosystem itself, and the niche may be influenced by all the factors included in its ecosystem. The niche of a species in a particular ecosystem helps to set up the features of its ecosystem that are important for its survival. According to reports, the incidence rates in Iraq and Egypt, for instance, might vary by 4-5 times depending on the region. This highlights how lifestyle, social, and environmental factors might affect the occurrence of *Brucella* (Hotez *et al*., 2012). The ecology of brucellosis was changing dramatically as a result of changes in lifestyle and the environment (Pappas, 2010), leading to an unequal distribution of the sickness around the world. It was possible to obtain soils with various organic matter contents by using samples from various sites from a single site where various organic matter contents have developed due to variations in crop management practices; or by using soil samples from a single site with various organic matter contents (Soane, 1990). Abbas and Aldeewan , (2009) showed that samples of cow, buffalo, and sheep milk were collected from various locations in the Basrah region of Iraq in comparison to the colder months, the prevalence of *Brucella* isolates was higher in the spring and summer, all *Brucella* isolates have shown the capacity to proliferate between the temperatures of (18-44^{0}C) and at pH 4-9. Bacterial growth and diversity were correlated with organic matter. The microbial diversity and number were very high in the top 10 cm of the soil and decrease with depth (Eilers *et al*., 2012). It was a pathogenic genus that was extremely

well-adapted to its hosts and could not survive in open circumstances for long periods of time and for this reason, it was known as a "facultative extracellular intracellular parasite," which means that *Brucella* occupies a niche in the intracellular milieu of host cells is unique to a single host cell (Gorvel and Moreno, 2002). Babur and Dindaroglu, (2020) examined seasonal variations in microbial activity and microbial biomass as well as the effects of seasonal variations to better understand the dynamics of nutrients in tree species (mainly temperature and water content). The availability and release of nutrients in an ecosystem were influenced by the decomposition of soil organic matter by soil microbes. Microbial population and activity levels varied significantly with the seasons, following a predictable pattern in microbial population and activity levels (summer>autumn>spring>winter).Improving these two important soil components could aid in climate change and mitigation in terms of soil aggregation, soil porosity, and aeration. There were notable differences in the geographic distribution of this disease in Iran, according to Pakzad *et al*., (2018).The prevalence of brucellosis was high in the west and north-western cities of the nation and this an illness was more common in the summer, residents of these regions have to pay more for healthcare. As a result, it was a necessary to precisely define the environmental, economic and social factors that contribute to the disease in order to identify the areas with the highest risk and the riskiest times of day. There was also a statistically significant difference across villages in the incidence of brucellosis, the peak period of incidence was in June and July. To prevent the spread of the epidemic, brucellosis should be closely monitored (Huang *et al*., 2012). The spring season had high seasonal incidence of *Brucella* infection (Haggag *et al*., 2016). Also ,the summer season had a higher rate of brucellosis($p<0.05$) (Alkahtani *et al*.,2020).Epidemiological research on this bacteria has revealed that it occurs in all seasons, most usually in spring and summer (Uluğ and Can- Uluğ, 2010), which was consistent with our findings.

Additionally, the findings corroborated those of Aune *et al*., (2012), who found that *Brucella* bacteria could survive in soil or vegetation for 21–81 days depending on the month, temperature, and exposure to sunlight. Since heavy metals are not biodegradable therefore persisting, their concentrations are constantly rising and posing a serious threat to the ecosystem (Sraieb *et al*., 2016). Activity and community composition of soil microorganisms could effectively serve as indicators of the soil's environmental quality (Igbinosa, 2015).The diversity and activity of soil microorganisms were extremely vulnerable to both inorganic and organic pollutants (Subrahmanyam *et al*., 2014). In industrialized countries, the most common causes of brucellosis were occupational exposures, veterinary medicine, farming, animal husbandry and butchering were high-risk occupations for the disease, presence of transmission in laboratory has also been reported (Mandel *et al*., 2005). Most adult cases of brucellosis (60.8%), according to epidemiological characteristics and clinical symptoms, originated in rural areas. 306 instances, or 66.3%, appeared in the spring or summer seasons (Roushan *et al*., 2004). During spring and summer seasons, brucellosis infections were most likely to develop, whereas the winter season was the least likely. Brucellosis cases are changed seasonally, increasing during the spring and summer seasons. This seasonal variation has been linked to springtime exposure to cows giving birth, an increase in travel to rural areas and consumption of raw milk products (Cooper,1991). pH range between 6.4 and 6.7 with a high of 8.7 and a minimum of 5.8 range was the optimal range for *Brucella* species to reproduce (Frobisher, 1968). According to studies by Estrada *et al*., (2005) , *Brucella abortus* was able to live for two days at pH 3.9 and for 34 days in milk with a pH range of 5.0 to 5.8 to lactic acid. Different subgroups were compared based on animal species, gender, age, sampling season, sampling locations, and the brucella screening method (Dadar *et al*., 2021).

Conclusions

&

Recommendations

Conclusions

1. In this study, it was noted that proportion of *Brucella* spp. presence in summer was high in summer and also environmental parameters which included (Air and Soil temperature, pH, EC, TDS and Salinity) were high except TOC was lower in summer while heavy metals included Cu, Fe, Cd were have lower concentrations in summer except Pb had higher concentration in summer, this indicated that differences of seasons affected to *Brucella* spp. ratio in soil with environmental factors.
2. *Brucella* spp. survived in local soils due to environmental conditions that being variations in *Brucella* species, which led to a change in their properties and make it resistance .
3. The transmission of *Brucella* species from its reservoir host, such as cows, to the rest of the livestock and herd present with them in same the barn, such as a sheep.
4. Recording new nucleotides sequencing in both soil and blood samples and presence of new isolates of *Brucella* in local soils and animals blood samples that do not exist previously when compared with *Brucella* species in the global soils and blood.
5. Registered new strains in soil and blood, 11 new species of *Brucella* (*B. pseudogrignonensis, B. rhizosphaerae, B. oryzae, B. intermedia, B. anthropic, B. ovis, B. inopinata, B. melitensis, B. lupini, B. pituitosa, B. thiophenivorans*) recorded in soil for 28 strains and four strains for two species of *Brucella* (*B.abortus and B.melitensis*) in blood, where noted that these strains were present in certain seasons and not present in other seasons.

6. The findings of soil demonstrated that *B. melitensis* was found in both the summer and autumn seasons, but *B. pseudogrignonensis* was found in the winter, spring and autumn seasons. *B. intermedia* in both the spring and fall seasons, *B. oryzae, B. anthropi, B. ovis* in the spring season while *B. thiophenivorans* and *B. pituitosa* in the autumn season. These types of *Brucella* have variations in sequencing. while results the sheep's blood showed presence of two types of *Brucella* spp. (*B. Melitensis and B. abortus*) have variations in sequence.
7. Also noted during phylogenetic analysis of the 16S rRNA sequences that *B. abortus* was nearest to Ukraine. While *B. melitensis* was nearest to with Greece in blood samples whereas the two strains of *B. pseudogrignonensis* one was nearest to India and the other was nearest to India and France, the two strains of *B.melitensis* one was nearest to India and China and the other was nearest to Mexico and USA, *B.intermedia* was nearest to Pakistan, *B.anthropi* was nearest to India: Pudungi and South Korea, *B.oryzae* was nearest to India: Maharashtra, *B.ovis* was nearest to India and USA , *B.inopinata* was nearest to Oman, *B.lupini* was nearest to Nigeria, *B.intermedia* was nearest to Russia, *B.pituitosa* was nearest to China and USA, *B.thiophenivorans* was nearest to India *and* Poland, *B.rhizosphaerae* was nearest to Ukraine and Pakistan in animals soils samples, these stated consider one o f the sources for the infection.

Recommendations

1. Studying indetails of each new isolate of *Brucella* to determine whether it is dangerous or unsatisfying or may be beneficial to soil and plants.

2- Studying of karyotype for knowledge of *Brucella* division phases.

3- Study of the genes responsible for pathogenicity and environmental factors resistance genes and also enzymes for *Brucella* spp.

4-Design a biosensor from *Brucella* species for monitoring of disease epidemiology and outbreak.

5-Examination of livestock imported from these countries Ukraine, Greece, India, India: Pudungi, India: Maharashtra, China, Pakistan, South Korea, India: Maharashtra, U.S.A, Oman, Nigeria, Mexico, France, Russia, Poland, which are considered one of the sources of animal infection with *Brucella* spp. and soil contamination with it.

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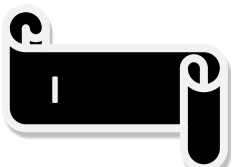
Appendices

Appendix 1: New nucleotides sequencing of *Brucella* isolates isolated from the sheep's blood samples (Sequence Results Analysis) and recorded in NCBI

16S ribosomal RNA gene						
No.	**Type of substitution**	**Location**	**Nucleotide**	**Sequence ID with compare**	**Source**	**Identities**
1	**Transition**	692	C\T	**ID: KF780870.1**	***Brucella abortus***	**99%**
2	**Transition**	692	C\T	**ID: KF780870.1**	***Brucella abortus***	**99%**
3	**Transition**	692	C\T	**ID: KF780870.1**	***Brucella abortus***	**99%**
4	**Transition**	524	T\C	**ID: KF780870.1**	***Brucella abortus***	**99%**
	Transition	692	C\T			
5	**Transition**	524	T\C	**ID: KF780870.1**	***Brucella abortus***	**99%**
	Transition	692	C\T			
7	**Transvertion**	590	A\T	**ID: KF780870.1**	***Brucella abortus***	**99%**
	Transition	866	T\C			
9	**Transition**	556	A\G	**ID: KF780870.1**	***Brucella abortus***	**99%**
	Transvertion	590	A\T			
	Transvertion	619	G\C			
	Transition	866	T\C			
10	**Transition**	556	A\G	**ID: KF780870.1**	***Brucella abortus***	**99%**
	Transvertion	590	A\T			
	Transition	866	T\C			
11	**Transvertion**	590	A\T	**ID: KF780870.1**	***Brucella abortus***	**99%**
	Transvertion	765	C\G			
	Transition	866	T\C			
12	**Transvertion**	590	A\T	**ID: KF780870.1**	***Brucella abortus***	**99%**
	Transition	831	A\G			
	Transition	868	T\C			
13	**Transition**	300	A\G	**ID: MK684240.1**	***Brucella melitensis***	**99%**
	Transvertion	335	T\G			
	Transition	488	C\T			
15	**Transition**	300	A\G	**ID: MK684240.1**	***Brucella melitensis***	**99%**
	Transvertion	335	T\G			
	Transition	488	C\T			
	Transition	890	C\T			
16	**Transition**	300	A\G	**ID: MK684240.1**	***Brucella melitensis***	**99%**
	Transvertion	335	T\G			
	Transition	488	C\T			
17	**Transition**	300	A\G	**ID: MK684240.1**	***Brucella melitensis***	**99%**
	Transvertion	335	T\G			
	Transition	488	C\T			
18	**Transition**	809	G\A	**ID: MK684240.1**	***Brucella melitensis***	**99%**
	Transition	1003	T\C			
19	**Transition**	809	G\A	**ID: MK684240.1**	***Brucella melitensis***	**99%**
	Transvertion	980	C\A			
	Transition	1003	T\C			
20	**Transition**	809	G\A	**ID: MK684240.1**	***Brucella melitensis***	**99%**
	Transvertion	980	C\A			
	Transition	1003	T\C			



1

Alignment of *Brucella abortus* strain S2 16S ribosomal RNA gene, partial sequence
Sequence ID: KF780870.1Length: 1383Number of Matches: 1
Range 1: 277 to 906GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1132 bits(1255)	0.0	629/630(99%)	0/630(0%)	Plus/Plus

```
Query  1    ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG  60
Sbjct  277  ............................................................  336
Query  61   TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA  120
Sbjct  337  ............................................................  396
Query  121  CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG  180
Sbjct  397  ............................................................  456
Query  181  TAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAA  240
Sbjct  457  ............................................................  516
Query  241  CTGCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAG  300
Sbjct  517  ............................................................  576
Query  301  GTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC  360
Sbjct  577  ............................................................  636
Query  361  TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCTACGC  420
Sbjct  637  ...........................................................C....  696
Query  421  CGTAAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATT  480
Sbjct  697  ............................................................  756
Query  481  AAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC  540
Sbjct  757  ............................................................  816
Query  541  CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCT  600
Sbjct  817  ............................................................  876
Query  601  TGACATCCCGGTCGCGGTTAGTGGAGACAC  630
Sbjct  877  ..............................  906
```

2

Alignment of *Brucella abortus* strain S2 16S ribosomal RNA gene, partial sequence
Sequence ID: KF780870.1Length: 1383Number of Matches: 1
Range 1: 277 to 906GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1132 bits(1255)	0.0	629/630(99%)	0/630(0%)	Plus/Plus

```
Query  1    ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG  60

Sbjct  277  ............................................................
336

Query  61   TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA
120

Sbjct  337  ............................................................
396

Query  121  CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG
180

Sbjct  397  ............................................................
456

Query  181  TAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAA
240

Sbjct  457  ............................................................
516

Query  241  CTGCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAG
300

Sbjct  517  ............................................................
576

Query  301  GTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC
360

Sbjct  577  ............................................................
636

Query  361  TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCTACGC
420

Sbjct  637  ............................................................C....
696

Query  421  CGTAAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATT
480

Sbjct  697  ............................................................
756

Query  481  AAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC
540

Sbjct  757  ............................................................
816

Query  541  CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCT
600

Sbjct  817  ............................................................
876

Query  601  TGACATCCCGGTCGCGGTTAGTGGAGACAC  630
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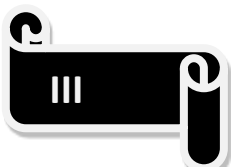

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Sbjct  877  ..........................  906
```

3

Alignment of *Brucella abortus* strain S2 16S ribosomal RNA gene, partial sequence
Sequence ID: KF780870.1Length: 1383Number of Matches: 1
Range 1: 277 to 906GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1132 bits(1255)	0.0	629/630(99%)	0/630(0%)	Plus/Plus

```
Query  1    ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG  60
Sbjct  277  ............................................................
336

Query  61   TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA
120

Sbjct  337  ............................................................
396

Query  121  CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG
180

Sbjct  397  ............................................................
456

Query  181  TAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAA
240

Sbjct  457  ............................................................
516

Query  241  CTGCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAG
300

Sbjct  517  ............................................................
576

Query  301  GTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC
360

Sbjct  577  ............................................................
636

Query  361  TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCTACGC
420

Sbjct  637  ............................................................c....
696

Query  421  CGTAAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATT
480

Sbjct  697  ............................................................
756

Query  481  AAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC
540

Sbjct  757  ............................................................
816

Query  541  CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCT
600

Sbjct  817  ............................................................
876
```

```
Query  601  TGACATCCCGGTCGCGGTTAGTGGAGACAC  630
Sbjct  877  ..............................  906
```

4

Alignment of *Brucella abortus* strain S2 16S ribosomal RNA gene, partial sequence
Sequence ID: KF780870.1Length: 1383Number of Matches: 1
Range 1: 277 to 906GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1128 bits(1250)	0.0	628/630(99%)	0/630(0%)	Plus/Plus

```
Query  1    ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG  60
Sbjct  277  ............................................................  336
Query  61   TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA  120
Sbjct  337  ............................................................  396
Query  121  CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG  180
Sbjct  397  ............................................................  456
Query  181  TAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAA  240
Sbjct  457  ............................................................  516
Query  241  CTGCCTTCGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAG  300
Sbjct  517  .......T....................................................  576
Query  301  GTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC  360
Sbjct  577  ............................................................  636
Query  361  TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCTACGC  420
Sbjct  637  ............................................................  696
Query  421  CGTAAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATT  480
Sbjct  697  ............................................................  756
Query  481  AAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC  540
Sbjct  757  ............................................................  816
Query  541  CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCT  600
```

```
Sbjct  817  ..........................................................
876

Query  601  TGACATCCCGGTCGCGGTTAGTGGAGACAC  630

Sbjct  877  ............................  906
```

5

Alignment of *Brucella abortus* strain S2 16S ribosomal RNA gene, partial sequence
Sequence ID: KF780870.1Length: 1383Number of Matches: 1
Range 1: 277 to 906GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1128 bits(1250)	0.0	628/630(99%)	0/630(0%)	Plus/Plus

```
Query  1    ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG  60

Sbjct  277  ..........................................................
336

Query  61   TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA
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Sbjct  337  ..........................................................
396

Query  121  CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG
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Sbjct  397  ..........................................................
456

Query  181  TAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAA
240

Sbjct  457  ..........................................................
516

Query  241  CTGCCTTCGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAG
300

Sbjct  517  .......T..................................................
576

Query  301  GTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC
360

Sbjct  577  ..........................................................
636

Query  361  TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCTACGC
420

Sbjct  637  ..........................................................C....
696

Query  421  CGTAAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATT
480

Sbjct  697  ..........................................................
756

Query  481  AAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC
540

Sbjct  757  ..........................................................
816
```

```
Query  541  CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCT
600

Sbjct  817  ............................................................
876

Query  601  TGACATCCCGGTCGCGGTTAGTGGAGACAC  630

Sbjct  877  ............................  906
```

7

Alignment of *Brucella abortus* strain S2 16S ribosomal RNA gene, partial sequence
Sequence ID: KF780870.1Length: 1383Number of Matches: 1
Range 1: 277 to 906GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1128 bits(1250)	0.0	628/630(99%)	0/630(0%)	Plus/Plus

```
Query  1    ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG  60

Sbjct  277  ............................................................
336

Query  61   TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA
120

Sbjct  337  ............................................................
396

Query  121  CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG
180

Sbjct  397  ............................................................
456

Query  181  TAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAA
240

Sbjct  457  ............................................................
516

Query  241  CTGCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAG
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Sbjct  517  ............................................................
576

Query  301  GTGAAATTCGTAGTTATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC
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Sbjct  577  ............A...........................................
636

Query  361  TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
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Sbjct  637  ............................................................
696

Query  421  CGTAAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATT
480

Sbjct  697  ............................................................
756

Query  481  AAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC
540
```

```
Sbjct  757  ..........................................................
816

Query  541  CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCCTACCAGCCCT
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Sbjct  817  ..........................................................T.........
876

Query  601  TGACATCCCGGTCGCGGTTAGTGGAGACAC  630

Sbjct  877  ..............................  906
```

9

Alignment of *Brucella abortus* strain S2 16S ribosomal RNA gene, partial sequence
Sequence ID: KF780870.1Length: 1383Number of Matches: 1
Range 1: 277 to 906GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1119 bits(1240)	0.0	626/630(99%)	0/630(0%)	Plus/Plus

```
Query  1    ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG  60

Sbjct  277  ..........................................................
336

Query  61   TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA
120

Sbjct  337  ..........................................................
396

Query  121  CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG
180

Sbjct  397  ..........................................................
456

Query  181  TAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAA
240

Sbjct  457  ..........................................................
516

Query  241  CTGCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGGGTGGAATTCCGAGTGTAGAG
300

Sbjct  517  ......................................A...................
576

Query  301  GTGAAATTCGTAGTTATTCGGAGGAACACCAGTGGCGAAGGCCGCTCACTGGACCATTAC
360

Sbjct  577  ............A............................G.................
636

Query  361  TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
420

Sbjct  637  ..........................................................
696

Query  421  CGTAAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATT
480

Sbjct  697  ..........................................................
756
```

```
Query  481  AAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC
540

Sbjct  757  ............................................................
816

Query  541  CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCCTACCAGCCCT
600

Sbjct  817  ...........................................................T..........
876

Query  601  TGACATCCCGGTCGCGGTTAGTGGAGACAC  630

Sbjct  877  ..............................  906
```

10

Alignment of *Brucella abortus* strain S2 16S ribosomal RNA gene, partial sequence
Sequence ID: KF780870.1Length: 1383Number of Matches: 1
Range 1: 277 to 906GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1123 bits(1245)	0.0	627/630(99%)	0/630(0%)	Plus/Plus

```
Query  1    ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG  60

Sbjct  277  ............................................................
336

Query  61   TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA
120

Sbjct  337  ............................................................
396

Query  121  CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG
180

Sbjct  397  ............................................................
456

Query  181  TAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAA
240

Sbjct  457  ............................................................
516

Query  241  CTGCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGGGTGGAATTCCGAGTGTAGAG
300

Sbjct  517  ........................................A...................
576

Query  301  GTGAAATTCGTAGTTATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC
360

Sbjct  577  ............A...............................................
636

Query  361  TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
420

Sbjct  637  ............................................................
696

Query  421  CGTAAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATT
480
```

```
Sbjct  697  ..........................................................
756

Query  481  AAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC
540

Sbjct  757  ..........................................................
816

Query  541  CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCCTACCAGCCCT
600

Sbjct  817  ..........................................................T.........
876

Query  601  TGACATCCCGGTCGCGGTTAGTGGAGACAC  630

Sbjct  877  ..............................  906
```

11

Alignment of *Brucella abortus* strain S2 16S ribosomal RNA gene, partial sequence
Sequence ID: KF780870.1Length: 1383Number of Matches: 1
Range 1: 277 to 906GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1123 bits(1245)	0.0	627/630(99%)	0/630(0%)	Plus/Plus

```
Query  1    ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG  60

Sbjct  277  ..........................................................
336

Query  61   TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA
120

Sbjct  337  ..........................................................
396

Query  121  CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG
180

Sbjct  397  ..........................................................
456

Query  181  TAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAA
240

Sbjct  457  ..........................................................
516

Query  241  CTGCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAG
300

Sbjct  517  ..........................................................
576

Query  301  GTGAAATTCGTAGTTATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC
360

Sbjct  577  ............A.............................................
636

Query  361  TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
420

Sbjct  637  ..........................................................
696
```

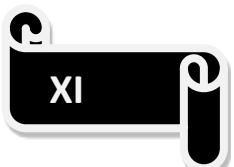
```
Query  421  CGTAAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATT
480

Sbjct  697  ............................................................
756

Query  481  AAACATTCGGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC
540

Sbjct  757  ........C...................................................
816

Query  541  CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCCTACCAGCCCT
600

Sbjct  817  ..................................................T.........
876

Query  601  TGACATCCCGGTCGCGGTTAGTGGAGACAC  630

Sbjct  877  ..............................  906
```

12

Alignment of Brucella abortus strain S2 16S ribosomal RNA gene, partial sequence
Sequence ID: KF780870.1Length: 1383Number of Matches: 1
Range 1: 277 to 906GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1123 bits(1245)	0.0	627/630(99%)	0/630(0%)	Plus/Plus

```
Query  1    ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG  60

Sbjct  277  ............................................................
336

Query  61   TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA
120

Sbjct  337  ............................................................
396

Query  121  CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG
180

Sbjct  397  ............................................................
456

Query  181  TAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAA
240

Sbjct  457  ............................................................
516

Query  241  CTGCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAG
300

Sbjct  517  ............................................................
576

Query  301  GTGAAATTCGTAGTTATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC
360

Sbjct  577  ............A...............................................
636

Query  361  TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
420
```



```
Sbjct  637  ..........................................................
696

Query  421  CGTAAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATT
480

Sbjct  697  ..........................................................
756

Query  481  AAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC
540

Sbjct  757  ..........................................................
816

Query  541  CGCACAAGCGGTGGGGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCCTACCAGCCCT
600

Sbjct  817  ..............A.................................T..........
876

Query  601  TGACATCCCGGTCGCGGTTAGTGGAGACAC  630

Sbjct  877  ..............................  906
```

13

Alignment of *Brucella melitensis* strain B2 16S ribosomal RNA gene, partial sequence
Sequence ID: MK684240.1Length: 1346Number of Matches: 1
Range 1: 211 to 1050GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1502 bits(1665)	0.0	837/840(99%)	0/840(0%)	Plus/Plus

```
Query  1    CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGC
60

Sbjct  211  ..........................................................
270

Query  61   AAGCCTGATCCAGCCATGCCGCGTGAGTGGTGAAGGCCCTAGGGTTGTAAAGCTCTTTCA
120

Sbjct  271  ............................A.............................
330

Query  121  CCGGGGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCC
180

Sbjct  331  ....T.....................................................
390

Query  181  GCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGC
240

Sbjct  391  ..........................................................
450

Query  241  GGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAATCCCGGAACTGCCTTTGATACTGG
300

Sbjct  451  ....................................C.....................
510

Query  301  AAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATA
360

Sbjct  511  ..........................................................
570
```

```
Query  361   TTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGA
420

Sbjct  571   ............................................................
630

Query  421   AAGCGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATG
480

Sbjct  631   ............................................................
690

Query  481   TTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGG
540

Sbjct  691   ............................................................
750

Query  541   GGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
600

Sbjct  751   ............................................................
810

Query  601   GCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGGTCGC
660

Sbjct  811   ............................................................
870

Query  661   GGTTAGTGGAGACACTATCCTTCAGTTAGGCTGGACCGGAGACAGGTGCTGCATGGCTGT
720

Sbjct  871   ............................................................
930

Query  721   CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCTT
780

Sbjct  931   ............................................................
990

Query  781   AGTTGCCAGCATTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGG
840

Sbjct  991   ............................................................
1050
```

15

Alignment of *Brucella melitensis* strain B2 16S ribosomal RNA gene, partial sequence
Sequence ID: MK684240.1Length: 1346Number of Matches: 1
Range 1: 211 to 1050GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1498 bits(1660)	**0.0**	**836/840(99%)**	**0/840(0%)**	**Plus/Plus**

```
Query  1     CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGC
60

Sbjct  211   ............................................................
270

Query  61    AAGCCTGATCCAGCCATGCCGCGTGAGTGGTGAAGGCCCTAGGGTTGTAAAGCTCTTTCA
120

Sbjct  271   ............................A...............................
330
```

```
Query  121   CCGGGGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCC
180

Sbjct  331   ....T.....................................................
390

Query  181   GCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGC
240

Sbjct  391   ............................................................
450

Query  241   GGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAATCCCGGAACTGCCTTTGATACTGG
300

Sbjct  451   ..................................C.........................
510

Query  301   AAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATA
360

Sbjct  511   ............................................................
570

Query  361   TTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGA
420

Sbjct  571   ............................................................
630

Query  421   AAGCGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATG
480

Sbjct  631   ............................................................
690

Query  481   TTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGG
540

Sbjct  691   ............................................................
750

Query  541   GGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
600

Sbjct  751   ............................................................
810

Query  601   GCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGGTCGC
660

Sbjct  811   ............................................................
870

Query  661   GGTTAGTGGAGACACTATCTTTCAGTTAGGCTGGACCGGAGACAGGTGCTGCATGGCTGT
720

Sbjct  871   ..................C.........................................
930

Query  721   CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCTT
780

Sbjct  931   ............................................................
990

Query  781   AGTTGCCAGCATTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGG
840

Sbjct  991   ............................................................
1050
```

Alignment of *Brucella melitensis* strain B2 16S ribosomal RNA gene, partial sequence
Sequence ID: MK684240.1Length: 1346Number of Matches: 1
Range 1: 211 to 1050GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1502 bits(1665)	**0.0**	**837/840(99%)**	**0/840(0%)**	**Plus/Plus**

```
Query  1    CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGC  60

Sbjct  211  ............................................................  270

Query  61   AAGCCTGATCCAGCCATGCCGCGTGAGTGGTGAAGGCCCTAGGGTTGTAAAGCTCTTTCA  120

Sbjct  271  ............................A...............................  330

Query  121  CCGGGGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCC  180

Sbjct  331  ....T.........................................................  390

Query  181  GCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGC  240

Sbjct  391  ............................................................  450

Query  241  GGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAATCCCGGAACTGCCTTTGATACTGG  300

Sbjct  451  .....................................C......................  510

Query  301  AAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATA  360

Sbjct  511  ............................................................  570

Query  361  TTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGA  420

Sbjct  571  ............................................................  630

Query  421  AAGCGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATG  480

Sbjct  631  ............................................................  690

Query  481  TTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGG  540

Sbjct  691  ............................................................  750

Query  541  GGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA  600

Sbjct  751  ............................................................  810

Query  601  GCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGGTCGC  660
```

```
Sbjct  811   ..........................................................
870

Query  661   GGTTAGTGGAGACACTATCCTTCAGTTAGGCTGGACCGGAGACAGGTGCTGCATGGCTGT
720

Sbjct  871   ..........................................................
930

Query  721   CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCTT
780

Sbjct  931   ..........................................................
990

Query  781   AGTTGCCAGCATTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGG
840

Sbjct  991   ..........................................................
1050
```

17

Alignment of *Brucella melitensis* strain B2 16S ribosomal RNA gene, partial sequence
Sequence ID: MK684240.1Length: 1346Number of Matches: 1
Range 1: 211 to 1050GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1502 bits(1665)	0.0	837/840(99%)	0/840(0%)	Plus/Plus

```
Query  1     CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGC
60

Sbjct  211   ..........................................................
270

Query  61    AAGCCTGATCCAGCCATGCCGCGTGAGTGGTGAAGGCCCTAGGGTTGTAAAGCTCTTTCA
120

Sbjct  271   ............................A.............................
330

Query  121   CCGGGGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCC
180

Sbjct  331   ....T.....................................................
390

Query  181   GCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGC
240

Sbjct  391   ..........................................................
450

Query  241   GGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAATCCCGGAACTGCCTTTGATACTGG
300

Sbjct  451   ....................................C.....................
510

Query  301   AAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATA
360

Sbjct  511   ..........................................................
570

Query  361   TTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGA
420
```

```
Sbjct  571   ..........................................................
630

Query  421   AAGCGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATG
480

Sbjct  631   ..........................................................
690

Query  481   TTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGG
540

Sbjct  691   ..........................................................
750

Query  541   GGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
600

Sbjct  751   ..........................................................
810

Query  601   GCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGGTCGC
660

Sbjct  811   ..........................................................
870

Query  661   GGTTAGTGGAGACACTATCCTTCAGTTAGGCTGGACCGGAGACAGGTGCTGCATGGCTGT
720

Sbjct  871   ..........................................................
930

Query  721   CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCTT
780

Sbjct  931   ..........................................................
990

Query  781   AGTTGCCAGCATTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGG
840

Sbjct  991   ..........................................................
1050
```

18

Alignment of *Brucella melitensis* strain B2 16S ribosomal RNA gene, partial sequence
Sequence ID: MK684240.1Length: 1346Number of Matches: 1
Range 1: 211 to 1050GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1507 bits(1670)	0.0	838/840(99%)	0/840(0%)	Plus/Plus

```
Query  1     CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGC
60

Sbjct  211   ..........................................................
270

Query  61    AAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCA
120

Sbjct  271   ..........................................................
330

Query  121   CCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCC
180
```

```
Sbjct  331   ..........................................................
390

Query  181   GCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGC
240

Sbjct  391   ..........................................................
450

Query  241   GGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTTTGATACTGG
300

Sbjct  451   ..........................................................
510

Query  301   AAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATA
360

Sbjct  511   ..........................................................
570

Query  361   TTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGA
420

Sbjct  571   ..........................................................
630

Query  421   AAGCGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATG
480

Sbjct  631   ..........................................................
690

Query  481   TTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGG
540

Sbjct  691   ..........................................................
750

Query  541   GGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGAA
600

Sbjct  751   ..........................................................G.
810

Query  601   GCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGGTCGC
660

Sbjct  811   ..........................................................
870

Query  661   GGTTAGTGGAGACACTATCCTTCAGTTAGGCTGGACCGGAGACAGGTGCTGCATGGCTGT
720

Sbjct  871   ..........................................................
930

Query  721   CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCTT
780

Sbjct  931   ..........................................................
990

Query  781   AGTTGCCAGCATCCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGG
840

Sbjct  991   ............T.............................................
1050
```

Alignment of *Brucella melitensis* strain B2 16S ribosomal RNA gene, partial sequence
Sequence ID: MK684240.1Length: 1346Number of Matches: 1
Range 1: 211 to 1050GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1502 bits(1665)	**0.0**	**837/840(99%)**	**0/840(0%)**	**Plus/Plus**

```
Query  1     CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGC  60

Sbjct  211   ............................................................  270

Query  61    AAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCA  120

Sbjct  271   ............................................................  330

Query  121   CCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCC  180

Sbjct  331   ............................................................  390

Query  181   GCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGC  240

Sbjct  391   ............................................................  450

Query  241   GGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTTTGATACTGG  300

Sbjct  451   ............................................................  510

Query  301   AAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATA  360

Sbjct  511   ............................................................  570

Query  361   TTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGA  420

Sbjct  571   ............................................................  630

Query  421   AAGCGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATG  480

Sbjct  631   ............................................................  690

Query  481   TTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGG  540

Sbjct  691   ............................................................  750

Query  541   GGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGAA  600

Sbjct  751   ............................................................G.  810

Query  601   GCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGGTCGC  660
```

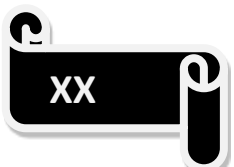
```
Sbjct  811   ..........................................................
870

Query  661   GGTTAGTGGAGACACTATCCTTCAGTTAGGCTGGACCGGAGACAGGTGCTGCATGGCTGT
720

Sbjct  871   ..........................................................
930

Query  721   CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAACCTCGCCCTT
780

Sbjct  931   ..........................................C.........
990

Query  781   AGTTGCCAGCATCCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGG
840

Sbjct  991   ............T..............................................
1050
```

20

Alignment of *Brucella melitensis* strain B2 16S ribosomal RNA gene, partial sequence
Sequence ID: MK684240.1Length: 1346Number of Matches: 1
Range 1: 211 to 1050GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1502 bits(1665)	**0.0**	**837/840(99%)**	**0/840(0%)**	**Plus/Plus**

```
Query  1     CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGC
60

Sbjct  211   ..........................................................
270

Query  61    AAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCA
120

Sbjct  271   ..........................................................
330

Query  121   CCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCC
180

Sbjct  331   ..........................................................
390

Query  181   GCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGC
240

Sbjct  391   ..........................................................
450

Query  241   GGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTTTGATACTGG
300

Sbjct  451   ..........................................................
510

Query  301   AAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATA
360

Sbjct  511   ..........................................................
570

Query  361   TTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGA
420
```



```
Sbjct  571   ..........................................................
630

Query  421   AAGCGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATG
480

Sbjct  631   ..........................................................
690

Query  481   TTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGG
540

Sbjct  691   ..........................................................
750

Query  541   GGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGAA
600

Sbjct  751   ..........................................................G.
810

Query  601   GCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGGTCGC
660

Sbjct  811   ..........................................................
870

Query  661   GGTTAGTGGAGACACTATCCTTCAGTTAGGCTGGACCGGAGACAGGTGCTGCATGGCTGT
720

Sbjct  871   ..........................................................
930

Query  721   CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAACCTCGCCCTT
780

Sbjct  931   ..................................................C.........
990

Query  781   AGTTGCCAGCATCCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGG
840

Sbjct  991   ............T.............................................
1050
```

Appendix 2: New nucleotides sequencing of *Brucella* isolates isolated from the animals soil's samples (Sequence Results Analysis) and recorded in NCBI

16S ribosomal RNA gene						
No.	**Type of substitution**	**Location**	**Nucleotide**	**Sequence ID with compare**	**Source**	**Identities**
2	**Transvertion**	**299**	**A\C**	**ID: OK178876.1**	***Brucella pseudogrignonensis***	**99%**
	Transition	**928**	**A\G**			
3	**Transvertion**	**299**	**A\C**	**ID: OK178876.1**	***Brucella pseudogrignonensis***	**99%**
	Transition	**928**	**A\G**			
4	**Transvertion**	**299**	**A\C**	**ID: OK178876.1**	***Brucella pseudogrignonensis***	**99%**
11	**Transition** **Transition** **Transition**	**373** 400 **607**	**C\T** **A\G** **A\G**	**ID: MZ165353.1**	***Brucella rhizosphaerae***	**99%**
13	**Transvertion**	**299**	**A\C**	**ID: OK178876.1**	***Brucella pseudogrignonensis***	**99%**
	Transvertion	**339**	**G\C**			
14	**Transvertion**	**299**	**A\C**	**ID: OK178876.1**	***Brucella pseudogrignonensis***	**99%**
	Transvertion	**339**	**G\C**			
	Transvertion	**972**	**T\G**			
15	**Transvertion**	**299**	**A\C**	**ID: OK178876.1**	***Brucella pseudogrignonensis***	**99%**
	Transvertion	**339**	**G\C**			
	Transvertion	**972**	**T\G**			
16	**Transition**	**71**	**A\G**	**ID: OL662885.1**	***Brucella oryzae***	**99%**
18	**Transition**	**71**	**A\G**	**ID: OL662885.1**	***Brucella oryzae***	**99%**
	Transition	**654**	**G\A**			
19	**Transition**	**71**	**A\G**	**ID: OL662885.1**	***Brucella oryzae***	**99%**
	Transvertion	**122**	**A\T**			
20	**Transvertion**	**32**	**A\C**	**ID: OL662885.1**	***Brucella oryzae***	**99%**
	Transition	**71**	**A\G**			
	Transvertion	**122**	**A\T**			
21	**Transition**	**461**	**A\G**	**ID: LC667796.1**	***Brucella intermedia***	**99%**
22	**Transvertion**	**518**	**A\C**	**ID: LC667796.1**	***Brucella intermedia***	**99%**

26	**Transvertion**	**518**	**A\C**	**ID: LC667796.1**	***Brucella intermedia***	**99%**
	Transition	**721**	**A\G**			
27	**Transition**	**403**	**T\C**	**ID: OK217265.1**	***Brucella anthropi***	**99%**
	Transition	**404**	**T\C**			
30	**Transvertion**	**556**	**G\C**	**ID: OK217265.1**	***Brucella anthropi***	**99%**
31	**Transvertion**	**1016**	**G\T**	**ID: MN990902.1**	***Brucella ovis***	**99%**
38	**Transition**	**668**	**A\G**	**ID: MN990902.1**	***Brucella ovis***	**99%**
	Transvertion	**979**	**A\T**			
39	**Transition**	**70**	**G\A**	**ID: KU587137.1**	***Brucella inopinata***	**99%**
	Transition	**280**	**T\C**			
43	**Transition**	**70**	**G\A**	**ID: KU587137.1**	***Brucella inopinata***	**99%**
	Transition	**280**	**T\C**			
	Transvertion	**404**	**C\G**			
44	**Transition**	**666**	**T\C**	**ID: KJ372229.1**	***Brucella melitensis***	**99%**
45	**Transvertion**	**524**	**T\A**	**ID: KJ372229.1**	***Brucella melitensis***	**99%**
46	**Transition**	**752**	**A\G**	**ID: OK655702.1**	***Brucella lupini***	**99%**
	Transition	**1060**	**A\G**			
49	**Transvertion**	**784**	**T\A**	**ID: OK655702.1**	***Brucella lupini***	**99%**
	Transition	**1055**	**A\G**			
50	**Transvertion**	**784**	**T\A**	**ID: OK655702.1**	***Brucella lupini***	**99%**
	Transition	**1055**	**A\G**			
51	**Transvertion**	**643**	**G\T**	**ID: OK655702.1**	***Brucella lupini***	**99%**
	Transvertion	**784**	**T\A**			
	Transition	**1055**	**A\G**			
52	**Transvertion**	**322**	**C\G**	**ID: MT275734.1**	***Brucella melitensis***	**99%**
	Transition	**342**	**A\G**			
53	**Transition**	**412587**	**T\C**	**ID: CP018782.1**	***Brucella pituitosa***	**99%**
54	**Transition**	**577**	**G\A**	**ID: KY880942.1**	***Brucella pseudogrignonensis***	**99%**
	Transvertion	**790**	**T\A**			
55	**Transvertion**	**911**	**C\A**	**ID: KY880942.1**	***Brucella pseudogrignonensis***	**99%**
56	**Transvertion**	**430**	**G\C**	**ID: MT611105.1**	***Brucella melitensis***	**99%**
	Transvertion	**579**	**T\A**			

57	**Transvertion**	**269**	**C\A**	**ID: OL360758.1**	***Brucella intermedia***	**99%**
	Transvertion	**683**	**C\G**			
58	**Transvertion**	**347**	**T\A**	**ID: MT611105.1**	***Brucella melitensis***	**99%**
59	**Transvertion**	**858**	**C\G**	**ID: OL360758.1**	***Brucella intermedia***	**99%**
	Transvertion	**859**	**C\G**			
61	**Transvertion**	**788**	**T\G**	**ID: MT611105.1**	***Brucella melitensis***	**99%**
62	**Transvertion**	**317**	**C\A**	**ID: OM729685.1**	***Brucella thiophenivorans***	**99%**
63	**Transition**	**727**	**T\C**	**ID: OL360758.1**	***Brucella intermedia***	**99%**
	Transvertion	**838**	**C\A**			
	Transition	**843**	**A\G**			

2

Alignment of *Brucella pseudogrignonensis* strain 2H7b 16S ribosomal RNA gene, partial sequence
Sequence ID: OK178876.1Length: 1341Number of Matches: 1
Range 1: 230 to 999GenBankGraphicsNext Match Previous Match

Score	Expect	Identities	Gaps	Strand
1385 bits(1535)	**0.0**	**769/770(99%)**	**0/770(0%)**	**Plus/Plus**

Query 1 GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAA 60

Sbjct 230 289

Query 61 GCCTGATCCCGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCACC 120

Sbjct 290 .....A..... 349

Query 121 GGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGC 180

Sbjct 350 409

Query 181 GGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGCGG 240

Sbjct 410 469

Query 241 ACTTTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGAA 300

Sbjct 470 529

Query 301 GTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATT 360

Sbjct 530 589

Query 361 CGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAAA 420

Sbjct 590 649

Query 421 GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTA 480

Sbjct 650 709

Query 481 GCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGA 540

Sbjct 710 769

Query 541 GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA 600

Sbjct 770 829

Query 601 TGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATACCGGTCGCGGA 660

Sbjct 830 889

Query 661 CACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTGCATGGCTGTCGTCA 720

Sbjct 890 949

Query 721 GCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCG 770

Sbjct 950 999

3

Alignment of *Brucella pseudogrignonensis* strain 2H7b 16S ribosomal RNA gene, partial sequence
Sequence ID: OK178876.1Length: 1341Number of Matches: 1
Range 1: 230 to 999GenBankGraphicsNext Match Previous Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1380 bits(1530)	**0.0**	**768/770(99%)**	**0/770(0%)**	**Plus/Plus**

Query 1 GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAA 60

Sbjct 230 289

Query 61 GCCTGATCCCGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCACC 120

Sbjct 290 .....A..... 349

Query 121 GGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGC 180

Sbjct 350 409

Query 181 GGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGCGG 240

Sbjct 410 469

Query 241 ACTTTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGAA 300

Sbjct 470 529

Query 301 GTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATT 360

Sbjct 530 589

Query 361 CGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAAA 420

Sbjct 590 649

Query 421 GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTA 480

Sbjct 650 709

Query 481 GCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGA 540

Sbjct 710 769

Query 541 GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA 600

Sbjct 770 829

Query 601 TGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATACCGGTCGCGGA 660

Sbjct 830 889

Query 661 CACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACGGGTGCTGCATGGCTGTCGTCA 720

Sbjct 890 .....A..... 949

Query 721 GCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCG 770

Sbjct 950 999

4

***Brucella pseudogrignonensis* strain 2H7b 16S ribosomal RNA gene, partial sequence**
Sequence ID: OK178876.1Length: 1341Number of Matches: 1
Range 1: 230 to 999GenBankGraphicsNext Match Previous Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1380 bits(1530)	**0.0**	**768/770(99%)**	**0/770(0%)**	**Plus/Plus**

Query 1 **GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAA 60**

Sbjct 230 289

Query 61 GCCTGATCCCGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCACC 120

Sbjct 290 .....A..... 349

Query 121 GGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGC 180

Sbjct 350 409

Query 181 GGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGCGG 240

Sbjct 410 469

Query 241 ACTTTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGAA 300

Sbjct 470 529

Query 301 GTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATT 360

Sbjct 530 589

Query 361 CGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAAA 420

Sbjct 590 649

Query 421 GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTA 480

Sbjct 650 709

Query 481 GCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGA 540

Sbjct 710 769

Query 541 GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA 600

Sbjct 770 829

Query 601 TGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATACCGGTCGCGGA 660

Sbjct 830 889

Query 661 CACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACGGGTGCTGCATGGCTGTCGTCA 720

Sbjct 890 .....A..... 949

Query 721 GCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCG 770

Sbjct 950 999

11

Alignment of *Brucella rhizosphaerae* strain K3 16S ribosomal RNA gene, partial sequence
Sequence ID: MZ165353.1Length: 1398Number of Matches: 1
Range 1: 132 to 971GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1502 bits(1665)	0.0	837/840(99%)	0/840(0%)	Plus/Plus

Query 1 GTGCCCTTCGGGGGAAAGATTTATCGGCAAAGGATGAGCCCGCGTTGGATTAGCTAGTTG 60

Sbjct 132 191

Query 61 GTGAGGTAAAGGCTCACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCAC 120

Sbjct 192 251

Query 121 ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAA 180

Sbjct 252 311

Query 181 TGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGC 240

Sbjct 312 371

Query 241 TTTTTCACCGGTGAAGATAATGACGGTAGCCGGAGAAGAAGCCCCGGCTAACTTCGTGCC 300

Sbjct 372 .C.....A..... 431

Query 301 AGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCA 360

Sbjct 432 491

Query 361 CGTAGGCGGATTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTTTG 420

Sbjct 492 551

Query 421 ATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAGATTC 480

Sbjct 552 .....A.... 611

Query 481 GTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGA 540

Sbjct 612 671

Query 541 GGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGA 600

Sbjct 672 731

Query 601 TGAATGTTAGCCGTCGGGGGGTTTACCTTTCGGTGGCGCAGCTAACGCATTAAACATTCC 660

Sbjct 732 791

Query 661 GCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC 720

Sbjct 792 851

Query 721 GGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATACC 780

Sbjct 852 911

Query 781 GGTCGCGGACACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTGCATGG 840

Sbjct 912 971

13

Alignment of *Brucella pseudogrignonensis* strain 2H7b 16S ribosomal RNA gene, partial sequence
Sequence ID: OK178876.1Length: 1341Number of Matches: 1
Range 1: 230 to 999GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1380 bits(1530)	**0.0**	**768/770(99%)**	**0/770(0%)**	**Plus/Plus**

Query 1 GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAA 60

Sbjct 230 289

Query 61 GCCTGATCCCGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAACCTCTTTCACC 120

Sbjct 290 .....A.....G..... 349

Query 121 GGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGC 180

Sbjct 350 409

Query 181 GGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGCGG 240

Sbjct 410 469

Query 241 ACTTTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGAA 300

Sbjct 470 529

Query 301 GTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATT 360

Sbjct 530 589

Query 361 CGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAAA 420

Sbjct 590 649

Query 421 GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTA 480

Sbjct 650 709

Query 481 GCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGA 540

Sbjct 710 769

Query 541 GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA 600

Sbjct 770 829

Query 601 TGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATACCGGTCGCGGA 660

Sbjct 830 889

Query 661 CACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTGCATGGCTGTCGTCA 720

Sbjct 890 949

Query 721 GCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCG 770

Sbjct 950 999

14

Alignment of *Brucella pseudogrignonensis* strain 2H7b 16S ribosomal RNA gene, partial sequence
Sequence ID: OK178876.1Length: 1341Number of Matches: 1
Range 1: 230 to 999GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1376 bits(1525)	**0.0**	**767/770(99%)**	**0/770(0%)**	**Plus/Plus**

Query 1 GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAA 60

Sbjct 230 289

Query 61 GCCTGATCCCGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAACCTCTTTCACC 120

Sbjct 290 .....A.....G..... 349

Query 121 GGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGC 180

Sbjct 350 409

Query 181 GGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGCGG 240

Sbjct 410 469

Query 241 ACTTTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGAA 300

Sbjct 470 529

Query 301 GTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATT 360

Sbjct 530 589

Query 361 CGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAAA 420

Sbjct 590 649

Query 421 GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTA 480

Sbjct 650 709

Query 481 GCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGA 540

Sbjct 710 769

Query 541 GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA 600

Sbjct 770 829

Query 601 TGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATACCGGTCGCGGA 660

Sbjct 830 889

Query 661 CACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTGCATGGCTGTCGTCA 720

Sbjct 890 949

Query 721 GCTCGTGTCGTGAGATGTTGGGGTAAGTCCCGCAACGAGCGCAACCCTCG 770

Sbjct 950 .....T..... 999

15

Alignment of *Brucella pseudogrignonensis* strain 2H7b 16S ribosomal RNA gene, partial sequence
Sequence ID: OK178876.1Length: 1341Number of Matches: 1
Range 1: 230 to 999GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1376 bits(1525)	**0.0**	**767/770(99%)**	**0/770(0%)**	**Plus/Plus**

Query 1 GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAA 60

Sbjct 230 289

Query 61 GCCTGATCCCGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAACCTCTTTCACC 120

Sbjct 290 .....A.....G..... 349

Query 121 GGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGC 180

Sbjct 350 409

Query 181 GGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGCGG 240

Sbjct 410 469

Query 241 ACTTTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGAA 300

Sbjct 470 529

Query 301 GTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATT 360

Sbjct 530 589

Query 361 CGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAAA 420

Sbjct 590 649

Query 421 GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTA 480

Sbjct 650 709

Query 481 GCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGA 540

Sbjct 710 769

Query 541 GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA 600

Sbjct 770 829

Query 601 TGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATACCGGTCGCGGA 660

Sbjct 830 889

Query 661 CACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTGCATGGCTGTCGTCA 720

Sbjct 890 949

Query 721 GCTCGTGTCGTGAGATGTTGGGGTAAGTCCCGCAACGAGCGCAACCCTCG 770

Sbjct 950 .....T..... 999

16

Alignment of *Brucella oryzae* strain C19-1 16S ribosomal RNA gene, partial sequence
Sequence ID: OL662885.1Length: 700Number of Matches: 1
Range 1: 1 to 700GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1259 bits(1395)	**0.0**	**699/700(99%)**	**0/700(0%)**	**Plus/Plus**

Query 1 GGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGAGTACGGTC 60

Sbjct 1 60

Query 61 GCAAGATTAAGACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT 120

Sbjct 61 .....A..... 120

Query 121 AATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGATCGCGGTTAGTGGAG 180

Sbjct 121 180

Query 181 ACACTATCCTTCAGTTCGGCTGGATCGGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGT 240

Sbjct 181 240

Query 241 GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCTTAGTTGCCAGCA 300

Sbjct 241 300

Query 301 TTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGGATGACG 360

Sbjct 301 360

Query 361 TCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTGACAGTG 420

Sbjct 361 420

Query 421 GGCAGCGAGCACGCGAGTGTGAGCTAATCTCCAAAAGCCATCTCAGTTCGGATTGCACTC 480

Sbjct 421 480

Query 481 TGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGA 540

Sbjct 481 540

Query 541 ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTTTTACCCGAA 600

Sbjct 541 600

Query 601 GGCGCTGTGCTAACCGCAAGGAGGCAGGCGACCACGGTAGGGTCAGCGACTGGGGTGAAG 660

Sbjct 601 660

Query 661 TCGTAACAAGGTAGCCGTAGGGGAACCTGCGGTTGGATCA 700

Sbjct 661 700

18

Alignment of *Brucella oryzae* strain C19-1 16S ribosomal RNA gene, partial sequence
Sequence ID: OL662885.1Length: 700Number of Matches: 1
Range 1: 1 to 700GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1254 bits(1390)	**0.0**	**698/700(99%)**	**0/700(0%)**	**Plus/Plus**

Query 1 GGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGAGTACGGTC 60

Sbjct 1 60

Query 61 GCAAGATTAAGACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT 120

Sbjct 61 .....A..... 120

Query 121 AATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGATCGCGGTTAGTGGAG 180

Sbjct 121 180

Query 181 ACACTATCCTTCAGTTCGGCTGGATCGGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGT 240

Sbjct 181 240

Query 241 GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCTTAGTTGCCAGCA 300

Sbjct 241 300

Query 301 TTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGGATGACG 360

Sbjct 301 360

Query 361 TCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTGACAGTG 420

Sbjct 361 420

Query 421 GGCAGCGAGCACGCGAGTGTGAGCTAATCTCCAAAAGCCATCTCAGTTCGGATTGCACTC 480

Sbjct 421 480

Query 481 TGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGA 540

Sbjct 481 540

Query 541 ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTTTTACCCGAA 600

Sbjct 541 600

Query 601 GGCGCTGTGCTAACCGCAAGGAGGCAGGCGACCACGGTAGGGTCAGCGACTGGAGTGAAG 660

Sbjct 601 .....G..... 660

Query 661 TCGTAACAAGGTAGCCGTAGGGGAACCTGCGGTTGGATCA 700

Sbjct 661 700

19

Alignment of *Brucella oryzae* strain C19-1 16S ribosomal RNA gene, partial sequence
Sequence ID: OL662885.1Length: 700Number of Matches: 1
Range 1: 1 to 700GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1254 bits(1390)	**0.0**	**698/700(99%)**	**0/700(0%)**	**Plus/Plus**

Query 1 GGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGAGTACGGTC 60

Sbjct 1 60

Query 61 GCAAGATTAAGACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT 120

Sbjct 61 .....A..... 120

Query 121 ATTTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGATCGCGGTTAGTGGAG 180

Sbjct 121 .A..... 180

Query 181 ACACTATCCTTCAGTTCGGCTGGATCGGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGT 240

Sbjct 181 240

Query 241 GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCTTAGTTGCCAGCA 300

Sbjct 241 300

Query 301 TTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGGATGACG 360

Sbjct 301 360

Query 361 TCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTGACAGTG 420

Sbjct 361 420

Query 421 GGCAGCGAGCACGCGAGTGTGAGCTAATCTCCAAAAGCCATCTCAGTTCGGATTGCACTC 480

Sbjct 421 480

Query 481 TGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGA 540

Sbjct 481 540

Query 541 ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTTTTACCCGAA 600

Sbjct 541 600

Query 601 GGCGCTGTGCTAACCGCAAGGAGGCAGGCGACCACGGTAGGGTCAGCGACTGGGGTGAAG 660

Sbjct 601 660

Query 661 TCGTAACAAGGTAGCCGTAGGGGAACCTGCGGTTGGATCA 700

Sbjct 661 700

20

Alignment of *Brucella oryzae* strain C19-1 16S ribosomal RNA gene, partial sequence
Sequence ID: OL662885.1Length: 700Number of Matches: 1
Range 1: 1 to 700GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1250 bits(1385)	**0.0**	**697/700(99%)**	**0/700(0%)**	**Plus/Plus**

Query 1 GGAGTTTACTCTTCGGTGGCGCAGCTAACGCCTTAAACATTCCGCCTGGGGAGTACGGTC 60

Sbjct 1 .....A..... 60

Query 61 GCAAGATTAAGACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT 120

Sbjct 61 .....A..... 120

Query 121 ATTTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGATCGCGGTTAGTGGAG 180

Sbjct 121 .A..... 180

Query 181 ACACTATCCTTCAGTTCGGCTGGATCGGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGT 240

Sbjct 181 240

Query 241 GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCTTAGTTGCCAGCA 300

Sbjct 241 300

Query 301 TTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGGATGACG 360

Sbjct 301 360

Query 361 TCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTGACAGTG 420

Sbjct 361 420

Query 421 GGCAGCGAGCACGCGAGTGTGAGCTAATCTCCAAAAGCCATCTCAGTTCGGATTGCACTC 480

Sbjct 421 480

Query 481 TGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGA 540

Sbjct 481 540

Query 541 ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTTTTACCCGAA 600

Sbjct 541 600

Query 601 GGCGCTGTGCTAACCGCAAGGAGGCAGGCGACCACGGTAGGGTCAGCGACTGGGGTGAAG 660

Sbjct 601 660

Query 661 TCGTAACAAGGTAGCCGTAGGGGAACCTGCGGTTGGATCA 700

Sbjct 661 700

21

Alignment of *Brucella intermedia* Cq-25 gene for 16S rRNA, partial sequence
Sequence ID: LC667796.1Length: 1070Number of Matches: 1
Range 1: 239 to 938GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1259 bits(1395)	**0.0**	**699/700(99%)**	**0/700(0%)**	**Plus/Plus**

Query 1 AATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGAC 60

Sbjct 239 298

Query 61 GCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA 120

Sbjct 299 358

Query 121 AACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAAC 180

Sbjct 359 418

Query 181 ATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGGATTGACGGGGGCCCGCA 240

Sbjct 419 .....A..... 478

Query 241 CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGAC 300

Sbjct 479 538

Query 301 ATCCCGATCGCGGTTAGTGGAGACACTATCCTTCAGTTCGGCTGGATCGGAGACAGGTGC 360

Sbjct 539 598

Query 361 TGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA 420

Sbjct 599 658

Query 421 CCCTCGCCCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGC 480

Sbjct 659 718

Query 481 CGAGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACG 540

Sbjct 719 778

Query 541 TGCTACAATGGTGGTGACAGTGGGCAGCGAGCACGCGAGTGTGAGCTAATCTCCAAAAGC 600

Sbjct 779 838

Query 601 CATCTCAGTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATC 660

Sbjct 839 898

Query 661 GCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG 700

Sbjct 899 938

22

Alignment of *Brucella intermedia* Cq-25 gene for 16S rRNA, partial sequence
Sequence ID: LC667796.1Length: 1070Number of Matches: 1
Range 1: 239 to 938GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1259 bits(1395)	**0.0**	**699/700(99%)**	**0/700(0%)**	**Plus/Plus**

Query 1 AATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGAC 60

Sbjct 239 298

Query 61 GCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA 120

Sbjct 299 358

Query 121 AACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAAC 180

Sbjct 359 418

Query 181 ATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCA 240

Sbjct 419 478

Query 241 CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCCGAACCTTACCAGCCCTTGAC 300

Sbjct 479 .....A..... 538

Query 301 ATCCCGATCGCGGTTAGTGGAGACACTATCCTTCAGTTCGGCTGGATCGGAGACAGGTGC 360

Sbjct 539 598

Query 361 TGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA 420

Sbjct 599 658

Query 421 CCCTCGCCCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGC 480

Sbjct 659 718

Query 481 CGAGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACG 540

Sbjct 719 778

Query 541 TGCTACAATGGTGGTGACAGTGGGCAGCGAGCACGCGAGTGTGAGCTAATCTCCAAAAGC 600

Sbjct 779 838

Query 601 CATCTCAGTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATC 660

Sbjct 839 898

Query 661 GCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG 700

Sbjct 899 938

26

Alignment of *Brucella intermedia* Cq-25 gene for 16S rRNA, partial sequence
Sequence ID: LC667796.1Length: 1070Number of Matches: 1
Range 1: 239 to 938GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1254 bits(1390)	**0.0**	**698/700(99%)**	**0/700(0%)**	**Plus/Plus**

Query 1 AATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGAC 60

Sbjct 239 298

Query 61 GCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA 120

Sbjct 299 358

Query 121 AACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAAC 180

Sbjct 359 418

Query 181 ATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCA 240

Sbjct 419 478

Query 241 CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCCGAACCTTACCAGCCCTTGAC 300

Sbjct 479 .....A..... 538

Query 301 ATCCCGATCGCGGTTAGTGGAGACACTATCCTTCAGTTCGGCTGGATCGGAGACAGGTGC 360

Sbjct 539 598

Query 361 TGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA 420

Sbjct 599 658

Query 421 CCCTCGCCCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGC 480

Sbjct 659 718

Query 481 CGGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACG 540

Sbjct 719 ..A..... 778

Query 541 TGCTACAATGGTGGTGACAGTGGGCAGCGAGCACGCGAGTGTGAGCTAATCTCCAAAAGC 600

Sbjct 779 838

Query 601 CATCTCAGTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATC 660

Sbjct 839 898

Query 661 GCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG 700

Sbjct 899 938

27

Alignment of *Brucella anthropi* strain OR31B43 16S ribosomal RNA gene, partial sequence
Sequence ID: OK217265.1 Length: 1034 Number of Matches: 1
Range 1: 211 to 910 GenBank Graphics Next Match Previous Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1254 bits(1390)	**0.0**	**698/700(99%)**	**0/700(0%)**	**Plus/Plus**

Query 1 AGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATG 60

Sbjct 211 270

Query 61 ATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT 120

Sbjct 271 330

Query 121 ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG 180

Sbjct 331 390

Query 181 TTGTAAAGCTCTCCCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA 240

Sbjct 391 .....TT..... 450

Query 241 CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG 300

Sbjct 451 510

Query 301 TAAAGCGCACGTAGGCGGGCTAATAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAA 360

Sbjct 511 570

Query 361 CTGCCTTTGATACTGTTAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAG 420

Sbjct 571 630

Query 421 GTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC 480

Sbjct 631 690

Query 481 TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC 540

Sbjct 691 750

Query 541 CGTAAACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATT 600

Sbjct 751 810

Query 601 AAACATTCCGCCTGGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGC 660

Sbjct 811 870

Query 661 CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAAGCAA 700

Sbjct 871 910

30

Alignment of *Brucella anthropi* strain OR31B43 16S ribosomal RNA gene, partial sequence
Sequence ID: OK217265.1Length: 1034Number of Matches: 1
Range 1: 211 to 910GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1259 bits(1395)	**0.0**	**699/700(99%)**	**0/700(0%)**	**Plus/Plus**

Query 1 AGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATG 60

Sbjct 211 270

Query 61 ATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT 120

Sbjct 271 330

Query 121 ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG 180

Sbjct 331 390

Query 181 TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA 240

Sbjct 391 450

Query 241 CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG 300

Sbjct 451 510

Query 301 TAAAGCGCACGTAGGCGGGCTAATAAGTCAGGGGTGAAATCCCGGCGCTCAACCCCGGAA 360

Sbjct 511 .....G..... 570

Query 361 CTGCCTTTGATACTGTTAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAG 420

Sbjct 571 630

Query 421 GTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC 480

Sbjct 631 690

Query 481 TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC 540

Sbjct 691 750

Query 541 CGTAAACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATT 600

Sbjct 751 810

Query 601 AAACATTCCGCCTGGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGC 660

Sbjct 811 870

Query 661 CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAAGCAA 700

Sbjct 871 910

31

Alignment of *Brucella ovis* 16S ribosomal RNA gene, partial sequence
Sequence ID: MN990902.1Length: 1453Number of Matches: 1
Range 1: 281 to 1120GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1511 bits(1675)	**0.0**	**839/840(99%)**	**0/840(0%)**	**Plus/Plus**

Query 1 TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATG 60
Sbjct 281 340

Query 61 GGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTC 120
Sbjct 341 400

Query 121 TTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAG 180
Sbjct 401 460

Query 181 CAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACG 240
Sbjct 461 520

Query 241 TAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTTTGAT 300
Sbjct 521 580

Query 301 ACTGGAAGTCTTGAGTATGGAAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGT 360
Sbjct 581 640

Query 361 AGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGTCCATTACTGACGCTGAGG 420
Sbjct 641 700

Query 421 TGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG 480
Sbjct 701 760

Query 481 AATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAACATTCCGC 540
Sbjct 761 820

Query 541 CTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGG 600
Sbjct 821 880

Query 601 TGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGA 660
Sbjct 881 940

Query 661 TCGCGGTTAGTGGAGACACTATCCTTCAGTTCGGCTGGATCGGAGACAGGTGCTGCATGG 720
Sbjct 941 1000

Query 721 CTGTCGTCAGCTCGTTTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGC 780

Sbjct 1001 .....G..... 1060

Query 781 CCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGG 840

Sbjct 1061 1120

38

Alignment of *Brucella ovis* 16S ribosomal RNA gene, partial sequence
Sequence ID: MN990902.1Length: 1453Number of Matches: 1
Range 1: 281 to 1120GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1507 bits(1670)	**0.0**	**838/840(99%)**	**0/840(0%)**	**Plus/Plus**

Query 1 TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATG 60

Sbjct 281 340

Query 61 GGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTC 120

Sbjct 341 400

Query 121 TTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAG 180

Sbjct 401 460

Query 181 CAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACG 240

Sbjct 461 520

Query 241 TAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTTTGAT 300

Sbjct 521 580

Query 301 ACTGGAAGTCTTGAGTATGGAAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGT 360

Sbjct 581 640

Query 361 AGATATTCGGAGGAACACCAGTGGCGAGGGCGGCTCACTGGTCCATTACTGACGCTGAGG 420

Sbjct 641 .....A..... 700

Query 421 TGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG 480

Sbjct 701 760

Query 481 AATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAACATTCCGC 540

Sbjct 761 820

Query 541 CTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGG 600

Sbjct 821 880

Query 601 TGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGA 660

Sbjct 881 940

Query 661 TCGCGGTTAGTGGAGACACTATCCTTCAGTTCGGCTGGTTCGGAGACAGGTGCTGCATGG 720

Sbjct 941 .....A..... 1000

Query 721 CTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGC 780

Sbjct 1001 1060

Query 781 CCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGG 840

Sbjct 1061 1120

39

Alignment of *Brucella inopinata* isolate Endo12 16S ribosomal RNA gene, partial sequence
Sequence ID: KU587137.1Length: 510Number of Matches: 1
Range 1: 37 to 490GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
810 bits(898)	**0.0**	**452/454(99%)**	**0/454(0%)**	**Plus/Plus**

Query 1 GAGCGCCCCGCAAGGGGAGCGGCAGACGGGTGAATAACGCGTGGGAACGTACCATTTGCT 60

Sbjct 37 .....G..... 96

Query 61 ACGGAATAACTCAGGGAAACTTGTGCTAATACCGTATGAGCCCGAAAGGGGAAAGATTTA 120

Sbjct 97 156

Query 121 TCGGGCAAATGATCGGCCCGCGTTGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGG 180

Sbjct 157 216

Query 181 CGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCA 240

Sbjct 217 276

Query 241 GACCCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCC 300

Sbjct 277 ...T..... 336

Query 301 ATGCCGCGTGAGTGATGAAGGCCCTAGGTTGTAAAGCTCTTTCACCGGTGAAGATAATGA 360

Sbjct 337 396

Query 361 CGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGG 420

Sbjct 397 456

Query 421 GCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCG 454

Sbjct 457 490

43

Alignment of *Brucella inopinata* isolate Endo12 16S ribosomal RNA gene, partial sequence
Sequence ID: KU587137.1Length: 510Number of Matches: 1
Range 1: 37 to 490GenBankGraphicsNext Match Previous Match

Score	**Expect**	**Identities**	**Gaps**	**Strand**
806 bits(893)	**0.0**	**451/454(99%)**	**0/454(0%)**	**Plus/Plus**

Query 1 GAGCGCCCCGCAAGGGGAGCGGCAGACGGGTGAATAACGCGTGGGAACGTACCATTTGCT 60

Sbjct 37 .....G..... 96

Query 61 ACGGAATAACTCAGGGAAACTTGTGCTAATACCGTATGAGCCCGAAAGGGGAAAGATTTA 120

Sbjct 97 156

Query 121 TCGGGCAAATGATCGGCCCGCGTTGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGG 180

Sbjct 157 216

Query 181 CGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCA 240

Sbjct 217 276

Query 241 GACCCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCC 300

Sbjct 277 ...T..... 336

Query 301 ATGCCGCGTGAGTGATGAAGGCCCTAGGTTGTAAAGCTCTTTCACCGGTGAAGATAATGA 360

Sbjct 337 396

Query 361 CGGTAACGGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGG 420

Sbjct 397 .....C..... 456

Query 421 GCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCG 454

Sbjct 457 490

44

Alignment of *Brucella melitensis* strain BHU08 16S ribosomal RNA gene, partial sequence
Sequence ID: KJ372229.1Length: 735Number of Matches: 1
Range 1: 71 to 700GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1132 bits(1255)	**0.0**	**629/630(99%)**	**0/630(0%)**	**Plus/Plus**

Query 1 CCTTCGGGAGAAAGATTTATCGGCAAAGGATGAGCCCGCGTTGGATTAGCTAGTTGGTGG 60

Sbjct 71 130

Query 61 GGTAAAGGCCCACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACACTG 120

Sbjct 131 190

Query 121 GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGG 180

Sbjct 191 250

Query 181 CGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTT 240

Sbjct 251 310

Query 241 TCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCA 300

Sbjct 311 370

Query 301 GCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTA 360

Sbjct 371 430

Query 361 GGCGGACTTTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATAC 420

Sbjct 431 490

Query 421 TGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAG 480

Sbjct 491 550

Query 481 ATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTG 540

Sbjct 551 610

Query 541 CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAA 600

Sbjct 611 670

Query 601 TGTTAGCCGTCGGGGTGTTTACACTCCGGT 630

Sbjct 671 .....T.... 700

45

Alignment of *Brucella melitensis* strain BHU08 16S ribosomal RNA gene, partial sequence
Sequence ID: KJ372229.1Length: 735Number of Matches: 1
Range 1: 71 to 700GenBankGraphicsNext Match Previous Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1132 bits(1255)	**0.0**	**629/630(99%)**	**0/630(0%)**	**Plus/Plus**

Query 1 CCTTCGGGAGAAAGATTTATCGGCAAAGGATGAGCCCGCGTTGGATTAGCTAGTTGGTGG 60

Sbjct 71 130

Query 61 GGTAAAGGCCCACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACACTG 120

Sbjct 131 190

Query 121 GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGG 180

Sbjct 191 250

Query 181 CGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTT 240

Sbjct 251 310

Query 241 TCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCA 300

Sbjct 311 370

Query 301 GCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTA 360

Sbjct 371 430

Query 361 GGCGGACTTTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATAC 420

Sbjct 431 490

Query 421 TGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAAATCCGAGTGTAGAGGTGAAATTCGTAG 480

Sbjct 491 .....T..... 550

Query 481 ATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTG 540

Sbjct 551 610

Query 541 CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAA 600

Sbjct 611 670

Query 601 TGTTAGCCGTCGGGGTGTTTACACTTCGGT 630

Sbjct 671 700

46

Alignment of *Brucella lupini* strain ARO19 16S ribosomal RNA gene, partial sequence
Sequence ID: OK655702.1Length: 1333Number of Matches: 1
Range 1: 335 to 1104GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1380 bits(1530)	**0.0**	**768/770(99%)**	**0/770(0%)**	**Plus/Plus**

Query 1 AAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTC 60

Sbjct 335 394

Query 61 GTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAA 120

Sbjct 395 454

Query 121 GCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGC 180

Sbjct 455 514

Query 181 CTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGA 240

Sbjct 515 574

Query 241 AATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGAC 300

Sbjct 575 634

Query 301 GCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA 360

Sbjct 635 694

Query 361 AACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAGAC 420

Sbjct 695 .....A.. 754

Query 421 ATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCA 480

Sbjct 755 814

Query 481 CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGAC 540

Sbjct 815 874

Query 541 ATACCGGTCGCGGACACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTG 600

Sbjct 875 934

Query 601 CATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC 660

Sbjct 935 994

Query 661 CTCGCCCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCG 720

Sbjct 995 1054

Query 721 AGAGGGAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGC 770

Sbjct 1055 .....A..... 1104

49

Alignment of *Brucella lupini* strain ARO19 16S ribosomal RNA gene, partial sequence
Sequence ID: OK655702.1Length: 1333Number of Matches: 1
Range 1: 335 to 1104GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1380 bits(1530)	**0.0**	**768/770(99%)**	**0/770(0%)**	**Plus/Plus**

Query 1 AAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTC 60

Sbjct 335 394

Query 61 GTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAA 120

Sbjct 395 454

Query 121 GCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGC 180

Sbjct 455 514

Query 181 CTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGA 240

Sbjct 515 574

Query 241 AATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGAC 300

Sbjct 575 634

Query 301 GCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA 360

Sbjct 635 694

Query 361 AACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAAC 420

Sbjct 695 754

Query 421 ATTCCGCCTGGGGAGTACGGTCGCAAGATAAAAACTCAAAGGAATTGACGGGGGCCCGCA 480

Sbjct 755 .....T..... 814

Query 481 CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGAC 540

Sbjct 815 874

Query 541 ATACCGGTCGCGGACACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTG 600

Sbjct 875 934

Query 601 CATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC 660

Sbjct 935 994

Query 661 CTCGCCCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCG 720

Sbjct 995 1054

Query 721 GGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGC 770

Sbjct 1055 A..... 1104

50

Alignment of *Brucella lupini* strain ARO19 16S ribosomal RNA gene, partial sequence
Sequence ID: OK655702.1Length: 1333Number of Matches: 1
Range 1: 335 to 1104GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1380 bits(1530)	**0.0**	**768/770(99%)**	**0/770(0%)**	**Plus/Plus**

Query 1 AAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTC 60

Sbjct 335 394

Query 61 GTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAA 120

Sbjct 395 454

Query 121 GCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGC 180

Sbjct 455 514

Query 181 CTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGA 240

Sbjct 515 574

Query 241 AATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGAC 300

Sbjct 575 634

Query 301 GCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA 360

Sbjct 635 694

Query 361 AACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAAC 420

Sbjct 695 754

Query 421 ATTCCGCCTGGGGAGTACGGTCGCAAGATAAAAACTCAAAGGAATTGACGGGGGCCCGCA 480

Sbjct 755 .....T..... 814

Query 481 CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGAC 540

Sbjct 815 874

Query 541 ATACCGGTCGCGGACACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTG 600

Sbjct 875 934

Query 601 CATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC 660

Sbjct 935 994

Query 661 CTCGCCCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCG 720

Sbjct 995 1054

Query 721 GGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGC 770

Sbjct 1055 A..... 1104

51

Alignment of *Brucella lupini* strain ARO19 16S ribosomal RNA gene, partial sequence
Sequence ID: OK655702.1Length: 1333Number of Matches: 1
Range 1: 335 to 1104GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1376 bits(1525)	**0.0**	**767/770(99%)**	**0/770(0%)**	**Plus/Plus**

Query 1 AAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTC 60

Sbjct 335 394

Query 61 GTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAA 120

Sbjct 395 454

Query 121 GCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGC 180

Sbjct 455 514

Query 181 CTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGA 240

Sbjct 515 574

Query 241 AATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGAC 300

Sbjct 575 634

Query 301 GCTGAGGTTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA 360

Sbjct 635 .....G..... 694

Query 361 AACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAAC 420

Sbjct 695 754

Query 421 ATTCCGCCTGGGGAGTACGGTCGCAAGATAAAAACTCAAAGGAATTGACGGGGGCCCGCA 480

Sbjct 755 .....T..... 814

Query 481 CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGAC 540

Sbjct 815 874

Query 541 ATACCGGTCGCGGACACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTG 600

Sbjct 875 934

Query 601 CATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC 660

Sbjct 935 994

Query 661 CTCGCCCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCG 720

Sbjct 995 1054

Query 721 GGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGC 770

Sbjct 1055 A..... 1104

52

Alignment of *Brucella melitensis* strain H1/SRLAAH/2019 16S ribosomal RNA gene, partial sequence
Sequence ID: MT275734.1Length: 1375Number of Matches: 1
Range 1: 242 to 1011GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1380 bits(1530)	**0.0**	**768/770(99%)**	**0/770(0%)**	**Plus/Plus**

Query 1 CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGG 60

Sbjct 242 301

Query 61 ACAATGGGCGCAAGCCTGATGCAGCCATGCCGCCTGAATGGTGAAGGCCCTAGGGTTGTA 120

Sbjct 302 .....C.....A..... 361

Query 121 AAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCG 180

Sbjct 362 421

Query 181 TGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAG 240

Sbjct 422 481

Query 241 CGCACGTAGGCGGGCTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCC 300

Sbjct 482 541

Query 301 TTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAA 360

Sbjct 542 601

Query 361 ATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACG 420

Sbjct 602 661

Query 421 CTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA 480

Sbjct 662 721

Query 481 ACGATGAATGTTAGCCGTTGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACA 540

Sbjct 722 781

Query 541 TTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCAC 600

Sbjct 782 841

Query 601 AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACA 660

Sbjct 842 901

Query 661 TCCCGATCGCGGTTAGTGGAGACACTATCCTTCAGTTAGGCTGGACCGGAGACAGGTGCT 720

Sbjct 902 961

Query 721 GCATGGCTGTCGTCAACTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA 770

Sbjct 962 1011

53

Alignment of *Brucella pituitosa* strain AA2 chromosome 2, complete sequence
Sequence ID: CP018782.1Length: 1716190Number of Matches: 2
Range 1: 412257 to 412956GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1259 bits(1395)	0.0	699/700(99%)	0/700(0%)	Plus/Plus

Query 1 CCCAACTAAATGATGGCAACTAAAGGCGAGGGTTGCGCTCGTTGCGGGACTTAACCCAAC 60

Sbjct 412257 412316

Query 61 ATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTATCCGGTCCAGCCGAACTGA 120

Sbjct 412317 412376

Query 121 AAGACACATCTCTGTGTCCGCGACCGGTATGTCAAGGGCTGGTAAGGTTCTGCGCGTTGC 180

Sbjct 412377 412436

Query 181 TTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTT 240

Sbjct 412437 412496

Query 241 AATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAATGCGTTAGCTGCGCCACCGAAGTG 300

Sbjct 412497 412556

Query 301 TAAACACCCCGACGGCTAACATTCATCGTTCACGGCGTGGACTACCAGGGTATCTAATCC 360

Sbjct 412557 .....T..... 412616

Query 361 TGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTAATGGTCCAGTGAGCCGCCTTCGC 420

Sbjct 412617 412676

Query 421 CACTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTACACTCGGAATTCCACTCACCT 480

Sbjct 412677 412736

Query 481 CTACCATACTCAAGACTTCCAGTATCAAAGGCAGTTCCAGAGTTGAGCTCTGGGATTTCA 540

Sbjct 412737 412796

Query 541 CCCCTGACTTAAAAGTCCGCCTACGTGCGCTTTACGCCCAGTAAATCCGAACAACGCTAG 600

Sbjct 412797 412856

Query 601 CCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTTCTTCTCCGGTTAC 660

Sbjct 412857 412916

Query 661 CGTCATTATCTTCACCGGTGAAAGAGCTTTACAACCCTAG 700

Sbjct 412917 412956

54

Alignment of *Brucella pseudogrignonensis* strain SitB363 16S ribosomal RNA gene, partial sequence
Sequence ID: KY880942.1Length: 1311Number of Matches: 1
Range 1: 304 to 1003GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1254 bits(1390)	0.0	698/700(99%)	0/700(0%)	Plus/Plus

Query 1 ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG 60

Sbjct 304 363

Query 61 TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA 120

Sbjct 364 423

Query 121 CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG 180

Sbjct 424 483

Query 181 TAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAA 240

Sbjct 484 543

Query 241 CTGCCTTTGATACTGGAAGTCTTGAGTATGGTAAAGGTGAGTGGAATTCCGAGTGTAGAG 300

Sbjct 544 .....G..... 603

Query 301 GTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC 360

Sbjct 604 663

Query 361 TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC 420

Sbjct 664 723

Query 421 CGTAAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATT 480

Sbjct 724 783

Query 481 AAACATACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC 540

Sbjct 784 .....T..... 843

Query 541 CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCT 600

Sbjct 844 903

Query 601 TGACATACCGGTCGCGGACACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGT 660

Sbjct 904 963

Query 661 GCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGG 700

Sbjct 964 1003

55

Alignment of *Brucella pseudogrignonensis* strain SitB363 16S ribosomal RNA gene, partial sequence
Sequence ID: KY880942.1Length: 1311Number of Matches: 1
Range 1: 304 to 1003GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1259 bits(1395)	0.0	699/700(99%)	0/700(0%)	Plus/Plus

Query 1 ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG 60

Sbjct 304 363

Query 61 TTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAA 120

Sbjct 364 423

Query 121 CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCG 180

Sbjct 424 483

Query 181 TAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAA 240

Sbjct 484 543

Query 241 CTGCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAG 300

Sbjct 544 603

Query 301 GTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTAC 360

Sbjct 604 663

Query 361 TGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC 420

Sbjct 664 723

Query 421 CGTAAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATT 480

Sbjct 724 783

Query 481 AAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC 540

Sbjct 784 843

Query 541 CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCT 600

Sbjct 844 903

Query 601 TGACATAACGGTCGCGGACACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGT 660

Sbjct 904 .....C..... 963

Query 661 GCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGG 700

Sbjct 964 1003

56

Alignment of *Brucella melitensis* strain IMHT4 16S ribosomal RNA gene, partial sequence
Sequence ID: MT611105.1Length: 1366Number of Matches: 1
Range 1: 231 to 790GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1002 bits(1110)	0.0	558/560(99%)	0/560(0%)	Plus/Plus

Query 1 ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGAC 60

Sbjct 231 290

Query 61 AATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAA 120

Sbjct 291 350

Query 121 GCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTG 180

Sbjct 351 410

Query 181 CCAGCAGCCGCGGTAATACCAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCG 240

Sbjct 411 .....G..... 470

Query 241 CACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTT 300

Sbjct 471 530

Query 301 TGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGAAGAGGTGAAAT 360

Sbjct 531 .....T..... 590

Query 361 TCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCT 420

Sbjct 591 650

Query 421 GAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC 480

Sbjct 651 710

Query 481 GATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATT 540

Sbjct 711 770

Query 541 CCGCCTGGGGAGTACGGTCG 560

Sbjct 771 790

57

Alignment of *Brucella intermedia* strain ANKI 16S ribosomal RNA gene, partial sequence
Sequence ID: OL360758.1Length: 1435Number of Matches: 1
Range 1: 265 to 1034GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1380 bits(1530)	0.0	768/770(99%)	0/770(0%)	Plus/Plus

Query 1 CCACCCTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGG 60

Sbjct 265 ....A..... 324

Query 61 ACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTA 120

Sbjct 325 384

Query 121 AAGCTCTTTCACCGGTGAAGATAATGACGGTACCCGGAGAAGAAGCCCCGGCTAACTTCG 180

Sbjct 385 444

Query 181 TGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAG 240

Sbjct 445 504

Query 241 CGCACGTAGGCGGGCTAATAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCC 300

Sbjct 505 564

Query 301 TTTGATACTGTTAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAA 360

Sbjct 565 624

Query 361 ATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGAGG 420

Sbjct 625 .....C. 684

Query 421 CTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA 480

Sbjct 685 744

Query 481 ACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAACA 540

Sbjct 745 804

Query 541 TTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCAC 600

Sbjct 805 864

Query 601 AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACA 660

Sbjct 865 924

Query 661 TCCCGATCGCGGTTAGTGGAGACACTATCCTTCAGTTCGGCTGGATCGGAGACAGGTGCT 720

Sbjct 925 984

Query 721 GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA 770

Sbjct 985 1034

58

Alignment of *Brucella melitensis* strain IMHT4 16S ribosomal RNA gene, partial sequence
Sequence ID: MT611105.1Length: 1366Number of Matches: 1
Range 1: 176 to 707GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
956 bits(1059)	0.0	531/532(99%)	0/532(0%)	Plus/Plus

Query 1 GGGTAAAGGCTCACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACACT 60

Sbjct 176 235

Query 61 GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGG 120

Sbjct 236 295

Query 121 GCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGAAAAGCTCT 180

Sbjct 296 .....T..... 355

Query 181 TTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGC 240

Sbjct 356 415

Query 241 AGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGT 300

Sbjct 416 475

Query 301 AGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTTTGATA 360

Sbjct 476 535

Query 361 CTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTA 420

Sbjct 536 595

Query 421 GATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGT 480

Sbjct 596 655

Query 481 GCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA 532

Sbjct 656 707

59

Alignment of *Brucella intermedia* strain ANKI 16S ribosomal RNA gene, partial sequence
Sequence ID: OL360758.1Length: 1435Number of Matches: 1
Range 1: 265 to 1034GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1380 bits(1530)	0.0	768/770(99%)	0/770(0%)	Plus/Plus

Query 1 CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGG 60

Sbjct 265 324

Query 61 ACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTA 120

Sbjct 325 384

Query 121 AAGCTCTTTCACCGGTGAAGATAATGACGGTACCCGGAGAAGAAGCCCCGGCTAACTTCG 180

Sbjct 385 444

Query 181 TGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAG 240

Sbjct 445 504

Query 241 CGCACGTAGGCGGGCTAATAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCC 300

Sbjct 505 564

Query 301 TTTGATACTGTTAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAA 360

Sbjct 565 624

Query 361 ATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACG 420

Sbjct 625 684

Query 421 CTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA 480

Sbjct 685 744

Query 481 ACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAACA 540

Sbjct 745 804

Query 541 TTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACgggggggCGCAC 600

Sbjct 805 .....CC..... 864

Query 601 AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACA 660

Sbjct 865 924

Query 661 TCCCGATCGCGGTTAGTGGAGACACTATCCTTCAGTTCGGCTGGATCGGAGACAGGTGCT 720

Sbjct 925 984

Query 721 GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA 770

Sbjct 985 1034

61

Alignment of *Brucella melitensis* strain IMHT4 16S ribosomal RNA gene, partial sequence
Sequence ID: MT611105.1Length: 1366Number of Matches: 1
Range 1: 231 to 790GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1006 bits(1115)	0.0	559/560(99%)	0/560(0%)	Plus/Plus

Query 1 ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGAC 60

Sbjct 231 290

Query 61 AATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAA 120

Sbjct 291 350

Query 121 GCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTG 180

Sbjct 351 410

Query 181 CCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCG 240

Sbjct 411 470

Query 241 CACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTT 300

Sbjct 471 530

Query 301 TGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAAT 360

Sbjct 531 590

Query 361 TCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCT 420

Sbjct 591 650

Query 421 GAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC 480

Sbjct 651 710

Query 481 GATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATT 540

Sbjct 711 770

Query 541 CCGCCTGGGGAGTACGGGCG 560

Sbjct 771 .....T.. 790

62

Alignment of *Brucella thiophenivorans* strain SSR3 16S ribosomal RNA gene, partial sequence
Sequence ID: OM729685.1Length: 1278Number of Matches: 1
Range 1: 70 to 615GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
976 bits(1081)	0.0	545/546(99%)	0/546(0%)	Plus/Plus

Query 1 TGGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCCATAGCTGGTCTGAG 60

Sbjct 70 129

Query 61 AGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTG 120

Sbjct 130 189

Query 121 GGAGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGT 180

Sbjct 190 249

Query 181 CTTAGGATTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCC 240

Sbjct 250 309

Query 241 CGGCTAAATTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTA 300

Sbjct 310 .....C..... 369

Query 301 CTGGGCGTAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAAC 360

Sbjct 370 429

Query 361 CCCGGAACTGCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAG 420

Sbjct 430 489

Query 421 TGTAGAGGTGAAATTCSTAKATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGA 480

Sbjct 490 549

Query 481 CCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAG 540

Sbjct 550 609

Query 541 TCCACG 546

Sbjct 610 615

63

Alignment of *Brucella intermedia* strain ANKI 16S ribosomal RNA gene, partial sequence
Sequence ID: OL360758.1Length: 1435Number of Matches: 1
Range 1: 262 to 866GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1078 bits(1195)	0.0	602/605(99%)	0/605(0%)	Plus/Plus

Query 1 CAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATAT 60

Sbjct 262 321

Query 61 TGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTT 120

Sbjct 322 381

Query 121 GTAAAGCTCTTTCACCGGTGAAGATAATGACGGTACCCGGAGAAGAAGCCCCGGCTAACT 180

Sbjct 382 441

Query 181 TCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTA 240

Sbjct 442 501

Query 241 AAGCGCACGTAGGCGGGCTAATAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACT 300

Sbjct 502 561

Query 301 GCCTTTGATACTGTTAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGT 360

Sbjct 562 621

Query 361 GAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTG 420

Sbjct 622 681

Query 421 ACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCCGGTAGTCCACGCCG 480

Sbjct 682 .....T..... 741

Query 481 TAAACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAA 540

Sbjct 742 801

Query 541 ACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAAATCAAGGGAATTGACGGGGGCCCG 600

Sbjct 802 .....C....A..... 861

Query 601 CACAA 605

Sbjct 862 866

الخلاصة

يهدف هذا البحث إلى معرفة وجود جنس البروسيلا في العينات من محافظة بابل والعلاقة مع العوامل البيئية حيث يتالف من جزئين من جمع العينات : احدهما من تربة الحيوانات اما الاخرى من دم الأغنام.

يتضمن الجزء الأول من الجمع الفصلي هوجمع ثلاث وستون من عينات الترب الحيوانية لقرى مختلفة التي بدأت من شهر شباط الى شهرتشرين الثاني،2021 في وسط وجنوب وشمال محافظة بابل،مدينة الحلة،العراق التي تضمنت العوامل الفيزيائية والكيميائية (درجة حرارة الهواء والتربة،المقياس اللوغارتمي للدالة الحامضية ،التوصيلية الكهربائية ،المواد الصلبة الذائبة الكلية ،الملوحة والمواد العضوية الكلية) كذلك قياس العناصرالثقيلة (الحديد،النحاس،الكادميوم والرصاص) بينما يتضمن الجزء الثاني من عينات الجمع هو سحب الدم من الوريد الوداجي لعشرين نموذج من الأغنام (ثمانية عشر عينة من الإناث وعينتين من الذكور) من قرية المهناوية في محافظة بابل، وسط الحلة، العراق في نهاية شهرايار2021 حيث فحصت النماذج اولا بواسطة جهاز عد خلايا الدم لتشخيص الاصابة في دم الاغنام وبعد ذلك شخص جنس البروسيلا بواسطة جهاز (real time PCR).

بينت النتائج وجود انواع من البروسيلا في بعض العينات البكتيرية المعزولة من التربة وتختلف نسبته باختلاف الفصول الاربعة حيث كانت أعلى نسبة تواجد البروسيلا في فصل الصيف وأقلها في فصل الشتاء وكانت هذه النسب مرتبطة بالمؤشرات البيئية الموسمية. فكان معدل النسب المئوية للبروسيلا (60% ،78.13% ،100% ،91.67%) ومعدل القيم للخواص الفيزيائية والكيميائية التي تضمنت درجة حرارة الهواء (27.40 ،30.69، 42.11، 32.83) م^0، درجة حرارة التربة (21.85، 26.17 ، 37.11 ، 23.01) م^0، المقياس اللوغارتمي للدالة الحامضية (7.62، 7.56 ، 7.81 ، 71.51)، التوصيلية الكهربائية (3957.0، 3768.34 ، 4958.89، 5123.33) مايكروسمنس\سم، المواد الصلبة الذائبة (2812.0، 2645.94 ، 3548.89، 3593.33) ملغم\لتر،الملوحة (2.53، 2.47 ، 3.17، 3.28) ‰ و المواد العضوية الكلية (42.44، 35.68 ، 34.57، 27.93) % في مواسم الشتاء ،الربيع ،الصيف والخريف على التوالي بينما كان معدل القيم للعناصر الثقيلة كالاتي النحاس (12.51، 5.36 ، 4.11 ، 5.95) ملغم \كغم ، الحديد (412.29 ، 304.08 ، 314.95، 392.44) ملغم\كغم

،الكادميوم (1.88، 0.52 ، 1.17 ، 1.77) ملغم\كغم والرصاص (647.99 ، 726.97 ، 731.77 ، 722.32) ملغم\كغم في فصول الشتاء ،الربيع ،الصيف والخريف على التوالي ،2021 حيث لوحظ بأن نسبة تواجد البروسيلا في الشتاء كان منخفضا بينما امتلك أعلى قيم للعناصر الثقيلة (النحاس، الحديد، الكادميوم) باستثناء الرصاص الذي كان منخفضا ، في حين امتلك النسب الأعلى في الصيف أقل القيم (النحاس،الحديد والكادميوم) باستثناء الرصاص الذي كان مرتفعًا. بينما المؤشرات الدموية لعينات دم الاغنام الذي كان معدلهم [(8.69(10^3\مايكروليتر) ،92.72 %،3.24 %] لعد كريات الدم البيض ،الخلايا اللمفاوية وخلايا الدم البيض الحبيبية على التوالي حيث اظهرت نتائج التحليل الإحصائي لهذه العينات أن جميع عينات الدم تحتوي على اصابة بسبب وجود فروق معنوية ($P<0.05$٪) في الخلايا الليمفاوية ولكن لا توجد فروق ($P>0.05$٪) في كريات الدم البيض و خلايا الدم البيض الحبيبية.

كذلك اظهرت النتائج بعد إرسال هذه العينات البكتيرية الموجبة لاجراء التحليل التسلسلي بأن البروسيلا المعزولة من التربة للمواسم الاربعة من العينات الموجبة لفحص PCR تحتوي على احدى عشر نوع من البروسيلا لـ (ثمانية وعشرون) سلالة جديدة مسجلة في بنك الجينات كلاتي : ست سلالات ل *Brucella melitensis* ، خمس سلالات ل *Brucella intermedia*، اربع سلالات ل *Brucella pseudogrignonensis* ، سلالتان ل *Brucella anthropi* ، سلالتان ل *Brucella ovis* ، سلالتان ل*Brucella inopinata*، سلالتان ل *Brucella oryzae* ، سلالتان ل *Brucella lupini*، سلالة واحدة ل *Brucella rhizosphaerae*، سلالة واحدة ل *Brucella pituitosa* ، سلالة واحدة ل *Brucella thiophenivorans*. في حين سجلت اربع سلالات جديدة لسلالتين من البروسلا الغنمية وسلالتين من البروسيلا المجهضة. حيث تم تسجيل هذه العزلات ونشرها في المركز الوطني لمعلومات التكنولوجيا الحيوية في قاعدة بيانات بنك الجينات والتي تم أخذها كمرجع لتحديد التغاير الوراثي و أيضا تم تحليل الشجرة لرسم شجرة النشوء والتطور وتحديد العلاقات التطورية للسلف المشترك لمعرفة درجة التقارب بين الانواع ومصدرها.

استنتجنا من خلال بحثنا بأن نسبة تواجد البروسيلا في فصل الصيف اعلى من فصل الشتاء وكذلك العوامل البيئية لتي تضمنت درجة حرارة الهواء ،درجة حرارة الماء،المقياس اللوغارتمي للدالة الحامضية،التوصيلية الكهربائية،المواد الصلبة الذائبة والملوحة ايضا مرتفعة خلال فصل الصيف ماعدا المواد العضوية الكلية اقل في فصل الصيف في حين كانت العناصر الثقيلة للنحاس،الحديد والكادميوم اقل في فصل الصيف ماعدا الرصاص كان اعلى، هذا يشير با اختلاف الفصول يؤثر على نسبة تواجد البروسيلافي التربة. تم ملاحظة تواجد عزلات البروسيلا

في فصول محددة للعينات البكتيرية المعزولة من التربة حيث وجدت السلالة (*B. melitensis*) في كلا فصلي الصيف والخريف والسلالة (*B.pseudogrignonensis*) في فصول الشتاء ،الربيع والخريف اما السلالة (*B.intermedia*) في كلا فصلي الربيع والخريف بينما السلالات (*B.oryzae*، *B.anthropi*، *B.ovis*) في فصل الربيع والسلالتان (*B.thiophenivorans* و *B. pituitosa*) في فصل الخريف حيث امتلكت كل من هذه العزلات وفقًا لتحليل البيانات تغايرات وراثية بينما وضحت نتائج العينات البكتيرية المعزولة من الدم وجود نوعين من البروسيلا (البروسيلا الغنمية والبروسيلا المجهضة) وامتلكت ايضا تغايرات وراثية. ايضا استنتج من خلال تحليل الشجرة التطورية لوحظ بان السلالة *B. abortus* اقرب الى اوكرانيا بينما السلالة *B. melitensis* اقرب الى اليونان في عينات الدم بينما السلالتان *B.pseudogrignonensis* احدهما اقرب الى الهند والاخرى قريبة الى الهند وفرنسا، السلالتان *B.melitensis* احدهما اقرب الى الهند والصين والاخرى اقرب الى المكسيك والولايات المتحدة الامريكية ،السلالة *B.intermedia* اقرب الى باكستان، السلالة *B.anthropi* قريبة الى الهند وجنوب كوريا، السلالة *B.oryzae* اقرب الى الهند، السلالة *B.ovis* قريبة الى الهند والولايات المتحدة الامريكية، السلالة *B.inopinata* اقرب الى سلطنة عمان،السلالة *B.lupini* اقرب الى نيجيريا،السلالة *B.intermedia* اقرب الى روسيا، السلالة *B.pituitosa* اقرب الى الصين والولايات المتحدة الامريكية، السلالة*B.thiophenivorans* اقرب الى الهند وبولندا، السلالة *B.rhizosphaerae* اقرب الى باكستان واوكرانيا في عينات ترب الحيوانات.

جمهـــــــــورية العـــــــــراق
وزارة التعليم والعالي والبحث العلمي
جامعـــــة بابـــــل / كلية العلوم
قســـــم علـــو م الحياة

العلاقة بين توزيع انواع البروسيلا وبعض العوامل البيئية في محافظة بابل ،العراق

اطروحة مقدمة إلى

مجلس كلية العلوم – جامعة بابل

وهي جزء من متطلبات نيل شهادة الدكتوراه فلسفة في العلوم \ علوم الحياة

من قبل

وسن محمد عبدالزهره حسين مرشدي

بكالوريوس علوم /علوم الحياة / بيئة

ماجستيرعلوم /علوم الحياة / بيئة

بإشراف

أ. د.أياد محمد جبر المعموري **أ. د.جايسون راو**

2023 م **1444 هـ**