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Plant-based immunocontraceptive control of wildlife—"potentials, limitations, and possums"

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Abstract

Possums (*Trichosurus vulpecula*), originally introduced from Australia, are spread over 90% of New Zealand and cause major economic and environmental damage. Immunocontraception has been suggested as a humane means to control them. Marsupial-specific reproductive antigens expressed at high levels in edible transgenic plant tissue might provide a safe, effective, and cheap oral delivery bait for immunocontraceptive control. As proof of concept, female possums vaccinated with immunocontraceptive antigens showed reduced fertility, and possums fed with potato-expressed heat labile toxin-B (LT-B) had mucosal and systemic immune responses to the antigen. This demonstrated that immunocontraception was effective in possums and that oral delivery in edible plant material might be possible. Nuclear transformation with reporter genes showed that transgenic carrot roots accumulate high levels of foreign protein in edible tissues, indicating their potential as a delivery vector. However, prior to attempts at large scale production, more effective immunocontraceptive antigen-adjuvant formulations are probably required before plant-based immunocontraception can become a major tool for immunocontraceptive control of overabundant vertebrate pests.

Keywords: Immunocontraception; Possums; Plant-derived vaccines

1. Introduction

Populations of both native and introduced vertebrate animals may become overabundant, generally as a result of ecosystem disturbances, such as alterations in resources or predation. A range of strategies has been used in attempts to control such population imbalances.

There are few documented examples of successful long-term biocontrol for widely dispersed vertebrate populations, with perhaps the greatest success being the use of myxomatosis and rabbit haemorrhagic disease to control rabbits in Australia [1]. Despite this success, the use of lethal microorganisms as a management tool may be risky and difficult to control once the organism is released.

Fertility control may provide a non-lethal, more humane alternative for population control of vertebrate animals. One strategy for long-term fertility control is immunocontraception, where vaccination is used to induce antibodies against reproductive self-antigens, resulting in reduced fertility [2]. Most commonly, whole zona pellucida (ZP) harvested from pigs at slaughter has been used as the vaccine antigen [3]. Although successful in many species, immunocontraceptive control using whole ZP harvested from animals carries the risk of cross-species viral transfer and has limited ability to be scaled up. Recombinant ZP proteins can also be effective and production can be easily scaled up. Many other reproductive, developmental, and hormone based antigens and epitopes have also been tested successfully in various animal species and humans [2].

Immunocontraceptive vaccines can be delivered by injection, bait, or infectious recombinant microbes [4]. Injection along with an adjuvant provides the gold standard for strong immunocontraceptive responses, and carries no risk of affecting non-target animals. However, it is by far the most expensive method. Disseminating infectious viral and bacterial

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recombinant organisms that express an immunocontraceptive antigen offers cheap and rapid delivery of immunocontraceptive vaccines in the field, but carries the risk of spread to non-target populations or species. Baits provide the potential for mass delivery without the risk of spread, although other species may take the bait, depending on the method of delivery.

Currently, practical immunocontraceptive control of wild life has been done on a limited scale, for example with wild horse populations on a number of islands [5]. The animals have maintained excellent health despite several years of treatment and fertility levels have been kept very low. Delivery is by aerial darting.

Immunocontraceptive control by injection is not feasible for large disperse populations such as the brushtail possum (*Trichosurus vulpecula*), originally introduced from Australia, and now spread over 90% of New Zealand. Possums cause major economic and environmental damage there and New Zealand management agencies spend more than NZ $90 million annually on possum control and research, and have extensively investigated potential control strategies [6]. Modelling has indicated that fertility control could be effective, either alone or as a supplement to lethal baiting, provided 50% or more of females can be sterilised and the immunocontraceptive vaccine can be economically delivered on a mass scale [7].

Edible transgenic plants expressing an orally active immunocontraceptive vaccine may provide a cheap and safe delivery system [8]. Transgenic plant-based vaccines can be economically produced using the mass-scale efficiencies of modern agriculture, especially if edible plants are used to avoid the cost of extraction. Plant-based immunocontraceptive vaccines delivered in bait stations or aerially could provide a relatively cost-effective control strategy with greater risk control than strategies based on lethal or infectious agents. Over 90% of possums can be targeted using aerial delivered edible baits [7,9].

The feasibility of plant-based immunocontraceptive control of possums was investigated. Possum reproductive antigens were cloned, sequenced, and characterised, and plant-based delivery systems for these antigens were evaluated. To demonstrate proof of concept, female possums were injected with reproductive antigen vaccines to demonstrate reduced fertility and possums were fed with a model plant-based vaccine to show mucosal immune responses to that vaccine. Carrots, currently used for poison baiting of possums, were tested as a potential vaccine mass delivery system by evaluating the levels of recombinant proteins in their roots. The results to date indicate that immunocontraception is effective for possums and that plant-based immunocontraceptive vaccines can probably be produced inexpensively in carrots and possibly other appropriate crops at practical levels. However, further research will probably be required to make the immunocontraceptive antigens more immunogenic when delivered orally in edible plant tissue.

2. Results and discussions

In both animals and humans, antibodies generated against some reproductive self-antigens or epitopes may reduce fertility [2]. Based on this effect, an immunocontraceptive approach has been researched for control of fertility in a wide range of vertebrates and is being developed for use in possum control.

2.1. Infertility in possums inoculated with porcine ZP and whole possum sperm

Prior to extensive research on possum immunocontraceptive antigens and delivery systems, it was necessary to first determine whether possum fertility could be reduced or abolished by inducing an immune response against gamete antigens.

Female possums were injected with whole porcine ZP in Freund's complete adjuvant, followed by two injections, 4 and 10 weeks later, with the porcine ZP in incomplete Freund's adjuvant. Immunisation against porcine ZP resulted in a more than 75% reduction in fertility compared with control groups. Antibodies to porcine ZP were detected in the serum from all immunised possums as well as in vaginal washings and ovarian follicular fluid. Treatment had no effect on the number of females that cycled and were mated, but significantly reduced conception rates and thus, the proportion of females that gave birth. Immunisation against porcine ZP had no apparent effect on the number of follicles present in the ovary and did not cause ovarian dysfunction [10].

Male and female possums were also vaccinated with whole possum sperm. While strong levels of serum antibodies were raised against sperm antigens, the males showed no reduction in fertility. In contrast, treated females showed a more than 80% reduction in fertility compared with control groups [11].

Clearly, these results indicated that possum fertility is amenable to immunocontraceptive control and females are the obvious targets for fertility reduction when gamete antigens are used. The possum's polygamous mating system also suggests that targeting females would be a more effective strategy [9].

2.2. Infertility in possums inoculated with synthetic possum ZP antigens and epitope

Several candidate possum genes for immunocontraceptive antigens have been cloned, sequenced, expressed, and characterised [10,12,13]. It was anticipated that a range of antigens and epitopes would allow development of combination vaccines targeting female possums, and possibly the development of marsupial-specific immunocontraceptive vaccines. The use of several contraceptive epitopes would also reduce the risk of non-recognition of an epitope by the immune system of some animals due to genotypic differences in an outbred population [9,14]. The coding se-