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Pathogenesis in lambs and sequence analysis of putative virulence genes of Brazilian orf virus isolates

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ABSTRACT

The parapoxvirus orf virus (ORFV) is the agent of contagious ecthyma, an ubiquitous mucocutaneous disease of sheep and goats that may present variable clinical presentations. We herein studied the pathogenesis of ORFV infection in lambs and analyzed three putative virulence genes of four Brazilian ORFV isolates. Lambs inoculated in the labial commissures with each ORFV isolate ($n = 4$, viral titer $10^{5.6}$ $TCID_{50}$/ml) developed classical orf lesions, characterized by a progressive course of erythema/macules, vesicles, pustules and proliferative scabs. Lesions lasted an average of 22.9 days (18–26) and virus shedding was detected for approximately 24.6 days (18–30). Two isolates (SV269/11 and SV820/10) produced more severe, long-lasting lesions resulting in highest clinical scores. Lambs inoculated with isolate SV581/11 developed lesions markedly milder (lower clinical scores [$p < 0.05$]) and more limited than the other groups. Virus shedding by SV581/11 group, however, lasted similarly or even longer than the other groups. Sequence analysis of three virulence genes (VEGF, VIR and IL-10v) revealed amino acid deletions and mutations in VEGF and IL-10v genes of SV581/11 and SV252/11, the isolate(s) producing milder lesions. Additionally, the VEGF gene of isolate SV581/11 presented the lowest amino acid identity with the other isolates and with ORFV standard strain OV-IA82. Thus, these results demonstrate that ORFV isolates may display differential virulence in lambs and these differences might be associated with genetic changes in putative virulence genes.

1. Introduction

Orf virus (ORFV) is the prototype member of the genus *Parapoxvirus*, subfamily *Chordopoxvirinae*, family *Poxviridae*, along with bovine papular stomatitis virus (BPSV), pseudocowpox virus (PCPV) and parapoxvirus of New Zealand red deer (Moss, 2007). ORFV is the causative agent of orf, a mucocutaneous, highly contagious disease of sheep and goats also known as contagious ecthyma, contagious pustular dermatitis or scabby mouth (Haig and Mercer, 1998). The disease is characterized by maculopapular and proliferative scabby lesions in the skin around the mouth, nostrils, oral mucosa and teats (Haig and Mercer, 1998). Virus transmission occurs by direct, indirect contact or through contaminated fomites and pastures (Fleming and Mercer, 2007). Virus penetration usually occurs through abrasions in the lips, around the mouth or nostrils and lesions typically evolve through stages of erythema, macules, vesicles, pustules and proliferative scabs (Fleming and Mercer, 2007). In the absence of secondary infections or myiasis, lesions are usually self-limiting and resolve within 4–6 weeks, yet prolonged

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clinical courses have been occasionally reported (Ndikuwera et al., 1992; de la Concha-Bermejillo et al., 2003; Guo et al., 2003). The mortality is usually low, but morbidity may reach >90% of the herd, affecting preferentially lambs (Mazur and Machado, 1989). Human infections have been occasionally reported and are characterized by maculopapular lesions in the hands and fingers of people handling affected sheep (Yerrell et al., 1989; McKeever et al., 1988; Haig and McInnes, 2002). Contagious ecthyma is ubiquitous in most sheep and goat raising countries and is responsible for important losses in these flocks (Robinson and Balassu, 1981). Vaccination has been used with relative success to reduce the losses associated with ORFV infection (Fleming and Mercer, 2007).

The ORFV genome is a double stranded, linear DNA molecule of approximately 138 kilobases (kb) and contains 131 putative genes, 89 of which are conserved among chordopoxviruses (Delhon et al., 2004; Smith, 2007). Several genes with potential virulence functions have been identified in the ORFV genome, including an interleukin 10 (IL-10) homologue (IL-10v), vascular endothelial growth factor (VEGF) and interferon (IFN)-resistance gene (VIR) (Fleming and Mercer, 2007). These genes are located near the genome termini and, in general, lack similarity with other poxvirus or cellular proteins (Delhon et al., 2004). The association of some of these genes with ORFV virulence has been demonstrated through the production of gene deletion mutants displaying marked attenuation and producing poorly proliferative, rapidly resolving lesions (Lyttle et al., 1994; Cottone et al., 1998; Savory et al., 2000; Haig, 2006).

ORFV infection is widespread in most sheep and goat flocks worldwide and has been associated with either enzootic or epizootic disease with variable clinical presentations and varied degrees of severity (Salles et al., 1992; Langoni et al., 1995; Catroxo et al., 2002; Nóbrega et al., 2008). The field descriptions range from hyperemia and small pustules around the mouth and muzzle (Schmidt et al., 2012a,b) to the presence of scabby, proliferative and exsudative multifocal skin and mucocutaneous lesions (Salles et al., 1992; de Oliveira et al., 2012; Schmidt et al., 2012a,b). Long lasting severe lesions affecting the esophagus, intestine and respiratory tract have also been reported (Leite-Browning, 2008). A goat outbreak with high lethality and the presence of multifocal nodular and proliferative lesions of varied size disseminated throughout the body, teats, udder, vulva, base of the tail, abdomen, around the hooves, lips and nostrils has been recently described in Northern Brazil (de Oliveira et al., 2012). Atypical presentation with predominance of lesions on the limbs and head persisting for more than two months have been described in sheep outbreaks in the USA (Smith et al., 2002). A long lasting outbreak affecting young kids which developed generalized proliferative lesions, sometimes presenting suppurative arthritis, pneumonia and premature involution of the thymus has been described in the USA (de la Concha-Bermejillo et al., 2003).

The factors influencing the clinical presentation, course and severity of the ORFV-associated disease are diverse and include the immunological status, age, genetic susceptibility and breeding of the host (Tan et al., 1991; Haig and Mercer, 1998; Yeruham et al., 2000; de la Concha-Bermejillo et al., 2003; Haig, 2006) and environmental factors such as stress and animal grazing on abrasive pastures (Salles et al., 1992). In addition, the function of virulence gene products, especially VEGF, may influence the pathogenesis and some aspects of the ORFV-induced disease (Savory et al., 2000).

In order to investigate the association of specific genetic traits with virulence we inoculated lambs with Brazilian ORFV isolates originated from four outbreaks with varied clinical presentations and performed sequence analysis of three putative virulence genes.

2. Materials and methods

2.1. Experimental design

Groups of lambs ($n = 4$) were inoculated in the labial commissures with homogenates of scabs obtained from four outbreaks of contagious ecthyma in sheep. Following inoculation, the lambs were submitted to clinical monitoring and scoring during 30 days. Swabs collected from the lesions were submitted to virus isolation. The coding regions of three ORFV genes (VIR, VEGF and IL-10v) of each isolate were submitted to PCR amplification and sequence analysis.

2.2. Viruses and cells

Virus isolation, multiplication and quantification were performed in primary ovine fetal turbinate cells (OFTu). Cells were grown in minimum essential medium (MEM) containing penicillin (1.6 mg/mL), streptomycin (0.4 mg/mL) and amphotericin B (2.25 mg/mL), supplemented with 10% fetal bovine serum (FBS). Homogenates of scabs obtained from four outbreaks of contagious ecthyma in Rio Grande do Sul state, Southern Brazil, were used for inoculation: SV269/11, SV252/11, SV581/11 e SV820/10 (Schmidt et al., 2012a,b). Scabs were macerated and homogenized with MEM in a proportion of 1:10 (weight/volume) and centrifuged at low speed ($3000 \times g$ for 10 min). The supernatant was collected and submitted to virus quantification by limiting dilution in OFTu cells. Virus titers were expressed as $Log_{10}TCID_{50}$/mL. Each animal was inoculated with approximately $10^{5.6}$ $TCID_{50}$, contained in a 300 μL volume.

2.3. Animals and virus inoculation

Twenty 4 to 6-months-old Pollwarth male lambs originated from a flock without history of contagious ecthyma in the last 10 years were used. Groups of four lambs each were inoculated with each of four ORFV isolates; the fifth group was inoculated with minimal essential medium (MEM). The animals were kept in separate barns and received alfalfa and water *ad libitum*. All experimental procedures were performed according to recommendations by the Brazilian College of Animal Ethics and Experimentation and were approved by an Institutional Ethics Committee (approval 101/2010).