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Serological profile of buffalo (*Bubalus bubalis*) female calves vaccinated with standard *Brucella abortus* strain 19 vaccine using rose bengal, 2-mercaptoethanol and complement fixation tests

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ABSTRACT

The serological profiles of 21 female buffaloes vaccinated between 3 and 8 months of age using *Brucella abortus* strain 19 (S19) were evaluated by rose bengal (RBT), 2-mercaptoethanol (2ME) and complement fixation (CFT) tests. The serum strains were collected in day zero, 15, 30, 45, 60th days and subsequently to each 30 months, until 720th day after vaccination. No animal showed reaction in day zero. In 15th day above 95% of animals revealed reaction in all tests. All the animals presented absence of reactions in CFT, RBT and 2ME tests at 270, 300 and 360 days after vaccination, respectively. Our finding highlighted early response in CFT compared than other conventional agglutination tests. None of animals presented oscillation of titers or reactions in any test after 360 day of study, which enables the use of these tests after this period without interference of antibodies from S19 vaccine origin between 3 and 8 months in buffalo heifers.

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1. Introduction

Buffalo present as peculiar characteristics the great hardiness and adaptability to different topography, soils and climatic factors, allied to ability to production of meat and milk, representing a good alternative for the breeding livestock especially in tropical countries. In South of America, buffalo population is estimated about 4 million of animals, and from these 3.5 million are raised in Brazil. The contact of buffaloes with bovine or other livestock and wildlife, and the access to several ecosystems, become this species exposed to different infectious diseases, including brucellosis [1].

Brucellosis remains one of the most common zoonotic diseases worldwide [2]. Beside the public health concern, *Brucella* infections in livestock species also represent a great economic impact particularly in developing countries due to reproductive problems, reduced milk production [3], and restrictions on animal movements and trade, imposed by international regulatory norms [4].

Brucellosis in buffalo is caused by *Brucella abortus* (*B. abortus*), characterized by abortion predominantly in third trimester of gestation, non viable calves, infertility and reduction of milk production [5].

In countries and regions with high prevalence of disease in livestock species, the most effective measures to controlling and eradicating the disease are based on vaccination of all susceptible host, serologic tests and elimination of positive animals [3,6,7]. Vaccination is a critical tool to control or eradicate bovine and buffalo brucellosis, because prevents abortion and consequent pasture contamination, recognized as major form to transmission of *B. abortus* to these species [7]. Despite recent approved RB51 strain for use in some countries, *B. abortus* strain 19 (S19) remains the most commonly vaccine recommended against bovine and buffalo brucellosis worldwide [8–11]. The use of S19 vaccine in bovine leads to protection in cattle approximately up to nine years of age, with 65–75% of protection in all vaccinated animals throughout their productive life. However, production of immunoglobulins (Ig) and persistence of titers induced by strain S19 depend particularly of age of animals at the time of vaccination [3].

In Brazil, brucellosis in livestock is considered endemic in several states or regions [9,12]. *B. abortus* infections in bovine and buffaloes in this country are associated mainly with presence of abortion [9,13]. A few regional serological studies have revealed the occurrence of disease in buffaloes in this country [14–16].

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Brazilian Program for Control and Eradication of Brucellosis - PNCEBT [17] in bovine and buffalos was started in 2001. The major strategies recommended of PNCEBT are summarized as follows: (1) compulsory vaccination of bovine and buffalo heifers aged 3–8 months using S19 (*B. abortus*); (2) voluntary monitoring of seroprevalence in beef and dairy herds based on a periodic sampling collection, using milk ring test, rose bengal, 2-mercaptoethanol and/or complement fixation tests; (3) regulatory tests for animals prior to interstate transport and participation on fairs and exhibitions; (4) compulsory slaughtered of positive animals in approved abattoirs; (5) standardization of testing procedures through courses for accredited veterinarians; (6) voluntary accreditations of free herds, in accordance with international standards. Furthermore, heifers vaccinated at the recommended age are not eligible for serologic testing up to 24-month-old to avoid interference with antibodies induced by the S19 vaccine [9].

Conventional serologic test for bovine and buffalo brucellosis detect antibody against the LPS antigens induced by vaccination with S19 or exposure to virulent field *Brucella*. Indeed, no single serological test can differentiate without any doubt animals vaccinated with S19 and animals infected with virulent field strains [4]. In this context, serodiagnosis of buffalo brucellosis in different countries [18–20] and in Brazil [21,22], have focused predominantly the comparison of different serologic tests in animals. However, little information is available about serological profile and persistance of Ig in buffalo calves vaccinated with standard S19 [1,23]. The persistence of Ig in buffalo calves vaccinated with standard S19 can cause difficulties in serodiagnosis of disease using conventional methods [5,24].

Thus, the purpose of this study was investigate the serologic profile anti-*B. abortus* in buffalo calves vaccinated between 3 and 8 months of age with the S19, using rose bengal, 2-mercaptoethanol and complement fixation tests.

2. Materials and methods

2.1. Animals

The study was conducted in a delimited area from Department of Animal Production, located at the Lageado Experimental-Farm on the School of Veterinary Medicine and Animal Science, UNESP-Univ Estadual Paulista, Botucatu, state of Sao Paulo, Brazil. The animals were kept in 15 ha paddocks of *Brachiaria brizantha* pasture throughout the experiment. Paddocks were separated by a single fence.

Twenty-one female buffalo calves (*Bubalus bubalis*), between 3 and 8 months old received *B. abortus* vaccine S19. All the heifers are feed with pasture, received mineral salt mixture and were submitted to same management conditions. None of heifers had been previously vaccinated with S19 strain. Non-vaccinated group of animals was not established due to compulsory vaccination of buffalo heifers imposed by Brazilian Program for Control and Eradication of Brucellosis [17].

2.2. Vaccination and serum samples

The vaccine used consisted of a commercial product, prepared with the standard strain S19 (*B. abortus*). Prior to vaccination, the commercial vaccine was evaluated for purity, dissociation, and number of viable cells [25]. The animals were vaccinated with a single 2 mL dose, by subcutaneous route, containing $5.0–8.0 \times 10^{10}$ viable cells [26,27], according to the manufacturer's recommendations.

Before brucellosis vaccination, blood samples of 21 animals were collected constituted day zero of study. Subsequently, all the animals were sampled every 15 days (15th, 30th, 45th and 60th days post-vaccination) and after in intervals of 30 days (90th, 120th, 150th, 180th, 210th, 240th, 270th, 300th, 330th, 360th, 390th, 420th, 450th, 480th, 510th, 540th, 570th, 600th, 630th, 660th, 690th and 720th day post-vaccination), resulting in the monitoring of animals for more than two years old. Serum samples were centrifuged, separated in aliquots, and stored at −20 °C. Furthermore, all the animals were vaccinated against foot-and-mouth disease in 120th, 300th, 480th and 660th days of monitoring, according to Brazilian Program for Control and Prevention of foot-and-mouth disease [28].

2.3. Serological diagnosis

The serological diagnosis was performed using 2-mercaptoethanol (2ME), complement fixation (CFT), and acidified antigen in plates stained with rose bengal (RBT) tests. 2-ME, CFT and RBT were performed using antigens and procedures as described previously [26,27,29–32] and according to the Brazilian Program for Control and Eradication of Brucellosis - PNCEBT [17]. The antigens were prepared using *B. abortus* 1119/3 strain produced by Instituto de Tecnologia do Paraná-TECPAR, Curitiba, Brazil. The RBT antigen was stained and buffered at pH 3.65 ± 0.05. The Brazilian official norm [17] considers an RBT positive or negative test when there is presence or absence of agglutination, respectively, after 4 min of initiate the test. The 2 ME was performed by adding 80, 40, 20 and 10 μL of serum to four tubes. Latter, one mL of 0.1 M mercaptoethanol in 0.85 g% NaCl and 1 mL of *Brucella* antigen in 0.85 g% NaCl were added [33]. The tubes were shaken, incubated by 48 h and reactions were read as recommended by PNCEBT [17]. Complete agglutination at 1:25 dilution or more in the 2 ME was considered positive. CFT positive test was considered when at least 50% hemolysis at a serum dilution ≥20 ICFTU (international complement fixation test units)/ml [25,27].

3. Results

Fig. 1 show the serological profile of heifers vaccinated between 3 and 8 months of age using S19, during 720 days after vaccination. In day zero (day of vaccination) the 21 animals were negative in all tests. On the 15th day post-vaccination, over 95% of the animals revealed reactions in all tests. Higher titers on 2 ME were obtained between 15th to 45th days after vaccination. Among 120 and 150

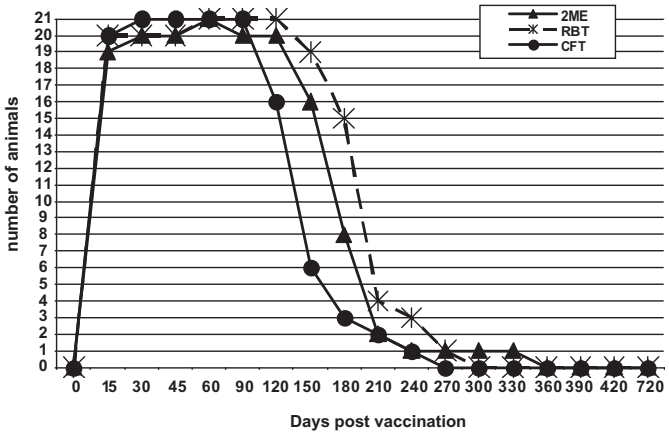

Fig. 1. Serological profile of buffalo heifers vaccinated with standard *Brucella abortus* S19 strain, 3 between 3 and 8 months of age, using rose bengal (RBT), 2-mercaptoethanol (2ME) and complement 4 fixation (CFT) tests, during 720 days of monitoring after vaccination. Ref. [28].

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