Original Article

The Occurrence Of Genetic Variation In B.Abortus Isolated From Sheep In Babylon Province, Iraq

Wasan Mohammed Abdulzahra[1]*, Ayad M.J. Al-mamoori[2], Jason Rao[3]

*[1]University of Babylon, Faculty of Basic Education ,E-mail: ayadmj77@gmail.com
[2]Biology Dept., College of Science, University of Babylon
[3]Cornell University U.S.A

***Corresponding Author: -** Wasan Mohammed Abdulzahra
Date Of Publication 22 October, 2022

DOI: 10.47750/pnr.2022.13.S08.163

Abstract

The samples collected from sheep's blood from(Al-Mihnawiya Village) in Babylon province , middle of Hilla , Iraq in the end of May month-2021. This investigation aim to knowledge presence types of *Brucella* spp. in the sheep's blood. *Brucella* is pathogen of zoonotic disease between animals and human. The bacteria isolated and identification by pcr. The results revealed presence the bacteria in all the blood samples and after senting these samples to DNA sequence noticed two species of bacteria included *B.melitensis* and *B.abortus* where compared these local samples with world researchs in NCBI by phylogenetic tree.DNA sequencing for *B.melitensis* and *B.abortus* include substituation mutation (transversion and transition) and at compared these results for genotype with phenotype that include (WBC, granulocyte and lymphocyte) by statistical analysis don't have significant differences (P>0.05%) in *B.melitensis* but there is significant differences(P<0.05%) in *B.abortus* , this mean existing genetic variety in this bacteria.

Keywords: genetic variation, *B.abortus*, sheep, Iraq

INTRODUCTION

Brucella is a gram-negative, intracellular, non-motile, none sporulating, non-toxigenic, no fermenting, and weakly acid-fast bacteria[1] . And it belong to the Brucellaceae family and is non-spore-forming diseases with a tiny size of (0.6–1.5)µm in length and (0.5–0.7)µm in diameter. Brucellosis is a zoonotic infection caused by the bacterial genus *Brucella.* The bacteria are transmitted from animals to humans by ingestion through infected food products, direct contact with an infected animal, or inhalation of aerosols, e disease is an old one that has been known by various names, including Mediterranean fever, Malta fever, gastric remittent fever, and undulant fever. Humans are accidental hosts, but brucellosis continues to be a major public health concern worldwide and is the most common zoonotic infection [2]. Brucellosis is a zoonotic disease that can be caused by four different *Brucella* species in humans:

B. suis, *B. melitensis*, *B. abortus*, and *B. canis*. As few as 10 to 100 organisms can cause the disease in humans. All Brucella species are gram-negative, nonmotile, facultative intracellular coccobacilli.

Brucella species do not form spores or toxins. The animal host of *B. suis* is swine; the hosts of *B. melitensis* are sheep and goats; the host of *B. abortus* is cattle, and the hosts of *B. canis* are dogs [3,4] . The most common zoonotic agent is *B. melitensis,* which is followed by *B. abortus*. Although human infection with *B. abortus* is usually minor, it can produce severe and intractable sickness. The incidence of *B. melitensis* is acute, often incapacitating infection in humans[5]. Animals are considered as natural reservoir of human Brucellosis. Genetic and immunological evidence indicates that all members of the Brucella genus are closely related Nevertheless, based on relevant differences in host preference and epidemiology displayed by the major variants, as well as molecular evidence of genomic variation, it has many virulence factor causing sever Pathogenicity[6]. *Brucella* is a genus with twelve species that infect various wildlife and domestic animal species[7]. *B. abortus* (cattle), *B. melitensis* (goats and sheep), B. ovis (rams), B. canis (dogs), B. suis (pigs), and B. neotomae (pigs) are the six Brucella species classified according to pathogenicity and preferred hosts (Common voles, desert wood rat). B. *melitensis*, *B. suis*, and *B. abortus* are the three most important pathogenic species in humans [8,9,10,7]. It is one of the most important infectious diseases that is transmitted to humans either by direct contact with infected animals or through the consumption of contaminated animal products, particularly unpasteurized milk and soft cheese[11] .

Brucella species are pathogenic bacteria that adapt to new hosts and are naturally transmitted to their primary hosts through direct or indirect contact, as well as inadvertently to other vulnerable hosts[12] . Mixed farming of cows, buffaloes, sheep, and goats has raised the danger of brucellosis, with small ruminants serving as primary hosts and cattle serving as an overflow host for B. melitensis[13] . Occupational illness is spread by abattoir employees and veterinarians coming into contact with infected animals, particularly aborted fetuses, fluid, membranes, or urine[5].

Brucella rods can enter via host cells by inhalation, ingestion, skin abrasion, or mucosal membranes[14]. After penetration into host, rods multiply in lymph nodes; afterward, they penetrate other organs[15] . It can modify immune response in host

cells; it has an affinity to the cells of specific tissues, e.g. placental trophoblast in fetal lung, pregnant females or reproductive system[16]. Brucellosis causes enlargement of lymph nodes, liver and spleen[17]. Pathogenicity of *Brucella* is dependent on their ability to multiply and survive within macrophages[18].

In 1886, *Brucella melitensis* first isolated by David Bruce from the spleen of a British soldier who died of a febrile illness, and known as Malta fever,The bacterium named *Micrococcus melitensis*, with 'melitensis' that derived from the Roman name for Malta, 'Melita'. In 1897, *Bacillus abortus* identified as the cause of contagious abortion in cattle by Bernhard Bang. Later, in 1917, it was found that the causes of the two diseases were identical, and renamed *Brucella* in honor of Bruce [17].

In Iraq, the small ruminant (sheep and goats) sector is very important for sustaining the country's food security. There are presently an estimated 7–8 million sheep and 1.5–2.0 million goats in Iraq, representing a valuable source of meat and milk production, and providing income and job security to people working across the agricultural sector[19]. An important challenge facing the small ruminant sector in Iraq is the challenging animal disease situation. Many endemic diseases are poorly managed and controlled as a consequence of the collapse of the veterinary infrastructure as a result of international economic sanctions and political and ethnic conflicts[20]. Among the many endemic animal diseases, *B. melitensis* continues to pose a threat to animal productivity and public health in Iraq.

Phylogenetically, Ochrobactrum, Daeguia, Crabtreella, Mycoplana, Pseudochrobactrum, and Paenochrobactrum are all members of the order Rhizobiales, which belongs to the class Alpha proteobacteria[21]. Isolation of "suspect" bacterial colonies from blood, bone marrow, or tissue samples, followed by bacteriological characterization, remains the unequivocal diagnosis ("gold standard") [22] . Brucellosis is a zoonotic disease that can infect both animals and people. In the United States, Brucellosis is still endemic[23]. According to OIE Terrestrial Manual[24], there is no single test by which a bacterium can be identified unequivocally as Brucella. A combination of growth characteristics, serological, bacteriological or molecular methods is required for a definitive identification. Ahmed *et al*., (2011)[25] investigated the prevalence of brucellosis disease in humans and sheep in Al- Hashymia district\ Babylon province depending upon data which was register in Babylon hospital and veterinary hospital in Babylon. All suspected cases infected with brucellosis. while Ashford & Whatmore, (2022)[26] founded that brucellosis, a zoonotic illness produced by members of the genus Brucella, is still a major zoonotic disease that affects both agricultural production and human health. the disease has been largely controlled in some regions of the world, it continues to have serious consequences in the Middle East, Africa, Asia, and Central and South America, among other places. *Brucella* organisms enter into their host through the mucosal membranes of the respiratory and digestive tracts[27]. Once inside, local professional phagocytes such as macrophages, dendritic cells, and neutrophils internalize the bacteria and move to the closest draining lymph nodes following the normal sampling of the immune system. This leads to subsequent dissemination to the different organs of the reticuloendothelial system, including lungs, spleen, liver, and bone marrow[28] .

The Blood Samples Collection

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 and were kept in freezer at −20 C^0 to hematological and molecular tests .

MATERIALS AND METHODS

Molecular And Haematological Study:

1- Haematological tests

Count blood cell (CBC) tests were done to find out of the bacterial infection in animals by CBC device .

2-Molecular Study

Molecular diagnostic methods are also currently being used for the detection of Brucella spp. in various samples[29] .

2.1 Brucella isolation from blood

1- Mixed blood samples by vortex

2- Took (1ml) from blood samples put it in (screw cap bottles) contain (4ml) of brain heart infusion and added horse serum to mixing.

3-Incubated for (21 days) for appearance turbidity

4- Subculture on brucella agar base by taking (100 microliter) from cultured broth by micropipette and published in Petridish and leaved in incubator for (14 days) until appearance of growth[30] .

2.2 Bacterial DNA Extraction:

Genomic DNA extracted from bacterial isolates cultured from the soil and blood according to the manufacturer's protocol FavorPrep™ Blood/ Cultured Cells Genomic DNA Extraction Mini Kit.

2.3 Conventional PCR

Conventional PCR were used to amplify the target bacterial DNA using specific primer pairs. It include 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 1), (table 2) and figure (1).

Table (1): The Sequence Of Primers That Used This Study

Primer	Sequence	Primer sequence	Tm (°C)	GC%	Size of Product (bp)
16s RNA	F	5'- AGAGTTTGATCCTGGCTCAG- 3'	54.3	50.0	1250
	R	5'- GGTTACCTTGTTACGACTT- 3'	49.4	42.1	Srinivasan *et al.*, (2015) [31]

Table (2): The Optimum Conditions (Amplification Condition) Of Detection The Blood's Isolated Bacteria (Stages And Temperature Of PCR For Gene 16srna) .

		Temperature	Time	cycle
Stage 1	Initial Denaturation	95ºC	5 sec	1
Stage 2	Denaturation2	95ºC	45 sec	35
	annealing	56ºC	45 sec	
	Extension	72ºC	1 sec	
Stage 3	Final Extension	72ºC	5 sec	1

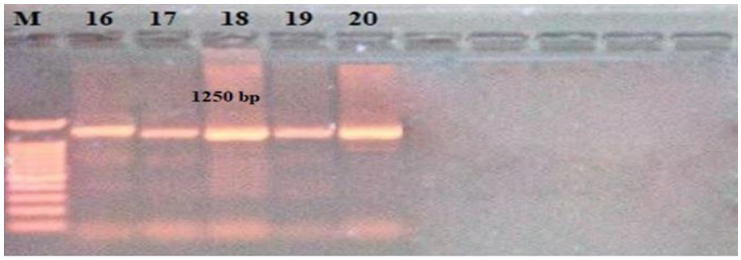

Figure (1): Agarose GEL ELECTROPHORESIS (2%) FOR 16Srna PRIMER-Blood (1250 BP) Primer Ta AT (57` C), (AT 65amp , 70 VOLTS, 60MIN.). Visualized UNDER U.V LIGHT AFTER STAINING WITH Gel STAIN., Lane M 100 BP Dna Ladder.

16S rRNA gene sequence analysis and Phylogenetic tree

Sanger sequencing was successful for 17 out of the 20 samples with an expected PCR product size (1250 bp) by 16S rRNA primers. The sequences were identified as belonging to *Brucella* spp. following similarity search by blast (sequence identity of 99%). DNA 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 deposed in GenBank with accession numbers.In addition, each sequence on blast search showed similarity and diversity with different *Brucella* spp. (*B. abortus, B. melitensis*) deposited in Genbank considering the percent identity. where 17 sequences were used for partial sequence alignment and phylogeny construction. Occurance types of substituations mutations Transvertion\Trasition) for (17 isolates) from the sheep's blood *Brucella* samples for two species of *Brucella* by compared local one strain with strains to one states 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 X) 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 . Phylogenetic analysis of the 16S rRNA gene sequences indicated that *B.* abortus compared with China, USA, India, Italy, South Africa, Russia, Nigeria, South Korea, Germany, Ukraine. While *B.* melitensis compared with Greece, China, India, USA, Norway, Croatia.as mentioned in figure (2).

.

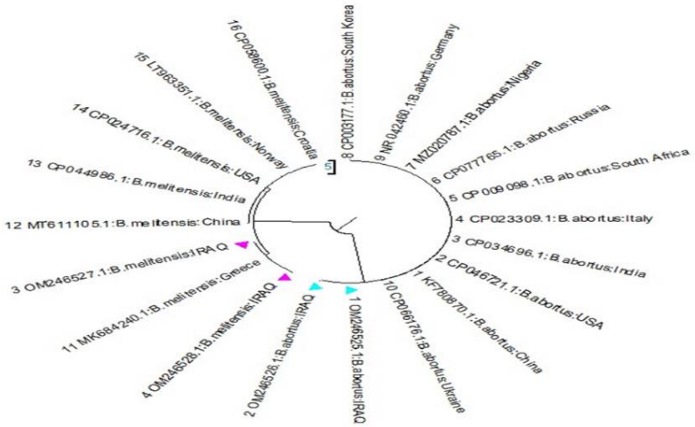

Figure (2): Evolutionary Analysis (Phylogenetic Tree) Of 16S Rrna Gene Sequences Of Brucella At Compared The Two Strains (*B.Abortus,B.Melitensis*) With Different States.

The results and discussion

The results of DNA sequencing revealed presence substituation mutations (Transvertion\Trasition) for (17 isolates) from the sheep's blood *Brucella* samples founded (4 new strains) for two species of *Brucella* , This evidence for presence genetic diversity with the isolates, This genetic variation mentioned in table (2).

	***Brucella abortus* Gene polymorphisms**					**P= value**
Blood parameters	**C\T**	**A\T**	**A\G**	**G\C**	**T\C**	
	Mean ±Standard error					
lymphocyte	91.91± 0.99	93.28± 1.00	92.07± 1.05	93.50± 2.36	93.01± 1.08	0.49
Granulocytes	2.20± 0.60	2.36± 1.08	7.10± 1.18	6.56± 1.36	6.66± 1.20	0.02*
WBC	8.50± 0.86	9.00± 0.91	8.25± 1.37	2.87± 0.42	6.52± 0.45	0.002*
***Brucella melitensis* Gene polymorphisms**						
	C\T	**G\A**	**A\G**	**T\G**	**C\A**	**T\C**	**P= value**
lymphocyte	91.66± 2.90	92.00± 1.20	94.00± 1.76	94.33± 1.97	95.00± 1.25	93.22± 1.50	0.72
Granulocytes	3.73± 1.90	2.56± 0.91	1.63± 0.27	1.83± 0.48	1.80± 0.27	2.66± 0.88	0.66
WBC	8.33± 0.88	7.33± 1.20	10.00± 1.73	6.66± 1.20	7.66± 1.33	8.16± 0.49	0.46

Table (3): The Relationship Between Genotype And Phenotype In Sheep's Blood Samples

This table show that *B.melitensis* don't have significant differences (p>0.05) between genotype and phenotype this indicate that genetic variation is present but don't effect on bacteria because of the reservoir host to *B. melitensis* is sheep and this genetic polymorphism may be form protective and resistence to this bacteria. While in *B.abortus* occurred substituation mutations and noted presence significant differences (p<0.05) between genotype and phenotype, this indicate to presence genetic variation in *B.abortus* , belong the reason to the reservoir host to *B.abortus* is cattles and translated to other host (sheep) that live with it in same place (animal barn) and contact with each other.

The present results was agreed with many studies, which done in Iraq and showed that *B. abortus* and *B.melitensis* represent the highest isolation percentages than other species[32].Brucella abortus has been reported as a cause of abortion in sheep in Nigeria[33] was coincide with study results where *B.abortus* readily infects cattle and spreads among pregnant animals, and can cause abortion. This means that schemes for the control and eradication of bovine brucellosis where infected sheep and goats are present must include its eradication from these animals as well[33]. Also with Ocholi *et al* ., (2005)[34] reported on a sporadic, naturally acquired infection of sheep with *B. abortus* on a privately owned farm in Toro near Bauchi, Nigeria. The abortions which occurred in a flock of 28 Yankassa sheep, involved five ewes at the third month of gestation. All isolates were identified and biotyped as *B. abortus* biovar 1. This biovar also isolated from cattle maintained on the farm in association with the sheep. The infection was attributed to the animal husbandry practices employed on the farm. All isolates were diagnosed as *B. melitensis* (Sheep and goats) are the natural reservoirs for *B. melitensis*. It is the dominant Brucella species in the Middle East and causes the majority of human cases[35,36]. Thus, the epidemiologic situation is coherent: there is a continuous spillover from a chronically infected sheep population to the consumers. *B. melitensis* in sheep poses a high potential risk for human infection and is a serious public health threat. It is also a significant threat to the livestock population, livestock owners, abattoir workers, meat vendors, and professional animal health workers[37] , All *Brucella* species are closely related and can be considered as pathovars of a single species[38]. Thus, it is not unexpected that host specificity of *Brucella* spp. is not 'absolute' but 'relative' , Although ruminants in general are susceptible to B. abortus, the infection in small ruminants is rare[39]. Experimental infection of pregnant ewes with *B.abortus* produced late term abortions. The aborted ovine fetuses developed lesions due to systemic infections similar to those reported in bovine fetuses after natural and experimental infections[40]. *B.abortus* infections have been reported in sheep in the USA[41]in Nigeria[34] and in Iran[42] , was the current research results also agreement with them . Detection of both, *B.abortus* and *B.melitensis* DNA, in one animal observed in this study demonstrated that one host can be infected with two different species of *Brucella* at the same time. The potential host range of Brucellae may also depend on breeding conditions[43]. *Brucella* species are pathogenic bacteria that adapt to new hosts and are naturally transmitted to their primary hosts through direct or indirect contact, as well as inadvertently to other vulnerable hosts[12]. Mixed farming of cows, buffaloes, sheep, and goats has raised the danger of brucellosis, with small ruminants serving as primary hosts and cattle serving as an overflow host for *B. melitensis*[13].

This study compared with Egypt , brucellosis was still endemic and infects a wide range of animal species causing tremendous economic losses[44]. *B.melitensis* was isolated from cattle, buffalo, sheep, goat and Nile catfish in the past[45] . In contrast, *B.abortus* was isolated from cattle, buffalo and camel[46], but was not recorded in small ruminant[47], was like with them in being *Brucella* species can infect the other host not only natural host by contact with them in same place. Moreover, in Iraq, there have been several remarkable achievements attributed to past mass vaccination campaigns. The most important outcome was the apparent decrease in incidence of human brucellosis, which declined to almost 17 cases/100,000 people in the middle and south of Iraq in 2009 compared with 27.23 cases/100,000 in 2002 and 88.55 cases/100,000 in 1995[48] .

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood – the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these results in the diagnosis and monitoring of disease[49]. Haemtological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood[50]. The genetic and non-genetic factors affecting haematological parameters of farm animals have been observed[51]. Several factors including physiological[52], environmental condition[53,54,] dietary content[55], affect the blood profile of healthy animal. Haematological parameters and its knowledge can be used to assess the health as well as the physiological status of farm animals under consideration. Changes of these parameters have been studied in cattle,

sheep and red Sokoto goats. There is great variation in the haematological parameters as observed between breeds, ages, sexes, management systems among others in farm animals[56]. *Brucella* spp. are facultative intracellular microorganisms and they settle in macrophages. After completing replications in these cells, they migrate to the lymphoid tissues of the reproductive system as a result of a primary bacteremia. The agent causes a secondary bacteremia from these tissues, which subsequently leads to a generalized infection and then abortion. Therefore, these persistent bacteremia phases, which almost always contain the microorganisms in the bloodstream, are repeated in the next gestational period[57], Modern PCR methods are capable of detecting both living bacteria and bacteria that have been phagocyted or killed by macrophages in different compartments of the blood during the periods of bacteremia[58,59]. Also, PCR techniques have the ability to determine the course of the infection. In this context, blood in which bacteria-laden macrophages are constantly circulating is a useful clinical material that can be used for diagnosis as a source of DNA belonging to the infectious agent [58] .

Several molecular typing methods have been applied to search for DNA polymorphisms between Brucella spp.[60]. Complete genome sequencing is ideal; they have the advantage that mutational changes are totally reflected in the sequence, an event that does not occur in proteins, due to the degenerate character of the genetic code; clarifying in this way the main evolutionary mechanims involved in the different processes of speciation, a example of this type of study was made in 2015 in where the authors sequenced the complete genome of several B. melitensis strains, achieving not only discrimination between vaccine and endemic species, but also performing a phylogenetic reconstruction of the history of the species and proposing a possible global distribution of the bacterium[61,62]. One of the genetic targets frequently used for strain identification and strain phylogeny is the rRNA, particularly the 16S rRNA gene used in this study. These genes are highly conserved and diverge very slowly. The DNA sequences from separate species within a genus will differ by only a few percent. Sequence identity among 16S rRNA sequences is typically interpreted as indicating a single species[63] .

The current study agreed with (Kaltungo et al.,2014)64 that performed two different diagnostic methods (serological test and PCR analysis). Based on the study results, the infection rate was 8.8% on serological testing and 1.4% on PCR analysis. Interestingly, the positive samples on PCR analysis were negative on serological testing, indicating that in some cases, PCR can detect Brucella genes in seronegative animals due to its ability to detect small amounts of the pathogen in body fluids of infected animals .

Polymorphisms are variations in DNA sequence that are frequent in the population. mutations or DNA alterations with a frequency of greater than 1% in a population and alterations with a frequency of greater than 1% in a population[65] .

A mutation, on the other hand, is defined as any deviation from the normal DNA sequence. This means that there is a common normal allele in the population, and that the mutation transforms it into a rare and aberrant variety[66].

Phenotypic qualities are influenced by these genetic factors , polymorphisms in a number of genes are linked to individual genetic susceptibility. They have the ability to alter the effects of environmental exposure as well as gene-environmentinteractions[67]

REFERENSES

1. Gwida M., Al Dahouk S., Melzer F., Rősler U., Neubauer H. and Tomaso H. (2010). Brucellosis – regionally emerging zoonotic disease? Croat. Med. J. 51(4): 289–295. [PMC free article] [PubMed] [Google Scholar.
2. Głowacka P, Żakowska D, Naylor K, Niemcewicz M, Bielawska-Drózd A. (2018). Brucella - Virulence Factors, Pathogenesis and Treatment. Pol J Microbiol. Jun 30;67(2):151-161. [PMC free article] [PubMed].
3. Bukhari EE. (2018).Pediatric brucellosis. An update review for the new millennium. Saudi Med J. Apr;39(4):336-341. [PMC free article] [PubMed].
4. Brown VR, Bowen RA, Bosco-Lauth AM. (2018)..Zoonotic pathogens from feral swine that pose a significant threat to public health. Transbound Emerg Dis. Jun;65(3):649-659. [PubMed].
5. Boon,N.A.; Colledge N.R.; Walker B. R., and Hunter, J. A. (2010). Davidsons principle and practice 21thed. London .U.K.pp:318-320.
6. Marie-Rose Abdo, Pascale Joseph, Jérémie Mortier, François Turtaut, JeanLouis Montero, Bernard Masereel, Stephan Köhler, Jean-Yves Winum.(2011).Anti-virulence Strategy against Brucella suis: Synthesis, Biological Evaluation and Molecular Modeling of Selective Histidinol Dehydrogenase Inhibitors. Org Biomol Chem. Apr 1;: 21461427.
7. Whatmore AM, Koylass MS, Muchowski J, EdwardsSmallbone J, Gopaul KK, Perrett LL. (2016). Extended multilocus sequence analysis to describe the global populationstructure of the genus Brucella: phylogeography and relationship to biovars.FrontMicrobiol.7(2049). https://doi.org/10.3389/fmicb.2016.02049.[PubMed], [Google Scholar].
8. Lindahl E, Sattorov N, Boqvist S, Sattori I, Magnusson U.(2014). Seropositivity and risk factors for Brucella in dairy cows in urban and peri-urban small-scale farming in Tajikistan. Trop Anim Health Prod. 46(3):563–569.
9. Wareth G, Melzer F, Elschner MC, Neubauer H, Roesler U. (2014). Detection of Brucella melitensis in bovine milk and milk products from apparently healthy animals in Egypt by real-time PCR. J Infect Dev Ctries. 8(10):1339- 1343.
10. Kaynak-Onurdag F, Okten S, Sen B. (2016). Screening Brucella spp. in bovine raw milk by real-time quantitative PCR and conventional methods in a pilot region of vaccination, Edirne, Turkey. J Dairy Sci. 99(5):3351–3357.
11. Akbarmehr J.(2011).The prevalence of Brucella abortus and Brucella melitensis in local cheese produced in Sarab city, Iran and its public health implication. African Journal of Microbiology Research, 5(12): 1500-1503 .
12. Moreno, E.(2014).Retrospective and prospective perspectives on zoonotic brucellosis. Front. Microbiol.5. [CrossRef] .
13. .ElWahab EWA, Hegazy Y, Wael F, Mikeal A, Kapaby AF, Abdelfatah M, Bruce M, Eltholth MM. (2019). Knowledge, attitudes and practices (KAPs) and risk factors of brucellosis at the human-animal interface in the Nile Delta, Egypt. BioRxiv.607655. [Google Scholar].
14. Franco M.P., Mulder M., Gilman R.H. and Smits H.L.(2007). Human brucellosis. Lancet. Infect. Dis. 7(12): 775–786. [PubMed] [Google Scholar]
15. Galińska E.M. and Zagórski J..(2013). Brucellosis in humans – etiology, diagnostics, clinical forms. Ann. Agric. Environ. Med. 20(2): 233–238. [PubMed] [Google Scholar].
16. de Figueiredo, P., Ficht, T. A., Rice-Ficht, A., Rossetti, C. A., & Adams, L. G. (2015). Pathogenesis and immunobiology of brucellosis: review of Brucella–Host Interactions. The American journal of pathology, 185(6), 1505-1517.
17. Perkins S.D., Smither S.J. and Atkins H.S..(2010). Towards a Brucella vaccine for humans. FEMS Microbiol. Rev. 34: 379–394. [PubMed] [Google Scholar].

18. Christopher S., Umapathy B.L. and Ravikumar K.L..(2010). Brucellosis: Review on the recent trends in pathogenicity and laboratory diagnosis. J. Lab. Physicians. 2(2): 55–60. [PMC free article] [PubMed] [Google Scholar].
19. Bechtol, D.; Carpenter, L.R.; Mosites, E.; Smalley, D.; Dunn, J.R.(2011).Brucella melitensis infection following military duty in Iraq. Zoonoses Public Health, 58, 489–492. [CrossRef] [PubMed].
20. FAO.(2009).Brucella melitensis in Eurasia and the Middle East. In FAO Animal Production and Health, WHO and OIE Technical Bulletin; FAO: Rome, Italy.
21. Leclercq SO, Cloeckaert A, Zygmunt MS.(2020). Taxonomic organization of the family Brucellaceae based on a phylogenomic approach. Front Microbiol. 10:3083. https:// doi.org/10.3389/fmicb.2019.03083.
22. Bricker BJ .(2002). PCR as a diagnostic tool for brucellosis. Vet. Microbiol. 90(1-4):435-46.
23. Fouskis, I., et al., (2018). The epidemiology of Brucellosis in Greece, 2007–2012: a 'One Health'approach, 112(3), pp. 124–135.
24. Ahmed W. A.,. Alasaddy M. K and Al-Hassany Y. Y. (2011). An epidemiological study of brullcelosis disease in Al- Hashymia district\ Babylon province. ISSN: 1999- 6527 .
25. OIE (World Organization for Animal Health).(2016). Infection with Brucella ovis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.
26. Ashford, Roland T., and Adrian M. Whatmore.(2022)."Brucella." Molecular Typing in Bacterial Infections, Volume II. Springer, Cham,. 217-245.
27. Von Bargen, K., Gagnaire, A., Arce-Gorvel, V., de Bovis, B., Baudimont, F., Chasson, L., ... & Gorvel, J. P. (2015). Cervical lymph nodes as a selective niche for Brucella during oral infections. PLoS One, 10(4), e0121790.
28. Moreno, E.; Barquero-Calvo, E. The Role of Neutrophils in Brucellosis. Microbiol. Mol. Biol. Rev.(2020) , 84, e00048-20. [CrossRef] 34. Anderson, T.D.; Meador, V.P.; Cheville, N.F. Pathogenesis of Placentitis in the Goat Inoculated with Brucella abortus. I. Gross and Histologic Lesions. Vet. Pathol. 1986, 23, 219–226. [CrossRef]
29. Şahin M, Unver A and Otlu S .(2008). Isolation and biotyping of B. melitensis from aborted sheep foetuses in Turkey. Bulletin of Veterinary Inst Pulawy, 52: 59-62.
30. Forbes B, Sahm D, Weissfeld A.(2007).Bailey and scott's diagnostic microbiology: A textbook for isolation and identification of pathogenic microorganisms. In: St. Louis , editor. The mosby company. C. V. Mosby . p. 378.
31. Srinivasan, R., Karaoz, U., Volegova, M., MacKichan, J., Kato-Maeda, M., Miller, S., ... & Lynch, S. V. (2015). Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. PloS one, 10(2), e0117617.
32. Seleem M N, Boyle S M and Sriranganathan N.(2008). Brucella pathogen without classic virulence genes. Vet. Microbiol. 129, 1-14.
33. Okoh A .(1980). Abortion in sheep near Kano, Nigeria. Trop Anim Health Prod 12(1):11–14.
34. Ocholi R, Kwaga J, Ajogi I, Bale J .(2005). Abortion due to Brucella abortus in sheep in Nigeria. Rev Sci Tech 24(3):973–979.
35. Mustafa, A.S.; Habibi, N.; Osman, A.; Shaheed, F.; Khan, M.W.(2017). Species identification and molecular typing of human Brucella isolates from Kuwait. PLoS ONE, 12, e0182111. [CrossRef] [PubMed].
36. Al-Adsani, W.; Ahmad, A.; Al-Mousa, M. A.(2018).case of Brucella melitensis endocarditis in a patient with cardiovascular implantable electronic device. Infect. Drug Resist., 11, 387–390. [CrossRef] [PubMed].
37. Seyman, D.; Asik, Z.; Sepin-Ozen, N.; Berk, H.(2015).Acute Brucellosis Following Accidental Exposure to Brucella melitensis Rev-1 Vaccine. West Indian Med. J., 65, 216–218. [CrossRef] [PubMed].
38. Martirosyan A, Moreno E, Gorvel J-P .(2011). An evolutionary strategy for a stealthy intracellular Brucella pathogen. Immunol Rev 240(1):211–234.
39. Díaz Aparicio E. (2013). Epidemiology of brucellosis in domestic animals caused by Brucella melitensis, Brucella suis and Brucella abortus. Rev Sci Tech. Apr;32(1):43-51, 53-60. Rev Sci Tech. 2013. PMID: 23837364 English, Spanish.
40. Gorham SL, Enright FM, Snider TG 3rd, Roberts ED. (1986).Morphologic lesions in Brucella abortus infected ovine fetuses. Vet Pathol 23(3):331–332.
41. Kreeger T, Cook W, Edwards W, Cornish T. (2004). Brucellosis in captive Rocky Mountain bighorn sheep (Ovis canadensis) caused by Brucella abortus biovar 4. J Wildl Dis 40(2):311–315.
42. Behroozikhah A, Bagheri NR, Amiri K, Bahonar A.(2012) .Identification at biovar level of Brucella isolates causing abortion in small ruminants of Iran. J Pathog. doi:10.1155/2012/357235.
43. Martirosyan A, Moreno E, Gorvel J-P.(2011). An evolutionary strategy for a stealthy intracellular Brucella pathogen. Immunol Rev 240(1):211–234.
44. Holt HR, Eltholth MM, Hegazy YM, El-Tras WF, Tayel AA, Guitian J .(2011). Brucella spp. infection in large ruminants in an endemic area of Egypt: cross-sectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). BMC Public Health 11:341. doi:10.1186/1471-2458-11-341.
45. El-Tras W, Tayel A, Eltholth M, Guitian J.(2010).Brucella infection in fresh water fish: evidence for natural infection of Nile catfish, Clarias gariepinus, with Brucella melitensis. Vet Microbiol 141:321–325.
46. Hamdy MER, Amin AS.(2002).Detection of Brucella species in the milk of infected cattle, sheep, goats and camels by PCR. Vet J 163:299–305.
47. Wareth G, Hikal A, Refai M, Melzer F, Roesler U, Neubauer H.(2014). Animal brucellosis in Egypt. J Infect Dev Ctries 8(11):1365–1373. doi:10.3855/ jidc.4872.
48. Mugabi, R.(2012). Brucellosis Epidemiology, Virulence Factors, Control and Molecular Targets to Prevent Bacterial Infectious Diseases. Master's Thesis, North Dakota State University of Agriculture and Applied Science, Fargo, ND, USA,.
49. Merck Manual.(2012).Haematologic reference ranges. Mareck Veterinary Manual. Retrieved from http://www.merckmanuals.com/.
50. Togun, V. A., Oseni, B. S. A., Ogundipe, J. A., Arewa, T. R., Hammed, A. A., Ajonijebu, D. C., . . . Mustapha, F. (2007). Effects of chronic lead administration on the haematological parameters of rabbits – a preliminary study (p. 341). Proceedings of the 41st Conferences of the Agricultural Society of Nigeria.
51. Xie, L., Xu, F., Liu, S., Ji, Y., Zhou, Q., Wu, Q., . . . Xie, P. (2013). Age- and Sex-Based Hematological and Biochemical Parameters for Macaca fascicularis. PLoS ONE, 8(6), e64892, doi:10.1371/journal.pone.0064892.
52. Alodan, M. A., & Mashaly, M. M.(1999). Effect of induced molting in laying hens on production and immune parameters. Poultry Science, 78(2), 171-177.
53. Vecerek, V., Strakova, E., Suchy, P., & Voslarova, E. (2002). Influence of high environmental temperature on production and haematological and biochemical indexes in broiler chickens. Czech Journal of Animal Science, 47(5), 176-182.
54. Graczyk, S., Pliszczak-Król, A., Kotonski, B., Wilczek, J., & Chmielak, Z. (2003). Examination of haematological and metabolic changes mechanism of acute stress in turkeys. Electronic Journal of Polish Agricultural Universities: Veterinary Medicine, 6(1), 1-10.
55. Kurtoğlu, F., Kurtoğlu, V., Celik, I., Keçeci, T., & Nizamlioğlu, M.(2005). Effects of dietary boron supplementation on some biochemical parameters, peripheral blood lymphocytes, splenic plasma cells and bone characteristics of broiler chicks given diets with adequate or inadequate cholecalciferol (vitamin D3) content. British Poultry Science, 46(1), 87-96.
56. Etim, N. N., Williams, M. E., Akpabio,U., & Offiong,E. E.(2014). Haematological parameters and factors affecting their values. Agricultural Science, 2(1), 37-47.
57. Pappas G, Papadimitriou P.(2007).Challenges in Brucella bacteraemia. Int J Antimicrob Agents, 30 (Suppl. 1), S29-S31,. DOI: 10.1016/j. ijantimicag.2007.06.011.
58. Zerva L, Bourantas K, Mitka S, Kansouzidou A, Legakis NJ.(2001). Serum is the preferred clinical specimen for diagnosis of human brucellosis by PCR. J Clin Microbiol, 39, 1661-1664, 2001. DOI: 10.1128/JCM.39.4.1661- 1664..

59. Khan MZ, Zahoor M.(2018).An overview of Brucellosis in cattle and humans, and its serological and molecular diagnosis in control strategies. Trop Med Infect Dis, 3(2):65,. DOI: 10.3390/tropicalmed3020065
60. Wattiau, P.; Whatmore, A.M.; Van Hessche, M.; Godfroid, J.; Fretin, D.(2011). Nucleotide polymorphism-based single-tube test for robust molecular identification of all currently described Brucella species. Appl. Environ. Microbiol. 77(18):6674-6679. [Links].
61. Whatmore, A.M.; Murphy, T.J.; Shankster, S.; Young, E.; Cutler, S.J.; Macmillan, A.P.(2005). Use of amplified fragment length polymorphism to identify and type Brucella isolates of medical and veterinary interest. J. Clin. Microbiol. 43(2):761-769.
62. Tan, K.-K.; Tan, Y.-C.; Chang, L.-Y.; Lee, K.W.; Nore, S.S.; Yee, W.-Y.(2015). ISA, M.N.M.; JAFAR, F.L.; HOH, C.- C.; ABUBAKAR, S. Full genome SNP-based phylogenetic analysis reveals the origin and global spread of Brucella melitensis. BMC Genomics. (England). 16(1):2-11.
63. Bricker BJ, Ewalt DR, MacMillan AP, Foster G and Brew S.(2000). Molecular characterization of Brucella strains isolated from marine mammals J Clin Microbiol, 38:1258-1262.
64. Kaltungo, B.Y.; Saidu, N.A.S.; Sackey, A.K.B.; Kazeem, H.M.(2014). A Review on Diagnostic Techniques for Brucellosis. Afr. J. Biotechnol., 13, 1–10. [CrossRef].
65. Teama, S. (2018). DNA polymorphisms: DNA-based molecular markers and their application in medicine. Genetic Diversity and Disease Susceptibility, 25.
66. Karki R, Pandya D, Elston RC, Ferlini C.(2015).Defining “mutation” and “polymorphism” in the era of personal genomics. BMC Med Genet 8:37. https://doi.org/10.1186/s12920-015-0115-z.
67. Rosero, J. A., Killer, J., Sechovcova, H., Mrázek, J., Benada, O., Fliegerová, K., ... & Kopečný, J.(2016).Reclassification of Eubacterium rectale (Hauduroy et al. 1937) Prévot 1938 in a new genus Agathobacter gen. nov. as Agathobacter rectalis comb. nov., and description of Agathobacter ruminis sp. nov., isolated from the rumen contents of sheep and cows. International Journal of Systematic and Evolutionary Microbiology, 66(2), 768-773.