Acta Tropica 160 (2016) 58–61

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Brucella abortus RB51 in milk of vaccinated adult cattle

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ARTICLE INFO

Article history:
Received 14 January 2016
Received in revised form 20 April 2016
Accepted 23 April 2016
Available online 30 April 2016

Keywords:
Cattle
Milk
Brucellosis
Vaccine
RB51
Vaccination

ABSTRACT

The aim of this study was to evaluate the shedding of *Brucella abortus* in the milk of cows vaccinated with a full dose of RB51 during lactation. Eighteen cows, nine previously vaccinated with S19 as calves and nine non-vaccinated, were immunized subcutaneously with 1.3×10^{10} CFU of *B. abortus* RB51, 30–60 days after parturition. Milk samples from all animals were collected daily until day 7, and at weekly interval for the next 9 weeks after vaccination. To evaluate the shedding of *B. abortus*, milk samples were submitted for culture and PCR. No *B. abortus* was isolated from any sample tested. Only one sample, collected on first day after vaccination from a cow previously vaccinated, was faintly positive in the PCR. In conclusion, the public health hazard associated with milk consumption from cows vaccinated with RB51 in post-partum is very low, despite vaccination with the full dose and regardless of previous S19 vaccination.

1. Introduction

Brucellosis caused by *Brucella abortus* is a chronic disease of cattle of worldwide economic and public health importance (Corbel et al., 2006). In Brazil, brucellosis is widely distributed (Poester et al., 2002), with herd prevalences ranging from 0.32% (Sikusawa et al., 2009) to 41.2% (Negreiros et al., 2009) among states. Vaccination of cattle is one of the most effective measure to reduce the prevalence of brucellosis, being successfully used in many control and eradication programs (Olsen and Stoffregen, 2005; Dorneles et al., 2015).

Brucella abortus RB51 vaccine strain is a lipopolysaccharide O-antigen deficient mutant derived from the virulent strain *B. abortus* 2308, which does not induce an antibody response detectable by routine serological tests (Schurig et al., 1991). This feature allows RB51 vaccination to be performed at any age, while vaccination with S19 is normally restricted to calves between 3 and 8 months of age (Brasil, 2006; Corbel et al., 2006). In Brazil, and in some other countries, RB51 is approved for vaccination of heifers older than 8 months (Poester and Gonçalves, 2006; Brasil, 2006, 2007). As the majority of the Brazilian adult cattle population was never vaccinated against brucellosis and the major current strategy of Programa Nacional de Controle e Erradicação de Brucelose e Tuberculose (National Program on the Control and Eradication of brucellosis and tuberculosis) (Brasil, 2006) is based on a vaccination program using Strains 19 and RB51, the vaccination of lactating cows with RB51 is prone to become frequent. Hence, many concerns on the public health safety of RB51 vaccination of adult animals are raised, as there are only scanty data on the shedding of RB51 in the milk (Samartino et al., 1999; Samartino and Fort, 2000; Uzal et al., 2000).

Additionally, the colonization of mammary gland and associated lymph nodes by *B. abortus* have been demonstrated (Carvalho-Neta et al., 2010), and organisms may be excreted in the milk (Xavier et al., 2009). Foodborne transmission of *Brucella* spp. is well known and is especially common through the consumption of raw milk and cheese made with unpasteurized milk (Godfroid and Cloeckaert, 2005).

Considering the enormous importance of vaccination with RB51 for the control of bovine brucellosis and its possible impacts on

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http://dx.doi.org/10.1016/j.actatropica.2016.04.012
0001-706X/

public health, and the scarcity of data available in the literature, the aim of this study was to evaluate, by culture and PCR, the shedding of *B. abortus* in the milk of cows vaccinated with full dose of RB51 during lactation.

2. Materials and methods

2.1. Animals

Eighteen Holstein-zebu crossbred cows from brucellosis-free herds, ranging in age from 2.1 to 5 years, and that calved 30–60 days previously, were used in the study. They were divided in two groups. Group 1 was composed of nine cows selected from the herd of the Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG), at Pedro Leopoldo, Minas Gerais State, Brazil. All animals were vaccinated with S19 as calves (between three and eight months of age) (Brasil, 2006). The nine cows in group 2 were selected from a serologically confirmed brucellosis-free herd at Lages, Santa Catarina State, Brazil. The State of Santa Catarina has the lowest prevalence of brucellosis in the country and vaccination with S19 is prohibited (Brasil, 2004; Sikusawa et al., 2009). All cows were raised semi-intensively and fed a balanced diet of corn silage, concentrate and mineral salt mixture. This study was approved by the Ethical Committee for the use of Experimental Animals of the Universidade Federal de Minas Gerais, Brazil (CETEA) under protocol 136/2010.

2.2. Vaccination

On day 0 of the experiment, the eighteen cows were subcutaneously vaccinated with 1.3×10^{10} CFU of viable *B. abortus* strain RB51 (Brasil, 2007), prepared according to OIE (2009).

2.3. Milk sampling

Milk samples were collected in sterile polypropylene tubes on days 1, 2, 3, 4 and 7, and thereafter once a week from second week (day 14) until ninth week (day 63) after vaccination. Teats were disinfected using 70% alcohol before sampling. The first milk streams of each teat were discarded, and then 50 mL of milk samples were manually collected from all quarters and stored at −20 °C.

2.4. Bacteriology of the milk

Samples were thawed and centrifuged at 2500*g*, for 15 min. The intermediate phase was discarded and the fat layer was mixed with the pellet. The mixtures were immediately inoculated onto tryptose agar (BD Difco, Franklin Lakes, New Jersey, USA) plates with antibiotics [Farrell's selective supplement (Oxoid, Basingstoke, Hampshire, UK)] in duplicates (Alton et al., 1988). Moreover, 1.0 mL of each mixture was diluted in 9.0 mL of enrichment media (tryptose broth with Farrell's selective supplement) and incubated with 5% CO_2 at 37 °C for 7 days, and then inoculated on tryptose agar plates with antibiotics (Minharro, 2009). All plates were incubated in 5% CO_2 at 37 °C for 9 days, before being discarded and considered negative. Any bacterial colony suspect of *Brucella* spp. was identified by biochemical tests (Alton et al., 1988) and PCR (Baily et al., 1992; Bricker and Halling, 1995).

2.5. PCR assays

Extraction of DNA from milk samples was performed according to Pitcher et al. (1989). PCR assay for detection of *Brucella* spp. (genus-specific PCR) was carried out as described by Baily et al. (1992). Positive results in genus-specific PCR were tested by the multiplex AMOS-enhanced PCR according to Bricker and Halling (1995), to confirm the presence of RB51.

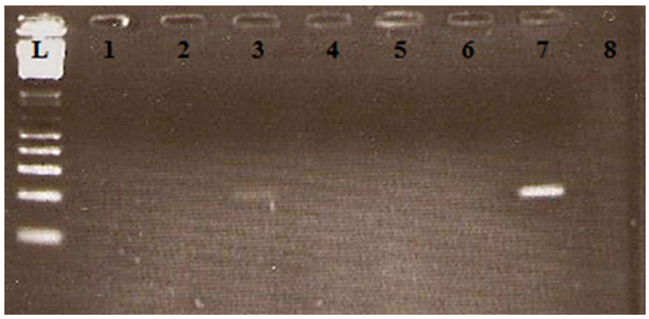

Fig. 1. Agarose gel (1%) stained with ethidium bromide (1%) of *Brucella* spp. specific PCR performed from milk samples of RB51 vaccinated cows. The PCR was performed in milk samples collected from cows that calved 30–60 days previously, on day 1 after RB51 vaccination (lanes 1–6). This cows were also vaccinated with S19 as calves (group 1). L – 1 Kb plus DNA Ladder marker (Invitrogen, USA). Positive (RB51 in milk) and negative control were shown in lanes 7 and 8, respectively.

2.6. Statistical analysis

To assess the statistical significance of the results a power analysis was performed as described by Cohen (1988), using the *pwr* package (Champely, 2015) on the R software version 3.2.2 (R Development Core Team, 2015).

3. Results

Brucella abortus RB51 vaccine strain was not recovered from any of the milk samples tested by conventional bacteriological methods.

A single milk sample from one animal from Group 1, previously vaccinated with S19 as calf, on day 1 presented a faint band in *Brucella* genus-specific PCR (Baily et al., 1992) (Fig. 1, lane 3). The sample amplified by the genus-specific PCR was further confirmed as Strain RB51 by AMOS-enhanced PCR (Bricker and Halling, 1995).

The power of the analysis was greater than 0.995.

4. Discussion

Strain RB51 is a live attenuated *B. abortus* vaccine approved in Brazil for use in adult cattle with full calf dose (1.3×10^{10} CFU/dose) (Brasil, 2007). As lactating cows will also be vaccinated, that creates a real concern of local authorities about the possible threat to public health. The present study investigated, using very sensitive and specific techniques, if vaccination of cows during lactation leads to excretion of viable RB51 vaccine strain in milk up to nine weeks post-vaccination, and concluded that the public health hazard associated with the consumption of milk from post-partum RB51-vaccinated cows is very low, since RB51 strain was not found in 233 out of the 234 milk samples (99.6%).

Our results showed that *B. abortus* strain RB51 was not isolated from any of the milk samples tested during nine weeks (63 days) post-vaccination. The use of selective media for direct plating of suspected samples could reduce the number of *Brucella* spp. colonies, and consequently could result in reduction of sensitivity of the diagnosis (Marin et al., 1996). In order to overcome that and improve the sensitivity of the culture, reducing false-negative results, enrichment in tryptose broth supplemented with antimicrobials (Farrell's supplement) was used before plating. This approach is reported to enhance the rate of isolation of *B. abortus* from infected animals by 50% (Minharro, 2009) and has an analytical sensitivity of 200 *B. abortus* CFUs/mL of milk (data not shown).