

Vaccines for Caprine Brucellosis: Status and Prospective

Abstract

Brucellosis is an endemic world wide zoonosis, affecting both human and animals. It is caused by bacteria belonging to genus *Brucella*. There is host specificity in *Brucella* species, however selective and restricted inter species transmission is also reported causing zoonosis. In human brucellosis is mainly caused by *Brucella melitensis* followed by *Brucella abortus*. *Brucella melitensis* is more virulent than *Brucella abortus* and first to cause human brucellosis. It is responsible for caprine brucellosis and goats residing in close vicinity to human are main source of infection. This can be avoided by the use of safe and effective vaccination of goat population. There is an effective vaccine for the caprine vaccination ie. Rev.1, however it is hazardous to human, thus its use is not recommended in many of the countries. Under such circumstances development and trials of various traditional and advanced vaccines have been attempted. These have been summarized and discussed on merit and demerit basis, in present paper with a possibility to obtain safe and effective vaccine against *Brucella melitensis*.

Keywords: Brucellosis; *Brucella melitensis*; Vaccine; Vaccination; Rev.1.

Mini Review

Volume 2 Issue 3 - 2016

Amit Kumar^{1*}, VK Gupta², AK Verma³, SK Yadav¹ and Anu Rahal⁴

¹Department of Veterinary Microbiology, College of Veterinary Sciences, Mathura, India

²CADRAD, Indian Veterinary Research Institute, Izatnagar, India

³Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Sciences, Mathura, India

⁴Division of Goat Health, Central Institute for Research on Goats, Farha, mathura, India

***Corresponding author:** Amit Kumar, Assistant Professor, Department of Veterinary Microbiology, College of Veterinary Sciences, DUVASU, Mathura-281001, India, Tel: +919412120813; Email: balyan74@gmail.com

Received: April 16, 2016 | **Published:** May 24, 2016

Introduction

Brucellosis in goats is mainly caused by *B. melitensis*, although this pathogen may also infect cattle and other ruminants [1]. This pathogen has three different biovars [2]. *B. melitensis* was the first species in the genus *Brucella* described and was first isolated by Bruce in 1887 [3] from the spleens of soldiers dying of Mediterranean fever on the island of Malta. The origin of the disease remained a mystery for nearly 20 years until Themistocles Zammit accidentally demonstrated the zoonotic nature of the disease in 1905 by isolating *B. melitensis* from goat's milk [4]. Initially it was believed that goats were not the source of infection since they did not become ill when inoculated with *Brucella* cultures [5]. However, the causing organism is mainly responsible for human brucellosis all over the world. Thus the prevention of human brucellosis largely depends upon the prevention and control of caprine brucellosis and similar to the control strategies against any infectious disease vaccination is the first and foremost step to the control animal brucellosis [6]. The most common methods for control of the ovine brucellosis are vaccination of animals and slaughter of infected flocks [7,8]. There is no human vaccine in contrast to animals where vaccination is supposed to be one of the most cost-effective measures to achieve eradication [9-11] and to improve human health in endemic areas [12,13].

Discussion

For animal use vaccines *B. abortus* S19, Cotton stain 45, RB51 and *B. melitensis* Rev.1 have been successfully used in large and small ruminants, respectively [14-16]. Effective well established *B. melitensis* strain Rev.1 vaccine is available for sheep and goats [15,17,18]. It protects animals for years together and protection has been evaluated after conjunctival and subcutaneous inoculation in kids, lambs and adults [1,19,20]. Despite the

controversial background of creating hindrance in serological detection of infection, shedding in secretions and virulence to human [21], it has been used in many developing and European countries to control the disease in animals as it protects large proportion of vaccinated animals against infection [22,23]. Many developed countries have eradicated the disease with the use of these vaccines, but vaccination induced abortions in pregnant animals, transmission of disease in humans through vaccinated animals [10,21,24] and resistance of Rev.1 (most pathogenic to humans) against the antibiotic streptomycin which is used to treat the disease had forced bans on these in many countries [10,25,26]. Further *B. melitensis* Rev.1 vaccine strain under standard conditions (i.e. full dose via the subcutaneous route in young animals) elicit a long lasting serological response against smooth lipopolysaccharide (sLPS) of the *Brucella* surface that interferes in serodiagnosis of infection as agglutination test and that seriously interferes with serological screening for infected due to similarity with wild strain of *B. melitensis* [15,27,28]. In comparison to full dose, the reduced dose elicits shorter and less intense antibody response following vaccination [29-31] and can be used safely in pregnant sheep and goats [30-35]. However, excretion of vaccine strain mainly in vaginal excretion and foetal contents during abortion in pregnant sheep and goats after field infections [36-39] further showed the necessity to put stress on different methods of vaccination.

The vaccination with lesser bacterial load in smaller volume through conjunctiva was also attempted with lesser serological response and protection almost similar to full and reduced dose in young calves and adult animals [19,39-43]. Conjunctival route produces lesser abortion and excretion of vaccine strain [39,41]

and these can be further reduced by the vaccination during early pregnancy [39]. However, the dose of vaccination and reimmunization depends upon the age, species and physiological status of animal [19,20,30]. Although the sero diagnosis problems can be partially solved by using the conjunctival route during calf hood and by avoiding adult vaccination [44] but serological follow up individual animals, the breeding conditions characteristic of small ruminants make these measures unrealistic. Therefore, effective brucellosis vaccines not interfering in diagnosis with minimum or no virulence for human would represent a major breakthrough [26,45].

To overcome these problems many other options were attempted in sheep and goats as *B. suis* S2 attenuated strain with smooth LPS [1,46,47], live attenuated rough (S-LPS lacking) *Brucella* strain, *B. abortus* RB51 [48,49], live rough strains obtained by transposon mutagenesis from smooth *B. melitensis* 16M strain, VTRM1 [50] and smooth *B. abortus* 2308 strain, RfbK strain [51] with limited success. Use of targeted and transposon mutagenesis through disruption of *per*, *wbo A* and so also *wbk A* (putative perosamine synthetase and glycosyltransferase genes) resulted in the development of R mutants that showed better results than RB51 under laboratory trials [52-54]. Rough (R) *brucella* mutants which lack the LPS immunodominant N-formylperosamine O-polysaccharide (O-PS) were also attempted for vaccination after attenuation [15,26,45]. Moreover, rough vaccines or spontaneous mutants were developed after repeated passage on antibiotic-containing media like RB51, a *B. abortus* R mutant that carries IS711-disrupted *wbo A* (putative glycosyl transferase gene). This has lightened a torch of hope to have good vaccine. These mutants resulted from the alteration in OPS precursor synthesis, its polymerization and transport or due to the many other possible defects in the inner core oligosaccharide [45,54]. However, RB51 yielded controversial results in cattle and was not effective in sheep. Furthermore it has resistant to the antibiotic rifampin used to treat brucellosis [15,45]. Many other mutants of *B. melitensis* viz. RBM9, RBM11, RBM15, RBM17 and RBM19 have been obtained by repeated passage over antibiotic containing media [55] but the presence of undefined LPS and resistance against rifampin, an antibiotic used for the treatment against brucellosis, render them ineffective [45,55]. Moreover, under controlled experimental and field conditions rough vaccine has been reported to be least equivalent to the Rev.1 vaccine [12].

In 2000, a vaccine prepared from a killed, whole cell suspension of *Brucella melitensis* was given without adjuvant or with added *Mycobacterium phlei* or bentonite clay in cattle and sheep revealed higher levels of both humoral and cell mediated immunity [56]. In china an attenuated *B. melitensis* vaccine M5-90 is being used for vaccination of sheep and goats [57,58]. However, the antibody responses raised by those two live vaccines are difficult to distinguish from naturally *Brucella* infected animals using the conventional serological tests.

BP26, Periplasmic protein of *Brucella* is reported to be a hope for better diagnosis in brucellosis [59-62] being most conserved in all the species of *brucella* with sensitivity and specificity for the diagnosis of animal *brucellosis* by enzyme immunoassays (EIAs) [60,61,63-66]. It also revealed excellent antibody and cellular responses [67,68]. However, the molecular feature of BP26

antigen remains unclear. A mutant obtained by deletion of BP26 in Rev.1 [69] revealed protection against *B. melitensis* in sheep or *B. ovis* in rams [70,71] while BP26-deleted M5-90 mutant lost its ability to induce protective immunity [72,73]. This BP26 antigen within Rev. 1 induces high IgG1 titers and cellular response of IFN- γ , IL-4, IL-5 and IL-6 [67,74]. BP26, TF (trigger factor) and omp31 are potent source of protective immunity against *Brucella* infections [63,75-77].

Recombinant BP26 has been investigated for diagnosis of brucellosis in sheep and goats [60,61,66,78]. A DNA vaccine encoding outer membrane protein (OMP31) of *Brucella melitensis* 16M has also been reported to induce immune response in mice [79]. Recently the NMP (membrane protein extracts) in comparison to rBP26 (rough BP26) are reported to be more sensitive and specific in ELISA for detection of antibodies to *Brucella* from sheep, and had 90% agreement with the combination of SAT and RBPT [80], has reported that an invasive *E. coli* vector platform can deliver antigens of *B. melitensis* to the immune system. In such conditions invasive *E. coli* may be an ideal vaccine since they are nonpathogenic, can deliver antigens to antigen-presenting cells, and contain natural adjuvant properties to promote cellular immune responses [81]. However, the vaccination results of these mutants are yet to be proven in the form of best vaccine with immune response at par to S-19 or Rev.1. The live attenuated *Brucella melitensis* vaccine strain Rev.1 is recognized worldwide as the best vaccine available against brucellosis in sheep and goats [10,11,82,83].

Conclusion

Due to this contraindication there is unavailability of universally adopted vaccine and vaccination strategy, making the eradication of the disease difficult [11,12]. WHO [12] has also agreed that "correctly standardized Elberg 101 strain Rev.1 vaccine should continue to be considered as the basis of brucellosis control in small ruminants where vaccination is applied, until new safer and effective versions of *B. abortus* and *B. melitensis* vaccines based on rough strains, have been tested under controlled experimental and field conditions and shown to be at least equivalent to the Rev.1 vaccine".

References

1. Verger JM, Grayon M, Zundel E (1995) Comparison of the efficacy of *Brucella suis* strain 2 and *Brucella melitensis* Rev. 1 live vaccines against a *Brucella melitensis* experimental infection in pregnant ewes. *Vaccine* 13(2): 191-196.
2. Bricker BJ, Halling SM (1994) Differentiation of *Brucella abortus* bv. 1, 2 and 4, *Brucella melitensis*, *Brucella ovis* and *Brucella suis* bv 1 by PCR. *J Clin Microbiol* 32(11): 2660-2666.
3. Alton GG (1990) *Brucella melitensis*. In: Nielsen K, Duncan R (Eds.), *Animal brucellosis*. CRC Press, USA, pp. 383-409.
4. Godfroid J, Cloeckert A, Liautard JP (2005) From the discovery of the Malta fever's agent to the discovery of a marine reservoir, brucellosis has continuously been a reemerging zoonosis. *Vet Res* 36(3): 313-326.
5. Pappas G, Papadimitriou P (2007) Challenges in *Brucella* bacteraemia. *Int Antimicrob Agents* 30(Suppl 1): 29-31.
6. Alton GG (1967) Rev 1 *Brucella melitensis* vaccine. Serological reactions in Maltese goats. *J Comp Pathol* 77(3): 327-329.

7. Blasco JM, Garin-Bastuji B, Marin CM, Gerbier G, Fanlo J, et al. (1994a) Efficacy of different rose Bengal and complement fixation antigens for the diagnosis of *Brucella melitensis* infection in sheep and goats. *Vet Rec* 134(16): 415-420.
8. Da Costa MR, Irache JM, Gamazo C (2012) A cellular vaccines for ovine brucellosis: A safer alternative against a worldwide disease. *Expert Rev Vaccines* 11(1): 87-95.
9. Nicoletti PL (1990) Vaccination. In: Nielsen KH, Duncan JR (Eds.), *Animal Brucellosis*. Boca Raton: CRC Press, USA, pp. 283-299.
10. Bastuji GB, Lasco JM, Grayon M, Verger JM (1998) *Brucella melitensis* infection in sheep: present and future. *Vet Res* 29(3-4): 255-274.
11. Office International des Epizooties (OIE) (2010) *Manual of standards for diagnostic tests and vaccines*. (3rd edn), Office International des Epizooties, Paris, France, p. 251.
12. WHO / MZCP (1998) *Human and Animal Brucellosis*. Report of a WHO/MZCP workshop, Damascus, Syria.
13. Zinsstag J, Schelling E, Roth F, Bonfoh B, Don de Savigny, et al. (2007) Human benefits of animal interventions for zoonosis control. *Emerg Infect Dis* 13(4): 527-531.
14. Alton GG (1968) Further studies on the duration of immunity produced in goats by the Rev-1 *Brucella melitensis* vaccine. *J Comp Pathol* 78(2): 173-178.
15. González D, Grilló MJ, De Miguel MJ, Ali T, Arce-Gorvel V, et al. (2008) *Brucellosis Vaccines: Assessment of Brucella melitensis Lipopolysaccharide Rough Mutants Defective in Core and O-Polysaccharide Synthesis and Export*. *PLoS ONE* 3(7): e2760.
16. Ebrahimi M, Nejad RB, Alamian S, Mokhberalsafa L, Abedini F, et al. (2012) Safety and efficacy of reduced doses of *Brucella melitensis* strain Rev. 1 vaccine in pregnant Iranian fat-tailed ewes. *Vet Ital* 48(4): 405-412.
17. Alton GG (1966) Duration of the immunity produced in goats by Rev.1 *Brucella melitensis* vaccine. *J Comp Pathol* 76(3): 241-253.
18. Blasco JM (1997) A review of the use of *B. melitensis* Rev 1 vaccine in adult sheep and goats. *Prev Vet Med* 31(3-4): 275-283.
19. Fensterbank R, Pardon P, Marly J (1985) Vaccination of ewes by a single conjunctival administration of *B. melitensis* Rev.1 vaccine. *Ann Rech Vet* 16(4): 351-356.
20. Ferrer DM (1998) Comparación entre métodos inmunológicos de diagnóstico de la brucellosis ovina por *Brucella melitensis* y eficacia de la inmunización de ovejas adultas con la vacuna Rev.1 por vía conjuntival. University of Murcia, Spain.
21. Blasco JM, Díaz R (1993) *Brucella melitensis* Rev1 vaccine as a cause of human brucellosis. *Lancet* 342(8874): 805.
22. Garrido F (1992) Rev 1 and B-19 vaccine control in Spain. Observations on the handling and effectiveness of Rev 1 vaccine and the immune response. In: Plommet M (Ed.), *Prevention of Brucellosis in the Mediterranean countries*. Pudoc Scientific Publishers, Wageningen, Netherlands, pp. 223-231.
23. Banai M (2002) Control of small ruminant brucellosis by use of *Brucella melitensis* Rev. 1 vaccine: laboratory aspects and field observations. *Vet Microbiol* 90(1-4): 497-519.
24. Spink WW (1956) *The nature of brucellosis*. Food and Agriculture Organization of the United Nations, USA.
25. Ariza J, Pellicer T, Pallares R, Foz A, Gudiol F (1992) Specific antibody profile in human brucellosis. *Clin Infect Dis* 14(1): 131-140.
26. Schurig GC, Sriranganathan N, Corbel MJ (2002) *Brucellosis vaccines: past, present and future*. *Vet Microbiol* 90(1-4): 479-496.
27. Alton GG, Elberg S (1967) Rev 1 *Brucella melitensis* Vaccine. A review of ten years study. *Vet Bull* 37: 793-800.
28. MacMillan AP (1997) Investigation of the performance of the Rose Bengal plate test in the diagnosis of *Brucella melitensis* infection of sheep and goats. *World Animal Review* 89: 57-60.
29. Blasco JM, Estrada A, Mercadal M (1984) A note on adult sheep vaccination with reduced dose of *Brucella melitensis* Rev.1. *Ann Rech Vet* 15(4): 553-556.
30. Gasca A, Jiménez JM, Díaz L (1985) Experiencias sobre vacunación antibrucelar de cabras adultas con la cepa Rev.1. BNE, p. 33-34.
31. Henriques H, Hueston WD, Hoblet KH, Shulaw WP (1992) Field trials evaluating the safety and serologic reactions of reduced dose *Brucella melitensis* Rev.1 vaccination in adult sheep. *Prev Vet Med* 13(3): 205-215.
32. Kolar J (1984) Diagnosis and control of brucellosis in small ruminants. *Prev Vet Med* 2(1-4): 215-225.
33. Al-Khalaf SA, Mohamad BT, Nicoletti P (1992) Control of brucellosis in Kuwait by vaccination of cattle, sheep and goats with *Brucella abortus* strain 19 or *Brucella melitensis* strain Rev 1. *Trop Anim Health Prod* 24(1): 45-49.
34. Kolar J (1995) Some experience from brucellosis control with Rev.1 vaccine in a heavily infected country - Mongolia. *FAO/WHO/OIE Round table on the use of Rev.1 vaccine in Small Ruminants and Cattle*. CNEVA, Alfort, France, p. 21-22.
35. Uysal Y (1995) Field experience with Rev 1 vaccine in Turkey. *FAO/WHO/OIE Round Table on the use of Rev.1 vaccine in small ruminant and cattle*. CNEVA, Alfort, France.
36. Alton GG (1970) Vaccination of goats with reduced doses of Rev 1 *Brucella melitensis* vaccine. *Res Vet Sci* 11(1): 54-59.
37. Crowther RW, Orphanides A, Polydorou K (1977) Vaccination of adult sheep with reduced doses of *Brucella melitensis* strain Rev.1. *Trop Anim Health Prod* 9(2): 85-91.
38. Fensterbank R, Pardon P, Marly J (1982) Comparison between subcutaneous and conjunctival route of vaccination of Rev.1 strain against *Brucella melitensis* infection in ewes. *Ann Rech Vet* 13(4): 295-301.
39. Bagués MP, Marín CM, Barberán M, Blasco JM (1989) Responses of ewes to *B. melitensis* Rev.1 vaccine administered by subcutaneous or conjunctival routes at different stages of pregnancy. *Ann Rech Vet* 20(2): 205-213.
40. Bagues MP, Marín CM, Blasco JM, Moriyón I, Gamazo C (1992) An ELISA with *Brucella* lipopolysaccharide antigen for the diagnosis of *B. Melitensis* infection in sheep and for the evaluation of serological responses following subcutaneous or conjunctival *B. melitensis* Rev 1 vaccination. *Vet Microbiol* 30(2-3): 233-241.
41. Zundel E, Verger JM, Grayon M, Michel R (1992) Conjunctival vaccination of pregnant ewes and goats with *Brucella melitensis* Rev.1 vaccine: safety and serological responses. *Ann Rech Vet* 23(2): 177-188.
42. Díaz AE, Marín C, Alonso B, Aragón V, Perez S, et al. (1994) Evaluation of serological tests for diagnosis of *B. melitensis* infection of goats. *J Clin Microbiol* 32(5): 1159-1165.
43. Marin CM, Moreno E, Moriyon I, Diaz R, Blasco JM (1999) Performance of competitive and indirect enzyme-linked immunosorbent assays,

- gel immunoprecipitation with native hapten polysaccharide and standard serological tests in diagnosis of sheep brucellosis. *Clin Diagn Lab Immunol* 6(2): 269-272.
44. De Frutos C, Durán FM, Leon M, Navarro A, Perales A, et al. (1994) Consideraciones sobre la epidemiología y el control de la brucelosis en pequeños rumiantes. *Proceedings Jornadas Internacionales sobre Brucelosis*, Madrid.
 45. Moriyon I, Grillo MJ, Monreal D, Gonzalez D, Marin CM, et al. (2004) Rough vaccines in animal brucellosis: structural and genetic basis and present status. *Vet Res* 35(1): 1-38.
 46. Xin X (1986) Orally administrable brucellosis vaccine: *B. suis* S2 vaccine. *Vaccine* 4(4): 212-216.
 47. Mustafa AA, Abusowa M (1993) Field-oriented trial of the Chinese *Brucella suis* strain 2 vaccine in sheep and goats in Lybia. *Vet Res* 24(5): 422-429.
 48. Schurig GG, Roop RM, Bagchi T, Boyle S, Buhrman D, et al. (1991) Biological properties of RB51; a stable rough strain of *Brucella abortus*. *Vet Microbiol* 28(2): 171-188.
 49. Bagues MP, Marin CM, Barberan M, Blasco JM (1993) Evaluation of vaccines and of antigen therapy in a mouse model for *Brucella ovis*. *Vaccine* 11(1): 61-66.
 50. Elzer P, Enright F, McQuinston, Boyle G, Schurig G (1998) Evaluation of a rough mutant of *Brucella melitensis* in pregnant goats. *Res Vet Sci* 64(3): 259-260.
 51. Adams G, Ficht T, Allen C (1998) Derivation and evaluation of the rough rfbK brucellosis vaccine in cattle. *Foro Nacional de Brucelosis*, Mexico, pp. 141-158.
 52. Winter AJ, Schurig GG, Boyle SM, Sriranganathan N, Bevins JS, et al. (1996) Protection of BALB/c mice against homologous and heterologous species of *Brucella* by rough strain vaccines derived from *Brucella melitensis* and *Brucella suis* biovar 4. *Am J Vet Res* 57(5): 677-683.
 53. Monreal D, Grillo MJ, Gonzalez D, Marin CM, de Miguel MJ, et al. (2003) Characterization of *Brucella abortus* O-polysaccharide and core lipopolysaccharide mutants and demonstration that a complete core is required for rough vaccines to be efficient against *Brucella abortus* and *Brucella ovis* in the mouse model. *Infect Immun* 71(6): 3261-3271.
 54. Mc Donagh MM, Ficht TA (2006) Evaluation of protection afforded by *Brucella abortus* and *Brucella melitensis* unmarked deletion mutants exhibiting different rates of clearance in BALB/c mice. *Infect Immun* 74(7): 4048-4057.
 55. Adone R, Ciuchini F, Marianelli C, Tarantino M, Pistoia C, et al. (2005) Protective properties of rifampin-resistant rough mutants of *Brucella melitensis*. *Infect Immun* 73(7): 4198-4204.
 56. Ram S, Krishnappa G, Sastry KNV, Honnegowda, Ramanatha KR (2000) Evaluation of killed *Brucella melitensis* vaccine adjuvanted with bentonite clay and *Mycobacterium phlei* in cattle and sheep. *Indian Veterinary Journal* 77(3): 189-192.
 57. Deqiu S, Donglou X, Jiming Y (2002) Epidemiology and control of brucellosis in China. *Vet Microbiol* 90(1-4): 165-182.
 58. Zhang WY, Guo WD, Sun SH, Jiang JF, Sun HL, et al. (2010) Human brucellosis, Inner Mongolia, China. *Emerg Infect Dis* 16(12): 2001-2003.
 59. Cloeckaert A, Debbbarh HSA, Vizca'ino N, Saman E, Dubary G, Zygmunt MS (1996) Cloning, nucleotide sequence, and expression of the *Brucella melitensis* bp26 gene coding for a protein immunogenic in infected sheep. *FEMS Microbiol Lett* 140(2-3): 139-144.
 60. Rossetti OL, Arese AI, Boschiroli ML, Cravero SL (1996) Cloning of *Brucella abortus* gene and characterization of expressed 26-kDa periplasmic protein: potential use for diagnosis. *J Clin Microbiol* 34(1): 165-169.
 61. Salih Alj, Debbbarh H, Zygmunt MS, Dubray G, Cloeckaert A (1996) Competitive enzyme-linked immunosorbent assay using monoclonal antibodies to the *Brucella melitensis* BP26 protein to evaluate antibody responses in infected and *B. melitensis* Rev.1 vaccinated sheep. *Vet Microbiol* 53(3-4): 325-337.
 62. Li Jia-Yun, Liu Y, GaoXiao-Xue, Gao X, Cai H (2014) TLR2 and TLR4 signaling pathways are required for recombinant *Brucella abortus* BCSP31-induced cytokine production, functional upregulation of mouse macrophages, and the Th1 immune response *in vivo* and *in vitro*. *Cellular and Molecular Immunology* 11: 477-494.
 63. Vizca'no N, Cloeckaert A, Zygmunt MS, Dubray G (1996) Cloning, nucleotide sequence, and expression of the *Brucella melitensis* omp31 gene coding for an immunogenic major outer membrane protein. *Infect Immun* 64(9): 3744-3751.
 64. Cloeckaert A, Vizca'no NJ, Paquet Y, Bowden RA, Elzer PH (2002) Major outer membrane proteins of *Brucella* spp. past, present and future. *Vet Microbiol* 90(1-4): 229-247.
 65. Mediavilla P, Verger JM, Grayon M, Cloeckaert A, Marín CM, et al. (2003) Epitope mapping of the *Brucella melitensis* BP26 immunogenic protein: usefulness for diagnosis of sheep brucellosis. *Clin Diagn Lab Immunol* 10(4): 647-651.
 66. Gupta VK, Kumari R, Vohra J, Singh SV, Vihan VS (2010) Comparative evaluation of recombinant BP26 protein for serological diagnosis of *Brucella melitensis* infection in goats. *Small Ruminant Research*. 93(2-3): 119-125.
 67. Clapp B, Walters N, Thornburg T, Hoyt T, Yang X, et al. (2011) DNA vaccination of bison to brucellar antigens elicits elevated antibody and IFN- γ responses. *J Wildl Dis* 47(3): 501-510.
 68. Yang X, Hudson M, Walters N, Bargatze RF, Pascual DW (2005) Selection of protective epitopes for *Brucella melitensis* by DNA vaccination. *Infect Immun* 73(11): 7297-7303.
 69. Cloeckaert A, Jacques I, Grilló MJ, Marín CM, Grayon M et al. (2004) Development and evaluation as vaccines in mice of *Brucella melitensis* Rev.1 single and double deletion mutants of the bp26 and omp31 genes coding for antigens of diagnostic significance in ovine brucellosis. *Vaccine* 22(21-22): 2827-2835.
 70. Jacques I, Verger JM, Laroucau K, Grayon M, Vizcaino N, et al. (2007) Immunological responses and protective efficacy against *Brucella melitensis* induced by bp26 and omp31 *B. melitensis* Rev 1 deletion mutants in sheep. *Vaccine* 25(5): 794-805.
 71. Grillo MJ, Marín CM, Barberán M, de Miguel MJ, Laroucau K, et al. (2009) Efficacy of bp26 and bp26/omp31 *B. melitensis* Rev.1 deletion mutants against *Brucella ovis* in rams. *Vaccine* 27(2): 187-191.
 72. Qu Q, Wang ZJ, Zhen Q, Huang LY, Yu YQ, et al. (2009) Effect of bp26 on immune response and protective efficacy of M 5 against *Brucella melitensis* in mice. *Journal of Jilin Agricultural University* 31(4): 438-446.
 73. Wang ZJ, Zhen Q, Qiao F, Wang YF, Du XY, et al. (2009) Construction of

- BP26 tagged vaccine strain and development of discriminating PCR for Brucella. *Wei Sheng Wu Xue Bao* 49(3): 405-409.
74. Yang X, Walters N, Robison A, Trunkle T, Pascual DW (2007) Nasal immunization with recombinant *Brucella melitensis* bp26 and trigger factor with cholera toxin reduces *B. melitensis* colonization. *Vaccine* 25(12): 2261-2268.
75. Cassataro J, Estein SM, Pasquevich KA, Velikovskiy CA, de la Barrera S, et al. (2005a) Vaccination with the recombinant *Brucella* outer membrane protein 31 or a derived 27-amino-acid synthetic peptide elicits a CD4⁺ T helper 1 response that protects against *Brucella melitensis* infection. *Infect Immun* 73(12): 8079-8088.
76. Cassataro J, Velikovskiy CA, de la Barrera S, Estein SM, Bruno L, et al. (2005b) A DNA vaccine coding for the *Brucella* outer membrane protein 31 confers protection against *B. melitensis* and *B. ovis* infection by eliciting a specific cytotoxic response. *Infect Immun* 73(10): 6537-6546.
77. Pasquevich KA, Estein SM, Samartino C, Zwerdling A, Coria LM, et al. (2009) Immunization with recombinant *Brucella* species outer membrane protein Omp16 or Omp19 in adjuvant induces specific CD4⁺ and CD8⁺ T cells as well as systemic and oral protection against *Brucella abortus* infection. *Infect Immun* 77(1): 436-445.
78. Liu WX, Hu S, Qiao ZJ, Chen WY, Liu LT, et al. (2011) Expression, purification, and improved antigenic specificity of a truncated recombinant bp26 protein of *Brucella melitensis* M5-90: a potential antigen for differential serodiagnosis of brucellosis in sheep and goats. *Biotechnol Appl Biochem* 58(1): 32-38.
79. Gupta VK, Rout PK, Vihan VS (2007) Induction of Immune Response in Mice with a DNA Vaccine Encoding Outer Membrane Protein (OMP31) of *Brucella melitensis* 16M. *Res Vet Sci* 82(3): 305-313.
80. Qui J, Wang W, Wu J, Zhang H, Wang Y, et al. (2012) Characterization of Periplasmic Protein BP26 Epitopes of *Brucella melitensis* reacting with Murine Monoclonal and Sheep Antibodies. *PLoS One* 7(3): e34246.
81. Gupta VK, Radhakrishnan G, Harms J, Splitter G (2012) Invasive *Escherichia coli* vaccines expressing *Brucella melitensis* outer membrane proteins 31 or 16 or periplasmic protein BP26 confer protection in mice challenged with *B. melitensis*. *Vaccine* 30(27): 4017-4022.
82. Blasco JM, Marin CM, de Bagues MP, Barberan M, Hernandez A, et al. (1994b) Evaluation of allergic and serological tests for diagnosis of *Brucella melitensis* infection in sheep. *J Clin Microbiol* 32(8): 1835-1840.
83. Elberg SS, Faunce K (1957) Immunization against *Brucella* infection. VI. Immunity conferred on goats by a nondependent mutant from a streptomycin-dependent mutant strain of *Brucella melitensis*. *J Bacteriol* 73(2): 211-217.