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Research To Develop Contraceptive Control of Brushtail Possums in New Zealand

Simon E. Jolly, Phil E. Cowan, and Janine A. Duckworth

Abstract: Common brushtail possums are serious pests in New Zealand, where they threaten the survival of native plants and animals and spread bovine tuberculosis. A National Science Strategy Committee established in 1991 to coordinate possum research gave high priority to research aimed at biological control of possums, particularly contraceptive control. Surveys are identifying pathogens and potential vectors, and research has begun on immunology, gene transcription, potential contraceptive targets, and sociobiology. As there are more than 60 million

possums in New Zealand, contraceptive vaccine delivery systems need to be cost effective, and they must be publicly acceptable. A vaccine could be included in a bait, but long-term cost-effective control will probably require a biological vector. Eventually the best control strategy will probably combine traditional control and immunocontraception.

Keywords: brushtail possums, immunocontraception, *Trichosurus vulpecula*, vectors

The Possum Problem

The common brushtail possum (*Trichosurus vulpecula*), a native Australian marsupial, was introduced into New Zealand in the last century to start a fur industry. Possums are folivorous and largely arboreal, and they average about 3 kg in weight. They are relatively widespread in Australia, although not often in high number. Like all marsupials, they are legally protected in Australia (How 1983). In contrast, possum population densities are 5 to 20 times higher (up to 25/ha [Green 1984]) in New Zealand, where the possum is acknowledged as the most serious vertebrate pest (Parliamentary Commissioner for the Environment 1994).

Possums established themselves so successfully in New Zealand partly because the forests evolved in the absence of any mammalian browser. Lacking specific chemical defenses against such an attack, many native trees and shrubs are highly palatable to introduced herbivores. There are also no significant possum predators in New Zealand. The large possum population is degrading the composition and structure of some forest types (Payton 1987, Stewart and Rose 1988, Allen et al. 1989). Populations of native animals are also affected because possums compete with them for fruit and nectar supplies (Cowan 1991). Predation, disturbance, and competition are reducing the survival and reproductive success of endemic and highly endangered species such as the kokako (*Callaeas cinerea*) and the short-tailed bat (*Mystacina tuberculata*) (Leathwick et al. 1983, Brown et al. 1993).

In addition to such impacts on conservation values, possums are the major wildlife host of bovine tuberculosis (Tb) in New Zealand. Possums maintain this disease and spread it to cattle and deer herds (Livingstone 1986, 1991), threatening the country's livestock exports. In the 1993–94 financial year, NZ\$17 million was spent on Tb eradication programs in cattle and deer herds and NZ\$18 million was spent on possum control in Tb endemic areas (Parliamentary Commissioner for the Environment 1994). Possum control is chiefly dependent on the use of Compound 1080 (sodium monofluoroacetate) baits, but public opposition to the widespread use of this poison is increasing with particular concern about possible environmental contamination.

In October 1991, the New Zealand Government recognized the seriousness of the possum threat to the economy and environment by establishing a National Science Strategy Committee (NSSC) to coordinate possum research. The NSSC has given a high priority to research on the biological control of possums, with contraceptive control as a major focus (Atkinson and Wright 1993). Biological control options for possums were discussed at a workshop hosted by the NSSC for New Zealand and Australian scientists in October 1992. There was general consensus that too little was known about some fundamental aspects of possum biology to embark on a focused project like that for rabbit and fox control in Australia (see Tyndale-Biscoe, this volume). It was agreed that basic research was required in seven priority areas

(table 1). This chapter describes some of the research that has been initiated and the philosophy underlying the research priorities.

Table 1. Priority research areas (not in order) for the biological control of common brushtail possums in New Zealand

1	<i>Screening for pathogens and potential vectors</i>	The National Science Strategy Committee is open to the possibility of identifying organisms that could be used for classical biological control as well as organisms that could be used to vector contraceptive vaccines.
2	<i>Immunology</i>	These are both vital for the development of contraceptive vaccines.
3	<i>Gene transcription</i>	
4	<i>Control of reproduction through the central endocrine system</i>	Studies in these fields aim to identify potential contraceptive vaccine targets.
5	<i>Biology of sperm, eggs, and the reproductive tract</i>	
6	<i>Control of lactation</i>	
7	<i>Sociobiology</i>	This is an important area of research in regard to the transmission of vectors and the behavioral consequences of contraceptive vaccines.

Research Priorities

Pathogens and Potential Vectors

Possum parasites and pathogens are being comprehensively surveyed in New Zealand and Australia to identify organisms for use as classical biological control agents or as vectors of contraceptive vaccines (Heath et al. 1994). Because New Zealand's possum population was founded from only a few hundred animals and some intermediate hosts are not present in New Zealand, possums in this country probably carry fewer types of parasites and pathogens than those in Australia (Cowan 1990). Also, because New Zealand's possum population has been isolated for more than 100 years, it may be more susceptible than the Australian population to the broader range of parasites and pathogens present in Australia.

In New Zealand, approximately 200 possums were trapped in 1993 and 1994 in each of eight locations close to the original possum release sites. These animals have been examined for the presence of ectoparasites and endoparasites, and samples from the heart, lung, liver, spleen, and gut have been screened for bacteria and viruses. Comparative studies will begin in Australia shortly.

Immunology

The New Zealand Pastoral and Agricultural Research Institute (AgResearch) is working on the immune response of possums. This research is primarily focused on the kinetics of antibody production in the possum against particulate and soluble proteins, and on determining optimal systems for stimulating high antibody responses at both the parenteral and secretory level. Early results indicate that cell-mediated responses are generally poor in the possum, which relies more on antibody responses. Responses to particulate antigens (e.g., whole sperm) are considerably stronger than responses to small soluble antigens (B. Buddle, pers. comm.). The cytokines that regulate immune responses in possums are being identified.

Gene Transcription

Although gene transcription is one of the research priorities, there appears to be little current research. AgResearch has started investigating how foreign DNA is expressed in nematodes and considering how nematodes might be used as vectors of contraceptive vaccines. A number of groups are preparing c-DNA libraries, including those for the pituitary (AgResearch, Wellington), the mammary gland (University of Otago, Dunedin), and the testis and female reproductive tract (Marsupial Cooperative Research Centre, Macquarie University, and La Trobe University, Australia).

Potential Contraceptive Targets

Central Endocrine System—Gonadotropin-releasing hormone (GnRH) is central to the regulation of reproduction and has therefore been suggested as one possible target of a contraceptive vaccine. Vaccines against GnRH are easily produced. Because the

hormone is present in the body in tiny amounts, antibodies produced by GnRH vaccines can readily remove the hormone from circulation.

A major disadvantage is that the structure of this hormone is identical in all mammals and birds, so GnRH vaccines would not be possum specific. It is possible, however, that species-specific regions may be present on GnRH receptors or that gene regulators may have species-specific regions. AgResearch in Wellington is investigating the regulation of GnRH gene expression.

Another disadvantage of GnRH vaccines is that they effectively neuter animals so that social behaviors that are dependent on sex steroids are likely to change in vaccinated animals. At Landcare Research, Christchurch, investigators are testing the behavioral effects on possums of a commercial GnRH vaccine registered for use in cattle in Australia (Hoskinson et al. 1990). Captive groups of one male and two female possums are observed regularly after the dominant female has been vaccinated with the GnRH vaccine. This work is still at an early stage, but the vaccine does not seem to affect the social status of the dominant possums.

Biology of Sperm, Eggs and the Reproductive Tract—At Landcare Research, we are focusing on sperm, eggs, and the fertilization process. We are investigating the immunological responses of possums to sperm vaccines and collaborating with Macquarie University, Australia, to characterize specific antigens of possum sperm and zona pellucida. Targeting gametes has advantages because many antigens are potentially possum specific, and an immunological attack on sperm or eggs should not affect behavior. Sperm vaccines are attractive. If sperm are attacked in the female tract before fertilization, this will disrupt fertility in females as well as in males. Repeated insemination of infertile females may also act as a booster vaccination and result in a long-lived immunity.

Vaccines have been prepared from whole sperm using Freund's Complete and Freund's Incomplete adjuvants. We are studying the effect of the vaccine on fertility and the relationship between fertility and

antibody titre. The Macquarie group is also working on in vitro fertilization (IVF) systems for possums using Tammar wallabies (*Macropus eugenii*) as a marsupial model (Mate and Rodger 1993). An IVF system will greatly facilitate the characterization of gamete antigens and ultimately be essential for screening potential vaccine antigens. Because possums are marsupials, we hope that the gamete antigens are different from those of eutherian mammals. However, there appears to be some homology between possum and eutherian zona pellucida proteins (J. Rodger, pers. comm.); detailed characterizations are yet to be done on the sperm surface proteins of the possum.

Secretions of the female reproductive tract are important for the transport of sperm, eggs, and early embryos, as well as being vital for growth and survival of the conceptus. The Macquarie University group plans to characterize some of the secretory proteins as an immunological attack directed at some of these may result in infertility. Workers at AgResearch in Dunedin are identifying functional aspects of the female reproductive tract that may be susceptible to disruption by estrogenic plant compounds. This group is considering the possibility of being able to genetically modify food plants favored by the possum so that they deliver contraceptive compounds (B. McLeod, pers. comm.). Initial work concentrates on normal function of the reproductive tract, looking at the cyclical changes in the ultrastructure of the tract, secretory organelles, and mucoid secretions.

Control of Lactation—Disruption of lactation is not true contraception, but is an option with possums, which, like all marsupials, invest in lactation rather than pregnancy. Pregnancy in possums lasts only 17 days; lactation lasts up to 230 days (Pilton and Sharman 1962). An immunological attack could suppress milk production or change the composition of the milk so that it does not provide the appropriate nutrients. In possums and some other marsupials, cessation of lactation is a natural method of population regulation: when conditions in the wild are unfavorable, lactation ceases and pouch young die. In some years, up to 50 percent of possum pouch young may die in this way. The pouch young has limited sensory

development until it is nearly 3 months old (Lyne and Verhagen 1957, Hughes and Hall 1984), so targeting lactation in the early stages of development is considered relatively humane.

The composition of the milk undergoes marked changes during the long suckling period in marsupials (Tyndale-Biscoe and Renfree 1987). At Landcare Research, we are investigating changes in the elemental composition of possum milk during the course of lactation. Sodium, potassium, iron, and copper all show marked changes. We are especially interested in milk calcium levels. Brushtail possums develop metastatic calcinosis when fed a diet high in calcium, and calcium metabolism is readily disrupted in possums by cholecalciferol (Eason 1991). Also in progress is work to model the effects of lactation disruption on pouch young using the prolactin inhibitor bromocriptine. The aim of this project is to quantify the relationship between milk production and growth and survival of the pouch young.

Otago University is characterizing possum milk proteins. Researchers at the university have identified some unusual proteins associated with certain stages of lactation that are analogous to late lactation protein in Tammar wallabies (*Macropus eugenii*) (Nicholas et al. 1987), but the significance of these to the developing pouch young has not yet been established (M. Grigor, pers. comm.).

Sociobiology

Behavioral changes caused by contraceptive vaccines could compromise their efficacy. Possum social organization is based around dominance hierarchies (Winter 1976, Biggins and Overstreet 1978). If sterilization affects social behavior, changes in social structure could allow subordinate individuals to increase their breeding success. Landcare Research has been studying whether sterilization will affect social status. Females are more aggressive than males (Winter 1976, Oldham 1986), and our trials have shown that in captivity females retain their dominance status even after ovariectomy. The status of males also appears unchanged by castration (P. McAllum, unpubl. data). We plan further work to

untangle the behavioral effects of sex steroids from learned behaviors and to ascertain that the results of pen trials are applicable to free-living populations.

Future Directions

Vectoring a Vaccine

As there are more than 60 million possums in New Zealand (Cowan 1991), vaccine delivery must be cost effective. A vaccine included in a bait is the likely research target for the medium term (6 or 7 years). However, long-term, cost-effective control will probably need a self-sustaining biological vector such as a genetically modified virus (as proposed for rabbit and fox control in Australia; Tyndale-Biscoe, this volume), bacterium, or nematode. At this early stage, the New Zealand research program is keeping all options open.

Any vector would need to meet rigid specifications to allay the concerns of the public and New Zealand's trading partners. It would need to be humane and unable to survive away from possums, so that it could not cross to Australia, where possums are protected. The vector must infect only possums, and its mode of action must also be possum specific. The last two criteria act as a double safeguard.

Expectations

A biologically vectored vaccine is unlikely to eradicate possums because its success would be inherently dependent on the density of the possum population. When a population is dense, the level of possum to possum contact is high, and a vectored vaccine would have a significant impact. As a population declines and possums become more sparsely distributed, transmission of the vectored vaccine would be reduced. The population would equilibrate at a new lower level but not decline to extinction. This density-dependent effect could largely be circumvented by the use of a sexually transmitted vector. Possums actively seek partners in the breeding season, so a venereal infection is likely to persist even at low population levels.

A venereal vector would have other advantages. Any contraceptive vaccine would probably rely on the mucosal immune system to cause infertility, so sexual transmission of the vector delivers the vaccine to exactly where it can be most effective. The use of a sexually transmitted organism would also allay the concern that the vector might cross to Australia and infect protected possums there.

Computer modeling is currently defining the ecological and epidemiological criteria that need to be met to minimize the impact of possums. Modeling suggests that the possum population must be reduced to about 40 percent of its present level for 8–10 years to eradicate bovine tuberculosis (Barlow 1991). Such a reduction is theoretically achievable with a vectored contraceptive vaccine. Target reductions in possum numbers to protect conservation values are more difficult to quantify because of the diversity of the conservation values society wants to protect. This is an area of active research. The likely timetable for research and development of effective biotechnological control strategies for possums is in the order of 10 to 20 years, so New Zealand will have to depend on traditional pest control technologies for some time yet. Eventually the best control strategy will probably combine traditional control and immunocontraception (Barlow 1994).

In the coming years, new technologies will offer new possibilities for the control of vertebrate pests. The future promises some exciting research. We believe that it is only through the use of biotechnologies such as vectored immunocontraception that control of this pest can become truly sustainable.

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