Microbial Pathogenesis 52 (2012) 192–199

Contents lists available at SciVerse ScienceDirect

Microbial Pathogenesis

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

Vaccinia viruses isolated from cutaneous disease in horses are highly virulent for rabbits

Juliana Felipetto Cargnelutti [a], Candice Schmidt [a], Eduardo Kenji Masuda [b], Lisiane Danusa Braum [a], Rudi Weiblen [a], Eduardo Furtado Flores [a,*]

[a] Setor de Virologia, Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria, Av. Roraima, 1000 Camobi, Santa Maria, Rio Grande do Sul, CEP 97105-900, Brazil
[b] Laboratório de Patologia Veterinária, Departamento de Patologia, Av. Roraima, 1000 Camobi, Santa Maria, Rio Grande do Sul, CEP 97105-900, Brazil

ARTICLE INFO

Article history:
Received 25 July 2011
Received in revised form
21 December 2011
Accepted 23 December 2011
Available online 29 December 2011

Keywords:
Orthopoxvirus
Pathogenesis
Virulence
Animal model

ABSTRACT

Two genotypically distinct *Vaccinia viruses* (VACV), named P1V and P2V, were isolated from an outbreak of cutaneous disease in horses in Southern Brazil. We herein investigated the susceptibility of rabbits, a proposed animal model, to P1V and P2V infection. Groups of weanling rabbits were inoculated intranasally (IN) with P1V or P2V at low ($10^{2.5}$ $TCID_{50}$), medium ($10^{4.5}TCID_{50}$), or high titer ($10^{6.5}TCID_{50}$). Rabbits inoculated with medium and high titers shed virus in nasal secretions and developed serous to hemorrhagic nasal discharge and severe respiratory distress, followed by progressive apathy and high lethality. Clinical signs appeared around days 3–6 post-inoculation (pi) and lasted up to the day of death or euthanasia (around days 5–10). Virus shedding and clinical signs were less frequent in rabbits inoculated with low virus titers. Viremia was detected in all groups, with different frequencies. Viral DNA was detected in the feces of a few animals inoculated with P1V and P2V, low titer, and with P2V at high titer. Gross necropsy findings and histological examination showed diffuse interstitial fibrousing pneumonia with necrosuppurative bronchopneumonia and intestinal liquid content. Neutralizing antibodies were detected in all inoculated animals surviving beyond day 9pi. These results show that rabbits are highly susceptible to VACV isolated from horses, and develop severe respiratory and systemic disease upon IN inoculation. Thus, rabbits may be used to study selected aspects of VACV infection and disease.

1. Introduction

Vaccinia virus (VACV) is an *Orthopoxvirus*, family Poxviridae, associated with exanthematic and vesicular cutaneous disease in cattle, buffaloes and humans [1]. The origin of VACV is still unknown and several hypotheses have been proposed. An evolution from the variola virus (VARV), evolution from cowpox virus (CPXV), recombination between VARV and CPXV and a possible origin from an extinct animal species or virus are among the speculated origins [2]. Due to its low virulence for humans and antigenic similarity with VARV, VACV strains were used during decades in the vaccine employed in the World Program of Eradication of Smallpox [3].

A number of outbreaks of exanthematic and vesicular disease affecting dairy cows and milkers has been reported in the last decade in Southeast Brazil [4–6]. The disease has assumed considerable importance in public and animal health in some rural communities [6–8]. VACV strains have been repeatedly isolated from these outbreaks [7–9], yet their origin and epidemiology remain uncertain. Characterization of VACV strains involved in these outbreaks has led to the identification of two distinct groups of viruses (VACV groups 1 and 2), differing from each other in genetic and biological aspects [10]. Although genetically distinct and displaying different virulence for mice, viruses from both VACV groups cause a clinically indistinguishable disease in cattle [9,11]. The surprising emergence of VACV in Brazil has suscited interest in elucidating their origin, ecology and epidemiology [11–13].

Our group reported an outbreak of cutaneous disease in horses in Southern Brazil (Pelotas county, Rio Grande do Sul state, Brazil), in which a mixed VACV infection was demonstrated [14,15]. Genetic and biological analysis of equine isolates revealed a co-infection with two VACV strains belonging to distinct groups, named thereafter Pelotas 1 (P1V) and Pelotas 2 virus (P2V) [15]. These findings were somewhat unexpected since natural VACV infections in horses are extremely rare [16]. Likewise, equine VACV infections have never been reported in Brazil, even in areas and herds

* Corresponding author. Tel./fax: +55 55 3220 8034.
E-mail address: eduardofurtadoflores@gmail.com (E. Furtado Flores).

0882-4010/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micpath.2011.12.005

experiencing cattle and human disease. In addition, the location of this outbreak was far distant from the previously reported VACV cases in cattle and human.

Thus, we decided to investigate some aspects of the biology, epidemiology and pathogenesis of these horse VACV strains. We first sought to investigate the susceptibility of rabbits to P1V and P2V after intranasally (IN) inoculation.

2. Material and methods

2.1. Cells and viruses

Vero cells (*African Green Monkey)* were used for virus amplification, quantitation and virus isolation. Cells were cultured in RPMI medium, containing ampicillin (1.6 mg/L), streptomycin (0.4 mg/L) and amphotericin B (2.25 mg/L), supplemented with 10% bovine fetal serum. P1V and P2V strains were isolated concomitantly from sick horses in an outbreak of cutaneous disease in Southern Brazil [14]. Preliminary characterization of PV1 and PV2 has been carried out by Brum et al. [14] and by Campos et al. [15]. Cell cultures and virus growth were performed at 37 °C with CO_2 at 5%.

2.2. Animals and virus inoculation

Thirty five weanling New Zealand white rabbits (30-40 days-old), weighing approximately 300–400 g were randomly allocated in seven groups. Animals of each group were inoculated with a different VACV strain (P1V or P2V), at three different titers, as follows: P1V - group 1 (low titer - $10^{2.5}$ $TCID_{50}$/animal); 2 (medium titer - $10^{4.5}$ $TCID_{50}$) and 3 (high titer - $10^{6.5}$ $TCID_{50}$); P2V – group 4 (low titer - $10^{2.5}$ $TCID_{50}$); 5 (medium titer - $10^{4.5}$ $TCID_{50}$) and 6 (high titer - $10^{6.5}$ $TCID_{50}$). Rabbits of group 7 were inoculated with RPMI and served as mock-infected controls. Prior to virus inoculation, rabbits were anesthetized by ketamine (50 mg/kg) and xylazine (5 mg/kg). The IN inoculation was performed into the paranasal sinuses [17]; each animal received 0.5 mL of the viral suspension in each nostril. Rabbits from different groups were housed in separate cages to avoid cross-contamination and were given food and water *ad libitum*. All procedures of animal handling and experimentation were performed under veterinary supervision and according to the recommendations of the Brazilian Committee on Animal Experimentation (COBEA, law # 6.638 of May, 8th, 1979). The experiments were approved by an Institutional Committee on Ethics and Animal Welfare and Experimentation (UFSM, Comitê de Ética e Experimentação Animal: process 97/2010).

2.3. Animal monitoring, sample collection and testing

Rabbits were monitored for clinical signs on a daily basis during 30 days pi (dpi) and weighted every two days up to day 12pi. Nasal swabs, peripheral blood and feces were collected for virological examination (virus isolation and quantitation) and for PCR. Virus isolation from swabs was performed in monolayer of Vero cells. Samples were considered negative after three passages of five days each without cytopathic effect (*cpe*). Virus titers in nasal secretions were quantitated by limiting dilution, calculated according to Reed & Munch [18] and expressed as $Log_{10}TCID_{50}$. Lung and gut samples collected at necropsy were submitted to virus isolation and quantitation in Vero cells. Tissue fragments were homogenized in MEM (10% w/v) and submitted to virus isolation as described above.

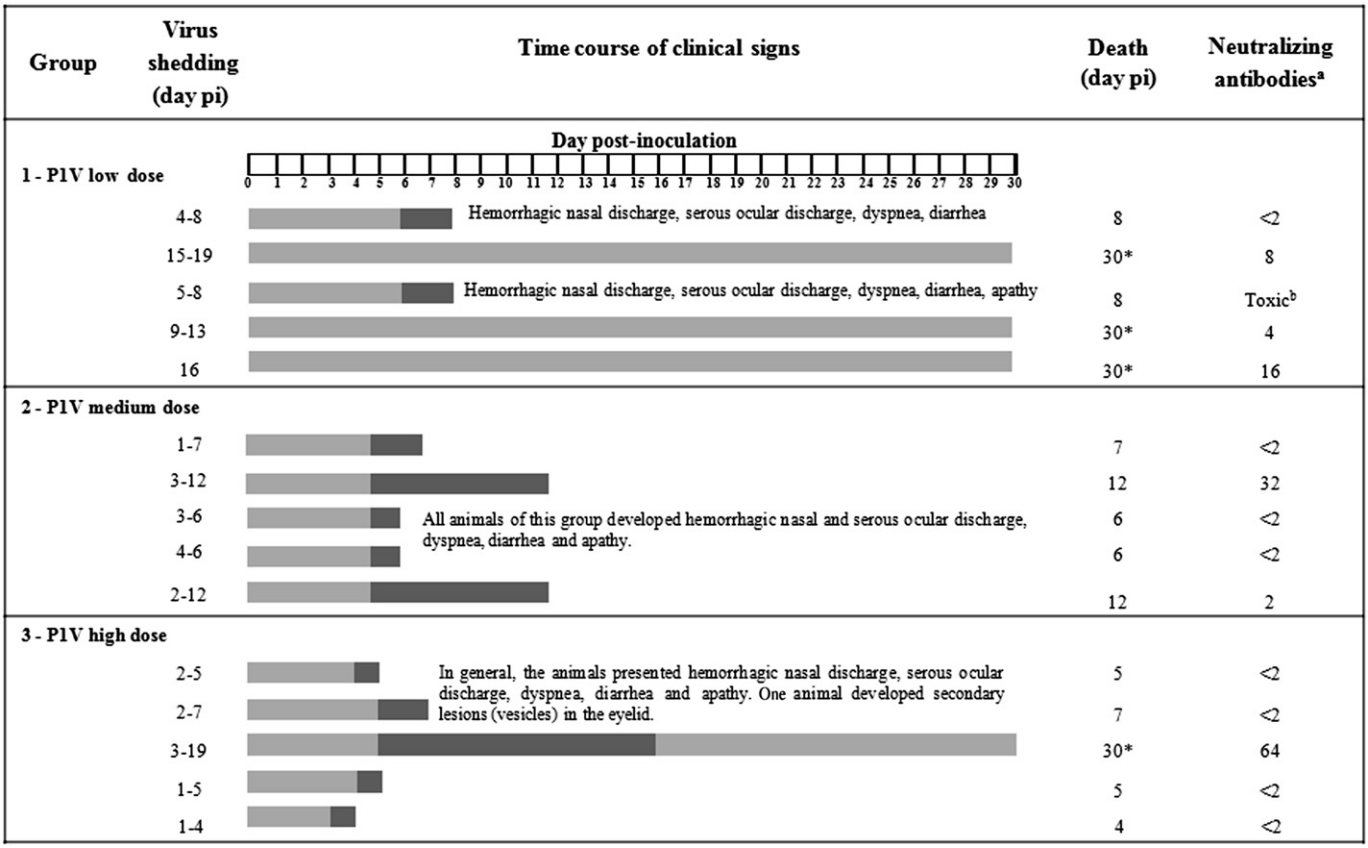

Fig. 1. Virus shedding, time course of the disease and serological response of rabbits inoculated intranasally with VACV P1V at three different doses. Dark bars show the duration of disease signs; light bars mean absence of clinical signs. *Animals were submitted to euthanasia at last day of experiment; [a]Titers of neutralizing antibodies were detected in serum samples collected at the day of death; [b]Serum was toxic for cell cultures.