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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 be the use of safe and effective vaccination of goat population. There is an effective vaccine for the caprine vaccination ie. Rev.1, however it has 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.

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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 20years 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 animals 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 glycosyltranferase 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 tranferase 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, anantibiotic 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.1vaccine.[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] Recently, it has been 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, the eradication of the disease is 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".

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Conflicts of interest

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