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Clinical, hematological and biochemical parameters of dairy cows experimentally infected with *Vaccinia virus*

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ABSTRACT

Vaccinia virus (VACV) is the etiological agent of bovine vaccinia (BV), an important zoonosis that affects dairy cattle. There are many aspects of the disease that remain unknown, and aiming to answer some of these questions, the clinical, hematological, and biochemical parameters of VACV experimentally infected cows were evaluated. In the first part of the study, lactating cows were infected with VACV-GP2 strain. In the second part, animals previously infected with VACV-GP2 were divided into two treatment groups: Group 1, immunosuppressed cows; and Group 2, re-infected cows. In this study, BV could be experimentally reproduced, with similar lesions as observed in natural infections. Moreover, a short incubation period and local lymphadenopathy were also observed. VACV could be detected by PCR and isolated from scabs taken from teat lesions of all inoculated and re-inoculated animals. Lymphocytosis and neutrophilia were observed in all animals from the first part of the experiment, and lymphopenia and relative neutrophilia were observed in the immunosuppressed animals. Detection of viral DNA in oral mucosa lesions suggests that viral reactivation might occur in immunosuppressed animals. Moreover, clinical disease with teat lesions may occur in previously VACV-infected cows under the experimental conditions of the present study.

1. Introduction

Vaccinia virus (VACV) is the prototype virus of the family *Poxviridae*, genus *Orthopoxvirus*, and it was used in smallpox vaccines in the last century. Since 1999, several outbreaks of VACV in Brazil, affecting cattle and humans, have resulted in serious economic losses and have had a major impact on public health (Lobato et al., 2005). The disease caused by VACV in cows is known as bovine vaccinia (BV), and it causes a significant reduction in milk production and increases susceptibility to secondary bacterial infections, such as mastitis (Trindade et al., 2003; Lobato et al., 2005). In naturally infected cows, BV lesions begin with red papules on the skin of teats and sometimes the udder, evolving to vesicles. Three to four days later, these lesions become umbilicated pustules surrounded by inflammatory tissue and then turn into ulcers, until complete wound healing occurs. Lesions on the lips, muzzle and mouth of the calves that suckled affected cows are often observed (Trindade et al., 2008). In humans, infection is characterized by the presence of ulcerative and pustular lesions on the hands, forearms and face, in addition to fever, pain and lymphadenopathy (Lobato et al., 2005).

Previous studies revealed the presence of VACV DNA as well as infectious virus in milk from naturally infected cows (Abrahão et al., 2009a). Moreover, infectious VACV was recovered from cheese and cheese whey produced with experimentally contaminated milk (de Oliveira et al., 2010). Although no cases of VACV human infection have been identified in Brazil through the consumption of milk from diseased cows, a strain of VACV, Buffalopox virus (BPXV), which affects buffaloes in India, was described as the etiological agent of oropharyngeal lesions in humans who consumed unpasteurized milk (reviewed by Berkelman, 2003).

Although some studies have addressed the molecular characteristics of VACV strains and some aspects of BV epidemiology, there is little information about other aspects of BV epidemiology and

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pathogenesis (Trindade et al., 2008). Due to the difficulties in assessing BV in the field, reports of outbreaks are sparse. Furthermore, when they do occur, the lesions are often in advanced stages or are already healed when a research team arrives at the farm. To our knowledge, a detailed investigation of the pathogenesis of bovine VACV infection has not been reported.

The aim of this work was to experimentally infect dairy cows with VACV strain Guarani P2 (VACV-GP2) and evaluate the clinical, hematological and biochemical parameters associated with primary infection. In addition, we evaluated the clinical, hematological and biochemical parameters after reinfection and after the administration of an immunosuppressive drug.

2. Materials and methods

2.1. Virus

The VACV Guarani P2 (VACV-GP2) strain, which was isolated from a cow in an outbreak of BV in Brazil (Trindade et al., 2006), was used to inoculate and re-inoculate the animals. The virus was multiplied and purified according to Campos and Kroon (1993).

2.2. Animals and inoculation

The study was divided into two parts.

2.2.1. First part

Eight crossbred, lactating dairy cows, seronegative for *Orthopoxvirus* and aged between three and ten years old, were used. The cows' teats were divided into cranial and caudal regions. Three teats of each cow had their epidermis abraded with sandpaper (number 4, JX-41, Lixas Doble A, Argentina) and were inoculated with 50 μl of 10^6 PFU/ml (plaque forming units per milliliter) of VACV-GP2. The left posterior teat (LPT) of each cow was abraded, but not inoculated. The animals were monitored daily for 32 days.

2.2.2. Second part

Nine cows previously infected with VACV-GP2 were divided into two groups. This study began 70 days after of the first inoculation.

2.2.2.1. Group 1 (G1). Four dairy cows from the first experiment were treated daily with dexamethasone (DMS) at 0.1 mg/kg IM for 5 days (Iketani et al., 2002), to study possible virus reactivation. The animals were monitored for 12 days.

2.2.2.2. Group 2 (G2). Five dairy cows, three from the first part of this study and two additional cows that were experimentally infected with the same strain of VACV, 240 days prior, were re-inoculated with VACV-GP2, to determine if re-infection was possible. The epidermal abrasion and inoculation were performed in the same way as described in the first part of the study. The only difference was that all teats were inoculated. The animals were monitored for 15 days after the second inoculation.

2.3. Experimental facilities

The animals were housed in pairs in four isolated rooms in microbiologically secure (NB-2) animal pens at the National Agricultural Laboratory – Ministry of Agriculture, Livestock and Food Supply in Pedro Leopoldo city, Minas Gerais, Brazil. The animals were fed a complete balanced diet and water *ad libitum*. All cows were slaughtered at the end of the second part of the study, after a full clinical recovery.

2.4. Clinical assessment and samples collection

2.4.1. First part

Clinical evaluation of the teat lesions was performed daily beginning on day 0 before the inoculation until day 32 post-inoculation. Systemic clinical evaluations were performed at day 0 before the inoculation and on alternate days until the 32nd day post-inoculation (d.p.i). The parameters that were observed were the following: body temperature, cardiac and respiratory rates, rumenal movements and a detailed analysis of the skin, external mucosa and superficial lymph nodes. Milk was collected once a day by hand milking and milk production from each cow was measured every day. The uninfected teat of each cow was milked first to avoid contamination. Serum samples were collected daily for antibody detection and twice a week to evaluate biochemical parameters and hepatic and kidney functions. Blood samples were collected twice a week to evaluate hematological parameters. Scabs collected from teat lesions from all animals were collected for VACV DNA detection by a PCR reaction and for viral isolation, as well as swabs taken from lesions observed in the oral mucosa.

2.4.2. Second part

2.4.2.1. Group 1 (G1). The animals were observed for 12 days after the first injection of DMS. A complete clinical examination (including vital parameters and a detailed analysis of the skin, mucosa and superficial lymph nodes) was performed daily from day one before immunosuppression to the 12th day post-treatment. The cows were milked every day. Serum samples were collected before the treatment, as well as every day after treatment for antibody detection, and to evaluate biochemical parameters and hepatic and kidney functions. A blood sample was collected daily to evaluate the hematological parameters to check if the cows were immunosuppressed. Swabs were taken from lesions observed in the oral mucosa for VACV DNA detection by PCR reaction.

2.4.2.2. Group 2 (G2). A complete clinical examination was performed one day before re-inoculation and on alternate days until the 15th d.p.i. Milk was collected once a day by hand milking. Serum samples were collected before the re-inoculation and daily after treatment, for antibody detection. Blood samples, including sera, were collected twice a week to evaluate hematological and biochemical parameters, as well as hepatic and kidney functions. Scabs taken from lesions on the teats were collected for VACV DNA detection by PCR and for virus isolation.

2.5. Milk quality tests

Somatic cells counts (SCC) were performed daily in collected milk samples in the first part of the study to detect clinical and subclinical mastitis and to evaluate milk quality. SCC tests were performed at the "Milk Analysis Laboratory" at the Veterinary School, Universidade Federal de Minas Gerais (UFMG).

2.6. Serology

Serum samples were submitted to an immunoperoxidase monolayer assay (IPMA) (Gerber et al., 2012) and a serum-neutralization (SN) test, to detect total antibodies (TA) and neutralizing antibodies (NA), respectively. The IPMA test used was optimized to detect total anti-VACV IgG antibodies at the "Research on Animal Virology Laboratory" at the Veterinary School, UFMG (Gerber et al., 2012). For SN, serum was heated at 56 °C for 30 min and used in a SN assay as described by Lobato et al. (2005). The neutralization titer was expressed as the reciprocal of the highest dilution that inhibited 50% of plaque formation compared to positive and negative controls.