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Review paper

# A review of the basis of the immunological diagnosis of ruminant brucellosis☆

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ABSTRACT

Bacteria of the genus *Brucella* cause brucellosis, the most common bacterial zoonosis worldwide. The diagnosis of *Brucella abortus* and *Brucella melitensis* ruminant brucellosis is based on bacteriological and immunological tests, the latter being routinely used in control and eradication and surveillance programs. Infections by smooth and rough *Brucella* spp., the use of smooth and rough vaccines, and the false-positive serological reactions caused by *Yersinia enterocolitica* O:9 and other cross-reacting bacteria represent the immunological contexts in which those tests are used. This complex context explains the large number of brucellosis tests that have been developed, and that vary in antigen type, antigen presentation, antibody and conditions for the reaction, the response detected and the sample required. This wealth of information and an imperfect understanding of *Brucella* antigens and of the peculiarities of the immunoresponse to *Brucella* has created confusion and led to several misconceptions on the usefulness and limitations of the brucellosis diagnostic tests. In this review, *Brucella* antigens are examined focusing on cellular topology, supramolecular properties, epitopic structure and lipopolysaccharide and protein cross-reactivity in the various contexts of the immune response in ruminants. Then, the significance of these features in diagnostic tests that use whole bacteria is discussed with respect to the activities of ruminant immunoglobulins, and the effect of pH on unspecific agglutinations, non-agglutinating and blocking antibodies, pseudoprozones and complement activation. Similarly, the bacterial surface lipopolysaccharides and cognate polysaccharides are discussed with regards to topological effects, epitope exposure, ionic strength and antibody avidity in immunoprecipitation, immunosorbent and fluorescence polarization assays. Finally, the search for immunodominant protein antigens and their use in immunological tests is reviewed. Critical review of the existing information is necessary both to select optimal tests according to the logistical means available and the epidemiological context, and to focus the development of new tests.



**Contents**



*Abbreviation:* CFT, complement fixation test; DTH, delayed type hypersensitivity; ELISA, enzyme-linked immunosorbent assay; c-ELISA, competitive ELISA; i-ELISA, indirect ELISA; FPA, fluorescence polarization assay; FPSR, false positive serological reactions; LFiC, lateral flow immunochromatography; LPS, lipopolysaccharide; NH, native hapten; Omp, outer membrane protein; R, rough; RBT, rose bengal test; S, smooth; SAT, standard agglutination test.

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## 1. Introduction

Brucellosis is a zoonosis caused by the bacteria of the genus *Brucella* that affects a variety of domestic and wildlife mammals. Classically, the genus includes six species: *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Brucella neotomae, Brucella canis* and *Brucella ovis.* Two more species, *Brucella ceti* and *Brucella pinnipedialis*, include strains isolated from marine mammals, and *Brucella microti* and *Brucella inopinata* have been proposed more recently for brucellae isolated from the common vole and a breast implant, respectively. Among domestic animals, ruminants are highly susceptible to brucellosis. *B. melitensis* and *B. abortus* preferentially infect small ruminants and cattle respectively, but the latter can also be infected by *B. melitensis* and by *B. suis*, the species found preferentially in swine and some wildlife mammals (Moreno, 2014). Although rarely reported, sheep are also susceptible to infections by *B. abortus* (Luchsinger and Anderson, 1979). Sheep, goat and cattle brucellosis, the focus of this review, are highly contagious conditions manifested clinically by abortions and infertility. Humans are not themselves a source of contagion for brucellosis, and domestic animals constitute the main reservoir for human infection (Moreno, 2014). Human brucellosis is grave and debilitating, requires prolonged antibiotic treatment and may lead to permanent sequelae (Zinsstag et al., 2011). *B. melitensis* is probably the most important cause of human brucellosis worldwide.

Brucellosis is regarded as an emerging disease and as one of the most widespread zoonoses worldwide. Although the epidemiological understanding of brucellosis in resource poor countries is limited and studies that have accurately defined the incidence of brucellosis in human and animals are rather scarce (Dean et al., 2012), different sources suggest that brucellosis is very important for the resource-poor country context and emerging economies (Anon, 2013; Moreno, 2014). If one considers the FAO figures for 2013, 85% of cattle (1.25 billion) and nearly 90% (1.9 billion) of sheep and goats are found in non-industrialized countries.[1] This puts into context the potential scale of this disease in domestic ruminants and the implication this has for public health.

Elucidation of the occurrence and epidemiology of animal brucellosis, as well as its control and eradication, are dependent on several factors, including the judicious use of diagnostic tests. Vaccination, identification of infected animals through use of diagnostic modalities and culling form the instrumental basis of control and eradication strategies (Moreno, 2014). The gold standard diagnostic method in terms of 100% specificity is bacteriological culture, but this method is unpractical for large-scale testing. Molecular methods based on the detection of specific DNA have not yet been proved useful for direct diagnosis (Yu and Nielsen, 2010). On the other hand, indirect 'immunological' diagnostic tests have found wide application.

Since the beginning of the history of brucellosis, almost all types of immunological tests have been investigated for its diagnosis in ruminants (Brinley Morgan, 1967; MacMillan, 1990; McGiven, 2013; Nielsen, 2002). The persistent quest for better diagnostics emphasizes the inexistence of the perfect test (easy and robust, affordable and able to identify all infected individuals while differentiating those that are not infected, have been vaccinated or show antibodies elicited by cross-reacting bacteria) but also the fact that brucellosis is a disease in which the results and applicability of diagnostic tests are affected both by technical issues and complex biological, epidemiological and socio-economic factors. These include the absence of pathognomonic clinical signs, the silent behavior of the pathogen towards the immune system, its intracellular niche, a complex antigenic structure shared by field and vaccine strains, the existence of largely different management conditions of the animal hosts, the facilities available for diagnosis and whether tests are used for control and eradication versus surveillance. The consequence has been the generation of an exceedingly large body of literature (Nielsen, 2002) of difficult interpretation, and a large number of tests and antigens (Table 1) of variable practical value. In this context, the purpose of the present work is to

[1] Figures calculated by totaling FAOSTAT population data for 2013 for the European Union, North America, Australia and New Zealand (industrialized countries) and subtracting this value from the world sheep and goat population (http://faostat3.fao.org/home/E; accessed 05.05.15.).