10. Biological Control of Rodents — the Case for Fertility Control Using Immunocontraception

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Abstract

Managing rodent pests on a broad scale using lethal methods is not an appropriate long-term strategy given their extraordinary breeding capacity and high mobility. Moreover, environmental, animal welfare and ethical concerns regarding the use of poisons and trapping has decreased the acceptance of mortality methods in recent times. Another reason for avoiding lethality is that it may promote a strong selective pressure for resistance to the lethal agent, be it a disease or a chemical. The addition of fertility control, specifically immunocontraception, to the armoury currently available to control rodent pests, is discussed in this chapter. Fertility control aims to reduce a specific population size by reducing the number of young produced and recruited into the population.

Existing fertility control techniques (e.g. steroids, synthetic hormones) are flawed because they require repeated administration to maintain the sterility level of the population, they have undesirable physiological and behavioural side effects and they are not specific to the target animal. Delivery of these sterilising agents is logistically difficult, time-consuming and expensive and therefore they are not suitable for controlling field populations of rodent pests that are often widespread and cryptic in their habits. An immunocontraceptive vaccine, either distributed in bait or disseminated in a species-specific viral vector, is a new tool that could be used to reduce the productivity of pest populations. The various components of this approach and 'proof of concept' laboratory experiments conducted in house mice in Australia are described. It must be recognised that to critically evaluate the efficacy of a viral-vectored immunocontraceptive agent requires a multi-disciplinary approach with a strong ecological and epidemiological focus. Only then can the impact of this control technique be assessed at the population level.

Keywords

Immunocontraception, biological control, rodents, viral-vectored, fertility control, genetically manipulated organism, murine cytomegalovirus, zona pellucida, ectromelia virus, reproductive antigens

INTRODUCTION

RODENTS HAVE gained the reputation as one of the most persistent and ubiquitous vertebrate pests affecting human populations. They cause economic problems because of the damage they inflict in agricultural systems (e.g. Caughley et al. 1994), environmental problems due to the chemicals used for their control (e.g. Saunders and Cooper 1981; Singleton and Redhead 1989), social problems associated with their close proximity to human habitation (e.g. Beckmann 1988) and health problems as carriers of zoonoses (Childs et al. 1994; Gratz 1994; also see Mills, Chapter 6). Of increasing concern is the impact of introduced rodents on the conservation of native wildlife (e.g. Wace 1986; Moors et al. 1992; Key et al. 1994; also see Dickman, Chapter 5). Rats have been reported as the major pest in rice crops in Southeast Asia (Geddes 1992; Singleton and Petch 1994), and cause significant problems in Africa (Leirs et al. 1997; also see Makundi et al., Chapter 22), Australia (Singleton and Redhead 1989; Caughley et al. 1994), China (see Zhang et al., Chapter 12) and elsewhere (Prakash 1988a; also see Buckle, Chapter 7).

Many species that become pests do so because of their reproductive potential. They often have several large litters in each breeding period, show early onset of sexual maturity and have a short life expectancy (Tyndale-Biscoe 1994). Rodent pests typically show these life history traits—for example, one breeding pair of house mice (*Mus domesticus*) is theoretically capable of producing over 600 offspring in six months and the average life expectancy in a field population is four to six months (Singleton 1989). A post-partum oestrus allows females to produce a litter every 21 days (Whittingham and Wood 1983). Therefore, curbing the reproductive potential of rodents may be a more appropriate control tactic than increasing their mortality (Singleton 1994; also see Krebs, Chapter 2).

Increasing community interest in environmental and animal welfare issues associated with conventional pest control techniques, such as poisoning and trapping, has focused interest on developing non-lethal, non-toxic alternatives (Bomford 1990). One such strategy is to focus on reducing reproduction, rather than increasing the mortality of the pest species. This is commonly referred to as fertility control.

In this chapter, we will examine why fertility control is theoretically superior in many respects to conventional methods of rodent control that rely on increasing mortality. We discuss the various methods of fertility control currently available for reducing rodent pest populations and then focus on immunocontraception, a relatively new approach to the problem of controlling wild pest populations.

Effective pest control requires a thorough understanding of the biology and population dynamics of the pest species (Howard 1967). Specifically, for effective fertility control, a reduction is required not only in the reproductive potential of the species, but also in the final population size (Bomford 1990; Bomford and O'Brien 1997) and in the potential damage inflicted (Bomford and O'Brien 1997). Thus, we emphasise in this chapter the importance of

an ecological framework for considering the use of immunocontraception and fertility control in general. We also stress that although the general principle of fertility control is similar for all mammals, the particular approach may be different for each pest and needs to consider the ecological and behavioural features of each species (Cowan and Tyndale-Biscoe 1997).

Conventional Methods of Control

Control of rodent pests currently relies on increasing their mortality. For large-scale control in agricultural systems, this typically involves the use of rodenticides such as anticoagulants and acute poisons (see Meehan 1984; Prakash 1988a for reviews). In small-scale domestic control, both rodenticides and traps are often employed. These methods are easily applied by farmers or householders and there is usually an immediate effect on population size and damage caused by rodents (Table 1) (Bomford 1990).

However, these conventional control methods are obviously not always effective in the long term (Table 1) as rodent pests are still a major problem. This may be because lethal methods are often used inefficiently as an ad hoc control approach when rodent populations have already reached high densities. Another major factor is the high reproductive capacity of the pest and the ability to re-invade treated areas from surrounding untreated sites (e.g. Emlen et al. 1948; Twigg et al. 1991). Also, because these methods are often labour intensive, they are rarely applied in areas with inaccessible terrain. The expense of poison-baiting large areas long-term can also be prohibitive, particularly if damage to crops is not reduced. For example, during a mouse plague in southern Australia in 1993, the cost of one bait application to 46,000 ha was approximately A$319,500 (Kearns 1993).

Table 1.
Advantages and disadvantages of rodenticides for the control of rodent pests (after Singleton and Redhead 1989; Bomford 1990; Chambers et al. 1997 unless indicated otherwise).

Advantages	Disadvantages
Immediate effect on population numbers and damage	Development of bait shyness if sublethal dose ingested (Prakash 1988b)
Permanent control method; removes animals for the whole of their expected life span	Non-target deaths due to primary and secondary poisoning—not species-specific
Cost effective for short-term control and reduction in damage caused	Inhumane
	May pollute the environment with poison residues
	Potential re-invasion of treated areas by rodents from neighbouring untreated sites
	Ineffective over the long-term for highly fecund or mobile species (e.g. rodents) (Caughley 1977, 1985)
	Expensive to apply over large areas long-term

Fertility control has the potential to overcome some of the inadequacies of conventional control techniques and a naturally disseminating immuno-contraceptive would reduce the need for manual delivery of the control agent.

Fertility Control as an Alternative to Conventional Methods

Fertility control has been suggested as a more appropriate control strategy than enhancing mortality under the following circumstances:

- for species with high fecundity (Caughley et al. 1992; Tyndale-Biscoe 1994);
- for species with high natural mortality rates and a rapid population turnover (Stenseth 1981; Bomford 1990; Hone 1992; Barlow 1994; Barlow et al. 1997);
- when a more humane method of population control is desired (Marsh and Howard 1973; Hutchins et al. 1982; Hutchins and Wemmer 1987);
- when the effects of sterilisation exceed any increases in juvenile or adult survival due to a lowering of birth rates (Sinclair 1997); and
- for preventing or reducing population growth after some other technique has reduced numbers, particularly in long-lived species (Bomford 1990; Barlow 1994).

The last point emphasises one of the main differences between these two control strategies—increasing mortality has an immediate effect on population numbers and damage, while reducing fertility has a delayed response until natural mortality reduces population size (Barlow et al. 1997). If sterile individuals inflict as much damage as fertile individuals, sterility is of little practical value to agriculturalists. Thus, in some instances, fertility control may need to be used in conjunction with another control method.

It has been suggested that the presence of a given number of sterile individuals in the population exerts a greater, more sustained biocontrol pressure than if the same number of animals were simply removed from the population (Howard 1967). Sterile individuals fail to contribute to the next generation as well as competing for space, food and social order. This in turn reduces the reproductive success and survival of fertile individuals and continues the suppression of breeding in subordinates if dominants are sterilised (Howard 1967). Therefore, fertility control could be used as a long-term strategy for slowing a population's growth rate and hence maintaining numbers at this lower level. Modelling the relative impact of culling versus sterilisation on populations with density-independent or exponential growth rates supports this argument (Bomford 1990). However, for populations with density-dependent or logistic growth rates, the relative efficiency of sterilisation will depend on the nature of the density-dependent regulation. Populations with density-dependent mortality appear to be reduced by sterilisation more quickly than those with density-dependent recruitment (Barlow et al. 1997).

General Aims of Fertility Control

Fertility control aims to reduce population size by reducing the number of young

produced and recruited into the population. This can be achieved by temporary, permanent or partial sterilisation.

A successful fertility control method therefore needs to (after Bomford 1990; Bomford and O'Brien 1997):

- cause temporary or permanent sterility leading to reduced recruitment in the population;
- be deliverable in a way that allows an adequate proportion of the target population to be treated, particularly for widespread and abundant species in areas with poor access;
- reduce the target population sufficiently to reduce damage caused by the pest species to an acceptable level (Braysher 1993);
- produce minimal side effects to the target species (e.g. behavioural changes, interference with social structure);
- be target-specific;
- be environmentally benign (Marsh and Howard 1973); and
- be cost effective compared with conventional methods of control.

In the following section, we explore the various options available for fertility control of rodents and examine how well each of these satisfy the criteria for a suitable fertility control agent for controlling wild populations.

Options for Fertility Control — Existing Technologies

Many techniques have been developed for managing or controlling the fertility of individual animals in captivity or in confined areas that are not subject to immigration. These methods include surgical sterilisation or castration, use of chemical sterilants, agonists that block the function of natural hormones, and inhibitors of lactation (Table 2). Most of these approaches are expensive and time-consuming to apply, often have undesirable side effects (e.g. chemosterilants can induce gastrointestinal problems, abnormal growth and dysfunction of the gonads), and affect non-target species. Many disrupt gonadal function and sexual behaviour. Further, their applicability and effectiveness for free-ranging populations is low due to the difficulties of delivering the sterilising agent on a broad scale and sustaining the inhibition of reproduction.

Immunocontraception for Controlling Pest Populations — the Concept

Immunocontraception uses the body's immune system to induce immune responses (circulating antibodies or cellular immune effector cells) against reproductive cells or proteins essential to successful gametogenesis, fertilisation or implantation, leading to infertility. The feasibility of immunocontraception was directly demonstrated when Baskin (1932) injected women with human sperm and no conceptions occurred during the one-year follow-up period.

Ideally, the immunocontraceptive prevents pregnancy but does not disrupt endocrine function (i.e. renders the animal infertile but not impotent) and therefore reproductive/social behaviour is unaffected. Animals continue to occupy territory,

Table 2.
Summary of potential techniques for fertility control of pest populations and assessment of their relevance for managing rodents. Sources: Singleton and Spratt 1986; Spratt and Singleton 1986; Marsh 1988; Vickery et al. 1989; Bomford 1990; Sankai et al. 1991; Gao and Short 1993; Tyndale-Biscoe 1994, 1997a; Marks et al. 1996; Becker and Katz 1997; Jochle 1997.

Technique for fertility control	Major advantages	Major disadvantages	Efficacy for rodent pest populations	
			Current	Future
Surgery	**Castration and ovariectomy**		Very low	Very low
	Permanent	Expensive and invasive		
	One treatment only, therefore costs recouped over time	Leads to behavioural changes		
	Vasectomy and tubal ligation		Very low	Very low
	Permanent	Expensive and invasive		
	One treatment only, therefore costs recouped over time	Impractical for high density field populations		
	No behavioural changes			
Disease	**For example, *Capillaria hepatica***		Field tested, but ineffective at low densities	Low
	Natural parasite of rodents	Insufficient level of persistence in Australian situation		
		Complex life cycle		
Chemicals	**Agonist and antagonists of GnRH[a] (disrupt natural hormone functions)**		Low, but untested	Low, but untested
	Both sexes infertile	Costly to administer		
		Not permanent, may only reach a proportion of the population		
		Side effects (dose dependent)		
		Not appropriate for promiscuous species		

[a] GnRH = gonadotrophin releasing hormone
[b] ISCOMs = immunostimulatory complexes.

Table 2. (Cont'd)

Summary of potential techniques for fertility control of pest populations and assessment of their relevance for managing rodents. Sources: Singleton and Spratt 1986; Spratt and Singleton 1986; Marsh 1988; Vickery et al. 1989; Bomford 1990; Sankai et al. 1991; Gao and Short 1993; Tyndale-Biscoe 1994, 1997a; Marks et al. 1996; Becker and Katz 1997; Jochle 1997.

Technique for fertility control	Major advantages	Major disadvantages	Efficacy for rodent pest populations	
			Current	Future
Chemicals	**Synthetic steroids, anti-steroids, anti-steroid receptor (e.g. Diethylstilbestrol, RU486)**		Low, but untested	Low, but untested
	Low cost	Side effects (dose dependent)		
	Bait or implant	Must be administered regularly		
		Non-target effects		
	Prolactin inhibitors (affect lactation and/or gestation (e.g. Bromocriptine, Cabergoline)		Low	Moderate
	Oral delivery	Not permanent		
	Low cost	May not be ethically acceptable as starves young or aborts foetuses		
		Must be regularly administered		
Immunocontraception	**Disseminating vector, non-disseminating vector, synthetic delivery systems (e.g. ISCOMs[b], microspheres)**		Moderate but untested	Expected high
	Long term reduction in fertility	Not yet available		
	Species-specific, humane, cost effective	May need to repeat application		
	Could be reversible	Includes use of genetically modified organisms		

[a] GnRH = gonadotrophin releasing hormone

[b] ISCOMs = immunostimulatory complexes.

maintain social status and may suppress the fecundity of subordinates. Such an approach is potentially species-specific, considered humane and could be cost effective in the long term (Tyndale-Biscoe 1994).

Unlike vaccines directed against infectious diseases, immunocontraceptive vaccines are directed against 'self' proteins that would not normally be recognised as foreign (Alexander and Bialy 1994; Jones 1994; Dunbar 1997). Therefore, the 'self' antigen to be used in the vaccine must be presented in a 'foreign' or 'non-self' form to elicit an immune response. In 1987, a new approach to fertility control was conceived—the concept that viruses could be used to deliver immunocontraceptives (Tyndale-Biscoe 1994) (see Figure 1). This could be achieved by delivering the immunocontraceptive vaccines through the agency of a virus or other contagious agents that spread naturally through the target pest population. Similarly, a non-disseminating agent in baits could be used to provoke an appropriate immune response.

Since 1992, this approach has been under development at the Cooperative Research Centre for Biological Control of Vertebrate Pest Populations (Vertebrate Biocontrol CRC) and its successor the Pest Animal Control CRC based in Canberra, Australia. The Centre's mission is "to contribute to the better management of Australia's biodiversity by limiting growth of vertebrate pest populations through fertility control".

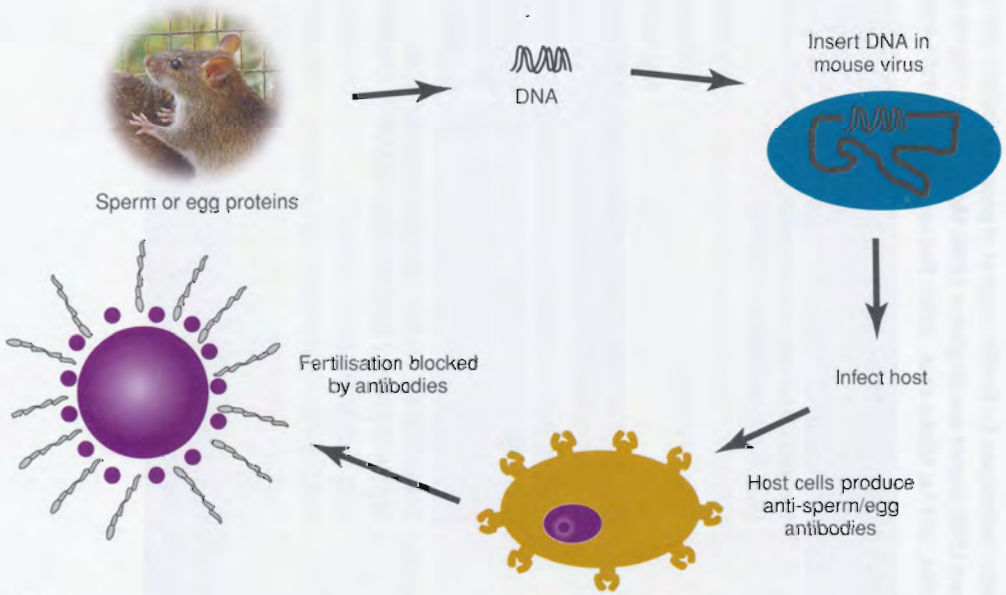

Figure 1.
The concept of viral-vectored immunocontraception. Genes encoding a reproductive protein(s) are incorporated into the genetic structure of a species-specific virus. This virus infects the host, expressing the reproductive protein(s) as well as viral proteins on the surface of infected cells. The host's immune system produces antibodies against the reproductive protein(s), as well as the virus, and these spread to the reproductive tract where they bind to either the egg or the sperm and block fertilisation. Redrawn with permission from the Vertebrate Biocontrol CRC.

IMMUNOCONTRACEPTION—ITS COMPONENTS

The choice of reproductive antigen(s)

Fertility control agents may be of two types: anti-gonadal or anti-gametic. By targeting the gametes there is likely to be less disruption of other reproductive functions, but sustained immune responses may be more difficult to achieve because the gamete proteins are produced in small quantities at specific sites and are not highly immunogenic (Alexander and Bialy 1994). Initial studies by many groups have focused on sperm proteins as candidate antigens. The view was that male antigens might be able to induce significant immune responses in the female reproductive tract because they were not expected to be auto-antigens in females. The potential of sperm proteins, such as SP-10 and testis-specific lactate dehydrogenase (LDH-C4) has been explored in humans, baboons and pigs (Goldberg and Shelton 1986; Herr et al. 1990a,b) and PH-20 in guinea pigs (Primakoff et al. 1988). Initial research in the Vertebrate Biocontrol CRC also focused on sperm antigens (Bradley 1994; Holland and Jackson 1994; Tyndale-Biscoe 1994). However, after several sperm antigens had been tested by direct immunisation without effects on the fertility of female rabbits or foxes (Bradley et al. 1997; Hardy et al. 1997; Holland et al. 1997), attention turned to the female gamete antigens, specifically the zona pellucida proteins forming the extracellular coat of the oocyte.

In the mouse, the zona pellucida comprises three non-covalently linked glycoproteins, ZPA, ZPB and ZPC, which are expressed by the growing oocytes in the ovary. ZPC is the receptor for sperm binding at the time of fertilisation (Florman and Wassarman 1985; Rosiere and Wassarman 1992). Passive immunisation with monoclonal antibodies to ZPC inhibit fertilisation in vivo (East et al. 1984, 1985), and active immunisation with synthetic peptides which include a B-cell epitope of ZPC also induce infertility for periods from 0–8 months (Millar et al. 1989; Lou et al. 1995). For these reasons, mouse ZPC (also known as ZP3) was the first candidate antigen to be tested in a mouse viral-vectored system.

Many of the zona pellucida proteins and sperm proteins show high identity between species (Harris et al. 1994; Bradley et al. 1997; Holland et al. 1997; Jackson et al. 1998). Therefore a key challenge is to identify or engineer the antigen to be species-specific. This may be achievable using specific peptides or epitopes. The difficulty then becomes whether such small peptides have the ability to block fertility. The use of epitopes alone or in combination with immunomodulatory molecules (such as cytokines or T-cell help epitopes) to enhance the species-specific immune responsiveness to these antigens are currently being investigated (Dalum et al. 1997; Ramsay and Ramshaw 1997).

Delivery of the immunocontraceptives

The delivery of an anti-fertility vaccine to populations of wild animals over large areas poses a number of unique problems. It is essential to consider the distribution of the species under study, whether large-scale or localised control is desired, and any possible consequences for non-target species. Another factor is the genetic heterogeneity of the

wildlife population, which is certain to generate significant individual variability in the immune responses to a vaccine (Klein 1979). Effective application of any vaccine requires that a high level of immunity can be achieved amongst individuals exposed to the vaccine (Alexander and Bialy 1994). As mentioned previously, it may therefore be necessary for the antigen(s) to be presented in conjunction with other highly immunogenic carrier proteins (e.g. cytokines and immunomodulatory molecules) to maintain a contraceptive level of immunity. In addition, multiple antigenic determinants could be included within a vaccine to stimulate a broad range of immune responses.

The three main delivery systems under development are (i) non-disseminating genetically modified organisms (GMOs) in baits, (ii) synthetic delivery systems and (iii) disseminating GMOs such as viruses or bacteria. For many rodent pests, particularly those that are native species, bait delivery may be the method of choice for political, social, economic and ecological reasons.

Non-disseminating agents

Non-replicating GMOs, such as attenuated *Salmonella*, are currently being developed and tested (Bradley 1994; Bradley et al. 1997). Selected mutant strains of *Salmonella* have the advantage that they are avirulent without decreasing their immunogenicity and they are not infective. Furthermore, the introduction of a 'suicide' plasmid into this system would have the added advantage of degrading the foreign deoxyribonucleic acid (DNA) and would make it more acceptable because the bait-delivered product would contain no foreign genetic material (Knudsen et al. 1995; Tedin et al. 1995).

Various gram negative bacteria (*Escherichia coli, Salmonella typhimurium, Vibrio cholerae, Klebsiella pneumoniae* and *Actinobacillus*) can be engineered to carry a gene (PhiX174 geneE) which, when induced, causes lysis and release of the cytoplasmic contents of the bacteria. This process produces a non-living vaccine delivery system. These bacteria can also be engineered to carry other genes (e.g. encoding reproductive proteins). After lysis, the 'ghost' bacteria contain only membrane-associated recombinant antigen. Bacterial ghosts are cheap to produce, can be stored for long periods and can contain multiple antigenic determinants that are present in a highly immunostimulatory environment (Szostak et al. 1996). Such features make bacterial ghosts an attractive delivery system for immunocontraceptive antigens. However, it remains to be seen whether these preparations produce immunity to reproductive antigens after oral delivery.

Synthetic delivery systems

Synthetic delivery systems for antigens include ISCOMs (immunostimulatory complexes—e.g. Quil A, cholesterol, phospholipid constructs), microspheres (polylactide-coglycolide polyphosphazenes), and liposome emulsions (Davis 1996).

The current high costs of production mean these systems are only suitable for human and companion animal vaccination and not for broad-scale application to a wildlife population. Nevertheless, the per unit production cost will decrease as these systems become more popular and production technology improves.

Disseminating GMOs

These currently have the greatest theoretical potential for use as vectors for immunocontraceptive agents. A viral vector could potentially overcome problems associated with the distribution of an immunocontraceptive to control wild populations (Bomford 1990). The advantages of a viral-vectored immunocontraceptive agent over a bait-delivered immunocontraceptive agent are summarised in Table 3. Clearly the selection of a viral vector requires careful consideration of its properties. The current vector of choice for delivery of a mouse immunocontraceptive is mouse cytomegalovirus and it possesses most of the essential and desirable characteristics required (Table 4), although additional research is required to confirm some of its features.

Viral-Vectored Immunocontraception (VVIC) — Laboratory Progress

Ectromelia

Ectromelia virus (ECTV: family Poxviridae, genus *Orthopoxvirus*) causes the disease known as mousepox and is a pathogen of laboratory mice (Fenner and Buller 1997). It is closely related to vaccinia virus and was investigated as a useful model system for the development of viral-vectored immunocontraception (VVIC). A recombinant ectromelia virus, with a thymidine kinase negative phenotype, expressing ZPC was constructed and then used to infect female inbred laboratory mice of the BALB/c strain, which are highly susceptible to mousepox (Jackson et al. 1998). Fertility was assessed by pairing females with males from three weeks post-infection and monitoring for

Table 3.
Viral-vectored versus bait-delivered immunocontraceptives (after Bomford 1990; Shellam 1994; Chambers et al. 1997)

Advantages of a viral-delivered immunocontraceptive
A replicating virus may induce a stronger immune response and greater immunological memory.
An infective agent can potentially spread a reproductive protein rapidly through a population.
A self-perpetuating, infectious agent is ultimately cheaper than baits which must be manually applied.
A viral vector is a species-specific carrier.
Overcomes problems associated with bait aversion or bait shyness.
Overcomes the precise timing necessary for bait delivery relative to the target animal's breeding cycle.
Reduces wastage associated with inadvertent multiple baiting of some individuals.
Advantages of a bait-delivered immunocontraceptive
More acceptable to the public than the use of a disseminating genetically modified organism.
Easier regulation of control activities—can be readily withheld or withdrawn from use.

Table 4.
Essential and desirable properties for a virus which will act as a vector of an immunocontraceptive agent for the biological control of rodents (after Shellam 1994). Does murine cytomegalovirus (MCMV) meet these requirements?

Essential properties		MCMV
• Species-specific and naturally infects target species	√	Native murids will be tested to verify this
• Readily transmitted in target species	√	Seroprevalence >90% in wild mice (Smith et al 1993)
• Insertion of foreign gene is stable and does not affect viral growth or transmission	√	Insertion sites identified (Manning and Mocarski 1988); recombinant constructed with beta-galactosidase gene. More research required on effects on transmission
• Stimulates long lived immune response and immunological memory	?	
• Recombinant virus can be introduced and maintained in the presence of existing immunity	?	Wild mice have been found with up to four strains; infection with multiple strains can be achieved in the laboratory (Booth et al 1993). Epidemiology of this needs to be examined in wild mice
• Panel of isolates available	√	
• Epidemiology of infection understood and site of viral growth known	√	Virus persists in submaxillary gland Weak knowledge of epidemiology outside laboratory
• Approval by regulatory authorities likely	√	Already in Australia

Desirable properties		MCMV
• Virus is already in the country	√	(see Smith et al. 1993)
• Virus establishes persistent and non-lethal latent infection	√	
• Good local immunoglobulinA response which does not interfere with transmission	√	
• Mechanism for any genetically determined host resistance is known	√	Ability of subsequent infections to stimulate immune response not known
• Genetically determined host resistance does not interfere with infection or transmission	√	
• Mechanism of transmission known	√	Close contact; sexual and via saliva
• Virus is sexually transmitted	√	Enhances species-specificity
• Knowledge of the epidemiology of infection and transmission of natural virus variants	?	
• A DNA rather than an RNA virus (greater genetic stability)	√	

evidence of pregnancy and birth of litters. Two major experiments were conducted, one to assess the immediate effects on fertility and the second to test the duration of the effects.

The immediate effects on fertility were a reduction in the number of litters produced by females infected with ECTV–ZPC compared to uninfected controls or females infected with recombinant ectromelia virus (ECTV-602) expressing a non-reproductive marker protein, *LacZ* (Table 5). The effects on fertility were long term, with mice infected with ECTV–ZPC infertile for periods of 5–9 months while those infected with ECTV-602 remained fertile. Mice became fertile as the anti-ZPC antibodies in the serum decreased, but when they were re-infected with the recombinant virus, antibody titres to ZPC increased and the animals returned to an infertile state (Jackson et al. 1998). Therefore, this study provided the first demonstration of VVIC in laboratory mice.

Examination of the ovaries of infertile females revealed two possible mechanisms for infertility. Half of the animals showed disruption in folliculogenesis, with an absence of mature follicles and oocytes as well as large clusters of luteinised cells (Jackson et al. 1998). There was no observable oophoritis. The remaining animals showed normal ovarian development of follicles and ovulation; antibody localisation studies indicated binding of ZPC antibodies to these oocytes, suggesting that after ovulation, sperm would not be able to bind and result in fertilisation (Jackson et al. 1998).

Murine cytomegalovirus

Ectromelia virus is not present naturally in the Australian environment and therefore, for ethical, political and social reasons, is not an ideal candidate for release as a viral vector of an immunocontraceptive agent. Moreover, its lethality would select for resistance more rapidly than a non-lethal agent. Other research is being conducted using murine cytomegalovirus (MCMV) which is highly prevalent in Australian mouse populations and possesses the desirable properties of a vector (Table 4) (Singleton et al. 1993; Smith et al. 1993; Shellam 1994). This large DNA virus (230 kb, ~200 genes) is a member of the Betaherpesvirinae sub-family of the Herpesviridae. It

Table 5.
Infertility in BALB/c mice infected with either recombinant ectromelia virus expressing zona pellucida glycoprotein C (ECTV–ZPC) or recombinant ECTV expressing a non-reproductive marker protein (ECTV–602) compared with uninfected controls (after Jackson et al. 1998), SE = standard error.

Ectromelia virus infection	No. of mice with litters/total mice	No. of implantations (mean ± SE)		Litter size (mean ± SE)	
		Animals with litters	All animals	Animals with litters	All animals
None	10/10	9.5 ± 0.8	9.5 ± 0.8	6.6 ± 0.8	6.6 ± 0.8
ECTV-602	12/15	8.5 ± 0.9	6.8 ± 1.1	7.3 ± 0.7	5.8 ± 1.0
ECTV-ZPC	4/13	2.5 ± 0.7	0.8 ± 0.4	1.8 ± 0.3	0.5 ± 0.2

shows strict species-specificity (Hudson 1994) and establishes a persistent infection in the salivary gland with latent infection apparently associated with ubiquitous elements such as macrophages (Koffron et al. 1995; Pollock et al. 1997), rather than with organ-specific cells (e.g. hepatocytes). Infection requires close contact and the virus is believed to be transmitted via secretions such as saliva and sexual secretions (see Shellam 1994).

Recombinant MCMV has been constructed by inserting the mouse ZPC gene into the immediate early 2 (*ie2*) gene. The effects on fertility of infecting with recombinant MCMV expressing either ZPC or a non-reproductive marker gene (*LacZ*) were assessed for several different inbred strains of mice (BALB/c, A/J, C57BL/6, ARC/s) with varying susceptibility to infection with MCMV (Grundy et al. 1981; Allan and Shellam 1984). Recombinant MCMV–ZPC induced a long-lasting, high-titred antibody response to ZPC in all mice tested. The fertility of uninfected controls was also determined. BALB/c females ($n = 9$) infected with recombinant MCMV–ZPC produced no litters for 200 days after infection, while the uninfected controls and the MCMV–*LacZ* infected group produced approximately 250–350 pups during the same period (Figure 2). The response was similar in A/J females although the overall productivity of this strain was lower. Contraceptive effects of lesser magnitude were observed in C57BL/6 and ARC/s strains. The ovaries of recombinant MCMV–ZPC infected females showed histological changes but the mechanism of infertility remains under investigation.

These results (M. Lawson et al., unpublished data) demonstrate that recombinant MCMV expressing the ZPC gene can elicit an immunocontraceptive response in mice. This response occurred in the absence of high levels of replication of the recombinant virus, since very low levels of virus were found in the salivary glands of mice relative to controls. Research is continuing on ways to enhance this response in less susceptible strains of mice as well as to demonstrate the transmissibility and competitiveness of the recombinant virus when confronted with prior MCMV infection in wild outbred mice (see next section).

Epidemiological Considerations

Manipulating the genetic structure of a virus to incorporate an immunocontraceptive antigen may effect its transmissibility, persistence and species-specificity. Thus, it is important to examine the epidemiological consequences of such a manipulation from both an ecological and a viral engineering perspective. The key questions that need to be addressed are:

- What is the transmission rate of the wild-type virus and the recombinant sterilising virus? Do they differ? If so, why?
- Do the characteristics of viral infection such as the immune response and site of replication differ between the wild-type and recombinant virus?
- What is the threshold population size required to maintain the viral infection at a specified prevalence? What influences this?

- Can a recombinant strain of the virus establish and generate an immune response in a rodent population that may have a pre-existing infection with the wild-type virus? Is the order of infection important?
- What is the persistence of the virus in the environment?

Many of these questions are difficult to test in wild populations, particularly for the recombinant viruses where thorough testing under contained conditions is required before release into a field population. A crucial experiment will be to examine if the impact of the sterilising, recombinant virus on breeding affects the transmissibility of the virus.

Experiments will be conducted to address these questions using large (2 m × 2 m) cages to house a simulated 'population' of mice. These cages are

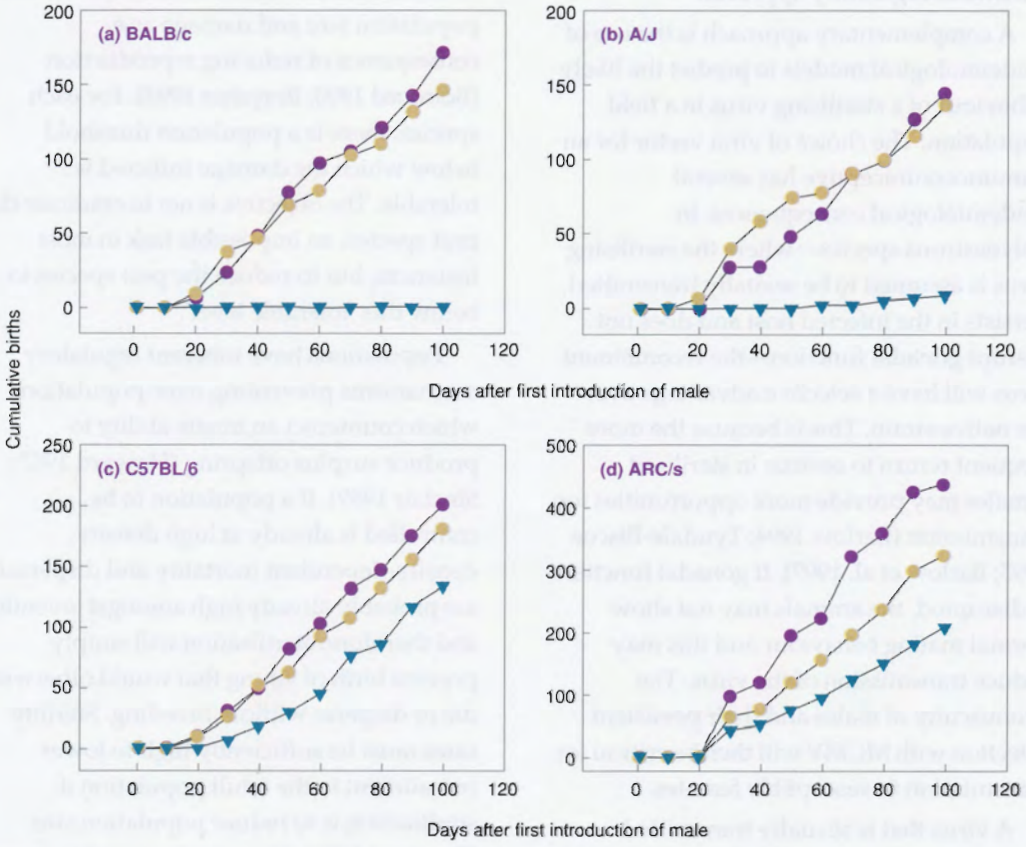

Figure 2.
Cumulative births in different strains of mice infected with either recombinant murine cytomegalovirus–zona pelucida glycoprotein C (MCMV–ZPC) (▼), or recombinant MCMV–*LacZ* (a non-reproductive marker protein gene (●) compared with uninfected controls (●). Groups of nine females were infected with 2×10^4 pfu (plaque forming units) of tissue culture-derived virus 21 days prior to the introduction of males to the breeding cages. Each cage contained three females and one male. Groups were checked for births several times per week.

internally complex so that mice can avoid each other within the cage if required. Uninfected mice will be released into this cage, a number of mice infected by intraperitoneal inoculation and the spread of the virus monitored. Further studies of MCMV in wild populations will still be necessary to assess the relevance of these cage results. The use of a virus strain expressing an innocuous, non-reproductive marker gene would be useful in this instance but awaits regulatory approval.

A complementary approach is the use of epidemiological models to predict the likely behaviour of a sterilising virus in a field population. The choice of viral vector for an immunocontraceptive has several epidemiological consequences. In polyoestrous species—where the sterilising virus is assumed to be sexually transmitted, persists in the infected host and does not disrupt gonadal function—the recombinant virus will have a selective advantage over the native strain. This is because the more frequent return to oestrus in sterilised females may provide more opportunities for transmission (Barlow 1994; Tyndale-Biscoe 1995; Barlow et al. 1997). If gonadal function is disrupted, the animals may not show normal mating behaviour and this may reduce transmission of the virus. The promiscuity of males and their persistent infection with MCMV will then be critical for transmission to susceptible females.

A virus that is sexually transmitted increases the chances of the VVIC agent contacting only the target pest population compared with a contagious or insect-borne virus. However, spatial modelling suggests that a sexually transmitted virus would be at a disadvantage when compared with a virus spread by insect vectors, as the requirement for close contact could potentially limit the rate of spread of the virus (G.M. Hood, unpublished data). These considerations need to be balanced when determining which viral vector is most appropriate in each pest control situation.

Ecological Implications

Immunocontraception can only be judged to be successful for rodent control if it reduces population size and damage as a consequence of reducing reproduction (Bomford 1990; Braysher 1993). For each species, there is a population threshold below which the damage inflicted is tolerable. The objective is not to eradicate the pest species, an impossible task in most instances, but to reduce the pest species to below this 'tolerable level'.

Populations have inherent regulatory mechanisms preventing over-population which counteract an innate ability to produce surplus offspring (Howard 1967; Sinclair 1989). If a population to be controlled is already at high density, density-dependent mortality and dispersal are probably already high amongst juveniles and therefore, sterilisation will simply prevent birth of young that would otherwise die or disperse without breeding. Sterility rates must be sufficiently high to lower recruitment to the adult population if sterilisation is to reduce population size (Bomford 1990). This emphasises the importance of gaining some understanding of the factors regulating populations and how these are affected by fertility control.

Fertility control may interact with other population processes to enhance the overall

effect on population numbers. For example, if a pest species is prevented from increasing its reproductive rate, predation may be able to maintain their population at a low level (Sinclair 1997). This may apply to house mouse populations in Australia in which avian predators are capable of regulating the population when mouse densities remain low but can not maintain this regulation when mouse densities increase to high levels (Sinclair et al. 1990).

Several ecological questions need to be addressed when assessing the potential of a particular immunocontraceptive agent:

- What proportion of a wild pest population needs to be sterilised to significantly reduce growth rate and population size? And can a delivery system be developed that will reach the required proportion of the population?
- Is the maintenance of social structure important and does it affect the efficiency of the immunocontraceptive?
- Is compensation a likely factor that could reduce the efficacy of an immunocontraceptive?

A further question is whether density-dependence plays a role in modifying the efficacy of a given sterility level. Will the proportion of sterilised individuals need to be increased in a high-density compared with a low-density population to have the same impact? Is compensation density-dependent? Some of the implications for density-dependent regulation on the applicability of sterilisation to control populations have been discussed in a previous section.

Level of sterility required

Modelling

Mathematical models have been used to estimate the level of fertility control required to produce a significant reduction in population size. Knipling and McGuire (1972) modelled the effects of permanent sterilisation of females or both sexes versus killing similar numbers in a rat population. Their model predicted that by sterilising 90% of both sexes, this had a greater effect than killing 90% of rats. However, they assumed that if 90% of males don't breed, 90% of females would not breed. In a species such as rodents with a promiscuous mating system, this is an invalid assumption (Kennelly et al. 1972; Pennycuik et al. 1978). There was also no allowance for compensatory changes in immigration and dispersal.

N. Stenseth et al. (unpublished data) have modelled empirical field data from populations of the multi-mammate rat *Mastomys natalensis*, in eastern Africa. The model simulated a permanent decrease in reproductive rate of this species and found that long-term reductions in population density were attained if between 50 and 75% of females were sterile.

A simple demographic model using life-history information obtained from laboratory, enclosure and field studies was used to examine the proportion of mice to be sterilised to produce a significant decrease in population size in enclosure populations (Chambers et al. 1997). This simulation was a useful precursor to a manipulative experiment (described below), assisting with experimental design and indicating the types of data that needed to be obtained. The

model examined two levels of sterilisation (67% and 75% of females) and compared the outcome against a non-sterilised population. The simulation found that both the 67% and 75% levels of sterility were sufficient to reduce population size and growth rate, relative to the unsterilised population (Figure 3).

However, models often overestimate the effectiveness of an immunocontraceptive as they are generally based on higher levels of fertility control than can be practically achieved in the field. They also tend to ignore or underestimate factors that may reduce the effects of fertility control, such as compensatory changes in behaviour, survival or fecundity (Bomford 1990). The latter applies to all of the models described above.

Manipulative experiments

Manipulative experiments can be used to examine empirically the sterility level suggested from mathematical models. Experiments involving surgical sterilisation allow the degree and nature of sterilisation required to reduce population size to be examined (Kennelly and Converse 1997). For example, when females in populations of house mice housed in outdoor enclosures (Figure 4) were surgically sterilised at a level of 67%, this significantly reduced population size. Over 18 weeks, populations were reduced from a mean abundance of 221 mice in two control populations to 104 mice in four sterilised populations (Chambers et al. 1999).

Kennelly et al. (1972) sterilised 85% of males in a Norway rat population and found no effect on population size when compared with an unsterilised population, confirming that the development of an immuno-contraceptive for males in a species with a promiscuous mating strategy is not effective.

Social structure

Many studies of wild mammals have shown that reproductive success is closely linked to an animal's rank in the social hierarchy. Lower ranking animals either do not breed or fail to rear their young to independence (Wasser and Barash 1983; Abbott 1988). Caughley et al. (1992) highlighted the need to have some understanding of the social structure and mating system of the species to be controlled by fertility control. They showed via modelling that for most scenarios, sterilisation would reduce population growth, irrespective of mating system or social structure. However, where the sterilisation of a single dominant female releases subordinates from breeding suppression, sterilisation actually enhanced the overall productivity of the population. This emphasises the need to sterilise individuals without compromising their social position (Chambers et al. 1997). This would maintain the breeding performance of subordinates at a low level, preventing compensation by these individuals for the reduction in population growth (Caughley et al. 1992; Barlow 1994; Tyndale-Biscoe 1994; Cowan and Tyndale-Biscoe 1997).

The importance of maintaining hormonal competence in surgically sterilised females in reducing the overall productivity of populations has been examined for mice housed in near-natural outdoor enclosures (Chambers et al. 1999). Female mice were either ovariectomised (hormonally incompetent) or tubally ligated (hormonally competent) at the rate of 67% per population

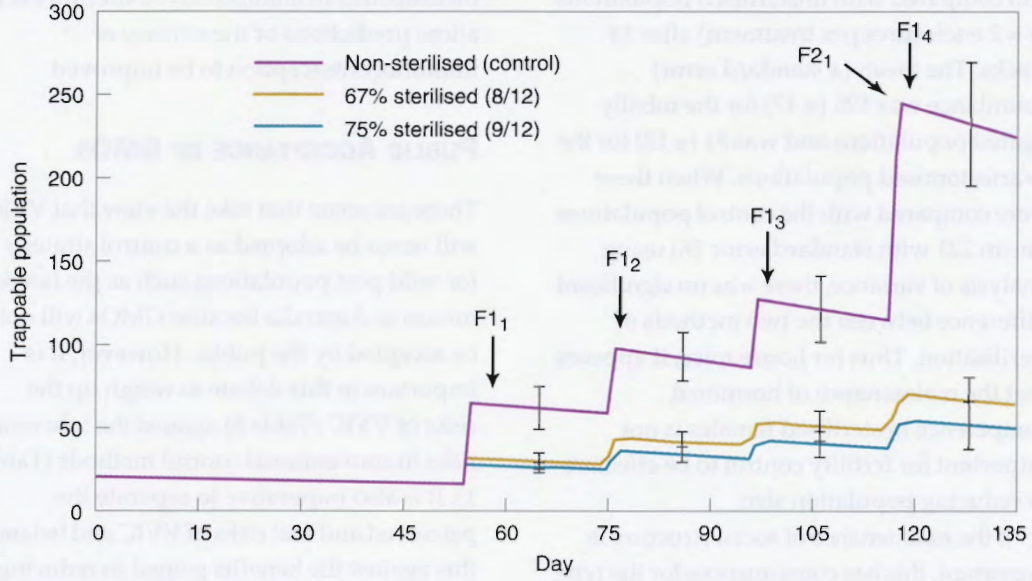

Figure 3.
Demographic model predicting the trappable population of mice housed in outdoor enclosures after 0%, 67% and 75% of females have been sterilised (see Chambers et al. 1997 for details of the model). Each plot is the mean (± standard deviation) of 10 runs of the model. F1$_1$ to F1$_4$ indicates when F1 generation litters (those produced by the founding population of mice) will enter the trappable population. F2$_1$ indicates when the first litter of the F2 generation (produced by the F1$_1$ litter) enters the trappable population (adapted from Chambers et al. 1997).

(a) (b)

Photos: G. Singleton, CSIRO.

Figure 4.
Outdoor enclosures used for manipulative experiments examining the effectiveness of fertility control to reduce mouse population abundance and rate of increase. Each enclosure is 15 m × 15 m in area and is protected from predators by wire mesh fencing. Mice are prevented from burrowing into or out of the enclosures by metal fences that are buried to a depth of 800 mm. Food and water are provided ad libitum. (a) Ground-level view; (b) Aerial view.

and compared with unsterilised populations (n = 2 enclosures per treatment) after 18 weeks. The mean (± standard error) abundance was 126 (± 17) for the tubally ligated populations and was 81 (± 12) for the ovariectomised populations. When these were compared with the control populations (mean 221 with standard error 26) using analysis of variance, there was no significant difference between the two methods of sterilisation. Thus for house mice, it appears that the maintenance of hormonal competence in sterilised females is not important for fertility control to be effective in reducing population size.

If the maintenance of social structure is important, this has consequences for the type of immunocontraceptive antigen to be used (Chambers et al. 1997). Some of these antigens are known to cause oophoritis or ovarian dysfunction that may affect the release of hormones controlling reproduction and social position (Skinner et al. 1984; Kirkpatrick et al. 1992; Rhim et al. 1992).

Compensation

If fertility is reduced, the average population size is also reduced, but only under certain conditions. If juvenile or adult survival improves with lower fertility or territoriality limits populations, the effects of lower birth rate will not change population size unless such reduction exceeds the effects of these processes (Sinclair 1997). If sterile individuals have increased survival, they may cause more damage than non-sterile animals (Bomford 1990).

As was discussed earlier, modelling the effects of fertility control on wildlife populations often ignores the effects of compensation. Therefore it is important to measure this in manipulative experiments to allow predictions of the efficacy of immunocontraception to be improved.

Public Acceptance of GMOs

There are some that take the view that VVIC will never be adopted as a control strategy for wild pest populations such as the house mouse in Australia because GMOs will not be accepted by the public. However, it is important in this debate to weigh up the risks of VVIC (Table 6) against the inherent risks in conventional control methods (Table 1). It is also imperative to separate the perceived and real risks of VVIC and balance this against the benefits gained in reducing the damage caused by the pests (Chambers et al. 1997). The strategy adopted by the Vertebrate Pest Animal Control CRC is that research should proceed incrementally with public discussion at each step so that its potential use can be weighed against the risks (Tyndale-Biscoe 1997b).

The risks of VVIC are discussed in detail in Tyndale-Biscoe (1994, 1995), Guynn (1997) and Williams (1997) and are summarised in Table 6. Most relate to the issues of public acceptability of GMOs and maintaining the species-specificity of the recombinant virus.

How species-specificity is achieved will depend on the target animal, the ecosystem, the delivery system, local non-target species and, in general, the aims of the fertility control program (Stohr and Meslin 1997). The important question to address is if a VVIC encounters a non-target species, will this cause infertility even though no productive infection occurs? Species-specificity operates at three levels: the viral vector, the reproductive protein and social