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Human–Wildlife Conflicts 3(1):20–29, Spring 2009

A review of the potential of fertility control to manage brushtail possums in New Zealand

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Abstract: Brushtail possums (*Trichosurus vulpecula*) were introduced into New Zealand from Australia in the mid-1800s and became a major invasive pest. They damage native biodiversity by browsing and predation, and they are a disease risk to the livestock industry by acting as vectors of bovine tuberculosis (TB). Management of possums includes their eradication from some offshore islands and control by trapping, shooting, and poisoning on the mainland. Possums have been eradicated successfully from some islands and greatly reduced in abundance in other areas of high conservation value or where they are infected with TB. However, possums are still at very high densities in many areas. Conventional control methods (i.e., poisoning and trapping) are expensive and may sometimes be contentious, and unless the population is eradicated, these methods must be applied in perpetuity. Biological control, especially immunocontraception, is being investigated as a more humane and cost-effective alternative that might avoid the need for ongoing control. Researchers have investigated the delivery systems of biological control agents and possums' responses to them. Possum zona pelucida and possum sperm vaccines have caused infertility in female possums, but the proportion of individuals sterilized varied. A possum-specific nematode is currently under investigation as a potential vector for biological control agents. However, there is concern, especially among Australians, whose possum populations are protected, about the safety of releasing a self-disseminating biocontrol system into the environment. Therefore, bait-delivered fertility control is likely to be used in the near future. A system that integrates various biological controls, including fertility control and improved conventional control methods, is likely to reduce the possum populations in New Zealand.

Key words: biocontrol, brushtail possum, human–wildlife conflicts, immunocontraception, invasive marsupial, New Zealand, *Trichosurus vulpecula*

The common brushtail possum (*Trichosurus vulpecula*) is a nocturnal, arboreal marsupial weighing about 2 to 4 kg. It is endemic to Australia and was introduced to New Zealand to establish a fur industry (Pracy 1974). The first successful introduction was made in 1858, and subsequently hundreds of liberations from Australia and New Zealand-bred possums were made by private acclimatization societies and government agencies (Pracy 1974, McDowall 1994, Clout and Ericksen 2000). Possums spread rapidly and are considered a successful invasive species in New Zealand (Clout and Eriksen 2000), having spread to 92% of the country by 1984 (Cowan 1990*a*).

The impact of possums on the native ecosystems is multifaceted. Damage by possums to native forests varies widely, depending on forest type (Zotov 1949, Holloway 1959, Wardle 1974, Dale and James 1977, Pekelharing 1979, Veblen and Stewart 1982). The selective browsing of possums has caused decline or extinction of some local plant species, such as kohehohe (*Dysoxylum spectabile*), southern rata (*Metrosideros umbellata*), and fuchsia (*Fuchsia excorticata*; Payton 2000). Where preferred foods are dominant, possums' selective browsing may lead to complete canopy collapse (Meads 1976, Batcheler 1983). However, the principal effect of possums on native forest is to cause gradual compositional changes (Coleman et al. 1980, Green 1984, Campbell 1990, Payton 2000).

Possums may compete with many native birds, whose diets overlap with theirs (Fitzgerald 1984, Cowan 1990*b*, Cowan and Waddington 1990). However, no studies have demonstrated actual competition or whether they have any effect on native bird populations (Sadleir 2000). Possums also prey on native fauna in New Zealand (Brown and Shorten 1993), including eggs and nestlings of native birds (Brown and Shorten 1993, Mclennan et al. 1996, Innes et al. 2004) and invertebrates, such as land snails (*Powelliphanta* spp.; Meads et al. 1984), stick insects (*Phasmatodea* spp.), and weta (*Stenopelmatidea, Rhaphidophorioae*; Cowan and Moeed 1987). Sweetapple et al. (2004) found that, whether by predation or competition, abundance of some species of native forest birds declined with the increasing length of

possum occupation in South Westland. Control of possums has resulted in population recovery of native pigeons (*Hemiphaga novaeseelandiae*; Innes et al. 2004).

Possums are the primary wildlife vector of bovine tuberculosis (TB) in New Zealand (Coleman 1988, Coleman and Caley 2000). TB in free-ranging possum populations was first recorded in 1967 (Ekdahl et al. 1970), and control of diseased populations began in 1971. Possum control has successfully reduced the numbers of infected livestock herds. However, the area with infected possums has, until recently, continued to increase (Batcheler and Cowan 1988, Coleman 1988, Clout and Ericksen 2000).

At present, primarily 2 techniques are being used for control of possums in New Zealand—toxic and nontoxic. The most commonly used poisons include sodium monofluoroacetate (1080), which was used in both aerial and ground-based poisoning (Eason et al. 2000, Morgan and Hickling 2000), and Feratox®, an encapsulated cyanide pellet bait (Thomas et al. 2003) that is more commonly used in current possum ground-control operations. Commonly used nontoxic techniques include shooting and trapping, mostly with leg-hold traps and kill-traps (Montague and Warburton 2000).

Possums have been successfully eradicated from many offshore islands, including some large islands, such as Rangitoto Island (2,311 ha) in Hauraki Gulf near Auckland (Miller and Anderson 1992) and Kapiti Island (1,965 ha) near Wellington (Cowan 1992). Possum control techniques have been greatly improved in recent years (Morgan 2004, Morgan 2006, Morgan et al. 2006, Coleman et al. 2007), leading to successful and sustained reduction in possum densities (Coleman and Livingstone 2000). However, sustaining the control of possums in other large areas on the mainland is difficult due to high cost, aversion behavior of possums to toxins induced by sublethal dosing (Hickling et al. 1999), and population recovery after the control operation for a combination of reasons, including immigration, increased breeding rate by survivors, and increased recruitment of young (Clout 1977, Green 1984, Green and Coleman 1984, Brockie et al. 1997, Cowan et al. 1997, Cowan and Clout 2000, Ji et al. 2004). There also is increasing public opposition toward control of possums by poisoning (Fitzgerald et al. 2000), which is the most effective control method available (Parkes and Murphy 2003). Much research effort and resources have been invested to seek alternative pest control methods that are more effective, less costly, and humane. Biological control, especially immunocontraception, is one option being explored for long-term possum management (Jolly 1993, Cowan 1996, Cowan 2000).

Biological control of possums

Approaches to biological control include investigations into control agents, delivery systems, and the potential behavioral responses of possums to biological controls, especially to fertility control. An ideal biological control should be affordable and safe. It should not affect the natural behavior of possums; it should be possum-specific, highly potent, permanently effective, and stable under different environmental conditions (McDowell et al. 2006). However, meeting all the above requirements is not an easy task.

Research to identify and assess the potential of lethal biological control agents for possums has investigated parasites, bacteria (Heath et al. 1998, Meers et al. 1998), and viruses (Mackintosh et al. 1995, Rice and Wilks 1996, Perrott et al. 2000, Baillie and Wilkins 2001). So far, however, no agent has been found suitable as a natural disease or predator for effective control of possums. Fertility control through immunocontraception is also under investigation as an alternative cost-effective and humane method for future management of possums in New Zealand (Cowan 2000).

Immunocontraception

Immunocontraception aims to elicit immune responses from the target animals against their own hormones essential to reproduction. The purpose is to prevent or reduce their reproduction rate (Tyndale-Biscoe 1991). Research into attacking key physiological processes includes interference with reproductive processes, including those listed below.

1. *Fertilization*. Immunization against sperm and egg proteins, resulting in blocking fertilization (Duckworth et al. 1998).
2. *Embryonic development.* Immunization against extracellular matrix proteins of

the developing embryo to coat proteins produced by the reproductive tract and a factor (LIF) important for implantation, resulting in death of the embryo (Selwood et al. 1998).

3. *Postnatal development.* Immunization against proteins essential for sex differentiation (Deakin et al. 1998) or the normal development of the ovary or testis, resulting in the offspring being born sterile (Eckery et al. 1998).
4. *Central endocrine control of reproduction.* Destruction of pituitary cells controlling the secretion of sex hormones or immunization against sex hormones, thus inhibiting breeding (Eckery et al. 1998).
5. *Passive immunity.* Immunization against a gut immunoglobulin receptor to prevent development of passive immunity, thus reducing survival of young (Eckery et al. 1998).
6. *Lactation*. Immunization against milk proteins or regulating factors to reduce pouch young survival or enhance the transfer of antibodies, resulting in the disruption of normal development (Demmer et al. 1998).

Interferences with the reproductive process after fertilization, especially during lactation, are likely to encounter ethical problems (Cowan 1996). Preventing fertilization will be the most socially acceptable form of immunocontraception to control possums. Protein from sperm and eggs has been investigated as potential antigens, with various successes (Duckworth et al. 1998, 1999). The most studied vaccine for fertility controls through interference against fertilization is zona pellucida (ZP), an extracellular matrix surrounding a mammalian egg (Harris et al. 1994). Injection of porcine ZP antigens has proven to induce infertility in a range of free-roaming animals (Turner et al. 2002, Gupta et al. 2004, Kirkpatrick and Frank 2005), including possums (Duckworth et al. 1999). Possum ZP has also been investigated as an antigen for possum fertility control, and immunization of possums with possum recombination ZP (rZP2 and rZP3) protein have resulted in infertility of 72 to 80% of females (Duckworth et al. 1999). To develop possum-specific vaccines, 3 epitopes on possum ZP2 protein relevant to infertility have been identified (Cui and Duckworth 2005). Duckworth et al. (2007) tested the contraceptive potential of these 3 peptides—Pep12, Pep31, and Pep44. Among these, only Pep44 had significant effect on possum fertility parameters. Immunization by conjugated Per44 through subcutaneous injection resulted in lowered egg fertilization rates and reduced number of embryos in superovulating and artificially inseminated possums.

Delivery systems

To date, the most successful delivery of a fertility control vaccine in captive mammals is through subcutaneous injection. Injection of porcine ZP to free-ranging horses (*Equus caballus*) resulted in 90% fertility (Turner et al. 2002). Fertility of a captive possum population was reduced by 75% after females were subcutaneously injected with whole possum ZP (Duckworth et al. 1998). However, it is not feasible to deliver vaccine by injection to widely-distributed free-ranging animals, especially in remote areas. Investigations have been carried out to identify potential vectors that can be genetically modified to carry the genes that encode fertility control protein as disseminating delivery systems for biological control agents. Potential vectors were identified from possums. Viruses found in possums include adenovirus, coronavirus, herpes virus (Rice and Wilks 1996), papillomavirus (Perrott et al. 2000), retrovirus (Baillie and Wilkins 2001), and wobbly possum virus (Mackintosh et al. 1995). Viruses that cause mortality, such as wobbly virus, will not be suitable as vectors. The potential of viruses as vectors for biological

The author feeds a trapped possum.

control is yet to be established. A possum-specific, naturally-occurring gut nematode, *Parastrongyloides trichosuri,* has been proposed as a promising vector for biological control of possums. *P. trichosuri* is widely spread in possum populations on North Island and has a discontinuous distribution on South Island (Cowan et al. 2005). Infection of the nematode to a parasite-naïve possum population has been successful. It spread across possums in a 400-ha area in 52 weeks and reached high prevalence in the population (Ralston et al. 2001). In about 135 weeks, the parasites had spread over an area of about 6,000 ha (Cowan et al. 2006). Genetically modifying this nematode to carry the genes encoding for proteins of immunocontraceptive antigens could be an efficient vector for possum control. *P. trichosuri* has a free-living stage during its reproductive cycle, making it uniquely suited for genetic analysis and manipulation (Grant et al. 2006).

Although a self-disseminating system would be a most effective and cost efficient for delivering a biocontrol agent to possum populations, it is likely to come across resistance during its development and release into the wild due to the issues surrounding infection of nontargeted animals and the risk of inadvertent or illegal transfer to Australia where brushtail possums are protected (Cooper 2004; Gilna et al. 2005; Hardy et al. 2006). Therefore, a disseminating delivery system is not likely to be available for possum management in New Zealand. Studies have been underway to investigate non-disseminating delivery systems. These include bacterial ghosts and genetically modified plants. Bacterial ghosts are nonliving, gram-negative bacterial cell envelopes devoid of cytoplasmic contents that maintain their cellular morphology and native surface antigenic structures (Jalava et al. 2003). The immune response of possums is low after oral administration of bacterial ghosts containing ZP antigen (Duckworth et al. 2001). Genetically modified plants that express immunocontraceptive vaccine were proposed as a promising non-disseminating delivery system for possum control (Smith et al. 1997). Possums fed with transgenic potatoes (*Solanum tuberosum*) carrying LT-B have showed immune responses to the antigen. This demonstrated that oral delivery of immunocontraceptive vaccine via edible plant is possible (Polkinghorne et al. 2005). Carrots (*Daucus carota*) prove to be a promising candidate. They currently are used as a bait for delivering poisons for possum control. The seed set of carrots occurs well after the maturation of roots, so the roots can be harvested before the seed set, preventing the escaping of the antigen encoding transgenic gene into the environment. Trials with green fluorescent protein (GFP) gene showed that transgenic carrots accumulated high levels of GFP in edible tissues (Polkinghorne et al. 2005). The combination of marsupial-specific immunocontraception vaccines, expressed in edible plants, such as carrots, could provide a relatively cheap, humane, and safe alternative for possum management (Polkinghorne et al. 2005).

Ear tagging a sedated possum.

Factors that might influence fertility control of possums

The ultimate goal of fertility control is to increase the proportion of infertile individuals in the possum populations. It is important to know how possums will respond to such infertility and whether their responses would affect the efficiency of the control. Ji et al. (2000) investigated the short-term response of possums to female sterility by imitating female sterility from immunocontraception by tubal ligation. A higher proportion of males was recorded in the study area after the sterilization treatment. This appears to be caused by the surrounding males moving into the study area in the presence of sterilized females that keep estrous cycling beyond the breeding season. Male body condition is significantly poorer in the post-mating season after the sterilization.

These results have positive implications for successful transmission of an infectious immunocontraceptive agent. First, females sterilized by such an agent could potentially infect not only local males, but also those from immediately surrounding areas. However, the radius of effect is unlikely to be very large since no more unmarked adult males than normal were attracted to the treatment zone. Second, poor body condition is generally known to result in increased risk of mortality among mammals (Hanks 1981, Fryxell 1987, Gorden et al. 1988, Choquenot 1991). In 1997, reduced body condition of male possums when sterilized females were present was presumably due to sexual activity continuing into the winter months. If this reduced condition resulted in a higher male mortality risk during winter, the effect would be similar to the vector-induced mortality of a biological control program (Barlow 1994), although it would be caused indirectly and would be sex-specific. In a recent study, Ramsey (2007) reported that sterilization of females by tubal ligation did not affect their ranging behavior. However, sterilization by gonadectomy had significantly reduced the breeding range of males, although it did not affect female ranging behavior. Gonadectomy has also reduced the transmission rate of a commonly-occurring pathogen, *Leptospira interrogans serovar balcanica.* This indicates that fertility control that interferes with endocrine control of reproduction may have reduced the contact rate among possums, and, therefore, may affect the transmission of such an infertility agent if it relies on close contacts among possums (Ramsey 2007).

Although bait-delivered immunocontraception is likely to be the method used in the near future, self-disseminating transmission of a control agent will be more efficient and cost-effective and warrants investigation for the future management of possums. Ecological modelling by Barlow (1997) of a sexually-transmitted, possum-specific virus that induces permanent sterility without suppressing reproductive cycles, indicated that the percentage of females sterilized and the contact rate between individuals are major factors affecting the success of such a biological control.

Possums are solitary nocturnal animals. Contacts among adults are mainly through mating, fighting, and co-denning (Caley et al. 1998). Studies through minisatellite DNA profiling (Sarre et al. 2000) and microsatellite DNA profiling (Taylor et al. 2000) indicate that the possum mating system is polygamous, with many males siring no young and some being successful. Such mating patterns did not change significantly when possum density was greatly reduced (Ji et al. 2001). A study on close interactions between female and male possums using proximity data loggers revealed that female possums can have close interactions with >1 male during breeding season, and the mating system of possums is likely to be polygamous (Ji et al. 2005). These findings imply that virus-vectored sterilizing agents that rely on possum contacts for spreading are likely to be successful.

Conclusions

The brushtail possum is one of many introduced mammals that causes severe damage to native ecosystems and the economy of New Zealand. Biological control, especially immunocontraception targeting female fertility, is potentially an inexpensive, efficient, and humane option for addressing the possum problem in New Zealand. Disseminating biological control, either traditional biocontrol using diseases or parasites or biotechnological biocontrol, such as fertility control through immunocontraception, depends on contact among possums for spread of a control agent (Jolly 1993, Barlow 1994). Polygamous mating patterns, promiscuity in close interaction of both sexes, and lack of behavioral responses that might negatively affect contact rates make a self-dissemination biocontrol system promising. However, due to concerns about the safety of releasing a self-disseminating biocontrol system into the environment, especially concerns about infection of nontarget species, bait-delivered fertility control is likely to be used in the near future. Although it is a promising alternative, so far, no immunocontraceptive antigen has achieved 100% response by possums. Such a non-response is likely to be, in part, genetic, and that will result in selection of non-responders (Cooper 2004). A system that integrates various

biological and conventional control methods will be more efficient for the future management of possums in New Zealand.

Acknowledgments

This paper was originally presented at "The IX International Mammalogical Congress" held in Sapporo, Japan. I thank Massey University both for funding the trip and producing the paper and 2 anonymous referees for their comments on the earlier draft.

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