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Olympica Sarma
Ph.D. Scholar, Department of Animal Genetics and Breeding, G B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Preetinder Singh
Ph.D. Scholar, Department of Veterinary Microbiology, G B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Corresponding Author
Olympica Sarma
Ph.D. Scholar, Department of Animal Genetics and Breeding, G B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Brucellosis: A major abortion causing disease of livestock with zoonotic potential

Olympica Sarma and Preetinder Singh

Abstract
Brucellosis is a bacterial disease caused by various species of Brucella which leads to abortion in pregnant animals. It mainly spreads through inhalation, fomites and sexual contact between the animals moreover raw or unpasteurized dairy products are the cause of infection in humans. The symptoms range from fever, joint pain, fatigue to abortion in the last trimester of pregnancy. As the organism has affinity for reproductive organs there are different symptoms in males and females. The diagnosis mainly based on detection of antibodies in the serum using the techniques like RBPT, Serum agglutination test and Indirect ELISA, molecular confirmation is done by PCR using genus specific primers. There are various vaccines available in the market for the prevention of the disease. These vaccines along with surveillance and monitoring plays the major role in control and prevention of the disease.

Keywords: Abortion, brucella, brucellosis, infection, reproductive organs, zoonotic

1. Introduction
Brucellosis is an acute or chronic contagious disease affecting cattle, swine, sheep, goat, camels, equines and dogs caused by various members of the family Brucellaceae. This disease is characterized by reproductive failure, abortions and placentitis. The other names by which the disease is known include: Bang's disease, Contagious abortion, infectious abortion, enzootic abortion, undulant fever, Malta fever and Mediterranean fever. Brucellosis is a bacterial infection that can spread to livestock and poses a major health and economic worldwide. *Brucella abortus* and *Brucella melitensis* are species that mostly frequently infect animals with brucellosis. Ovine/Caprine brucellosis which is caused by *Brucella melitensis* is by far the most significant clinically evident disease in humans.
Brucellosis is one of the most important zoonotic diseases occurring worldwide although all species of Brucella are pathogenic. However, *Brucella melitensis* is the principal cause of brucellosis in humans (Corbel, 1997) [1]. Animal infection frequently leads to abortion 1and reduced milk production. The animals well acknowledged to be a cause of human infection includes goats, sheep, cattle and swine. In humans the majority of instances of brucellosis occurred as a occupational disease mainly affecting people that work in abattoirs, vets, farmers, hunters and livestock producer (Maadi *et al*., 2011) [2]. Brucellosis is one of the most significant and re-emerging zoonotic disease worldwide. It causes decreased calving percentage, delayed calving, miscarriages, still birth and loss of man hours among infected individuals contributing to significant economic losses (Mai *et al*., 2012) [3].

2. History background
The first member of this genus was *Brucella melitensis* which was isolated by Sir David Bruce in 1887 from this spleen of the patients who died from Mediterranean fever and later it was named as Malta fever. The genus Brucella and the disease brucellosis are named after Sir David Bruce. The second organism was identified and isolated and identified ten years later by Danish veterinarian Frederick Bang in 1897. It was identified from aborted bovine fetuses and fetal membranes and was named as *Brucella abortus*. Since then, it has been named as Bangs disease and it is found that it not only infects cattle but also cause disease in human, horses, dogs, sheep and fowl. Cow's milk is one of the most important sources of infection for humans. It is also named as undulant fever because there are recurrent fever patterns seen in humans. In 1914 Traum identified the third organism from aborted pig fetuses and named the organism as *Brucella suis*. This was also isolated from horses, cattles, pigs, dogs and fowls. *Brucella ovis* and *Brucella canis* were identified in 1950s and 1960s respectively. *Brucella ovis* is the causative agent of epididymitis in rams and *Brucella canis* caused abortion in dogs.

In the year 1850 during Crimean war Brucellosis was came to attention of British Medical Officers in Malta and also referred as Malta fever. David Bruce in 1887 was the first scientist to establish the relation between organism and the disease. Another scientist Danish veterinarian Bernhard Bang in 1897 isolated an organism from the cases of spontaneous abortion in cattles and named that condition as Bang's disease. In 1905 Maltese scientist discovered that unpasteurized goat milk was major etiological factor for undulant fever. Finally, in late 1910 American bacteriologist Alice C. Evans compared Bang's bacillus and Bruce's coccus and concluded that they were indistinguishable and gave them a common term called coccobacillus. The name Brucella was given to the organism in the honor of scientist Bruce. There are other names like Crimean fever, Cyprus fever, Gibraltar fever, Goat fever, Italian fever and Neapolitan fever are also been applied to brucellosis.

In 1897, Bang discovered *Brucella abortus* in Denmark. The distribution of brucellosis is worldwide in animals. Most commonly occurring in developing countries like South America, Central Asia, the Mediterranean and the Middle East. Also, the disease is widely prevalent in almost all the states of India which is considered as one of the major zoonotic diseases in India. According to WHO, 20% of rural people suffer from brucellosis where pyrexia of unknown origin (PUO) is classical sign and brucellosis has a major economic impact due to the annual economic loss of rupees 240 million contributed by cattle and buffalo affected with Brucella infections. A survey has been conducted during the year 1994-2002 using software avidin-biotin ELISA from 23 states and one Union Territory implying 53,518 bovines (Cattle and Buffalo) which showed prevalence rate of 72% in Cattle and 5.2% in buffalo. Random samples were collected and tested from ovine and caprine (sheep and goat) in a nation survey which were tested and showed cumulative incidence of 7.9% in sheep and 2.2% in goats.

In a recent survey of veterinarians and Para veterinarians using Indirect ELISA to detect brucellosis was found to be very high prevalence rate i.e.; 1.5%. Strict eradication programs have been introduced to reduce the prevalence of the diseases in various countries like Austria Germany, Netherland, Poland, Taiwan, New Zealand, USA and Japan and also the disease has been successfully eradicated from the following countries Japan, Romania, Czechoslovakia, Austria and Switzerland.

3. Etiology

Brucella are small Gram negative cocco-bacilli that are 0.6 µm in length and 0.6 to 1.5 µm in width. They are non-motile, non-spore forming, non-capsulated and non haemolytic organisms. The biochemical profile shows that they are catalase positive, urease positive (except *Brucella ovis*) and oxidase positive (except *Brucella ovis* and *Brucella neotomae*). They are stained red with modified Ziehl-Neelsen staining. *Brucella ovis* and some biotypes of *Brucella abortus* require 5-10% CO_2 for primary isolation. They are capnophilic organism i.e., their growth increases in the presence of 5-10% CO_2. They are intracellular pathogens i.e.; they can remain viable inside the macrophages and this ability helps them to evade post immune system and spread to various organs. They have affinity for male and female reproductive organs and infected animals act as reservoir of infection for indefinite period of time. Viable organisms are shed by infected animals for many months. Further there are seven biotypes of *Brucella abortus*, five biotypes of *Brucella suis*, three biotypes of *Brucella melitensis* and one biotype of *Brucella ovis* and *Brucella canis* each. There are many species of Brucella which causes disease to animals and humans. The species includes *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Brucella ovis*, *Brucella canis*. *Brucella abortus* mainly produce infection to bovine species, *Brucella melitensis* causes infection in goats and sheeps, *Brucella suis* infects hogs. Among *Brucella abortus* nine biotypes have been recognize for causing diseases in cattle and buffalo. Although each species of Brucella is relatively specific for individual species of animals but they can also infect other species of animals and humans.

Brucella organisms are small Gram-negative rods and coccobacilli. They are non-motile and non-sporing. They are aerobic but some species may require added CO_2 for growth.

4. Transmission

The bacteria can enter body through various routes including host skin wounds, mucous membrane, conjunctiva and through inhalation. They can also enter through coitus and infected fetus; fetal membrane and genitalia are source of transmission of infection in the animals where the Brucella organisms are shedded in high concentration. Flies, ticks, rats can also act as a source of transmission.

In humans a healthy person can get infected through consumption of unpasteurized dairy products. People working at laboratory, slaughter house and meat packing industries are more prone to infection. Veterinarians especially gynecologist are at high risk of infection and antibodies have been detected in their blood in various clinical investigations.

Canine brucellosis has been reported to transmit through close contact between the animals and moreover there have been suggestion of transmission of brucellosis through contaminated urine (Carmichael and Joubert 1988) [4]. Unpasteurized animal products caused a great risk of transmission of brucellosis mainly the specific products like milk, butter milk and cheese are major cause of transmission (Cooper 1992) [5]. In 2006 two children of seven months and two months of age were diagnosed with brucellosis and the transmission was through breast milk (Arroyo *et al*. 2006) [6]. Studies showed that human to human transmission of Brucella can occur through breast feeding, sexual transmission and moreover through blood transfusion, bone marrow transplantation and aerosol route (Tuon *et al.* 2017) [7].

Susceptible host: Brucella affects many wild and domestic animals as well as domestic animals including cattle, buffalo, sheep, goats, pigs, dogs, cats, camels. Moreover, they have high zoonotic potential does can infect humans.

A wide variety of marine animals have been reported of Brucella infection. Moreover, a wildlife species such as bison, feral pigs, wild boar, foxes, water buck, reindeer and European hares has been found infected with *Brucella abortus* and *Brucella suis* (Godfroid, 2002) [8]. Brucella organism can be used as bioterrorism and agro terrorism attacks moreover, Brucella can jump from one host to another and thus cause a greater risk to human as well as animal health (Godfriod *et al*., 2011) [9]. Antibodies against various Brucella species were found in pre-ranging African wild ungulates in Zimbabwe (Motsi *et al*., 2013) [10].

5. Pathogenesis

There are several factors upon which the outcome of infection depends. These factors can be host related, pathogen related

or environmental factors. The number of organisms, virulence of the organisms and immune system of the host play a significant role in deciding the outcome. After the entry the organism localize in regional lymph nodes where they proliferate within the reticuloendothelial cells. As they are intracellular pathogens, they survived within macrophages are carried to other organs via blood stream (intermittent bacteraemia). They have particular affinity for male and female reproductive organ, placenta, fetus and mammary gland. Moreover, they can also localize in other organs such as lymph nodes, spleen, liver, joints and bones leading to various pathological condition. The course of infection varies in male and female. In male organism affects reproductive organs like seminal vessel, ampullae, testes and epididymitis and cause infertility. Male animal become carrier and always shed organism in semen.

Organism mainly enters matured animal/ adult animal by ingestion. This virulent bacterium is engulfed by phagocytes and transported to regional lymph nodes. In female the course of infection varies significantly in pregnant and non-pregnant animal. In non-pregnant animal Brucella remains localized in spleen, supra mammary lymph node and other lymphatic tissue but transmission can also occur through coitus, skin abrasion and transplacental route. In pregnant animal it affects uterus and mammary gland. Organism is intermittently shed in milk. In initial phase of pregnancy there is placentitis and abortion in end of third trimester (usually after 5 month). Brucella organism is present in fetus, placenta, fetal fluids and uterine discharge. In subsequent pregnancies there is no abortion but shedding of organism occurs during parturition. The main reason of localization of organism in reproductive organ is due to presence of erythritol which act as growth stimulant for Brucella and is found in high concentration in placenta, mammary gland and epididymis of cattle, sheep, goat and pigs. In chronic cases the organism localized in joints or intravertebral discs. *Brucella abortus* has ability to invade phagocytic and non-phagocytic host cell. Moreover, it invades intestinal mucosa through M cells. The intracellular survival of *Brucella abortus* depends upon its ability to inhibit phagosome-lysosome fusion (Neta *et al*., 2010) [11]. Brucella can replicate within macrophages, dendritic cells and placental trophoblast. Brucella has strong tissue tropism or reproductive system and lymphoreticular system. Brucella causes apoptosis inhibition of mononuclear cells and prevent maturation of dendritic cells (de Figueiredo *et al*., 2015) [12]. Brucella avoids the fusion of phagosome-lysome with the help of cyclic beta 1,2 glucan which is secreted into periplasm of Brucella (Poester *et al*., 2013) [13]. *Brucella suis*, *Brucella melitensis* and *Brucella abortus* required the genes that encode urease for the establishment of the infection. The initial survival of bacteria in macrophages is due to lipopolysaccharide (N Xavier *et al*., 2010) [14]. If Brucella enter through respiratory route it spread from lungs to other peripheral organs. Moreover, pulmonary immune response plays a major role and providing the immunity (Ferrero *et al*., 2020) [15].

6. Clinical Signs

Bovine brucellosis is mainly caused by *Brucella abortus* leads to form of abortion in third trimester in pregnant animals. Subsequent pregnancies are normal but there can be retention of placenta in endemic herds. Organisms persist in mammary gland and associated lymph nodes for many years and are excreted intermittently in milk. In bulls infected organs are seminal vesicles, ampullae, testicles and epididymides (Fig 3). In tropical countries hygroma involving lymph joints are often observed when disease is endemic. Mastitis in goats and ewes and orchitis in bucks and rams are reported along with osteoarthritis, synovitis and spondylitis.

In Cattle and Buffalo, brucellosis is known to be the most common cause of abortion (Fig 1) which usually occurs in the last trimester of pregnancy. The common sequelae to abortion are metritis and retained placenta. Mucopurulent discharge is another common clinical sign in infected animal. Arthritis is another common clinical sign in Brucella infected animals. In infected bulls, the common clinical signs reported are epididymitis and orchitis.

The main clinical manifestations in sows are abortion or birth of weak piglets. However, sterility, orchitis and lameness has also been reported in boars. In horse, the major clinical findings are development of fistulous withers and poll evil.

In Western Sudan 97 adult cattles suffering from hygroma, 32 with arthritis and two cattles showing long calving interval were screened for brucellosis and result showed that 92% hygroma cases, 62% arthritis and both the cattles showing long calving intervals was sero positive for brucellosis (Musa *et al*., 1990) [16]. Interferon gamma promotes abortion due to Brucella infection in pregnant mice (Kim *et al*., 2005) [17]. The brucellosis was characterized by clinical signs including abortion, retention of placenta, low fertility rate, embryonic and neonatal death (Megid *et al*., 2010) [18]. Abortion storm occurred in a flock of sheep where five ewes aborted at third month of gestation. They were examined and milk samples from the five ewes were MRT positive. A total of seven isolates of *Brucella abortus* were isolated from milk sample and vaginal swabs collected from aborting ewes (Ocholi *et al*., 2005) [19]. In a 45-year-old human splenic abscess was found which was caused by *Brucella melitensis* however, splenic abscess due to brucellosis is a rare event (Yilmaz *et al*., 2014) [20].

Fig 1: Aborted fetus due to *Brucella abortus* biovar 1 infection (Megid *et al*. 2010)

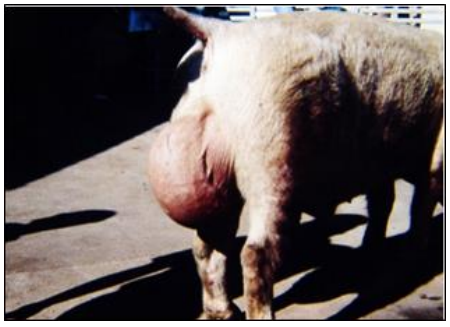

Fig 2: Unilateral testicular enlargement due to Brucella suis infection in Boar (Megid *et al*. 2010)

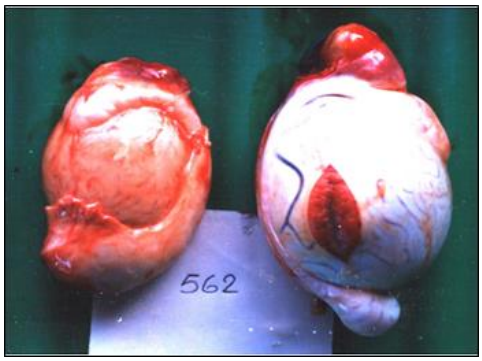

Fig 3: Epididymitis in rams due to *Brucella ovis* infection (Megid *et al*. 2010)

7. Necropsy findings

In tissues there are small granuloma as granuloma grows caseous necrosis occurs in the centre and large number of organisms enter the lesions specially in *Brucella suis*, granuloma occurs in all tissues. In horses it causes necrotizing and purulent lesions involving ligamentum nuchae. The bovine mammary gland and supra mammary lymph nodes are common sites of localization of *Brucella abortus*. This results in hardening of lymph nodes. Edema is observed in the maternal parts of placenta along with leathery plaques on external surface of the chorion may be seen. Enlargement of spleen, liver and superficial lymph nodes are common, necrotic changes are visible in cotyledons with dirty yellow in colour and presence of grayish yellow deposits, the subcutaneous and intramuscular tissues of the affected fetus are infiltrated with reddish serous fluid, occasionally, the expelled fetus is completely covered with pus like maternal along with swollen umbilicus and also sometimes, fetus may turn mummified.

In 1968 meningomyelitis due to brucellosis was reported (Sahadevan *et al*., 1968) [21]. Pathological findings in neonatal were suspected with co-infection of Brucella and *morbilli* virus included non-suppurative meningitis, pulmonary bronchopneumonia, presence of neutrophils and macrophages in alveoli. The tissues used for screening of Brucella included lungs, brain, lymph nodes and umbilicus (West *et al*., 2015) [22]. In infected goats histopathological findings includes endometritis, lymphoid hyperplasia in spleen and lymph node, lymphocytic mastitis. The interstitial pneumonia was the main finding in aborted fetus (Meador *et al*., 1988) [23]. The post mortem changes were found in beef cattle infected with brucellosis and these changes include contamination, blood aspiration and pulmonary emphysema (da Silva *et al*., 2022) [24]. Necropsy finding of a Bison fetus included capsular tear in liver, peritoneal cavity contains a small amount of blood, microscopic lesions include mild multifocal interstitial pneumonia and mild purulent bronchopneumonia. Fetal abomasal fluid, fetal lung fluid and fetal heart blood were used to isolate *Brucella abortus* biovar 1 (Rhayan *et al*., 1994) [25].

8. Diagnosis

It is mainly based on clinical sign and history.

8.1 Direct examination

It moreover depends on serological testing and isolation identification of the causative organism. The identification is based on demonstration of the causative agent by modified Ziehl-Neelsen staining and Koster's stain which are useful in demonstrating the bacteria from the impression smears made from aborted fetal material, placenta in case of abortion. Moreover, organisms can also be directly demonstrated from vaginal mucous, semen and various tissue. Organisms appeared to be small rods or coccobacillary, non-spore forming arranged in cluster or pairs.

For the purpose of identifying Brucella organism in formalin fixed paraffin embedded tissue under a live microscope and avidin-biotin peroxidase complex immunoenzymatic staining technique was employed (Meador *et al*., 1986) [26]. Gram staining revealed small gram negative cocco bacilli in 50 blood culture with high index of suspicion for brucellosis. Moreover, on this blood culture broth stamp's modified called Ziehl-Neelsen staining was carried out and result Showed being coccobacilli in the stain which were immediately recognized as Brucella species (Tilak *et al*., 2016) [27].

8.2 Indirect examination

RBPT is a qualitative serological test that is used to detect antibodies in the serum of suspected animal. It is internationally prescribed test for diagnosis of brucellosis. Further quantitative test like standard tube agglutination test, serum agglutination test and tube agglutination test can be performed for estimating the titer. This test is more effective and usually performed after positive quantitative test. Sero prevalence of brucellosis among the veterinarians, Para veterinarians, shepherds, butchers, animal owners were studied using Indirect ELISA, conventional and serological test like RBPT and STAT. It was found that Indirect ELISA were more sensitive as compared to another conventional test (Agasthya *et al*., 2007) [28]. Sero prevalence of brucellosis in South east Ethiopian pastoral livestock was studied using RBPT further all RBPT positive animals were confirmed by ELISA (Gumi *et al*., 2013) [29].

8.3 Isolation and Cultivation

Fecal stomach contents is considered best material for the isolation of the organism. It usually grows between 5-10% tension and potato medium show satisfactory result for pigment production. Brucella produces smooth colonies or rough colonies based on different species. These smooth and rough colonies are used for vaccine preparation. The colonies are round, pinpoint smooth, glistening and translucent. They are one mm in diameter and usually grow up to 2 mm to 3 mm. Various biochemical test like catalase, oxidase and urease are performed for the confirmation of the organism. Castaneda medium was used for isolation of brucella and identification was done by inoculating these colonies into Brucella agar containing thionin and Fuschin (Farrel, 1974) [30]. Culturing of animal samples suspected of brucellosis can be done on Farrell medium and modified Thayer Martin medium (De Miguel *et al*., 2011) [31].

8.4 Serological and Molecular techniques

Various techniques like FAT, Indirect ELISA, Direct ELISA, CFT and Coomb's Test can be used for serological identification of disease. Molecular techniques like PCR can also be used. For the screening of animals in a herd, Milk Ring Test or Abortus Bang Test is employed. It is a quick test for testing a herd that is suspected to have brucellosis.

FAT is usually performed for generic identification. For chronically affected animals CFT is considered more effective than other serological tests.

For the detection of antibodies related to *Brucella abortus* in cattle serum. The Fluorescence Polarization Assay and Competitive ELISA was used moreover it was seen that Fluorescent Polarization Assay was better as compared to Indirect ELISA, Competitive ELISA, CFT and SAT (McGiven *et al*., 2003) [32]. Antibodies focused primarily on smooth lipopolysaccharide are detected by RBPT and CFT. However, false positive serological reaction cannot be avoided using this test so, an ELISA based on R strain of Brucella was developed to measure specific IGg antibodies and avoid false positive serological reaction (Trotta *et al*., 2020) [33].

Genus specific Brucella species primers encoding for BCSP 31, outer membrane protein (*onp2b*, *onp2a* and *onp31*) are used for molecular detection of Brucella (Yu and Nielsen, 2010) [34]. The 16S rRNA sequence of *Brucella abortus* was used to developed primers for molecular detection of Brucella (Romero *et al*., 1995) [35].

8.5 Differential diagnosis

It is important in brucellosis as abortion is a common clinical finding in other livestock diseases. So, it is important to differentiate from other abortion causing diseases such as vibriosis, leptospirosis and infectious bovine rhinotracheitis.

9. Treatment

As such there is no cure for brucellosis in domestic animals. Only symptomatic treatment can be done. Vaccination is only the effective strategy in prevention of the disease. Although no antibiotic is fully effective in curing the disease however, antibiotics like chlortetracycline, penicillin, streptomycin and oxytetracycline have been found useful to certain extent. Oxytetracycline was injected intraperitoneal in Brucella infected cows and found that there was less severe infection as compared to non-infected animals and four cattles were found infection free (Fensterbank and Souriau, 1976) [36]. Research was conducted on therapeutic management of bovine brucellosis in endemically infected dairy cattle herd of Sahiwal breed. For this study 27 Sahiwal cattle were selected which were having history of abortion and still birth and *Brucella abortus* were isolated from vaginal discharges from aborted cows. They were divided into two groups. First group was given streptomycin, isoniazide and rifampicin with long-acting tetracycline and in the second group was given isoniazide and rifampicin for 15 days with one shot of bayrocin. The result shows that there was reduction in titres of anti-brucella antibodies and moreover twelve cows became pregnant and ten animals had normal calving (Singh *et al*., 2014) [37]. Multi drug therapy is preferred as compared to mono drug therapy in the treatment of brucellosis (Tuon *et al*., 2017) [38]. In Brazil *Brucella abortus* isolates from cattle showed reduced susceptibility to rifampicin and were multidrug resistant. However, they showed 100% sensitivity to doxycycline and ofloxacin (Barbosa *et al*., 2015) [39]. *Brucella melitensis* isolated from humans were susceptible to doxycycline, tetracycline and streptomycin. However, there was increased in resistance against rifampicin (Deshmukh *et al*., 2015) [40]. Similarly decrease sensitivity of Brucella isolates towards rifampicin was reported. So, this drug should be prescribed with caution (Ariza *et al*. 2007) [41]. Combination of doxycycline for 6 weeks with rifampicin was recommended for treatment of brucellosis (Ariza *et al*.,1992) [42]. Doxycycline and streptomycin can be best therapeutic solution for the treatment (Seleem *et al*., 2009) [43].

10. Prevention and Control

There are various national eradication schemes based on the detection and slaughter of infected animals. Vaccination is done in young heifers and cell mediated immunity is formed. Mainly there are three types of vaccines that are used in cattle. S19 vaccines is a cotton strain 19 vaccine and a smooth strain of *Brucella abortus* which is non-virulent but immunogenic. It is administered to female calves up to five months of age and is avoided in male calves as it may localize in testes. Strain 45/20 bacterin is a rough strain killed vaccine. Its main advantage is that it does not stimulate agglutinin production against smooth antigens of *Brucella abortus*. RB51 strain is a natural stable rough mutant and provides effective protection against abortion and does not induce serological response hence, it does not interfere in brucellosis surveillance programs. In Caprine and Ovine modified live Rev.1 strain vaccine is given to kids and lambs under six months of age which provides lifelong immunity. In pigs the modified live *Brucella suis* vaccine is used. In Dogs no commercial vaccine is available but combination of tetracycline and aminoglycoside may be effective. The effective dose of S19 vaccine is 10^9cfuand for RV51 vaccine the effective dose is 10^{10}cfu (De Oliveria *et al*., 2021) [44]. The efficacy of strain RV51 vaccine was tested and abortion rates were reduced to 25% as compared to unvaccinated group where abortion rates were 62% moreover RB51 vaccine did not cause any abortion neither the RB51 strain was isolated from any sample (Poester *et al*., 2006) [45]. Immune response of calves vaccinated with *Brucella abortus* S19 and RB51 strain was recorded. Results showed that both the vaccines induced immune response which was characterized by proliferation of cd8 + t cells and cd4 + t cells, production of interferon gamma and interleukin 17A by cd4 + t cells. Moreover, there was production of mammary cells and antibodies of IgG 1 class. It was also reported that *Brucella abortus* S19 vaccine induced a strong immune response as compared to Brucella RB51 vaccine (Dorneless *et al*., 2015) [46]. A full dose of *Brucella abortus* S19 calf hood vaccine in water buffaloes was capable of elicting of antigen specific immune response with secretion of interleukin 12, tumor necrosis factor alpha and interferon gamma. Moreover, immune response was found to be dose dependent (Shome *et al*., 2020) [47]. Immune response produced by *Brucella abortus* RB51 strain was compared and found that there was satisfactory humoral immune response however booster dose was capable of enhancing the immune response in both cattle and buffalo (Mohamud *et al*., 2020) [48].

Some of the precautions are enlisted which can reduce the risk of brucellosis. It is advised to maintain proper hygiene and biosecurity to prevent the occurrence of brucellosis as prevention is major factor in controlling the outbreak of disease, veterinarian should wear proper protective clothing and gloves while handling the sick or diseases animals and maximum precautions should be taken during the calving of the animal. In places like laboratory while handling samples which are highly suspected of Brucella/other zoonotic disease proper biosecurity should be maintained time to time, fumigation and disinfection should be done and discard the samples according to the recommended ways. Vaccination plays a key role in prevention of brucellosis and proper vaccination of animal should be done to ensure the safety of animal as well as worker or handler which comes in contact with animal on daily basis.

Prevention is the only strategy in elimination of infectious diseases. Surveillance is considered as most effective way to prevent brucellosis in an organized farm. Quarantine is a best strategy to avoid this spread of the disease. Milk should be pasteurized before consumption. Use of raw milk in making dairy products should be avoided. Time to time screening of herd should be there.

Mass vaccination is an effective strategy to control brucellosis in a herd or livestock moreover, zoonotic prevalence can also be reduced. Other strategies include restriction of animal movement and improvement in sanitization facility in farm to decrease the spread of disease. Ideal way to control brucellosis is improved the diagnosis, vaccination, effective treatment along within crease awareness among livestock owners. Awareness and knowledge regarding the causative agent and disease are major factors in prevention of any disease (Smits and Cutler, 2004) [49].

The spread of disease increases due to little feedback on occurring illness and symptoms. Therefore, constant monitoring and surveillance programme are required along with vaccination (Hassan *et al*., 2020) [50].

11. Zoonotic importance

Humans are susceptible to *Brucella abortus*, *Brucella suis* and *Brucella melitensis* and to some extent *Brucella canis*. Brucellosis caused a wide range of symptoms including fever, anorexia, headache, pain in muscle, joint or back and fatigue however, in severe cases or immune-compromised patients where can be arthritis, endocarditis, swelling of liver or spleen. In males there may be swelling of testicle and scrotum area whereas in females' abortion may occur. The treatment includes antimicrobial therapy which should be started during the early stages of infection. Moreover, humans can also develop severe hypersensitivity reaction following infection or after accidental inoculation with attenuated vaccine strain.

In Kenya *Brucella abortus* and *Brucella melitensis* were isolated from cattle and human patient and moreover, human cases were related to a group of people that were occupationally or domestically exposed to livestock (Njeru *et al*., 2016) [51]. Veterinarians, veterinary technician, insemination service employee, farmer, slaughter house worker and zoo technician are more prone to Brucella infection. There has been evidence of sexual transmission of brucellosis in humans. Two cases has been reported in which Brucella was transmitted from husband to wife. In one case there were orchiepididymitis and in second case there were no genital symptoms (Meltzer *et al*., 2010) [52]. According to world health organization annually there has been total of five lakh species of brucellosis per year. The highest incidence is found in Fyria, Mongolia and Tajikistan. Moreover, majority of outbreak has been related to *Brucella abortus* (Hull and Schumaker, 2018) [53]. In Kyrgyzsean a total of 1774 human serum samples were tested for brucellosis and sero prevalence were found to be 8.8% in humans (Bonfoh *et al*., 2012) [54]. In Tanzania a total of 230 humans were screened for brucellosis and 14 individuals were found to be sero positive for brucellosis moreover, the probability of brucellosis was more in young individuals and those who were in contact with sheep, cattle and goat in previous twelve months (Bodenham *et al*., 2020) [55]. In and around Ludhiana, India a total 241 blood samples were collected from the humans that were in contact with livestock and it was found that 24.5% were positive by RBT and 26.6% were positive via STAT with titre range between 80 and 1280 IU/ml (Yohannes and Paul, 2011) [56].

12. Conclusion

Brucellosis is becoming a re-emerging disease which has a large effect of livestock leading to morbidity and mortality and economic loss to the farmers. Moreover, being a zoonotic disease, it acts as source of infection and adverse conditions in humans specially those who are in frequent contact with the animals like farmers, veterinarians, para veterinarians etc. Moreover, with change in environment there has been increase in multi drug resistance among Brucella species which is becoming a global concern among scientist. So, there should be more focused towards surveillance and monitoring of the disease for early diagnosis and control. Various diagnostic technologies and preventive measures will become the key factors in control and may be in eradication of brucellosis in upcoming future.

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