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## Proceedings of the Vertebrate Pest Conference

### Title

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### Permalink

<https://escholarship.org/uc/item/2bh760z0>

### Journal

Proceedings of the Vertebrate Pest Conference, 22(22)

### ISSN

0507-6773

### Author

Hinds, Lyn A.

### Publication Date

2006

### DOI

10.5070/V422110254

# Immunocontraception of Small Mammals: Case Study for the Wild House Mouse in Australia

Lyn A. Hinds

CSIRO Entomology, and Invasive Animals Cooperative Research Centre, University of Canberra, ACT, Australia

**ABSTRACT:** Many exotic vertebrate species have been introduced either accidentally or intentionally into the Australian environment. Native species have also become pests due to changes in landscape use. Management of these pest populations, whether introduced or indigenous, is of concern both nationally and internationally. Public approval of existing pest control techniques involving lethal agents (poisoning, shooting, trapping, or disease) has declined in favour of alternative techniques, which are more humane, cost effective, species-specific, environmentally friendly, and suitable for delivery on a continental scale. Population management through disruption of reproduction rather than through the use of mortality agents has gained acceptance in the past 10-15 years. This has led to considerable research into the development of immunocontraceptive agents, for example to prevent mouse populations irrupting to plague proportions in cereal-growing regions of Australia. We have examined whether mouse populations can be managed with fertility control delivered using a transmissible mouse-specific virus (mouse cytomegalovirus, MCMV), engineered to carry an infertility agent. Field and laboratory results, as well as computer modelling, show excellent prospects for the use of vaccines based on MCMV. However, the efficiency of transmission of infertility remains a major challenge for the current research program. The public acceptability of the technology is yet to be confirmed. The issues of species specificity, delivery system stability, transmission efficiency, and other potential or perceived risks require open and wide-ranging debate, nationally and internationally, before trial field experiments of a genetically modified virus for controlling field populations of mammals could occur.

**KEY WORDS:** ectromelia, fertility control, house mouse, immune responses, MCMV, mouse cytomegalovirus, *Mus domesticus*, pest mammals, reproductive proteins, rodents, viral-vectored immunocontraceptives, zona pellucida, ZP3

Proc. 22<sup>nd</sup> Vertebr. Pest Conf. (R. M. Timm and J. M. O'Brien, Eds.)  
Published at Univ. of Calif., Davis. 2006. Pp. 96-101.

## INTRODUCTION

Many exotic vertebrate species have been introduced either accidentally or intentionally into the Australian environment. Some of these have adapted and flourished in their new habitats in the absence of their natural predators and diseases (Braysher 1993, Cowan *et al.* 2003). Native species, such as kangaroos and koalas, have also become pests due to their introduction into different habitats, man-made changes in their natural habitats, and/or the influence of agricultural and pastoral practices (Calaby and Grigg 1989, Cooper and Herbert 2001).

Pest plants, insects, or mammals have major impacts on agricultural production and for the natural environment. Currently, chemical control is the primary method of management with trapping, shooting, and exclusion also being used for some species. However, while these methods often provide effective control in the short term, they require repeated application, can affect non-target species, and may not be cost-effective in the long term. With increasing concerns about the use of chemicals in the environment, alternative approaches to pest management are being explored. For mammalian pests, fertility control using contraception is being developed (see Kirkpatrick and Frank 2005). This paper describes recent developments in fertility control for the wild house mice in Australia.

## Impact of Rodent Pests

Losses to vertebrate pests are a major concern in agricultural regions throughout the world, with rodents

being a major cause of food crop losses in many developing countries in Asia (Singleton and Petch 1994, Singleton 2003). In Australia, the principal rodent pest to agricultural crops is the house mouse, *Mus domesticus*, the same species as the well-studied laboratory mouse. Much is known about its biology and population dynamics in the Australian grain-growing regions, where it causes devastation to crops (Singleton 1989, Singleton and Redhead 1990, Pech *et al.* 1999, Singleton *et al.* 2005). Mouse populations irrupt irregularly, causing losses in the range of USD\$50-150 million (Singleton and Redhead 1989, Caughley *et al.* 1994). This translates to average annual losses of around USD\$10 million.

## Current Methods – Chemical and Biological Control

There is a growing demand in many countries for environmentally-friendly control strategies that do not rely only on chemical control, reduce costs, and minimise non-target effects. In addition, the domestic and international markets increasingly require clean agricultural goods that are produced in a sustainable manner.

Alternatives to poisons include the use of biological methods to increase mortality, or the use of chemicals or biological agents to reduce fertility (Gao and Short, 1993, Singleton, 1994). Chambers *et al.* (1999a) reviewed the relative advantages and disadvantages of reducing fertility versus increasing mortality for managing rodent populations. On balance, fertility control was favoured if it could be shown to be species specific, humane, and cost-effective.

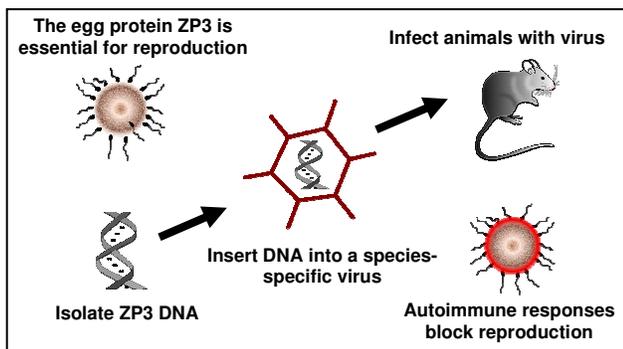
The concept of using viruses as vectors to deliver

fertility control originated in the early 1990s (see Tyndale-Biscoe 1994). In Australia, the house mouse, European rabbit (*Oryctolagus cuniculus*) and the European fox (*Vulpes vulpes*) have been used to test the concept of viral vectored immunocontraception (VVIC).

### The Concept of Immunocontraception

Immunocontraception occurs when an animal develops an immune response against a reproductive protein and consequently becomes infertile. At the population level, immunocontraceptive vaccines could be delivered using either a non-infectious agent in oral baits, or an infectious disseminating virus as the delivery vector.

There have been two lines of research for introduced vertebrate pests in Australia: fertility control using either a non-infectious agent delivered in non-toxic oral baits (foxes: Bradley *et al.* 1997, Reubel *et al.* 2005), or infectious viruses as carriers of an infertility agent (rabbits: Kerr *et al.* 1999, Mackenzie *et al.* 2006; mice: Chambers *et al.* 1999a, Jackson *et al.* 1998, Lloyd *et al.* 2003; Figure 1). In both cases, the aim has been to vaccinate the animal by delivering an antigen (a reproductive protein) that generates an immune response, with antibodies in the female host blocking fertilisation or oocyte development in the follicles of the ovary. Such an immunocontraceptive approach is potentially highly species specific, is considered humane, and could be cost effective in the long term (Tyndale-Biscoe 1994, Seamark 2001).



**Figure 1. Schematic of the concept of viral vectored immunocontraception for the wild house mouse. The immunocontraceptive vaccine is created by genetically modifying a carrier virus to include DNA for key reproductive proteins. The product is a modified virus, which during infection of the mouse, causes an immune response which attacks the animal's own eggs and prevents reproduction.**

## DEVELOPMENT OF VIRAL VECTORED IMMUNOCONTRACEPTION (VVIC)

### Choice of Reproductive Antigen

Initial research on reproductive antigens focused on gamete antigens, particularly sperm antigens (Tyndale-Biscoe 1994), but with little success (rabbits: Holland *et al.* 1997, Hardy *et al.* 1997; foxes: Bradley *et al.* 1997; mice: Hardy and Mobbs 1999). Recent research has focused on the female gamete antigens, particularly the zona pellucida proteins forming the extracellular coat of the oocyte; mouse ZP3 has become the antigen of choice

(Hardy *et al.* 2003, Clydesdale *et al.* 2004). A range of other female reproductive proteins, specific peptides, and epitopes have been assessed but with limited success (Hardy *et al.* 2006).

### Choice of Viral Vector

The use of infectious viral agents to deliver an anti-fertility vaccine was first proposed by Tyndale-Biscoe and Robbins (Tyndale-Biscoe 1994). The aim was a “release and forget” strategy for biological control. However, there are a number of essential and desirable prerequisites that would need to be met before a recombinant virus could be considered for such a role. These include a combination of biological (e.g., low-pathogenicity to mice, ability to develop a persistent immune response) and socio-political (e.g., species-specificity, stability of the virus – DNA rather than an RNA virus) requirements (Shellam 1994, Chambers *et al.* 1999a). Mouse cytomegalovirus (MCMV) has met each of the essential properties and most of the desirable properties identified for such a vector. Although there are some data wanting, especially on the epidemiology and immunology of MCMV strains isolated from wild mice in Australia (Shellam 1994), MCMV emerged as the strongest candidate vector for delivery of an infertility agent.

### Proof of Concept of VVIC in the Laboratory Context

Laboratory studies on the mouse confirmed the potential of using a recombinant virus, carrying a reproductive antigen, to sterilise mice. Proof of concept was achieved using ectromelia virus, an orthopoxvirus. Jackson *et al.* (1998) demonstrated that a recombinant ectromelia virus carrying the mouse zona pellucida 3 (ZP3) protein caused a significant reduction in the number of litters produced by infected females compared to uninfected controls. These mice remained infertile for periods of 5-9 months. While ectromelia offered an excellent laboratory model, virtually nothing is known about its epidemiology outside the laboratory; it is not present naturally in the Australian environment, and there are concerns about its impact on valuable laboratory colonies (see Bhatt and Jacoby 1986). Therefore, parallel research began using MCMV, which does occur naturally in 60-80% of Australian mice (Singleton *et al.* 1993, 2000, Smith *et al.* 1993) and appears to be species-specific (Shellam 1994).

Recombinant MCMV containing the ZP3 gene (recMCMV-mZP3) under the control of an immediate early promoter was generated through homologous recombination into the non-essential ie2 gene of MCMV (Cardin *et al.* 1995). Infection of BALB/c mice and of wild specific pathogen free mice induced long term infertility (>250 days) after direct intraperitoneal injection (Chambers *et al.* 1999a, Lloyd *et al.* 2003, Redwood *et al.* 2005). The infertility is due to the elimination of developing follicles in the ovary (Lloyd *et al.* 2003). The mechanism leading to infertility appears to be due to anti-mZP3 antibodies. Immunofluorescence studies demonstrate binding of serum antibodies from infertile mice to the zona pellucida of oocytes of normal ovaries. Passive immunisation of mice with serum antibodies from mice

immunized with recMCMV-mZP3 leads to a delay in breeding, presumably as the antibodies fall below a critical level (Lloyd *et al.* 2003). No inflammatory responses, such as oophoritis, have been reported in the ovaries of recMCMV-mZP3 infected mice.

Whilst some laboratory strains of mice other than BALB/c appeared less susceptible to contraception (Chambers *et al.* 1999a), specific pathogen-free outbred wild mice from Australia showed low prevalence of genetic resistance to MCMV infection (Scalzo *et al.* 2005) and were effectively sterilised following inoculation with recMCMV-mZP3 (M. Lloyd, pers. commun.; L. A. Hinds, unpubl. results). These results indicate that wild populations of Australian mice should be susceptible to contraceptive MCMV.

Demonstration of species specificity of recMCMV-mZP3 has been provided in studies using rats (Smith *et al.* 2005). Direct inoculation of laboratory rats with high doses of recMCMV-mZP3 induced strong immune responses to both MCMV and mZP3, but no replicating virus was found and there was no effect on fertility.

One remaining challenge to overcome is whether the recMCMV will transmit between mice and lead to infertility. Early studies on the transmission of recMCMV-ZP3 suggest that while it transmits across 3 generations, the infection does not result in infertility (S. Nikolovski and L.A. Hinds, unpubl. results). This has led to the search for more transmissible wild type MCMV strains from south-eastern Australia. One isolate selected for its transmission characteristics has been plaque-purified and engineered to carry the mZP3 gene in a different region of the genome (T. Strive, unpubl. results). This new recMCMV-mZP3 is currently being tested to determine its effects on fertility. We will also assess whether transmission occurs and leads to an induction of infertility in contact mice (L.A. Hinds, unpubl. results).

If these trials are successful, further studies of the species specificity, stability, and persistence of the recombinant virus must be undertaken in the laboratory before field studies can be considered.

## **ECOLOGICAL CONTEXT OF IMMUNO-CONTRACEPTION**

### **How Many Mice to Sterilise in the Field?**

Simple population models indicate that if more than two-thirds of females are sterilised, there will be a significant reduction in the abundance of mice at the end of 4 months (Chambers *et al.* 1997). This scenario corresponds to the use of a virus as a vector for an immunocontraceptive vaccine and is consistent with models that indicate that fertility control is more effective in reducing population densities than culling when populations have high rates of increase (Barlow 1997). Chambers *et al.* (1999b) demonstrated that a 67% level of sterility, achieved either by ovariectomy or tubal ligation, was sufficient to significantly reduce the growth rate of mouse populations housed in outdoor enclosures. However, hormonally competent, sterile females were unable to prevent fertile females from breeding. The treated populations were 45 to 65% of the control populations at the end of 18 weeks (expect 35% if no

compensation). The treated populations appeared able to compensate through increasing their breeding performance (increased percentage of females breeding, and slightly higher litter size) relative to the controls.

Other models have suggested that levels of infertility could range from 30 to 90% (Hone 1999, Davis *et al.* 2003, Arthur *et al.* 2006). When the experimental data of Chambers *et al.* (1999b) are included in Arthur *et al.*'s (2006) model, a level of 70% infertility is required. This estimate assumes that there is no competitive disadvantage to the introduced recMCMV compared to existing wild type field viruses. However, if wild mice are already infected with wild type strains and this prevents development of infertility when they become infected with recMCMV, then there is a significant reduction in the effectiveness of VVIC; indeed, close to 100% of susceptible mice must be made infertile in this scenario. Ongoing success will also require high levels of transmission of the recMCMV.

## **SOCIO-POLITICAL ASPECTS OF IMMUNO-CONTRACEPTION**

The growing demand for technologies that are cost effective, environmentally friendly, and politically and socially acceptable has accelerated advances in, and applications of, biotechnology. Genetically modified organisms (GMOs) are being developed to provide solutions to problems in medicine, agriculture, and the environment, and more recently for bioremediation in the mining sector (Tyndale-Biscoe 1995).

With the recent introduction of various GMOs, there is increasing public pressure to ensure that the potential risks imposed by release of GMOs are fully assessed. The assessment of environmental risks or impacts, real or perceived, requires a rigorous process which leads to clear statements of the probability of identified hazards arising. For the public, the process must impart an excellent understanding of the technology, its safety, and costs versus benefits, because ultimately it is the social and political reaction, acceptance or rejection, which will determine the outcome for release. Generally the first question asked by the public about fertility control using recombinant viruses is whether it will affect humans or other species. Such rational or irrational reactions must be dealt with in an incremental discussion with all facets of society, including the political arena. For fertility control agents, this can only be achieved by ongoing, extensive national and international debate (Tyndale-Biscoe 1994, Oogjes 1997, Stohr and Meslin 1997, Williams 1997, 2002).

The major concern with immunocontraceptive vaccines is their species specificity. Australian regulations (principally the Gene Technology Act 2000, administered through the Office of the Gene Technology Regulator; <http://www.ogtr.gov.au/>), with which all VVIC research has conformed, are designed to ensure public accountability and will constrain field-testing until all risks to non-target animals have been thoroughly explored and mitigated.

Other concerns about fertility control agents delivered by GMOs that must be addressed are animal welfare and natural selection against the agent. Concerns for animal

welfare reflect existing issues requiring that management of individuals and populations be humane. Guynn (1997) argues there is potential for behavioural/hormonal disruptions to cause ill effects in sterilised individuals. It has been also argued that natural selection against a fertility control agent may select for animals with poor immune systems, therefore favouring immunodeficient animals and thus increasing their susceptibility to pathogens (Guynn 1997, Nettles 1997). Furthermore, infertile animals may live longer and suffer the diseases of old age (Nettles 1997). However, Oogies (1997) and Singer (1997) argue that fertility control delivered by immunocontraceptive vaccines may be more humane than existing control techniques. Whether natural selection will also diminish the effectiveness of the immunocontraceptive vaccine itself needs to be assessed but remains a current issue in the debate.

## CONCLUSION

Progress towards developing fertility control of mice using immunocontraceptive vaccines has been very encouraging. The long breeding season of mice in the grain-growing regions in Australia means that viral-vectored vaccines offer the most promise for managing eruptions of mouse populations. This promise has been strengthened by the demonstration under laboratory conditions that genetically engineered MCMV can deliver an immunocontraception vaccine successfully. However, transmission of recMCMV leading to infertility has not been achieved so far.

There are two strong messages to emerge from this research. The nature of this research is that it is long term and requires significant infrastructure and strong collaboration across several fields of biological research—this has been possible through Vertebrate Biocontrol CRC and its successors, the Pest Animal Control CRC, and now the Invasive Animals CRC. The second is the necessity of developing a strong ecological understanding of the pest population one is aiming to manage. In the case of immunocontraceptive vaccines, this includes the epidemiology of the vector and the population dynamics of the host.

While the scientific progress with these new methods for fertility control is highly promising, the public acceptability of the technology is yet to be confirmed. The issues of species specificity, delivery system stability, and other potential risks require open and wide-ranging debate, nationally and internationally, before a field release of a genetically modified virus for controlling field populations of mammals goes ahead.

In summary, developments in biotechnology and ecology have led to good progress towards realising the concept of controlling rodent pest populations through the use of immunocontraceptive vaccines delivered by a non-toxic bait or with a virus that spreads naturally through the target pest population.

## ACKNOWLEDGMENTS

The research on immunocontraceptive vaccines is jointly funded by the Grains Research and Development Corporation and the Pest Animal Control Cooperative Research Centre, and its successor, the

Invasive Animals CRC. The efforts of many colleagues have contributed to this multidisciplinary project: Grant Singleton, Chris Hardy, and Roger Pech at CSIRO Sustainable Ecosystems, Canberra, ACT, Australia; and Malcolm Lawson, Megan Lloyd, Sonia Nikolovski, Alec Redwood, Geoff Shellam, and Lee Smith at Discipline of Microbiology, QEII Medical Centre, School of Biomedical and Chemical Sciences, The University of Western Australia, Nedlands, Western Australia. The author also acknowledges the invaluable contributions of many research support staff involved in this research program. Ms. Alice Kenney prepared the figure.

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