Brucellosis: The Second Most Important, Yet Neglected, Zoonotic Disease

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Abstract: Brucellosis is the second most important zoonotic disease after Rabies. Yet it is one of the neglected tropical diseases. It is prevalent worldwide and endemic in many countries. It affects several domestic animal species like cattle, dog, pig, sheep, goat, camel etc and can spread to humans through contact or consumption of contaminated milk and milk products, meat etc. The currently available vaccines and antibiotics have not succeeded in eradication of the disease. The infected animals can become carrier of the disease for the rest of their lives. Here we review certain important clinical, microbiological and pathological aspects of the disease and control measures against Brucellosis.

Keywords: Brucellosis, Brucella, Zoonotic disease, Diagnosis, Vaccines, Therapy, Phage.

INTRODUCTION

Brucellosis is a major re-emerging bacterial zoonosis of global importance affecting different animal species and man worldwide and is of economic, public health and bio-hazard importance. It is also known as “undulant fever”, “Mediterranean fever” and “Malta fever”. *Brucella*, the causative agent of Brucellosis, is a facultative, intracellular bacteria with marked tropism for the reproductive tract of pregnant domestic animals. *Brucella* organisms of all species cause persistent infection in the reticuloendothelial system of the host animal [1]. It causes chronic infectious disease of livestock, rodents, marine animals and human beings. The occurrence of the disease in humans depends largely on the occurrence of Brucellosis in an animal reservoir, including wildlife [2].

Brucellosis affects humans of all age groups and of both sexes. After Rabies, Brucellosis is considered to be the second most important zoonotic disease in the world. It causes disease in bovines, ovines, caprines, swines, canines etc. The disease in animals is also called as “Bang’s disease”, “enzootic abortion”, “epizootic abortion”, “slinking of calves”, “Ram’s epididymitis” and “contagious abortion”.

Symptoms of Brucellosis

Brucellosis in Humans

In man, infection is associated with characteristically recurrent febrile episodes that led to the naming of this disease as ‘undulant fever’ [3]. Symptoms of this disease in humans include undulant (rising and falling) fever, tiredness, sweating, night sweats, weakness, muscular pain, anaemia, headache, miscarriage, depression and body pain.

Brucellosis in Animals

Symptoms of this disease in animals include undulant fever, weak offsprings, delayed conception, retention of placenta, abortion in females and orchitis and epididymitis in males. Secretion of the organisms occurs in milk, uterine discharges and semen [2]. Once infected, the animal may continue to shed the organism and remain a source of infection for long period [4]. Mammary gland of the animal and the lymph nodes associated with it may also get infected. Subsequent pregnancies are usually carried to term, but uterine and mammary infection recurs, with reduced numbers of organisms in pregnancy products and milk.

Outbreaks of bovine Brucellosis are associated with abortion in the last trimester of gestation, delayed conception, temporary or permanent infertility in the affected animals, and weak newborn calves. The outcome of infection in cattle is dependent on age, reproductive and immunological status, natural resistance, route of infection, infectious challenge and virulence of infective strain [5]. Adult male cattle develops orchitis and infertility results in both sexes. Hygromas, involving leg joints, are a common manifestation of Brucellosis. Testicular abscesses and longstanding infections result in arthritic joints in cattle.

In acute infections, the organism is present in most major body lymph nodes. Adult male cattle may develop orchitis and Brucellosis may be a cause of infertility in both sexes. Hygromas in leg joints are a common sign of Brucellosis. It may be the only obvious

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manifestation of the infection; the hygroma fluid often contains *Brucella* organisms [6]. Brucella organisms localize in the placentas of goats, cows, and pigs due to the presence of erythritol at this site. Accumulation of large numbers of bacteria may eventually lead to abortion [7]. The severity of the disease depends upon many factors such as previous vaccination, age, sex and management such as herd or flock size and density. Abortions are more prevalent in unvaccinated animals and numbers of organisms shed are much greater.

ETIOLOGY OF BRUCELLOSIS

The causative agent of Brucellosis was isolated in 1987 by Sir Bruce from the spleen of fatally infected soldiers in Malta and was placed in genus *Micrococcus*. Bang discovered *Brucella abortus*, the cause of abortion in cattle and of Brucellosis (undulant fever) in human beings. *Brucella* organisms are non motile, non spore forming, Gram negative, small, facultative intracellular coccobacilli of genus *Brucella.* There are nine species of *Brucella* classified on the basis of their host specificity, seven of them that affect the terrestrial animals are: *B. abortus* (cattle), *B. melitensis* (goats), *B. suis* (hogs), *B. ovis* (sheep), *B. canis* (dogs), and *B. neotomae* (wood rat) [8]. The other species affecting aquatic animals are: *B. ceti (Cetaceans) B. pinnipedialis* (seals), and *B. microti* (voles) [2]. Brucellosis in bovines is usually caused by *Brucella abortus*, less frequently by *B. melitensis*, and occasionally by *B. suis*.

EPIDEMIOLOGY OF BRUCELLOSIS

Brucellosis is an important disease in many parts of the world especially in the Mediterranean countries of Europe, North and East Africa, the Middle East, South and Central Asia and Central and South America and yet it is often unrecognized and frequently goes unreported [9]. It is widespread in the world and endemic in most countries where proper measures for the prevention and mitigation of the disease are ignored. Countries like Australia, New Zealand and Israel are believed to be free from the disease [10]. In these countries the disease was eradicated by implementation of stringent disease control strategies that included test and slaughter policies.

Brucellosis has not yet been controlled in regions such as Africa, the Mediterranean, Middle East, parts of Asia and Latin America which are highly endemic. Several countries in Northern and Central Europe, Canada, Japan, Australia and New Zealand are believed to be free from the disease [11].

Brucellosis is endemic in India among the bovine population both in farms and in the villages. It causes heavy economic loss to the animal industry. It was estimated that Brucellosis causes an annual loss equivalent to US$58.8 millions in India [12]. The occurrence of this disease varies from 10% in marginal herds to 50% in organized farms and the socio-economic impact of the disease was estimated to run over Rs.500 Crores annually. An overall prevalence of 17.7% was reported in cattle and buffaloes in Punjab state of India [13, 4]. The long-term serological studies have indicated that 5% of cattle and 3% of buffaloes, 7.9% sheep and 2.2% goats in India are infected with Brucellosis [14]. In Punjab 20.67% of prevalence in cattle and 16.45% in buffaloes respectively was reported [15].

Transmission of Brucellosis

Transmission among Animals

Brucellosis affects many animal species, particularly the food producing animals like sheep (especially milk-producing), goats, cattle and pigs and, in addition camels, buffaloes, yaks and reindeer. Bovine Brucellosis can be transmitted through milk, aborted fetuses, fetal membranes and vaginal discharges from infected animals. The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, water and licking after birth, fetuses, and newborn calves. Bulls can act as a source of Brucellosis because they excrete the organism through their semen [16]. Seminal vesicles, ampullae, testicles, and epididymis may be infected in bulls; therefore, organisms are present in the semen. Agglutinating antibodies may be present in seminal plasma from infected bulls. *B. suis* and *B. canis*, cause infection in pigs and dogs, respectively.

Transmission to Humans

Although there has been a great progress in controlling the disease in many countries, still transmission to the human population frequently occurs [17]. Four species of *Brucella* cause disease in humans also: *B. melitensis, B. suis, B. abortus* and *B. canis* in descending order of pathogenicity. The types associated with marine animals may also have the capacity to cause human disease. Consumption of unpasteurized milk and soft cheeses made from the

milk of infected animals, primarily goats, infected with *Brucella melitensis* and occupational exposure of veterinarians, slaughter house workers and laboratory staff may cause Brucellosis in humans [18].

Mode of Transmission

B. abortus is transmitted mainly through contact with the placenta, fetus, fetal fluids and vaginal discharges from animals affected with Brucellosis. Animals are found to be infectious after abortion or parturition at full-term. *B. abortus* organisms may be found in the milk, urine, semen, feces and hygroma fluids of the infected animals. Brucellosis occurs mainly by direct and indirect contact of the mucous membranes with infective excretors [19]. Organisms may be shed in milk intermittently for a long time or lifelong. Many infected cattle become chronic carriers. *B. abortus* can be transmitted through broken skin. Although the mammary gland is usually colonized during the course of an infection, it can also be infected by direct contact, with subsequent shedding of the organisms in the milk. *In utero* infections also occur. Transmission by artificial insemination is reported to occur when contaminated semen is deposited in the uterus but not in the mid cervix. The disease is transmitted in man by consumption of unpasteurized milk [20].

PATHOGENESIS OF BRUCELLOSIS

Brucellae are intracellular parasites. They gain entry into the body through cuts and abraisons in the oral mucosa, nasopharynx, conjuctivae or genitalia and even unbroken skin. After entry into the body they survive in the cells of reticuloendothelial system, particularly in monocytes and macrophages. They can survive in the cells of ectodermal and mesodermal origin, but are not capable of replicating in cells of endodermal origin [21]. They evade the bactericidal activity of phagocytic cells and replicate within them. They are then carried to the lymph nodes where macrophages and polymorphonuclear cells die and lead to release of more bacteria.

In animals where acute infection is not controlled, bacteria disseminate and eventually localize in spleen and liver [22]. Localization in the reproductive organs or mammary glands is associated with the most severe pathology and capability to transmit infection. *Brucella* gain access to the uterus and fetus via a hematogenous route, and the bacteria initially localize within erythrophagocytic trophoblasts of the placentome.

DIAGNOSIS OF BRUCELLOSIS

Isolation of *Brucella*

Samples for *Brucella* species from cattle include fetal membranes, especially the placental cotyledons where the number of organisms tends to be very high. In addition, fetal organs such as the lungs, bronchial lymph nodes, spleen and liver, as well as foetal gastric contents, milk, vaginal secretions preferably after abortion or parturition and semen are samples of choice for isolation. Milk samples should be in pool from the mammary glands. Non pasteurized dairy products can also be sampled for isolation [23].

Brucella spp. was isolated from milk samples collected during several lactations from serologically positive females that had recently aborted [24]. *B. melitensis* was recovered from milk samples from *Brucella* positive animals and it was demonstrated that microorganisms can remain in latency, most commonly in udder tissues and in supra mammary lymph nodes [25]. Joint fluid was aseptically aspirated from nine seropositive cows from five herds with hygromas in the carpi / hocks. Colonies of *Brucella*-like organisms were purified and isolates of Brucella organisms were recovered from cows from three different herds [26].

Serological Assays

Agglutination Tests

Agglutination tests like the Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT), Microagglutination Test (MAT), and Indirect Haemagglutination Assay (IHA) are being used for qualitative and quantitative analysis of antibodies against *Brucella* organisms in serum samples.

RBPT holds a greater promise for animal screening. They found that RBPT had higher specificity and sensitivity compared with other serological tests [27]. It was reported that only 5.55% of suspected samples were found positive by STAT, 50% samples were positive by RBPT and Dot ELISA could detect antibodies in all (100%) samples. They reported a new superagglutination test for diagnosis of Brucellosis [28].

Plate agglutination test was described as a valid screening method for *Brucella canis* agglutinins. It was concluded that the micro method provided an economical means of screening sera for presence of antibodies [29]. On evaluation of canine Brucellosis by MAT, it was reported that MAT was more sensitive,

simpler to perform and easier than Tube Agglutination Test. It allows handling of large number of samples at once [30].

Indirect Haemagglutination (IHA) test was employed to diagnose human and animal Brucellosis. It was shown that the use of sheep erythrocytes sensitized with a specific lipopolysaccharide antigen in the IHA test provided a specific method, which is more sensitive than the agglutination test, for the diagnosis of Brucellosis in humans and livestock. It was opined that the IHA test was more specific and sensitive than the agglutination test and justified its use in diagnosis of human Brucellosis, the study of immunological status of the population and the examination of animals for Brucellosis [31].

Enzyme Linked Immunosorbent Assays (ELISA)

Various types of ELISAs are being used for qualitative and quantitative analysis of antibodies against *Brucella* organisms in serum samples.

In a study [32], ELISA was compared with Rose Bengal Plate Test, standard tube agglutination test and Coombs' test for detection of antibodies to *Brucella* organisms in the diagnosis of Brucellosis. Sera tested were from 208 patients from whom *Brucella melitensis* had been isolated, 177 patients with significant results in at least two conventional tests, and 107 patients with fever in whom all conventional tests were negative and from whom no *Brucella* spp. had been isolated. ELISA had the highest sensitivity (97%), greater specificity (96%) and good positive and negative predictive values (98% and 94%, respectively). ELISA was the only positive test in 6% of patients in whom Brucellosis had been confirmed by culture.

A study was carried out to compare the efficacy of RBPT, STAT and Dot ELISA in immunological detection of antibodies to *Brucella abortus* in sera. The study revealed that Dot ELISA was the most sensitive of the three tests used. The authors suggested that in order to get confirmatory diagnosis of *Brucella* infection, a combination of RBPT and Dot ELISA should be used, especially for the samples which are found negative by RBPT or STAT used alone or in combination [33].

ELISA has been claimed to be more sensitive followed by RBPT and STAT when applied to cattle sera, whereas RBPT was found to be more sensitive followed by STAT and ELISA when applied to buffalo sera. Comparison of dot-ELISA and ELISA for diagnosis of bovine Brucellosis proved dot-ELISA to be more sensitive [34].

The use of ELISA in comparison to RBPT and STAT was advocated for assessing the situations of Brucellosis in cattle, to have better results because it is less likely that an infected animal may go undetected by ELISA [35]. ELISA can be used to eliminate false positive results amongst RBPT positive sera [36]. The i-ELISA, RBPT, MAT and PCR were evaluated for diagnosis of Brucellosis in buffaloes and it was concluded that indirect ELISA detected more samples as positive among these tests. They suggested that i-ELISA can be used for routine serodiagnosis of *Brucella* infection in buffaloes [37].

IDENTIFICATION OF *BRUCELLA* BY POLYMERASE CHAIN REACTION (PCR)

PCR for *Brucella* does not cross react with closely related bacterial species except *Orchobactrum anthropic*, the closest known relative to *Brucella* [38]. The automation of PCR renders this technique very promising for bacterial identification [39].

A multiplex PCR (AMOS) [40] is applicable to differentiate between *B. abortus* biovars 1, 2 and 4, *B. melitensis*, *B. ovis,* and *B. suis* biovar 1 by specific PCR products based on unique chromosomal loci of the mobile genetic element IS711 in their genome. This PCR was later improved by other researchers by adding specific primers for *B. abortus* biovars 5, 6, 9 and genotype 3b of biovar 3 for their identification.

PCR and indirect ELISA were found to have 100% specificity in milk samples of bovines. A PCR assay was developed using the genus specific primer pair derived from the 16s rRNA sequence of *B. abortus*. DNA from all the representative strains of *Brucella* species and its biovars from 23 isolates yielded the 905 bp sequence [41]. A random amplified polymorphic DNA PCR assay was developed to differentiate all recognized *Brucella* species, including the species *B. ceti* and *B. pinnipedialis* infecting marine mammals [42]. *B. abortus* was detected from blood, milk and lymph tissue of serologically positive cows by conventional and real time PCR assays. They amplified various regions of the *Brucella* genome, IS711 genetic element, gene for 31 kDa outer membrane protein and 16s RNA [43]. A real-time PCR assay was developed for differentiating *B. abortus* from other *Brucella* species based on a single nucleotide polymorphism [44].

Brucella melitensis and *B. abortus* were detected by polymerase chain reaction with the primers chosen from within the coded sequence of a gene encoding 31 kDa *B. abortus* antigen. *Brucella meltensis* and *B. abortus* showed no difference in sensitivity of the reaction or in the size of amplified product (223 bp) [45]. A study was conducted to use PCR in diagnosis of sheep Brucellosis using serum as sample and the results were compared with those of RBPT. Out of 36 samples tested, 19 were positive by RBPT and PCR detected 13 positive samples [46]. PCR and cultural method of diagnosis of human Brucellosis from blood samples were compared and three different PCR methods were also compared for detection of *Brucella* and it was found that PCR is the most sensitive technique [47].

Genus-specific assays are simple and robust. The main genetic targets utilized for these applications are the *Brucella BCSP31* gene and the 16S–23S rRNA operon. Three independent *Brucella* specific nucleotide sequences encoding bcsp, omp2 and 16S rRNA gene were used in PCR assay which resulted in the amplification of 223, 193 and 905 bp amplicons respectively. The bovine blood samples were insensitive to 16S rRNA PCR [48].

Brucella Milk Ring Test and three different polymerase chain reaction techniques were conducted to identify infection in bovine milk. PCR on animal's filtered milk was found to be the best procedure to make the diagnosis of *B. abortus* infections [49].

Brucella was isolated from samples of vaginal swabs, aborted materials and placenta and confirmed by PCR using genus specific primer pairs B4/B5 primer pair, F4/R2 and JPF/JPR [50]. The sensitivity of three pairs of primers amplifying three different fragments including a gene encoding BCSP 31 (B4/B5), a sequence of 16S rRNA of *Brucella abortus* (F4/r2) and a gene encoding omp 2 (JPF/JPR) was compared. The sensitivity of the B4/B5 primer pair was reportedly more (98%), followed by JPF/JPR primer pair and F4/R2 primer pair (88.4% and 53.1%) respectively [51, 52].

IDENTIFICATION OF *BRUCELLA* BY REVERSE TRANSCRIPTION PCR (RT-PCR)

The classical methods for the determination of bacterial viability rely on the ability of cells to actively grow and form visible colonies on solid media. But under some circumstances, the number of viable organisms may be under represented by such methods as sublethally damaged organisms, fastidious and, uncultivable bacteria and viable cells that have lost the ability to form colonies under the test conditions will not be detected [53].

The presence of intact DNA sequences was initially considered as an indicator of cell viability [54]. However correlation of cell viability with detection of DNA was shown to be poor, with DNA persisting in killed cells for significant periods of time [55]. DNA was also demonstrated to persist in a PCR-detectable form in culture-negative environmental and clinical samples [56, 57].

RNA has been used as mostly positively correlated with viability and the most commonly used amplification techniques for detecting RNA are RT-PCR and nucleic acid sequence based amplification [58]. RT-PCR has historically been the amplification method of choice when analyzing RNA [53].

The DNA and RNA detection PCR methods for identifying *Brucella* species in human blood samples were compared in a study. Serum and blood analysis by DNA and RNA detection assays was reported to be convenient and safe method for rapid and accurate diagnosis of Brucellosis [59]. We have used RNA as a biomarker of Brucella for non-invasive monitoring and assessment of efficacy of anti-Brucella therapy [60].

CONTROL OF BRUCELLOSIS

The prevalence of the disease is a basic element which affects the choice of a mode of control, whether by disease control measures alone, medical (immunization) measures alone, or a combination of the two. Type of husbandry, patterns of commerce, the grouping or dispersion of farms, size of herds, proportion of animals vaccinated, and segregation of calving cows are all important parameters, which in some countries are given the same importance as the control of animal imports and the control of movements of animals; these measures are strictly applied in most countries in which control is compulsory [61]. The emergence of *Brucella* is aggravated by its isolates which are resistant to some clinically resistant anibiotics [62].

In Brucellosis free countries "Test and Slaughter" of positive animals has proved effective. However in India, "Test and Segregation" in combination with vaccination is the only practical and feasible method [63]. The treatment of Brucellosis in the cow has been

unsuccessful because of the intracellular sequestration of the organisms in the lymph nodes, mammary gland, and reproductive system. Treatment failures occur due to inability of the drug to penetrate the cell membrane barrier [64].

In India, effective control of Brucellosis is a national problem. A major obstacle in the control of this disease has been the disposal of the positive animals. In Brucellosis - free countries, test and slaughter of positive animals has proved effective. However, in India the existing socioeconomic conditions do not advocate this policy. The alternative method of "test and segregation" has been speculated to be the only method, which is practical and feasible in our country [12].

The development of an efficacious vaccine for Brucellosis has been a challenge for scientists for many years. Despite the availability of two live attenuated vaccine strains S19 and RB51 for cattle and strain Rev1 for small ruminants, improved vaccines are still awaited [3]. An appropriate antibiotic therapy for animals and human beings is still disputed and it is too expensive in most of the animal species.

CONVENTIONAL VACCINES

Brucella abortus Strain 19 Vaccine

Brucella abortus S19 vaccine is the most widely used vaccine against Brucellosis in cattle. It is the reference vaccine to which other vaccines are compared. It is used as a live vaccine and is normally given to female calves aged between 3 and 6 months as a single subcutaneous dose of 5–8 $\times 10^{10}$ viable organisms. A reduced dose can be administered subcutaneously to adult cattle, but some of these animals may develop persistent antibody titres and may abort and excrete the vaccine strain organisms in the milk. Alternatively, it can be administered to cattle of any age as either one or two doses of 5 $\times 10^{9}$ viable organisms, given by the conjunctival route. When vaccinating adult cattle such vaccination gives protection without a persistent antibody level and reduces the chances of abortion and excretion of organisms in milk.

Brucella Abortus RB51

Vaccine strain RB51 (S-RB51) is an attenuated organism which lacks the O-side chain of the LPS. Due to the lack of O-side chain this vaccine can be given single or multiple times without inducing antibodies which interfere with serodiagnostic tests [65]. Vaccine S-RB51 is the official vaccine in the USA, Chile, Mexico, Venezuela, Colombia, and Argentina.

Since 1996, *B. abortus* strain RB51 is the official vaccine for prevention of Brucellosis in cattle in several countries [66]. In USA, calves are vaccinated subcutaneously between the ages of 4 and 12 months with 1–3.4× 10^{10} viable strain RB51 organisms. In other countries, cattle are vaccinated at 4-12 months of age with a dose of 1–3.4 × 10^{10} organisms, with a booster revaccination from 12 months of age onwards with a similar dose. Full doses of RB51 administered intravenously in cattle may induce severe placentitis and placental infection in most vaccinated cattle [67].

RB51 vaccine can induce abortion in some pregnant cattle. Hence, vaccination of pregnant cattle with RB51 should be avoided. The side effects of RB51 can be reduced by reducing the dose. When using the reduced dose of this vaccine (1 × 10^{9} colony forming units [CFU]), on late pregnant cattle, no abortions or placentitis lesions are produced in subcutaneously vaccinated cattle [67].

Approaches for Vaccine Development against Brucellosis

DNA Vaccines

DNA vaccines using pc DNA and p6 vector containing the gene for ribosomal protein L7/L12 was created by Kurar and Splitter [68]. The study revealed that constructs could induce both specific antibodies and T-cell mediated immune responses. Th1 type of immune response was produced by DNA vaccine expressing the *Brucella* GroEL heat shock protein [69]. Induction of immune response in mice with a DNA vaccine encoding outer membrane protein (omp 31) of *Brucella melitensis* was studied. 16M eukaryotic expression vectors called pTargeTomp31, and found that pTargeTomp31 elicited a T-cell proliferative response and also induced a strong gamma interferon production upon restimulation with either omp 31 antigen or *B. melitensis* 16M extract [70].

Live Vector Vaccine

It has been shown that recombinant vaccinia virus expressing *Brucella* antigen induced specific immune response to these antigens in mice but protection was not significant [71]. *S. typhimurium* 4064 expresing 3 kDa *Brucella* protein was used for oral immunization of mice resulting in the production of local and serum antibodies though CMI response was poor [72].

Ribosomal Preparation

Brucella ribosomal preparations have been shown to give protection equal to the current *Brucella abortus* strain 19 vaccine [73]. L7/L12 ribosomal protein gene has been expressed in *Lactobcillus lacti*, which is the first step towards Food-Grade live *Brucella* vaccines against Brucellosis [74].

Subunit Vaccine

Recombinant p39 bacterioferritin and L7/L12 protein have been purified and tested as subunit vaccine with adjuvant [75, 76]. Mice immunized with these proteins showed a certain level of protective immunity.

Synthetic Peptide Vaccine

Only a few attempts have been made to investigate potential of synthetic peptide as vaccine candidate. Three peptides derived from the primary structure of *Brucella abortus* Cu-Zn superoxide dismutase were synthesized, but it failed to produce protective response [77].

Phage Lysate Vaccines

Phage lysates comprise a means of effectively killing bacteria while minimally altering their antigenicity [78]. A lysate preparation containing complete range of structurally unaltered antigenic moieties of the bacterial cell mimics the antigenic profile of the intact live organism and induces the desirable protective response at a significantly lower dose and circumvents most of the drawbacks of killed, subunit or live attenuated vaccines. This can also provide cross-protective immunogenic antigens. The phage lysis does not denature macromolecules, which is how heat treatment kills bacteria. Thus, phage lysates are potential candidates for vaccine development and improvement.

We have reported successful immunotherapy of bovine Brucellosis by phage lysates of RB51 (RL) and S19 (SL) strains of *Brucella abortus* organisms. The SL induced strong antibody response while RL stimulated cell mediated immunity against *Brucella* organisms. A single dose of 2 ml of cocktail lysate (having both RL and SL) injected subcutaneously, removed live *Brucella* organisms from cattle affected with Brucellosis [60].

Phage Therapy of Brucellosis

Application of phage has been recommended to be an effective means to reduce the colonization of *Brucella* in the spleen of mice [79]. The effect of phage therapy on immune responses in Brucellosis affected cattle was studied and it was found that phage therapy stimulates immune responses in cattle initially which decline slowly after 45 days [80, 81].

Phage Lysate Immunotherapy of Bovine Brucellosis

In a recent study [82] live attenuated *Brucella abortus* vaccine strain S19 organisms were employed to deliver a lytic brucellaphage *in vivo* to reach the virulent *Brucella* hiding intracellularly. The phage - pulsed S19 organisms sustainably induced significantly high titers of anti-*Brucella* antibodies.

CONCLUSION

Brucellosis is an important zoonotic disease affecting several species of animals and human beings. The current immunological and molecular diagnostic assays provide accurate diagnosis of the disease however, isolation of the organism from clinical samples is still the gold standard. Antibiotics alone are neither hundred percent effective nor economic in animal treatment. Available live attenuated vaccines Brucella abortus strains S19 and RB 51 are effective but have their own limitations and pitfalls. Bacteriophages offer an attractive option for therapy and can be used to make lysates of Brucella for immunotherapy and immunoprophylaxis of Brucellosis in animals. However, there is still a long way to go for applying phages in routine.

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