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A bacterial engineered glycoprotein as a novel antigen for diagnosis of bovine brucellosis

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ABSTRACT

Brucellosis is a highly contagious zoonosis that affects livestock and human beings. Laboratory diagnosis of bovine brucellosis mainly relies on serological diagnosis using serum and/or milk samples. Although there are several serological tests with different diagnostic performance and capacity to differentiate vaccinated from infected animals, there is still no standardized reference antigen for the disease. Here we validate the first recombinant glycoprotein antigen, an *N*-formylperosamine *O*-polysaccharide-protein conjugate (OAg-AcrA), for diagnosis of bovine brucellosis. This antigen can be produced in homogeneous batches without the need of culturing pathogenic brucellae; all characteristics that make it appropriate for standardization. An indirect immunoassay based on the detection of anti *O*-polysaccharide IgG antibodies in bovine samples was developed coupling OAg-AcrA to magnetic beads or ELISA plates. As a proof of concept and to validate the antigen, we analyzed serum, whole blood and milk samples obtained from non-infected, experimentally infected and vaccinated animals included in a vaccination/infection trial performed in our laboratory as well as more than 1000 serum and milk samples obtained from naturally infected and S19-vaccinated animals from Argentina. Our results demonstrate that OAg-AcrA-based assays are highly accurate for diagnosis of bovine brucellosis, even in vaccinated herds, using different types of samples and in different platforms. We propose this novel recombinant glycoprotein as an antigen suitable for the development of new standard immunological tests for screening and confirmatory diagnosis of bovine brucellosis in regions or countries with brucellosis-control programs.

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1. Introduction

Brucellosis is a highly contagious zoonosis caused by Gram-negative bacteria of the genus *Brucella* that affects livestock, wild animal species and humans. The main pathogenic species worldwide are *B. abortus*, responsible for bovine brucellosis; *B. melitensis*, the major etiologic agent of ovine and caprine brucellosis; and *B. suis*, responsible for swine brucellosis (Corbel, 1997). These three *Brucella* species remain an important cause of veterinary morbidity and mortality. Brucellosis causes important economic losses not only because it affects animal production (reduced milk, abortion, delayed conception and impaired fertility) but also because detection of the disease in a region or country imposes, due to international veterinary regulations, restrictions on animal movements and trade (Seleem et al., 2010). Brucellosis in humans can be severely debilitating and disabling and remains an important public health concern (Young, 1995). In the absence of a human brucellosis vaccine, prevention of the disease depends predominantly on control of brucellosis in animals that constitute the natural disease's reservoirs (Godfroid et al., 2010). For these reasons, many programs have been implemented worldwide to control and/or eradicate brucellosis mainly in cattle, small ruminants, and pigs.

Control of bovine brucellosis depends on vaccination and/or detection of infected animals and slaughter. Due to the lack of pathognomonic signs for the clinical diagnosis of brucellosis in animals, laboratory confirmation of bovine brucellosis by isolation of the pathogen or demonstration of specific antibodies is essential. Bacteriological isolation of the microorganism confirms the diagnosis; however, slow growth of brucellae primary cultures (up to 7 days), the risk involved in their handling and poor sensitivity makes diagnosis based exclusively on isolation of brucellae not always feasible and effective. Therefore, laboratory diagnosis mainly relies on serological diagnosis using serum and/or milk samples (Godfroid et al., 2010; Saegerman et al., 2010).

The most commonly used serological tests for diagnosis of bovine brucellosis are the agglutination tests such as the buffered plate agglutination test (BPAT), rose Bengal plate agglutination test (RBPT) and serum agglutination test (SAT), and the complement fixation test (CFT) (Aznar et al., 2012; DILAB-SENASA, 2009; Godfroid et al., 2010; OIE, 2012). Indirect enzyme-linked immunosorbent assays (iELISA) have also been developed for serum and m. For analyzing individual and bulk tank milk samples the milk ring test (MRT) is the most widely used test (Gall and Nielsen, 2004). All these assays use as antigen the whole bacteria, bacterial extracts containing high concentrations of smooth lipopolysaccharide (sLPS) or purified sLPS. These tests suffer from false positive reactions and, because S19 vaccine is antigenically similar to virulent strains, does not allow a precise differentiation of vaccinated from infected animals. Consequently, other tests have been developed including the competitive ELISA (CELISA) and the fluorescence polarization assay (FPA), which have eliminated most reactions due to cross-reacting antibodies and residual antibodies produced in response to S19 vaccination (McGiven et al., 2003; Nielsen, 1990; Nielsen et al., 1995, 1996). Both assays measure specific antibodies against the immunodominant *O*-polysaccharide section of the LPS (Caroff et al., 1984b). Additionally, a common problem to all serological tests currently used for diagnosis of brucellosis is the lack of a standardized reference antigen, and one of the main reasons for this is the source of the antigens as well as the preparation methods used (Al Dahouk et al., 2003). Therefore, for harmonization of the serological diagnosis of brucellosis there is a need to develop a new reference antigen easy to standardize.

Previously, we have produced and characterized a recombinant glycoprotein consisting of a homopolymer of *N*-formylperosamine, the *O*-polysaccharide of *B. abortus* (OAg) covalently linked to the carrier protein AcrA (hereinafter OAg-AcrA) (Iwashkiw et al., 2012). OAg-AcrA was produced using an in vivo engineered bacterial glycosylation system based on the combination of the LPS biosynthesis pathway of *Yersinia enterocolitica O:9*, whose O-polysaccharide is identical to the *B. abortus* OAg, and the *N*-glycosylation pathway of *Campylobacter jejuni* (Feldman et al., 2005). OAg-AcrA was applied to the development and validation of a new indirect immunoassay for diagnosis of human brucellosis using magnetic beads (Ciocchini et al., 2013). We have demonstrated that the assay has an excellent diagnostic performance allowing the detection of infection caused by the three main human brucellosis agents; *B. abortus*, *B. melitensis* and *B. suis.*

Here we validate for the first time a recombinant glycoprotein antigen for diagnosis of bovine brucellosis using serum, whole blood and milk samples under two different platforms. Our results demonstrate that OAg-AcrA is a new standardizable antigen that allows the development of highly accurate glycoprotein-based immunodiagnostics for bovine brucellosis.

2. Materials and methods

2.1. Production and purification of the O:9-polysaccharide-protein conjugate (OAg-AcrA)

Production and purification of the recombinant glycoprotein OAg-AcrA was performed as previously described (Iwashkiw et al., 2012). *Y. enterocolitica O:9* wild type strain transformed with the plasmids pMAF10 (encoding the *C. jejuni* oligosaccharyltransferase OTase PglB) and pMH5 (encoding the *C. jejuni* carrier protein AcrA fused to an histidine tag) was grown overnight at 37 °C in LB media, grown at 37 °C for 2.5 h ($OD_{600} \sim 0.5$) and PglBCj expression was induced with arabinose 0.2% (w/v). Four hours after induction at 37 °C, PglBCj was re-induced by a second addition of arabinose to maximize glycosylation of AcrA. Cells were harvested by centrifugation after 20 h of induction and periplasmic extracts were prepared by lysozyme treatment as described in Feldman et al. (2005) and subjected to Ni^{2+} affinity chromatography.

2.2. OAg-AcrA magnetic beads-based immunoassay (glyco-beads assay) development and optimization

Superparamagnetic COOH-modified microbeads (Bangs Laboratories, Inc.) were activated in one step