Assessing recombinant vaccinia virus as a delivery system for fertility control vaccines in the brushtail possum (*Trichosurus vulpecula*)

Duckworth, J.¹, Cross, M.¹, Fleming, S.², Scobie, S.¹, Whelan, E.², Prada, D.³, Mercer, A.², Cowan, P.⁴

¹Landcare Research, PO Box 40, Lincoln 7640, New Zealand, duckworthj@landcareresearch.co.nz
²Virus Research Unit, University of Otago, PO Box 56, Dunedin, New Zealand
³Ecogene Laboratory, Landcare Research Auckland 1142, New Zealand
⁴Landcare Research, PO Palmerston North 4442, New Zealand

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Abstract

The introduced brushtail possum (*Trichosurus vulpecula*) is a major threat to native biodiversity and agricultural production in New Zealand. Research on non-lethal management methods is focussed on fertility control, and aims to develop zona pellucida (ZP) vaccines suitable for bait delivery to free-living possums. Vaccine delivery remains a challenge. One highly successful oral wildlife vaccine which has been widely used to control rabies in wildlife in the US and Europe, is based on a replication-limited recombinant vaccinia virus (rVV). The potential of rVV as a vaccine delivery system has yet to be tested in any Australian marsupial species. In the present study we evaluated the infectivity of rVV, as well as cell-mediated and antibody immune responses, in the marsupial brushtail possum. Possums were exposed to a model recombinant vaccinia construct (rVV399, which expresses the Eg95 antigen of the hydatid disease parasite *Echinococcus granulosus*) or the parent vaccinia virus strain (*Lister*) by applying 10⁸ pfu of virus, drop wise onto the external surface of the nose and into the oral cavity. Both the recombinant and parent viruses persisted in the mucosal epithelium around the palatine tonsils for up to 2 weeks post-exposure. The parent vaccinia and the rVV399 construct induced peripheral blood lymphocyte reactivity against viral antigens in possums by 4 weeks post-exposure, and were still detectable at 4 months post-exposure. Serum antibody reactivity to the antigen Eg95 was recorded in 7/8 possums which received a single dose of the rVV399 construct and in 7/7 animals which received triple-dose delivery, with titre end-points in the latter case exceeding 1/4000 dilution. This study demonstrates that vaccinia virus will readily infect possums via an oronasal route and is capable of generating immune reactivity against both viral and heterologous antigens. This highlights the potential of recombinant vaccinia as a wildlife delivery system for fertility control vaccines for the brushtail possum and potentially other marsupial species.

Keywords: bait delivered vaccines, brushtail possum, fertility control, marsupial, vaccinia, wildlife

Introduction

The introduced brushtail possum (*Trichosurus vulpecula*) is a major threat to native biodiversity and agricultural production in New Zealand. Research on non-lethal management methods is focussed on fertility control, and aims to develop zona pellucida (ZP) vaccines suitable for bait delivery to free-living possums. We have shown that bacterial ghosts (BG) (particulate vaccines derived from non-living empty cell envelopes of gram-negative bacteria) expressing the c-terminal residues of possum ZP protein 2 (ZP2-C) significantly reduced the fertilisation rate of artificially inseminated possums and conception rates of naturally bred possums when delivered by oral or eye/nose routes (Walcher et al., 2008). Despite developing new constructs capable of expressing the ZP antigen at higher levels and encapsulated formulations to prevent proteolysis and gastric acid degradation in the gastrointestinal tract, we have not been able to improve immune response intensity and longevity sufficiently to make the vaccines practical for field application. A review of potential delivery systems for fertility control vaccines in possum (Cross et al., 2011) identified replication-limited poxviruses such as recombinant vaccinia virus (rVV) as the basis of a potential oral wildlife vaccine for possums. rVV has been widely used to control rabies in wildlife in the US and Europe, but has never been evaluated in an Australian marsupial. In the present study we evaluated the infectivity of rVV as well as cell-mediated and humoral immune responses, in the marsupial brushtail possum.
**Materials and methods**

Adult female possums, housed under Physical Containment Level 2 conditions, were exposed to a model recombinant vaccinia construct (rVV399, expressing the Eg95 antigen from the hydatid disease parasite *Echinococcus granulosus*, Marsland et al., 2003) by applying $10^8$ pfu of virus drop wise onto the external surface of the nose and into the oral cavity. Under ketamine anesthesia, eight possums were treated with a single dose of rVV339; seven animals with three weekly doses of rVV339 and eight with a single dose of the parent-strain vaccinia virus (Lister). The mucosa around the palatine tonsil was swabbed for DNA prior to treatment and at week 1, 2, and 4 post-infection to assess viral persistence by PCR analysis (Reubel et al., 2005; Sandvik et al., 1998). Blood samples were collected at week 0, 4, 8, 12 and 16 to measure immune reactions. Thymidine incorporation was used to assess lymphocyte proliferation responses to viral antigens, and possum serum antibody binding to Eg95 protein was measured by ELISA (Duckworth et al., 2007).

**Results**

A single dose of rVV339 infected 3/8 possums, and 7/7 animals were infected in the triple-dose group. No rVV339-treated animals showed overt signs of viral pathlogy. Following a single dose with the parent-strain vaccinia virus, 8/8 treated possums were infected and two animals exhibited small facial lesions that were positive for presence of vaccinia virus by PCR and *in vitro* culture. Both the recombinant and parent viruses persisted in the mucosal epithelium around the palatine tonsils for up to 2 weeks post-exposure. Parent strain vaccinia and the rVV399 construct induced peripheral blood lymphocyte reactivity against viral antigens, first apparent at 4 weeks post-exposure and still detectable at 4 months post-exposure. Serum antibody reactivity to Eg95 was recorded in 7/8 possums which received a single dose of the rVV399 construct, and in 7/7 animals which received triple-dose delivery and was detectable for at least 4 months. Maximum antibody titers were 1:2048 for possums which received a single dose or rVV339 and 1:4096 for animals that received multi-doses of the construct. Possums that received parent-strain vaccinia virus and non-exposed control possums remained non-responsive to Eg95.

**Discussion**

This study demonstrates that vaccinia virus can establish an infection in brushtail possums following application into the oral cavity and onto the external surface of the nose; a route of delivery designed to simulate the natural feeding behavior of possums. This is the first report in an Australian marsupial species demonstrating an immune response to a recombinant antigen in a poxvirus construct. While the potency and longevity of vaccinia-based vaccines expressing an immunocontraceptive antigen in possums is yet to be confirmed, these initial results, and the extensive safety and efficacy precedent set by the Raboral™ oral rabies vaccine in eutherian wildlife, provide encouragement for such an approach to be used as an oral vaccine delivery system for possums and potentially other marsupial species.

**References**


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