4

Brucellosis in Cattle and Buffaloes

AUTHORS DETAIL

Sahar Mustafa*[1], Anas Sarwar Qureshi[2], Farrah Deeba[3], Imaad Rashid[4], and Muhammad Usman[5]

[1]Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan
[2]Department of Anatomy, University of Agriculture, Faisalabad, 38040 Pakistan
[3]Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, 38040 Pakistan
[4]Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, 38000 Pakistan
[5]Department of Basic Sciences, KBCMA College of Veterinary and Animal Sciences, Narowal, Sub-campus UVAS-Lahore, Pakistan
*Corresponding author: saharmustafa30@gmail.com

Received: Sept 18, 2022 Accepted: Nov 25, 2022

INTRODUCTION

Brucellosis is one of the most cardinal infectious and zoonotic disease in developing countries all over the world that hampers livestock production and *human* vigour (Franc et al. 2018; Dadar et al. 2020). In humans, brucellosis is known as Gibraltar fever, Undulant fever, Maltese fever, Goat fever, Crimean fever, remitting fever, and Mediterranean fever, while in cattle and buffaloes it is called Bhang's disease (or contagious abortion) (Rossetti et al. 2017; Khurana et al. 2021). Various brucella strains are responsible for this disease. Brucella is a genus of gram-negative bacteria named after Sir David Bruce (Seleem et al. 2008). Brucella has a tendency to adapt to new hosts. It causes supplementary severe complications in humans because they are a dead-end host for this pathogen (Moreno 2014). Almost 50,000 expositions of disease transmission from animals were investigated in humans around the world each year (Pappas et al. 2006). Brucellosis in bovines was first declared in Zimbabwe (Africa) in 1906 (Kiros et al. 2016). Brucellosis in cattle and buffaloes was eradicated in Ireland (1980) (Khurana et al. 2021). It is still present in India due to its lack of awareness (Machavarapu et al. 2019). In China, the ubiquity of bovine brucellosis was 1.9% (Khurana et al. 2021). 6.3% of RBPT (Rose Bengal plate test) samples and 18.6% of herds are positive for bovine brucellosis in Pakistan (Ali et al. 2017). If proper control measures and vaccines are not adopted, then the ubiquity of disease will be higher because of a poor immune system (Ali et al. 2014; Dorneles et al. 2015). Only the Brucella vaccine cannot eradicate this disease. Along with the vaccine, undeniable preventive measures should be practiced (Seleem et al. 2010; Awah-Ndukum et al. 2018). Successful control procedures should be adopted to control brucellosis such as culling. Culling prevents disease transmission and inspection (Rahman et al. 2011; Durrani et al. 2020). Certain countries have restricted brucellosis by adopting control measures such as valid diagnostic appliances, smooth live immunization, and mass vaccination of huge populations (Moreno 2014). However, the elimination program is very costly, but Americans consume $1 on this program and save $7. In the USA, the elimination program's cost was $3.5 billion in 1934-1997 but in 1952, the price of abortion and decreased milk yield was about $600 million. The prevalence of brucellosis in humans varies between 0.03 and 160 per million people. In America, a $600 million loss is recorded due to brucellosis (Acha and Szyfres 2003; Pappas et al. 2006; Sriranganathan et al. 2009). In Pakistan, losses due to brucellosis, in dairy cattle were estimated at $3.4 billion and in India it was $58.8 million due to effective surveillance programs (Jamil et al. 2021). Around the world, economic losses effect the animal productivity and public health (Acha and Szyfres 2003; Pappas et al. 2006 Sriranganathan et al. 2009).

Brucella Organisms

Brucella species are pathogenic, facultative, non-motile, gram-negative coccobacillus, having a size of 0.6-1.5 µm length, 0.5-0.7 µm diameter that belongs to the menage of Brucellaceae (Moreno 2014). The menage Bruceallaceae consists of Brucella and 6 other genera (Leclercq et al. 2020). Recently, 12 species of Brucella have been identified (Whatmore et al. 2016). *B. abortus, B. suis, B. melitensis, B. canis, B. neotomae* and *B. ovis* are pathogenic Brucella species that affect cattle, pigs, goats, and sheep, as well as dogs, small rodents, desert rats, and rams, respectively. *B. abortus, B. melitensis, and B. suis* affect humans (De Bolle et al. 2015). These Brucella species affect wild and domestic animals (Lindahl et al. 2014; Wareth et al. 2014). In marine animals, 2 more species are reported: *B. inopinata* (isolated from humans) and *B. papionis* (isolated from baboons) (Olsen and Palmer 2014; Whatmore et al. 2014).
From frogs, 36 brucella species are isolated. Brucella also has biovars, 7 for *B. abortus*, 5 for *B. suis*, and 3 for *B. melitensis*. The remaining species have not been classified into biovars yet. Brucella belongs to the Proteobacteria phylum. It is gram negative, nitrate reductase and have partial acid fast, urease and catalase activity. This organism has the ability to survive

Citation: Mustafa S, Qureshi AS, Deeba F, Rashid I and Usman M, 2023. Brucellosis in Cattle and Buffaloes. In: Abbas RZ, Saeed NM, Younus M, Aguilar-Marcelino L and Khan A (eds), One Health Triad, Unique Scientific Publishers, Faisalabad, Pakistan, Vol. 2, pp: 25-33. https://doi.org/10.47278/book.oht/2023.37

in freezing conditions. It has high sensitivity to most common disinfectants. It can survive in cool and moist environment. Pasteurization can kill bacteria. So, pasteurization of milk is necessary for the prevention of bacteria. These organisms are non-motile and do not have any genes for flagella (Olsen and Palmer 2014; Scholz et al. 2016). The nomenclature of *Brucella* spp. Is based on the principal host species (Khurana et al. 2021). Entire brucella species have same genome atlas and magnitude (Sriranganathan et al. 2009). The genome size of brucella is 3.29Mb, which is divided into 2 chromosomes. Chromosome l has a genome size of 2.11 Mb and a G+C placid of 57.2%, while chromosome ll has an genome size of 1.18 Mb and a G+C placid of 57.3%. An allele sequence SKN13 of *B. abortus* was withdrawn from cattle's placenta in India, which is very helpful for comparative genetic data analysis. In some species, some virulence genes are absent (plasmids, pili, capsules) (Chauhan et al. 2016). Sankarasubramanian et al. (2017) explained that *B. abortus* has 143 vintage-specific single nucleotide polymorphisms (SNPs) in 311 alleles. Of these, 141 are significant SNPs. While in *B. melitensis*, from 132 alleles, there are 383 vintage-specific single nucleotide polymorphisms and of these, 379 are significant SNPs. These SNPs can bias host adaptation. Brucella's outer membrane is related to gram-negative bacteria. It has A and M LPS surface antigens. In *B. abortus* and *B. suis*, A dander is the main antigen, while in *B. melitensis*, M is the main dander. LPS is the main virulence factor in brucella. For diagnostic tests, outer membrane amino acids (proteins) are very important (Khurana et al. 2021).

Significance in Livestock

Brucellosis is still neglected in many areas, which causes serious health problems and effects the economy of livestock (Santos et al. 2013; Singh et al. 2015). In livestock, Brucella causes abortion and infertility (in both sexes), which is the major reason for economic loss (Sulima and Venkataraman 2010; Deka et al. 2018; Franc et al. 2018). Abortions, decreased milk production, obstacles in animal transport and marketing, decreased productive capacity, increased veterinary drug costs, control program expenses, missed reproductive cycles, and low market price are all major economic losses (Georgios et al. 2005; Blasco and Molina-Flores 2011; Dadar et al. 2020). If abortion occurs in the cow once in its life, then the infection remains asymptomatic in that animal throughout its life (Godfroid et al. 2010). The Brucellosis threat is increased due to animals' mixed farming (cows, buffaloes, sheep, and goats) (El-Wahab et al. 2019).

Pakistan is an Asian country in which agriculture plays a very vital role in the economy. In agriculture, the livestock sector plays a very important role. According to the economic survey of Pakistan in 2019, Pakistan has 90.8 million large ruminants, 109.4 million small ruminants, and 1.1 million camel population. The contribution of livestock to the national GDP is 11.7 percent, and in agriculture, it is 60.6 percent (2019-2020). In Pakistan, a major population of large ruminants is allocated around the areas of Punjab and Sindh, while a major distribution of sheep and goats is found in AJK, Baluchistan, KPK, and Gilgit-Baltistan. Pakistan ranked 4th in milk production in 2019 and its milk production is 61.7 million tonnes (Jamil et al. 2021). Nowadays, the cost of medication has decreased due to safety practices. Unintentionally, exposure of milk to *B. abortus* causes major economic losses and increases the human brucellosis threat (Singh et al. 2015). In Pakistan, exotic cattle, buffaloes, and crossbred cattle have higher brucellosis acceptance. Sahiwal cows have more resistance to brucellosis. Males have a lower occurrence of brucellosis than females (Jamil et al. 2021).

Punjab has the largest human and animal population. Rivers and the monsoon season (rain) provide excellent agricultural yield opportunities (July, August, and September). Due to traditional and best farming methods and the best veterinary diagnosis and inspection system, weather remains extreme from extreme winter (-37 °C) to extreme summer (46-54 °C). A high number of cases of brucellosis have been reported in Punjab. Brucellosis cases are highly variable and their variability depends upon diagnostic tests, environmental conditions, animal type and farming techniques (0–69% in bovines, 4.4–20% in equines, and 7–35% in sheep and goats). High brucellosis prevalence occurs in confined farming systems (Jamil et al. 2021). Khan et al. (2021) conducted an experiment with 300 cattle from Punjab (Sargodha, Sahiwal, and Chiniot). They take their blood samples for serum analysis through different tests (RBPT, iELISA, RT-PCR). The result indicates that 12.7% of cattle are seropositive. All PT-PCR-tested samples are positive for Brucella infection. Sindh ranks second in terms of human and animal (livestock) population and third in terms of area. In Sindh, Brucellosis cases are usually seen in large ruminants and camels but not seen in small ruminants. Pathogen levels in camels range from 12-32.4%, while in cattle they range from 17-25%. Khyber Pakhtunkhwa comes in 3rd place in livestock and human's population. In this province, bovines are found in larger numbers than small ruminants, but brucellosis is seen in both of these i.e., 0–13% in cattle and 3.2-16.7 % in small ruminants. Balochistan has the least population and the largest area of any province in Pakistan. In Balochistan, small ruminant cases are more commonly reported than large ruminant cases. Seroprevalence in bovines is 0.3-6%, while in small ruminants it is 2-2.7%. In Islamabad, brucellosis cases have been reported in large ruminants (1.6-8.3%) and small ruminants (2.2-13%). In Gilgit-Baltistan, brucellosis is reported in wild animals and in cattle (10.9%). In Azad Jamu and Kashmir, brucellosis is usually reported in goats at 13.3%. Almost 17 field types of *B. abortus* have been reported from bovines (Punjab) and one isolate of *B. melitensis* from goats (KPK). Confirmation of *B. melitensis* has been done by polymerase chain reaction (real-time PCR). Moreover, detection of *B. abortus* has been done by real-time PCR in canines, camels, small ruminants, and horses.

Lipopolysaccharide (LPS) antibodies of brucella have not been detected by any polymerase chain reaction. Brucellosis is also transmitted in animals genetically. According to one theory, an allele (nramp1) can decrease the occurrence of brucellosis in cows (sahiwal). On farms, the occurrence of brucellosis in females is more due to increasing A.I practices. The risk of brucellosis increases with age in situations such as foetal membrane retention, abortion, herd size, environment, management and the introduction of carrier animals into the herd (Ali et al. 2014; Jamil et al. 2021).

Zoonotic Importance

Zoonotic diseases are those diseases that can be transferred from animals (vertebrate) to humans and brucellosis is considered as a zoonotic infection (Rahman et al. 2020). In central Asia and Middle East, brucellosis in human is increasing day by day (Khurana et al. 2021). Human brucellosis occurs mostly in those areas where this disease is endemic, or humans come back from endemic areas (Hull and Schumaker 2018). Brucella can spread by direct and indirect contact to another susceptible host (Moreno 2014). Uterus (brucella vitiated animal), aborted fetus, infected bulla, uterine discharge, placental membranes and contaminated feed or water are the main sources of dissemination (Acha and Szyfres 2001; Addis 2015; Dadar et al. 2020). Due to cross-species dissemination, its host range is very vast (Horizontal dissemination) (Moreno 2014). Through milk and milk products (butter, ice-cream, cheese, whey and yogurt) *B. abortus* can easily be transmitted in humans (Dhanashekar et al. 2012). Abattoir workers, veterinarians, meat inspectors, farmers, butchers, animal keeper, and lab workers have greater chances to get exposed by the infection. Mostly those countries are at risk where pasteurization is not used properly and hygienic practices of animal husbandry is very low (Addis 2015). Due to absence of proper administration, poor hygiene, deficient of sanitation, intensive farming and poor political will, brucellosis spread is very high (Pal et al. 2017). If accidental utilization of live vaccines such as Strain 9 of *B. abortus* and Rev.1of *B. melitensis* occur, then it can cause infections in humans (Addis 2015). *B. melitensis* is considered to be highly endemic globally due to its re-emergence (Gwida et al. 2010). Its zoonotic importance increases due to increase global dealings of animal products, enlarge international tours, rapid deforestation, urbanization, migrating animal husbandry and unendurable development (Bayeleyegn 2007). Firm surveillance and restriction initiatives of milk pasteurization and dairy by-products could deplete the contingency of brucellosis in humans (Moreno 2014; Mailles et al. 2016; Dadar et al. 2020). Signs of human brucellosis are depression, chills, undulant fever, night sweats, nervousness, constipation, weight loss, joint pain, lack of appetite, myalgia, insomnia, loss of weight, inflammation of reproductive organs and some other body parts (epididymis, brain, spinal cord, vertebra, testes, bones and prostate gland) and sexual impotence (Acha and Szyfres 2003; Kochar et al. 2007; Mantur and Amarnath 2008; Kiros et al. 2016). Brucella affects all age group of humans and if brucellosis is not detected at the proper time, it can become chronic in nature (Addis 2015).

Pathogenesis

The main pathogenic factors in brucellosis are guanine monophosphate, lipopolysaccharide, adenine monophosphate, 42-KDa protein and urease. Brucella genetic material is lacking certain pathogenic genes that are encoded for capsules, plasmids, exotoxins and pili (Seleem et al. 2008). Their modes of transmission are wounds, feed intake, inhalation, and eyelid. After entering the host body, *B. abortus* starts multiplication in phagocytic cells (macrophagic and dendritic cells). When a female becomes pregnant, the *B. abortus*, through circulation, enters the blastocytic cells and mammary glands. It enters the placenta by blood (Moreno 2014; de Figueiredo et al. 2015) and transfers to the fetus. Allantoic fluid increases the growth of bacteria; thus, it makes the reproductive site as a predilection site. If the erythritol levels increases from the 5^{th} month of pregnancy, it may cause abortion. After ulcer formation and breakage of cells in the chorio-allantoic membrane, erythrophagocytic trophoblasts are found in the placentome. Damage in placentome, due to bacteria and stress, causes abortion (Khurana et al. 2021). Fig. 1 illustrate the pathogenesis of brucellosis (Acha and Szyfres 2001; Neta et al. 2010; Poester et al. 2013; Roset et al. 2014; Kiros et al. 2016; Khan et al. 2021; Khurana et al. 2021). Except in pregnant animals, this bacteria spreads in the environment through secretions and metabolic wastes. From one study it was analyzed that changes are seen in adenosine deaminase pursuit and the oxidative stress levels in serologically positive brucellosis cows in Brazil. Adenosine deaminase and catalase activity are decreased, whereas oxidative stress levels are increased in *B. abortus* animals. As brucella species are intracellular pathogens, they can live within neutrophils and monocytes (phagocytic cells) by different escape mechanisms and changes into acute, chronic and carrier forms (Khurana et al. 2021).

Clinical Signs

Clinical signs due to *B. abortus* include retention of fetal membranes, abortion (30–80% in vulnerable animals), birth of weak young ones, inflammation of the uterus, low fertility, fibrinous pleuritis, chronic mastitis, interstitial pneumonia, swelling of joints, edema in inter-cotyledonary placenta, leathery texture placenta and necrosis of cotyledons (Neta et al. 2010; Kiros et al. 2016). It mostly infects the reproductive system. Calves can show signs of infection at a mature age if infected with *B. abortus*. *B. abortus* incubation period varies from a few weeks to months (Kiros et al. 2016; Abdisa 2018). In male animals, *B. abortus* causes inflammation of the testes (orchitis), necrosis of the testis, inflammation of the epididymis (epididymitis), hygroma (in chronic cases),

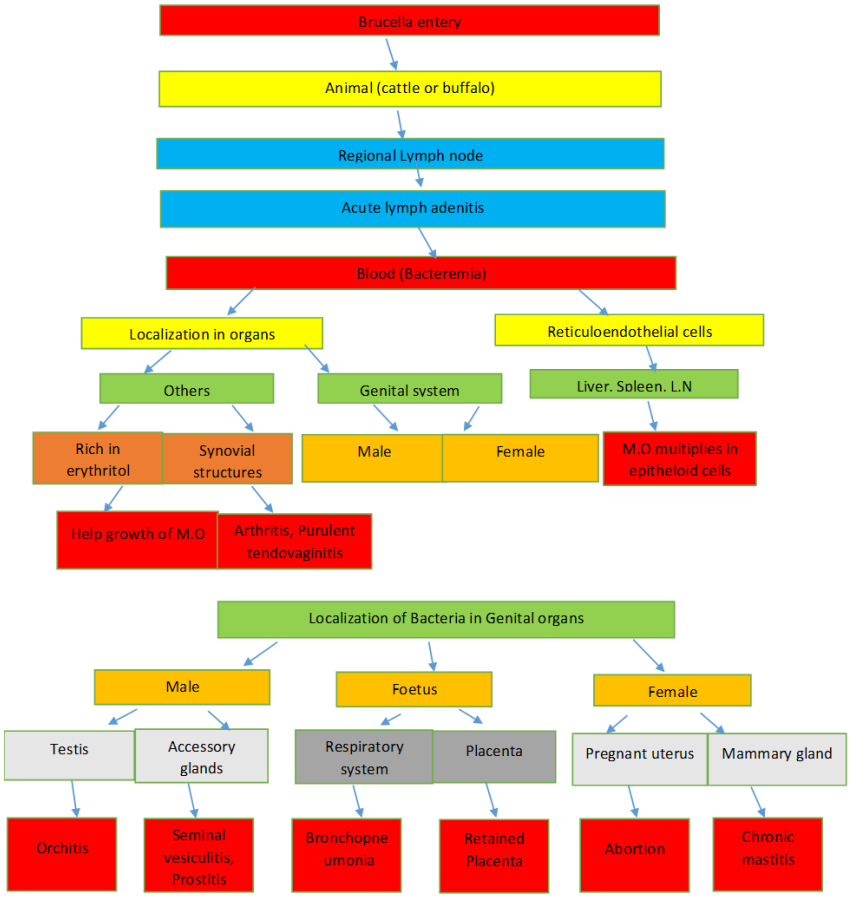

Fig. 1: Pathogenesis of Brucellosis

cervical bursitis, seminal vesiculitis and smaller testis than normal. Soft lesions containing thin and watery purulent exudate may soften the testis at times (Kiros et al. 2016; de Macedo et al. 2019).

Clinical Pathology

In lymphatic tissues and organs, small areas of inflammation are seen that are called granulomas. In the general stage of infection, pathogen is localized in tissues persistently. In the cases of abortion (female) and infertility (male), *Brucella* is seen in the reproductive tracts (Enright 1990; Acha and Szyfres 2001; Neta et al. 2010). An encroached bovine uterus with necrotic placentitis causes the fetus to die, and then abortion occurs. If abortion does not occur, then this kind of uterus and placenta lead to the birth of the infected calf. The cotyledons are swollen and enveloped by a yellowish or tenacious brown ooze. The inter-cotyledonary regions are condensed, cloudy and leathry, from which oozing of reddish fluid occurs. From a miscarried fetus, the body cavity is enlarged due to fluid accumulation and enlargement of organs (liver, spleen). From 4 months to a year, the fetus may be hairless (Khurana et al. 2021). In the lungs, pneumonitis is seen. Sometimes, fibrinous granulation and congestion may also occur. In several cases, infiltration of bronchioles, perialveolar tissue and peribronchial tissues is noted. Cobblestone bruises on the lungs are the main characteristic sign of brucellosis. In the mammary glands, macrophages accumulate in the interstitial spaces (Stableforth and Galloway 1959; Neta et al. 2010).

Bacterial Cell Cycle and Chromosomal Replication

Brucella growth is asymmetric and starts from one pole of the division site, but its lateral replication cannot be seen yet (Brown et al. 2012). The asymmetric replication of bacteria in intra-cellular nuclei is not properly seen but unipolar replication can seen by Texas Red Succinimidyl ester (TRSE). The amines on the bacterial surface are covalently bound by TRSE and this label is stationary. That's why replication (growth) is seen by increasing fragments of the cell body (unlabelled) (Khurana et al. 2021). Fluorescent d-AA (amino acid) can form spots on the growing region of peptidoglycan. Until now, it has not been applied to *B. abortus*. Polar growth of *B. abortus* is associated with the polar system's location. This system produces Cgs (cyclic-beta-1, 2-glucan), which has 17 to 25 glucose remnants (cyclic polymer) that are essential for pathogenicity and as an osmotic shock resistant. This polymer Cgs also disrupts the cholesterol-rich plasma

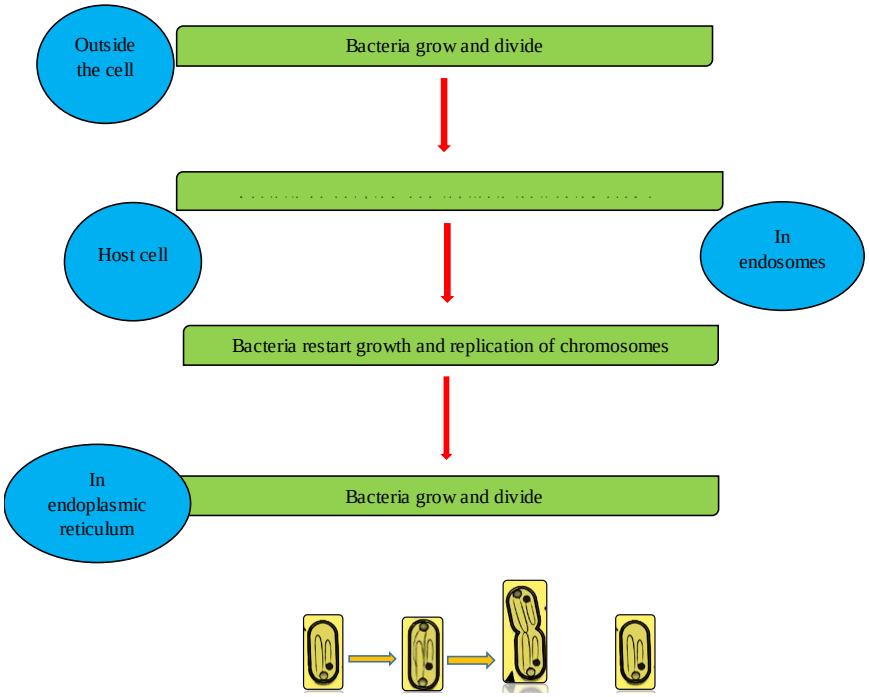

Fig. 2: Integration of Brucella's cell cycle with intracellular piracy

membrane domains of the host cell. Afterward, this polymer (Cgs) is transported (Cgt) into periplasmic space (Bundle et al. 1988; Arellano-Reynoso et al. 2005; Roset et al. 2014; Guidolin et al. 2015; Kuru et al. 2015). In the non-polar growth of bacteria, Cgs diffuses into the periplasm by Cgm. This complex (Cgs-Cgt) shows that other periplasmic complexes are also manufactured at the pole. In fact, peptidoglycan synthesis mechanisms and LPS export processes can be started at the division site's growth pole (De Bolle et al. 2015). Because of its growth and division outside the host, G1 bacteria have a high chance of host cell entry. After entering the host cells, these bacteria remain in endosomes. In endosomes, these bacteria remain in the G1 phase for many hours (depending on the host cell type). During this time, they do not grow and replicate. After the beginning of DNA replication, bacteria convert into their rBCV (replicative phase). This stage (replicative stage) is found in Endoplasmic reticulum. They restart the expansion and reproduction of chromosome No. 1 (chr.1). Then eBCV converts into rBCV. For this conversion, Vir B is required. Vir B production is influenced by the circumstances that occur in eBCV (acidic pH, starvation), as shown in Fig. 2 (De Bolle et al. 2015).

B. abortus has two chromosomes, Chr.I and Chr.II. However, the magnitudes and numbers of chromosomes differ between *Brucella* species. Chromosome 1 (chr. I) is long (2 Mb), discoid, and the place where its replication starts is detected 115kb from dnaA gene (genes that are made up of DNA), near to the parAB operon which is called orill. Chromosome II (chr. II) is smaller than chromosome I (1.2 MB). It is called a chromid (De Bolle et al. 2015). Both chromosomes have similar G+C content, but their duplication and division processes are different from each other. Chromosome II has genes that code for a network called the RepABC system. In RepABC system, Rep C initiates the DNA replication, but the other two (Rep A and Rep B) isolate the duplicated synchronization origin of orill (chr. II) that is thought to be placed inside the genes of repABC system (Pinto et al. 2012). While *B. suis* has two fused chromosomes, and when a cell divides from *B. abortus*, it gives rise to two daughter cells (De Bolle et al. 2015). This process occurs in 210 minutes (in a rich medium). This division occurs in G_2-phase (175 minutes after the initiation of this separation), whereas chromosome replication starts in S-phase (175 minutes after the initiation of this separation). Orill replication and segregation starts after 105 minutes of this division. And constriction begins 35 minutes prior to this division. These types of bacteria (constricting) are called pre-divisional bacteria, as shown in Fig. 3 (Hallez et al. 2007).

Diagnosis

For diagnosis of Brucellosis epidemiological patterns and history plays a very important role. There are different methods for diagnosis of brucellosis as illustrated in Fig. 4 (Lucero et al. 2003; O'Leary et al. 2006; Glynn and Lynn 2008; OIE 2009; Godfroid et al. 2010; Ali et al. 2014; Durrani et al. 2015; Arif et al. 2018; Khurana et al. 2021).

For isolation, bacteria can be collected from the uterus, milk, aborted fetus, iliac and supra-mammary lymph nodes, and spleen. Other body parts, such as the eyes, bones, brain, and joints, can also become infected. In male animals, bacteria can be obtained from genital organs and lymph nodes. In the acute phase of brucellosis, bacteria can be obtained from the semen as well. In the chronic phase, the bacteria level

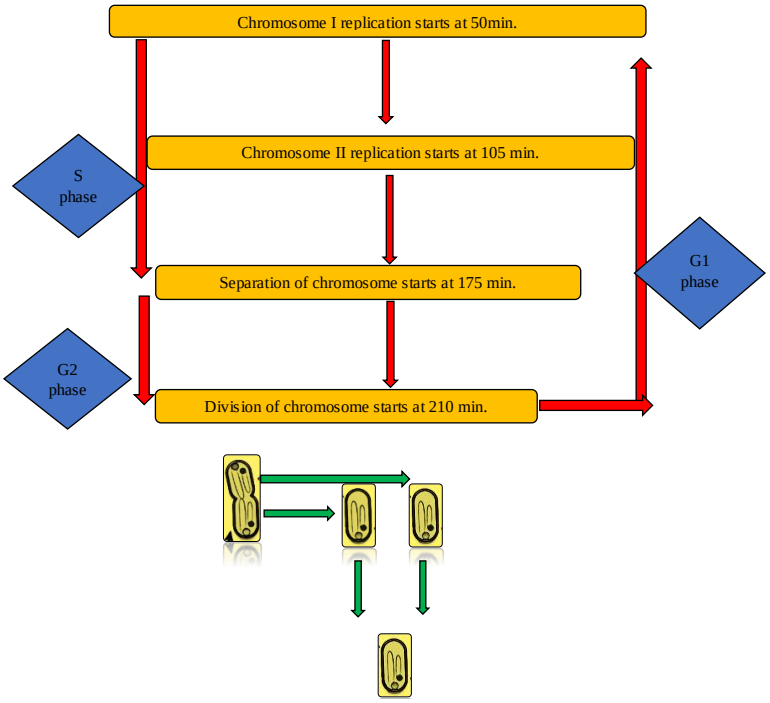

Fig. 3: Cell cycle of *B. abortus*

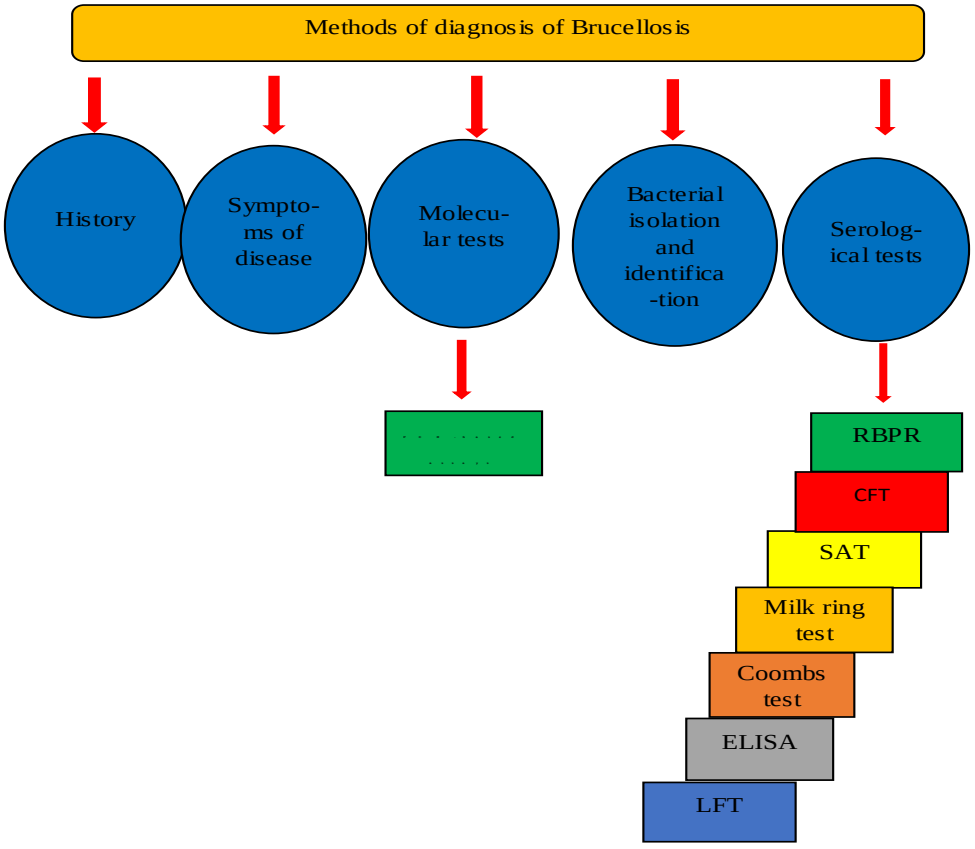

Fig. 4: Methods of diagnosis of brucellosis
PCR=Polymerase chain reaction, RBPT =Rose Bengal Plate Test, SAT=Standard Plate Agglutination Test, CFT= Complement Fiation Test, ELISA= Enzyme-linked immunosorbent assay, LFT= Lateral Flow Assay

decreases in excretion. The media that are used for brucella are TSA (trypticase-soya agar) and Columbia agar. *B. abortus* needs CO_2 and sera for development. A polymerase chain reaction assay (PCR) is a fast diagnostic procedure (Khurana et al. 2021). This procedure is also useful for diagnosis of biovars, analysis of treatment potency, and relapsing brucellosis (Christopher 2010). Real-time polymerase chain reaction (PCR) is used for an accurate

diagnosis of *B. abortus* (Redkar et al. 2001). This PCR observes different regions of *Brucella* genetic material (16S rRNA, IS711, and 31-KDA). An IS711-based PCR is used for the identification of *Brucella* from lympathic tissues, milk, and blood (O'Leary et al. 2006). PCR-based methods differentiate between the S19 vaccine and toxic strains of *B. abortus* (Kaynak-onurdag et al. 2016). IHC (immunohistochemistry) was used for the confirmative analysis of brucellosis in placental cotyledons and aborted tissues (Safari et al. 2019). Serological tests are used in eradication, inspection, and control strategies worldwide (Lucero et al. 2003). RBPT is useful for validation of swelling in the scrotum (hydrocele), neuro-brucellosis, swelling of joints (arthritis), inflammation of the epididymis (epididymitis), and swelling of the testis (epididymitis) (Mantur et al. 2006). CFT (complement fixation test) is used for exact detection of antibodies (IgM, IgG1). It can also be used in the serum of vaccinated (PB51) bovines. SAT (standard tube agglutination test) is an economical test. ELISA (enzyme linked immune-sorbent assay) is used for *Brucella* antigen sensing. It is considered the most accurate test (100% sensitivity and 99% specificity) for *Brucella* antigen. CFT is also best for persistent brucellosis. ELISA gives better results than acute brucellosis in conventional assays. Indirect ELISA is helpful in clinical brucellosis detection and is more accurate than SAT. It is very sensitive for CNS brucellosis detection and measures antibody (IgG, IgM, or IgA) levels in serum. For milking animals, indirect ELISA and milk ring tests are mostly used. The Coombs test and Burnet's intradermal tests are used to observe the condition of intolerance of contaminated essence to *B. abortus* (Khurana et al. 2021). Rose Bengal plate test (RBPT) gives most accurate detection (99%-99.5%). RBPT is an economical test used for sera screening (Arif et al. 2018).

Treatment

Utilization of antibiotics (streptomycin, tetracyclines, aureomycin and terramycin) can cause reduction in abortions in infected cattle. These antibiotics may be used in combination or alone. Due to cost of treatment, existence of antibiotic residuses in milk and treatment failure, this therapy is not satisfactory for bovine brucellosis. Oxytetracycline alone or with streptomycin is successful because it decreases the scattering of bacteria and terminating the signs of brucellosis at the time of calving. Oxytetracyline 20 mg/kg, I/M for 14 days (every 3rd day) along with streptomycin 20mg/kg, I/M, for 7 days (every day) may be used. Effective management is also necessary for bovine brucellosis treatment (Singh et al. 2014).

Control

S19 (live attenuated vaccine) and RB 51 (rough, rifampicin-resistant strain) vaccines are mostly used in bovine brucellosis. Under field conditions, S19 gives good results in cattle (80-95%). It provides immunity for entire life span of the cattle, if cattle is immunized in early age. In male calves, S19 does not give good results. If female calves are vaccinated with S19 then at mature age (heifers) abortion does not occur as no bacteria found in their reproductive system. Being infective for humans, S19 vaccine requires special preventive measures before use (gloves, protective glasses, masks, and sleeve coats). RB51 vaccine is best in cattle for control of abortion. Calves are protected against *B. abortus* sepsis and abortion, if they are immunized with RB51 (at 3, 5 and 7 months of age). It is successful in field conditions. Heifers can be immunized at the age of 10-12 months with *B. abortus* 2308 (virulent) strain. Some experiment shows that RB51 can be given in pregnancy when vaccination is not done in these cattle at early age. If cattle are vaccinated with strain 19 at early age, than RB51 can be given in pregnancy (Poester et al. 2006; Dorneles et al. 2015).

Conclusion

Brucellosis is considered as one of the most important and widespread zoonotic disease worldwide. *B. abortus, B. suis, B. melitensis, B. canis, B. neotomae*, and *B. ovis* are pathogenic *Brucella* species. The demand of milk increases with increase in population and the farmer does not know well about this disease. It is most prevalent in humans due to less adaptation of proper control strategies in Pakistan. The prevalence of this disease is on the rise owing to numerous hygienic, social, economic and cultural factors. Once entered in the host body, *B. abortus* multiplies in intracellular milieu of phagocytic cells such as macrophages and dendritic cells. When female conceives, the bacteria reach trophoblasts and the mammary gland through circulation where they multiplies to induce abortion. Brucellosis control programs mainly depends on early, accurate and precise diagnosis of the disease. It is diagnosed by history, disease symptoms, bacteriological isolation and identification, serological tests and various molecular tests. For control of this disease two vaccines, S19 and RB51 are given. For treatment combination of antibiotics are used. Main problems due to this bacteria are epididymitis, abortions, endometritis, retention of fetal membranes etc. Due to this disease major losses occur that includes animal health problems, production and effects on public health.

REFERENCES

Abdisa T, 2018. Review on the reproductive health problem of dairy cattle. Journal of Dairy and Veterinary Sciences 5: 1-12.

Acha NP and Szyfres B, 2001. Brucellosis in zoonosis and communicable diseases common to humans and animals. Pan Amer Health Organization Washington DC, USA 2001: 40-62.

Acha NP and Szyfres B, 2003. Zoonoses and Communicable Diseases Common to Man and Animals, 3rd Ed., Pan American Health Organization (PAHO), Washington, DC, USA.

Addis M, 2015. Public health and economic importance of brucellosis: A review. Public Health 5: 68-84.

Ali S et al., 2014. Isolation and identification of bovine Brucella isolates from Pakistan by biochemical tests and PCR. Tropical animal Health and Production 46: 73-78.

Ali S et al., 2017. Seroprevalence and risk factors associated with bovine brucellosis in the Potohar Plateau, Pakistan. BMC Research Notes 10(1): 1-11.

Arellano-Reynoso et al., 2005. Cyclic β-1, 2-glucan is a Brucella virulence factor required for intracellular survival. Nature Immunology 6: 618-625.

Arif S et al., 2018. Evaluation of three serological tests for diagnosis of bovine brucellosis in smallholder farms in Pakistan by estimating sensitivity and specificity using Bayesian latent class analysis. Preventive Veterinary Medicine 149: 21-28.

Awah-Ndukum J et al., 2018. Seroprevalence and associated risk factors of brucellosis among indigenous cattle in the Adamawa and North Regions of Cameroon. Veterinary Medicine International 2018: Article # 3468596.

Bayeleyegn M, 2007. Advanced veterinary public health lecture note. FVM, AAU, Debre-Zeit, Ethiopia 2007: 10–32.

Blasco JM and Molina-Flores B, 2011. Control and eradication of Brucella melitensis infection in sheep and goats. Veterinary Clinics of North America: Food Animal Practice 27: 95–104.

Brown PJ et al., 2012. Polar growth in the Alphaproteobac- terial order Rhizobiales. Proceedings of the National Academy of Sciences of the United States of America 109: 1697–1701.

Bundle DR et al., 1988. Characterization of Brucella polysaccharide B. Infection and immunity 56: 1101-1106.

Chauhan HC et al., 2016. Complete genome sequence of Brucella abortus SKN 13 isolated from placenta of aborted cattle in Gujarat, India. Genome Announcements 4: Article # 1123.

Christopher S, 2010. Brucellosis: review on the recent trends in pathogenicity and laboratory diagnosis. Journal of Laboratory Physicians 2: 55-60.

Dadar M et al., 2020. Contamination of milk and dairy products by Brucella species: a global systematic review and meta-analysis. Food Research International 128: Article # 108775.

De Bolle X et al., 2015. Brucella abortus cell cycle and infection are coordinated. Trends in Microbiology 23: 812-821.

De Figueiredo P et al., 2015. Pathogenesis and immunobiology of brucellosis: review of Brucella–Host Interactions. The American Journal of Pathology 185: 1505-1517.

de Macedo AA et al., 2019. Brucella-associated cervical bursitis in cattle. Tropical Animal Health and Production 51: 697–702.

Deka RP et al., 2018. Bovine brucellosis: prevalence, risk factors, economic cost and control options with particular reference to India-a review. Infection Ecology and Epidemiology 8: Article # 1556548.

Dhanashekar R et al., 2012. Milk-borne infections. An analysis of their potential effect on the milk industry. Germs 2: 101–109.

Dorneles EM et al., 2015. Recent advances in Brucella abortus vaccines. Veterinary Research 46: 1-10

Durrani AZ et al., 2020. Evaluation of therapeutic trials in bovines. In: Ranjbar M, Nojomi M and Mascellino MT, editors. New insight into brucella infection and foodborne diseases: Intech Open, UK; pp: 1-5.

Durrani NU et al., 2015. Prevalence survey of bovine brucellosis in apparently healthy dairy animals in Karachi, Pakistan. Journal of Animal Health and Production 3: 35-38.

El-Wahab EWA et al., 2019. Knowledge, attitudes and practices (KAPs) and risk factors of brucellosis at the human-animal interface in the Nile Delta, Egypt. BioRxiv: Article # 607655.

Enright FM, 1990. The pathogenesis and pathobiology of Brucella infection in domestic animals. In: Nielson K, Duncan J, editors. Robert animal brucellosis. Boca Raton (FL): CRC Press, Inc., Boca Raton, Fla; pp: 301-320.

Franc K et al., 2018. Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. BMC Public Health 18: 1-9

Georgios P et al., 2005. Brucellosis. The New England Journal of Medicine 352: 2325–2336.

Glynn MK and Lynn TV, 2008. Zoonosis update. American Veterinary Medical Association 233: 900-908.

Godfroid J et al., 2010. Diagnosis of brucellosis in livestock and wildlife. Croatian Medical Journal 51: 296–305.

Guidolin LS et al., 2015. Interaction network and localization of Brucella abortus membrane proteins involved in the synthesis, transport, and succinylation of cyclic β-1, 2-glucans. Journal of Bacteriology 197: 1640-1648.

Gwida M et al., 2010. Brucellosis – regionally emerging zoonotic disease? Croatian Medical Journal 51: 289-295.

Hallez R et al., 2007. The asymmetric distribution of the essential histidine kinase PdhS indicates a differentiation event in Brucella abortus. The EMBO Journal 26: 1444-1455.

Hull NC and Schumaker BA, 2018. Comparisons of brucellosis between human and veterinary medicine. Infection Ecology and Epidemiology 8: Article # 1500846.

Jamil T et al., 2021. Animal and Human Brucellosis in Pakistan. Frontiers in Public Health 9: Article # 660508.

Kaynak-Onurdag F et al., 2016. Screening Brucella spp. in bovine raw milk by real-time quantitative PCR and conventional methods in a pilot region of vaccination, Edirne, Turkey. Journal of Dairy Science 99: 3351–3357.

Khan I et al., 2021. Serosurvey and potential risk factors of brucellosis in dairy cattle in peri-urban production system in Punjab, Pakistan. Pakistan Veterinary Journal 10: 459-62.

Khurana SK et al., 2021. Bovine brucellosis–a comprehensive review. Veterinary Quarterly 41: 61-88.

Kiros A et al., 2016. A review on bovine brucellosis: epidemiology, diagnosis and control options. ARC Journal of Animal and Veterinary Sciences 2: 8–21.

Kochar DK et al., 2007. Hospital-based case series of 175 cases of serologically confirmed brucellosis in Bikaner. Journal of the Association of Physicians of India 55: 271–275.

Kuru E et al., 2015. Synthesis of fluorescent D-amino acids and their use for probing peptidoglycan synthesis and bacterial growth in situ. Nature Protocols 10: 33-52.

Leclercq SO et al., 2020. Taxonomicorganization of the family Brucellaceae based on a phylogenomicapproach. Frontiers in Microbiology 10: Article # 3083.

Lindahl E et al., 2014. Seropositivity and risk factors for Brucella in dairy cows in urban and peri-urban small-scale farming in Tajikistan. Tropical Animal Health and Production 46: 563–569.

Lucero NE et al., 2003. Fluorescence polarization assay for diagnosis of human brucellosis. Journal of Medical Microbiology 52: 883–887.

Machavarapu M et al., 2019. Endemic brucellosis in Indian animal and human populations: a billion dollar issue. Current Trends in Biotechnology and Pharmacy 13: 112–123.

Mailles A et al., 2016. Human brucellosis in France in the 21st century: results from national surveillance 2004–2013. Médecine et Maladies Infectieuses 46: 411–418.

Mantur BG and Amarnath SF, 2008. Brucellosis in India – a review. Journal of Biosciences 33: 539–547.

Mantur BG et al., 2006. Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. Journal of Medical Microbiology 55: 897–903.

Moreno E, 2014. Retrospective and prospective perspectives on zoonotic brucellosis. Frontiers in Microbiology 5: 213.

Neta AVC et al., 2010. Pathogenesis of bovine brucellosis. Journal of Veterinary Science 184: 146–155.

O'Leary S et al., 2006. Brucella abortus detection by PCR assay in blood, milk and lymph tissue of serologically positive cows. Research in Veterinary Science 81: 170-176.

OIE, 2009. Bovine brucellosis in terrestrial manual, 7th Ed., vol. 1. OIE, Paris.

Olsen S and Palmer M, 2014. Advancement of knowledge of Brucella over the past 50 years. Veterinary Pathology 51: 1076–1089.

Pal M et al., 2017. Public health and economic importance of bovine Brucellosis: an overview. American Journal of Epidemiology 5: 27-34.

Pappas G et al., 2006. The new global map of human brucellosis. The Lancet Infectious Diseases 6: 91–99.

Pinto UM et al., 2012. The ABCs of plasmid replication and segregation. Nature Reviews Microbiology 10: 755–765.

Poester FP et al., 2006. Efficacy of strain RB51 vaccine in heifers against experimental brucellosis. Vaccine 24: 5327–5334

Poester FP et al., 2013. Pathogenesis and pathobiology of brucellosis in livestock. Rev Sci Tech 32: 105-15.

Rahman M et al., 2020. Zoonotic diseases: etiology, impact, and control. Microorganisms 8: 1405.

Rahman MS et al., 2011. Prevalence of brucellosis in ruminants in Bangladesh. Veterinary Medicine 56: 379–385.

Redkar R et al., 2001. Real-time detection of Brucella abortus, Brucella melitensis and Brucella suis. Molecular and Cellular Probes 15: 43–52.

Roset MS et al., 2014. Brucella cyclic b-1,2-glucan plays a critical role in the induction of splenomegaly in mice. PLoS ONE 9: Article # 101279.

Rossetti CA et al., 2017. Caprine brucellosis: A historically neglected disease with significant impact on public health. PLoS Neglected Tropical Diseases 11: Article # 0005692.

Safari et al; 2019. In silco design and in vitro development of a highly accurate test to detect Brucella species. Turk J Med Sci 49: 368-374.

Sankarasubramanian J et al., 2017. Identification of genetic variants of Brucella spp. through genome-wide association studies. Infection, Genetics and Evolution 56: 92-98.

Santos RL et al., 2013. Economic losses due to bovine brucellosis in Brazil. Pesquisa Veterinaria Brasileira 33: 759-764.

Scholz HC et al., 2016. The change of a medically important genus: worldwide occurrence of genetically diverse novel Brucella species in exotic frogs. PLoS One 11: Article # 168872.

Seleem MN et al., 2008. Brucella: a pathogen without classic virulence genes. Veterinary Microbiology 129: 1-14.

Seleem MN et al., 2010. Brucellosis: a re-emerging zoonosis. Veterinary Microbiology 140: 392–398.

Singh B et al., 2015. Economic losses occurring due to brucellosis in Indian livestock populations. Preventive Veterinary Medicine 119: 211–215.

Singh SV et al., 2014. Therapeutic management of bovine brucellosis in endemically infected dairy cattle herd of native Sahiwal breed. Advances in Animal and Veterinary Sciences 2: 32-36.

Sriranganathan N et al., 2009. Brucella. Genome mapping and genomics in animal-associated microbes 2009: 1-64.

Stableforth AW and Galloway IA, 1959. Infectious diseases of animals, Vol. 1, Butterworths Scientific Publications, London, UK.

Sulima M and Venkataraman K, 2010. Economic losses due to Brucella melitensis infection in sheep and goats Tamilnadu. Journal of Veterinary and Animal Sciences 6: 191-192.

Wareth G et al., 2014. Detection of Brucella melitensis in bovine milk and milk products from apparently healthy animals in Egypt by real-time PCR. Journal of Infection in Developing Countries 8: 1339-1343.

Whatmore AM et al., 2014. Brucella papionis sp. nov., isolated from baboons (Papio spp.). International Journal of Systematic and Evolutionary Microbiology 64: 4120–412.

Whatmore AM et al., 2016. Extended multilocus sequence analysis to describe the global population structure of the genus Brucella: phylogeography and relationship to biovars. Frontiers in Microbiology 7: Article # 2049.