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Abstract: Control of wildlife pest populations by sterilization could be more effective than conventional mortality agents, provided that two conditions are met: (1) the endocrine function of affected animals is not compromised, so as to exploit the natural suppression of reproduction of subordinate members of the population that occurs in many species; and (2) the incidence of sterility is sufficient to lower population recruitment and growth. Both conditions could, theoretically, be met by use of an infectious recombinant virus, expressing genes for specific reproductive antigens. Using the rabbit, I describe the research required to test the concept and discuss the legal and ethical consequences that may arise from a positive outcome to the research.

Keywords: Gamete antigens, population ecology, recombinant virus, risk assessment, social hierarchies

Introduction

The idea that fertility control has a potential for the management of wild species has been recognized in recent years, as shown by a 1990 meeting in Melbourne, Australia (Tyndale–Biscoe 1991) and a 1993 meeting in Denver, CO. While considerable support for this concept exists on grounds of humane control of wildlife, several important and as yet unresolved matters remain regarding its safety. The biological and ethical issues largely resolve into whether we are concerned with controlling populations of desirable species at appropriate levels with the option for reversing the effects in future years, or whether we are concerned to control populations of undesirable species of wildlife at very reduced levels indefinitely and at minimum cost. In North America, the first concern is the overriding one; in Australia and New Zealand, the second is.

The European wild rabbit, *Oryctolagus cuniculus,* is an excellent example with which to explore some of the biological and ethical questions surrounding the use of sterility as a means of management of wildlife. In some countries, it is a highly regarded wildlife species while in others it is the most serious and intractable of all pests (Thompson and King 1994). In addition, there are many other species of lagomorph around the world that are well regarded, and some are endangered (Chapman and Flux 1993). Clearly, methods developed for the control of the common rabbit must not affect these other relatives.

The rabbit is indigenous to southern Europe, where it is regarded as a desirable element of the fauna. In Spain, it is the main prey of eight raptors, two snakes, and six species of mammal, including the endangered lynx, and it is also a prized game animal. It was domesticated by French monks and taken by the Normans to Britain (Rogers et al. 1994). Domesticated varieties of the rabbit are raised throughout the world for meat, skin, and fur. It is also an important laboratory species, and there is a strong culture of breeding distinct varieties by rabbit fanciers. This range of interests in the rabbit involves a large and worldwide trade and distribution of rabbits and rabbit products. The nature of this interchange was dramatically demonstrated by the newly recognized rabbit calicivirus, which causes a rapidly fatal disease in rabbits (viral/rabbit hemorrhagic disease, VHD/RHD), after its discovery in China in 1984 (Liu et al. 1984) and its spread to southern Europe in 1988 and to Mexico in 1990.

In Britain, the long-term impact of the rabbit on vegetation was not appreciated until the demise of rabbits after the myxomatosis epizootic of the 1950's (Thompson 1994). Since the rabbit's subsequent recovery, the value of the damage rabbits now cause has been estimated at $180 million each year.
In the nineteenth century, the rabbit was released on many islands as a source of emergency food for castaways (Flux 1994), and it was also introduced by British colonists to Australia and New Zealand for a variety of reasons, including sentimental ones of enjoying the presence of a familiar animal in an alien country. In many cases, these introductions had severely deleterious consequences to the vegetation and indigenous fauna. In recent years, some of the islands under Australian and New Zealand control have been cleared of rabbits at very great expense (Burbidge 1989, Towns et al. 1990), and the vegetation is recovering. However, this is not a practical strategy for larger areas or for continental Australia.

The Rabbit Problem in Australia

Within 20 years of the introduction of rabbits to Australia, it was evident that they were a serious problem. Their explosive spread across the continent was complete by the early years of this century and resulted in gross overgrazing of the native grasslands, permanent degradation of the semiarid region, and widespread soil erosion. The long-term damage that the rabbit is doing in this large region of Australia is serious because it is preventing the regeneration of the long-lived plant species. When the old plants die, therefore, the whole ecosystem is irrevocably changed. The rabbit has been a major factor in the extinction of the small to medium-sized marsupials that were indigenous to this region and the value of losses to the pastoral industry each year has been estimated to be $500 million. The rabbit is acknowledged to be the most serious of all animal pest species in Australia and its control the most urgent.

In 1888, the Intercolonial Rabbit Commission was set up ". . . to rid the country of this menace." In the century since, many schemes to control the rabbit have been proposed, but only one has come near to accomplishing it. At the end of 1950, the myxoma virus was released. It spread rapidly across the southern half of Australia, causing massive mortality among rabbit populations. This was the single most effective control of a pest mammal ever achieved, and the effects of it are still apparent in most regions of Australia (Parer et al. 1985). Within a few years of the release, attenuated strains of the virus had evolved, and resistance to the virus had developed in the rabbit populations (Marshall and Fenner 1958, Fenner and Ratcliffe 1965). In order to counter this apparent decline in the effectiveness of the virus, the highly virulent Lausanne strain was imported from Europe and for 20 years was regularly released by land managers and owners for rabbit control. It probably caused high mortality at the site and time of each introduction but did not persist or spread very far. Recent evidence (P. J. Kerr, pers. comm.) suggests that the Lausanne strain has not displaced the preexisting strains, and its value for rabbit control is unclear.

The efficacy of myxoma virus for broad-scale rabbit control is critically dependent on insect vectors, and in Australia the initial epizootic was effected by two species of mosquito. In Europe, however, the spread of the virus was largely due to the rabbit flea, *Spilopsylla cuniculi.* In 1960, this species was introduced to Australia and has become an important additional vector in the higher rainfall regions of the continent. A third vector, the Spanish flea *(Xenopsylla cunicularis),* which can survive in more arid environments, was released in South Australia in 1992. Hopefully, the Spanish flea will be an effective vector of the myxoma virus in the arid regions of the country, where rabbits are abundant in years of high rainfall.

Rabbit calicivirus was also being assessed in 1995 for possible release in Australia as another mortality agent. Unfortunately, it escaped from quarantine and is now widely dispersed. In addition to these measures, substantial resources have been directed by the Australian Government, through the Cooperative Research Centre for Biological Control of Vertebrate Pest Populations (1993, 1994), to investigate the potential for fertility control. In this approach, the myxoma virus would be used as a vector to introduce an immunocontraceptive to populations of wild rabbits.

Fertility Control of Rabbits

All previous attempts to control the rabbit have depended on developing methods to enhance mortality, such as disease, natural predators, commercial trapping, and shooting or poisoning with strychnine, arsenic, phosphorus, or sodium fluoroacetate (Compound 1080). Most of these methods are now illegal because of the pain they inflict on the animals in the process of killing them. In a recent study to compare the efficacy of these different methods, Williams and Moore (1995) found that the destruction of rabbit warrens was far more effective and long lasting than poisoning. When the warrens were left intact after fumigation or poisoning, rabbit populations recovered very rapidly because the warrens could be reoccupied and breeding could recommence. Similarly, after the

initial highly successful reduction of rabbits in 1951–54 with the myxoma virus, rabbit populations recovered in some areas because warrens were left intact, reproduction of the survivors was not curtailed, and resistance to the virus thus evolved rapidly (Marshall and Fenner 1958). Clearly, the most important factor in rabbit control is the rate of recovery after a treatment has been applied: if that could be curtailed, as with warren ripping, the effect of all methods would be enhanced.

Fertility control is sometimes regarded as mortality applied at an earlier stage of the life cycle, but for many species it could be much more than this. Many studies on wild mammals have shown that reproductive success is closely linked to high rank in the social hierarchy of the population. Lower ranking animals either do not breed or fail to rear their young to independence (Wasser and Barash 1983, Abbot 1988). Failure to breed has been shown in wild foxes in Britain to be effected by the dominant members of the group (McDonald 1987). In the wild rabbit, there is no evidence that dominant females suppress breeding by subordinates, but the survival of the kittens of dominant females is significantly greater than for those of subordinate females (Mykytowycz 1959, Mykytowycz and Fullager 1973, Cowan 1987). Thus, sterilization of dominant members could affect fecundity of the population disproportionately, provided that the sterilized individuals remain sexually active and retain or improve their status in the social hierarchy of the population, and that a sufficiently high proportion of the population is sterilized (Caughley et al. 1992, Barlow 1994). While these conditions have long been recognized (Knipling 1959, Davies 1961), the problem has been how to achieve them for control of a wildlife species.

For many species with strong social structures, it is therefore important that a sterilizing agent not compromise the hormonal function of the gonads of the target animal. Because rank order is related to levels of sex hormones, a castrated animal is rapidly replaced in the social hierarchy and exerts little or no influence on the reproductive potential of other members of the population. Methods of fertility control that rely on exposing the target animal to steroids of one sort or another usually affect endocrine functions and consequently the sexual and social behavior of the animal (Bomford 1990 and this volume). Likewise, the use of agents that immunize the animal against gonadotropin hormone-releasing hormone (GnRH) or block receptors for GnRH in the pituitary seriously affect the steroidal functions of the gonad as well as its gametogenic function. While these methods have wide application in livestock management, and could be useful for the control of breeding in local populations of wildlife or for those species in which breeding suppression does not occur, they are of little potential use for pest species where social and sexual behavior must not be compromised. Agents that affect gametes, fertilization, or implantation must be sought and then presented to the target animal in such a way as to induce a strong and persistent immune response that prevents or compromises pregnancy.

A second requirement for a widespread pest species like the rabbit is a means of delivering the agent to a large proportion of the population. The concept that we are investigating for the rabbit is to clone the genes encoding proteins that are critically involved in fertilization or implantation and insert them into the myxoma virus. Rabbits infected with the recombinant myxoma virus would simultaneously raise antibodies to the virus and to the reproductive antigen, and fertilization or implantation would be prevented. Because this immunization would not affect the hormonal status of the rabbit, it would not affect its sexual activity or social status in the population. For the wild rabbit, five key questions derive from this concept:

1. What proportion of females in a wild population must be sterile in order to reduce significantly the rate of growth of the population?

2. Can gamete-specific proteins be presented to the animal in such a way as to provoke an effective and long-lasting immune response that interferes with fertilization or fetal development?

3. Can recombinant myxoma viruses that express the genes encoding the gamete proteins be constructed in such a way that they can act as vectors to

immunosterilize the proportion of the wild population identified in the first question?

4. How and when will selection forces diminish the effect of the recombinant virus?

5. Can this be achieved in a way that does not put at risk other species in Australia or rabbits in other countries?

Effects of Sterility on Rabbit Population Dynamics

In Western Australia and in New South Wales, two large experiments on wild rabbits were begun in 1993 to test the effect of sterilizing various proportions of the female population on rate of increase and survival of young. In each experiment, 12 free-range populations, each initially of 50 to 100 adult rabbits, were isolated by combinations of fences and buffer strips, and each was allocated randomly to 1 of 4 treatments. On each site, all the rabbits were caught and marked, and 80 percent of the adult females were subjected to surgery, either laparotomy or ligation of the oviducts. The proportions sterilized on three sites each were 0 percent, 40 percent, 60 percent, or 80 percent. In addition, the impact on the European flea, *Spilopsyllus cuniculi,* and the incidence of infection with myxoma virus are being investigated. This flea is being studied because its life cycle is intimately tied to the reproductive cycle of the rabbit, particularly females in late pregnancy and newborn kittens. With a high proportion of the females sterile, will the flea population be able to survive and transmit myxoma virus?

These experiments have been run for 3 years to determine how the productivity of the populations is affected by the different levels of sterilization. Preliminary results from the first year suggest that, while the number of kittens was reduced on sites where females were sterilized, survival of these kittens was higher than on the control sites, so that, by the start of the next breeding season, the net production between sites was not different. However, survival of sterilized females appears to have been higher than for intact females and males, and sterilized females entered the next breeding season heavier than intact females (Williams and Twigg 1996). In the second and third years, the treatments were repeated on new recruits to the populations. In both experiments, climatic vagaries during the 2 years affected reproduction and survival. In general, however, the effect of sterilization followed the pattern of the first year. The most notable result was that the higher levels of sterility reduced the annual cohort of recruits; populations with 80-percent sterility tended to have a flat trajectory over time, unlike the fluctuating pattern in the experimental controls (L. Twigg and C. K. Williams, pers. comm). Knowledge of the longer term effects of sterility must await detailed analyses of these experiments and their application to mathematical models for extrapolation. This information will be crucially important in deciding the requirements for an effective recombinant virus.

Gamete Antigens for a Rabbit Immunocontraceptive

Effort is presently concentrated on interfering with fertilization by identifying those proteins present on the surface of the sperm and the ovum, which are involved in the processes leading to fusion of the male and female nuclei. These are

(1) locomotion of the sperm, which brings it into the vicinity of the of the ovum;

(2) the first contact between the sperm head and the zona pellucida, which induces the acrosome reaction of the sperm;

(3) release from the acrosome of enzymes that break down the zona and allow the sperm to pass through and lie against the plasma membrane of the ovum; and

(4) proteins on the equator of the sperm head that are thought to be critically important in causing the fusion of the sperm and egg plasma membranes, so that the sperm nucleus can enter the egg and fuse with its nucleus.

The approach being used to identify and isolate selected antigens is to develop polyclonal and monoclonal antibodies to gamete antigens and, if possible, assess the effect of these antibodies on sperm–egg binding, on in vitro fertilization, and on fertility in intact animals.

To date, 35 monoclonal antibodies have been prepared against rabbit sperm, and some of them have been shown to block sperm–egg binding and prevent fertilization in vitro. A cDNA library has been prepared from rabbit testis, and the gene encoding for one of these proteins (Pop1) has been sequenced and appears to be a novel testis-specific protein (M. Holland, pers. comm.). In addition, DNA probes, derived from sperm antigen genes of other species, are being used to isolate the rabbit homologues from the rabbit testis cDNA library. The first of these to be characterized are the homologues of the genes for the guinea-pig sperm antigens PH20 (Holland et al. 1997) and PH30 (Hardy and Holland 1996).

For effective immunocontraception, the antigen must provoke a strong and sustained immune response that will interfere with the functions of the gametes. This process involves the appropriate presentation of the antigen to the immune system to induce strong memory and the development of high titres of appropriate antibodies at the time that fertilization is most likely to occur. Gamete antigens are self proteins and therefore may not induce a strong immune response alone. However, spermatozoa, which are normally not presented to the immune system of the male because of the blood–testis barrier, may provoke an immune response when presented to the systemic circulation of males.

Effective application of a vaccine for fertility control requires that a high level of immunity be achieved amongst individuals exposed to the vaccine. In outbred populations of wild mammals, heterogeneity of the immune response between individuals may make it hard to reach or sustain that level. It may therefore be necessary to include several antigenic determinants together, so as to stimulate a broad range of immune responses within the population. In addition, the antigen(s) may have to be presented in conjunction with other highly immunogenic carrier proteins in order to induce a strong and lasting immunity. This could include species-specific cytokines, such as interleukin–6, which has recently been shown to enhance the immune response in vivo in mice (Ramsay et al. 1994).

Molecular Virology and Antigen Delivery

For immunosterilization to be effective in a wild population, the gamete antigens must reach a large proportion of the exposed population. Delivery systems can utilize

(1) direct presentation of the antigen (in baits or by projectiles, which is costly but safe),

(2) oral administration of nondisseminating recombinant micro-organisms (which carry the genes encoding the gamete antigens and immunogenic carrier proteins), or

(3) a recombinant micro-organism that spreads through the target population by sexual transmission, contagion, or arthropod vector.

The delivery of immunosterilizing agents by bait has considerable value in circumstances where the target population is restricted in distribution or in time. However, the control of rabbits in Australia calls for a more cost-effective means of delivery that would spread the agent through the population independently. This of course, brings with it a much higher degree of risk—but not so high that the concept should not even be investigated or contemplated.

Over the past decade, recombinant viruses, carrying gene sequences derived from other organisms, have been constructed to function as living vaccines. The best example is the vaccinia virus recombinant expressing a portion of the rabies virus genome, which has been used very successfully in Europe to immunize populations of wild foxes (Brochier et al. 1990). The success of this project is due to the ability of the recombinant virus to replicate in the oral cavity of the infected fox and because the rabies glycoprotein expressed by the inserted gene is highly immunogenic.

The myxoma virus has been circulating in the rabbit populations of Australia for more than 40 years, and no evidence has been found to indicate that it infects species other than rabbits. Myxoma virus is a large DNA leporipoxvirus, related to vaccinia virus, so technologies already developed for preparing recombinant poxviruses can be adapted for the construction of recombinant myxoma viruses. During the past 9

years, the structure of the virus genome has been investigated (Russell and Robbins 1989), and a number of open reading frames in the central region of the genome have been identified (Jackson and Bults 1990 and 1992a, R. J. Jackson et al. 1996), including insertion sites homologous to those used in the vaccinia virus work. The aim here was to preserve the viability and infectivity of the native myxoma virus by inserting foreign DNA at intergenic sites, and not to compromise the virus by inserting foreign DNA intragenically.

Plasmid transfer vectors, based on the myxoma virus, have been constructed. These vectors contain a multiple cloning site adjacent to vaccinia virus promoter elements inserted in an intergenic region between the myxoma virus *tk* gene and open reading frame MV8a (Jackson and Bults 1992b). These vectors were used to generate recombinant myxoma viruses in cell culture and demonstrated that the myxoma virus can carry additional DNA and express the product in a culture system (Jackson and Bults 1992b), and without associated attenuation (R. J. Jackson et al. 1996).

Other recombinant myxoma viruses have been constructed using an attenuated strain, which express the hemagglutinin antigen of influenza virus (Kerr and Jackson 1995). These recombinants express cell-membrane-bound hemagglutinin, which can be detected by immunofluorescence. In live rabbits, the recombinants provoke strong antibody titres to the myxoma virus and very strong antibody titres to the hemagglutinin antigen. This demonstration opens the way for the insertion of other genes, such as those encoding for reproductive antigens.

Competition Between Recombinant and Native Strains of Virus

Concurrently with the reproductive and viral programs, it is important to determine the conditions under which dissemination of the recombinant virus, expressing sterilizing genes, may be able to outcompete existing field strains. To assess such potential competition, the pathogenesis and epidemiology of the recombinant virus must be compared to that of purified strains of the virus and, in the case of myxoma virus, the various field strains that have evolved over the past 40 years in Australia. Using the techniques of restriction length fragment polymorphism and polymerase chain reaction, P. J. Kerr (pers. comm.) has been able to identify field strains of myxoma virus by criteria that are independent of the virulence or pathogenesis of the strain. This work is not only providing a far greater appreciation of the regional differences in the virus and its host but will enable the selection of strains with which to prepare recombinants much more effectively targeted to rabbit populations.

The other question here is the fitness of the sterilizing recombinant virus to survive in the host population. In preliminary modelling for a recombinant myxoma virus expressing a sterilizing gene, being undertaken by R. Pech and G. Hood (pers. comm.), persistence of strains depends on the rate at which new susceptible rabbits enter the population. Even virulent strains can persist in the population if the birth rate is high; at lower birth rates, however, only avirulent strains with a long period of infectivity survive. The persistence of sterilizing strains may be even more constrained because there will be fewer opportunities for transmission to new, susceptible rabbits. In a different model, in which the sterilizing virus is assumed to be sexually transmitted and to persist in the infected host, Barlow (1994) has estimated that the recombinant virus will be at a selective advantage over the native strain because the more frequent return to estrus by sterilized females will provide more opportunities for transmission. In this situation, he concludes that the recombinant virus could persist in a population that stabilizes at a substantially lower level.

Legal and Ethical Issues of Viral-Vectored Immunosterilization

The development of immunosterilizing vaccines that can be delivered to wild animals raises a number of important issues about the international consequences of the impact of an agent designed for a species that is a pest in one country but a desirable or even endangered species in another. One view is that the outcome of the concept is so uncertain and the risks are so great that approval for release will never be

given; therefore, the research should not proceed at all. Implicit in this view is the inference that these other issues will always outweigh the problem of the rabbit in Australia, a problem of great magnitude. An opposing view, which we favor, is that the research should proceed incrementally with public discussion and proper scrutiny at each step, so that, if the concept is shown to be valid, its potential use can be assessed properly against the risks. This view recognizes that understanding of the control processes in gene expression is advancing so fast that difficulties that now seem insuperable may not be so in a few years. However, if we wait until then before embarking on the basic research required to develop the concept, we will have delayed the time when it can be used. Delay may lead to further deterioration of threatened ecosystems, which is an issue of great concern in Australia. The debate is just beginning, and it is too early to establish rigid directives on this matter. Rather, it is important to establish first, whether it is possible to control populations by immunosterilization and second, if it is, to explore the range of options for delivery of the immunogen. Options range from the direct delivery of a nontransmissible immunogen, which is costly but has a low risk, to delivery by a disseminating microorganism in which the unit cost is very low but the risk is higher.

The risks relate to (1) the effect on species other than the prime target in the country with the pest problem, which are primarily national risks, and (2) the risks to the target species and related species in another part of the world, where they are valued highly. The latter risks are mainly international.

National Aspects—The important questions relate to species-specificity of the reproductive antigen complex, specificity of the virus to be used as the vector, and specificity of the means by which it will be transmitted.

Gamete antigens that have been characterized in recent years show considerable homology between species at the genomic level, and researchers need to know whether antigenic epitopes can be identified that affect fertilization only in the target species. It is likely that such epitopes, if they exist, may not provoke a strong immune response by themselves but may do so when coupled with some other protein or when expressed in conjunction with cytokines. If the cytokines themselves are specific to the target species, both specificity and immunogenicity of the antigen would be enhanced. Alternatively, if species-specific gamete antigens cannot be identified, then other antigens involved in reproduction may need to be considered. In the case of the rabbit, the protein uteroglobin, which is associated with implantation (Beier 1982), is specific to the rabbit and the gene encoding it has been cloned (Bailly et al. 1983). However, the evidence that it is essential for implantation is not strong, and the case against gamete antigens would have to be very strong before attempting to exploit uteroglobin for immunosterilization.

In the choice of vectors for the delivery of sterilizing antigens, the important aspect is the degree to which the viral vector is specific to the target species and is incapable of replicating and provoking an immune response in nontarget species. For myxoma virus in Australia, the case is strong that it is specific to the rabbit. In the past 40 years, as mentioned earlier, no evidence has been produced to indicate that the virus affects any species other than the rabbit, and humans exposed in an outbreak of myxomatosis did not seroconvert (E. W. Jackson et al. 1996). However, no critical tests have been done to determine that it does not undergo minimal replication in nontarget species, and no nontarget species has yet been screened for seroconversion. That can now be done for myxoma virus, using an enzyme-linked immunosorbent assay (ELISA) developed in our Center (Kerr 1997).

The third level of specificity is the way in which the virus is conveyed from one member of the target species to another. Contagious or insect-borne transmission involves a risk of cross-species infection, unless the insect vector is specific to the target species. Neither of the two species of rabbit flea introduced to Australia feeds on species of vertebrate other than the rabbit, and *Spilopsyllus cuniculi* can complete its own life cycle only on rabbits. However, the myxoma virus can be transmitted by other biting insects, a fact that reduces its specificity. Transmis-

sion of a viral vector by sexual contact or placental transfer would confer the greatest degree of specificity. In polyestrous species, sexual transmission would also confer a selective advantage on the recombinant over the native virus because, as mentioned above, females infected with the recombinant would undergo estrus more often and hence provide more opportunities for virus transmission (Barlow 1994). In this regard, herpesviruses that are sexually transmitted, show persistent infection, and are species specific may be particularly suitable candidates as viral vectors (Shellam 1994).

International Aspects—Another major concern about immunosterilization is international, centering on the risk posed by immunosterilization to a target species in countries where it is indigenous and well regarded. The concern is the risk of accidental or malicious export of the agent from a country where it is used for pest control to another country. For the rabbit, these concerns relate not only to the impact that the recombinant virus might have on *Oryctolagus cuniculus* but on other leporids that are susceptible to infection with leporipoxviruses. In North America, there are 17 species of *Sylvilagus* that could be infected, some of which are considered to be endangered species (Chapman and Flux 1993). In addition, there are other rare leporids in Mexico, Indonesia, and Japan that might be susceptible. The International Union for Conservation of Nature/Status Survey and Conservation Lagomorph Specialist Group has expressed strong reservation about the use of genetically engineered myxoma virus because of the potential risk to these species. What steps would be required to address this strongly expressed concern? A risk assessment needs to be undertaken that would place probability values on (1) transfer from the user country, (2) establishment in a second country, and (3) means to contain its spread, should an outbreak occur. These are similar to the concerns of international agencies that currently deal with other infectious organisms.

Probability of Transfer From the User Country—This probability would only include accidental or illegal transfer because presumably the power of legislation and its implementation with regard to the export of micro-organisms would be exercised under current international obligations. If the user country were Australia and a recombinant myxoma virus were being considered, risk assessment should include the past history of the virus in the country, the conditions required for initial establishment of the virus, and the evidence, if any, of the occurrence of genetically identifiable Australian strains of the virus anywhere else. The present evidence is that Australia is the only country that used the Standard Laboratory Strain (SLS) of the myxoma virus, and all field strains in Australia appear to be derived from it (P. J. Kerr, pers. comm.). There is no evidence that any strain of myxoma virus from Australia has been deliberately or accidentally spread to any other country. With techniques now developed, it would be possible to determine whether SLS-derived strains of the virus occur elsewhere.

It is perhaps significant that several attempts to release the virus in Australia before 1950 failed. The successful outbreak was due to the coincidence of widespread rains and large populations of two species of mosquito (Fenner and Ratcliffe 1965, Fenner and Ross 1994). In the early 1950's, attempts were also made to introduce the SLS myxoma strain to New Zealand, but they failed. At the time, investigators concluded that this happened because the appropriate insect vectors were not available to transmit the virus (Gibb and Williams 1994). More recently, there were moves to introduce the rabbit flea to New Zealand so as to provide a suitable vector, but the New Zealand Government decided in 1993 to forbid the release of the flea and the virus. There are several potential insect vectors of myxoma virus in New Zealand and many people there who would like to see the virus used. Despite this, legislative controls have been a sufficient barrier to its entry for 44 years.

Probability That the Agent Could Become Established in a Country Where the Target Species or Related Species Are Endemic—Whether leporipoxviruses are already present in the nontarget populations would influence whether the new virus could become established. Where related viruses or the same virus is already present, the probability of the recombinant strain becoming established may be low because of the so-called founder effect and also

because genetic manipulation may render the recombinant less competitive. In Australia, despite massive and repeated release of the Lausanne strain since 1957, particularly in Victoria (Fenner and Ross 1994), all field isolates so far examined are genetically derived from SLS (P. J. Kerr, pers. comm.). This finding suggests that the Lausanne strain has been unable to establish in the face of preexisting strains. In an analogous way, Japanese B encephalitis flavivirus has never become established in Australia, where Murray Valley encephalitis virus occurs, despite annual introductions in migratory birds from Japan (Davey et al. 1982).

In Europe, where the Lausanne strain of myxoma virus was released, it underwent a parallel but wholly independent evolution of attenuation (Fenner and Ross 1994). If the founder effect applies to virus strains, Australian SLS-derived strains would be unable to establish in Europe. This idea could be tested on Macquarie Island, where Lausanne was the only strain released, by introducing genetically distinct SLS strains.

The same principle applies to establishment of an immunosterilizing virus in Australian wild rabbit populations. Competition with field strains probably will pose a severe barrier to introduction and transmission, and special strategies for seeding and timing will probably need to be adopted. Such conditions would suggest a very low probability of transmission by limited events, such as accidental or malicious release in another country. Nevertheless, if research enables a highly competitive immunosterilizing strain to be produced and/or foreign populations or species are shown to be at risk, careful consideration must be given to the strains used and the safeguards to be built into the genetic manipulations and to whether these safeguards would provide adequate protection.

In America, various strains of myxoma virus are endemic in species of *Sylvilagus* in the Western United States and several countries in Central and South America, but the incidence of seropositivity in wild populations has been determined in a limited way for *S. bachmani* in California only (Regnery and Miller 1972). This strain was shown to be capable of infecting four other species of *Sylvilagus*, but it could not be transmitted by mosquito from any of the primary hosts because the amount of virus was insufficient for effective mosquito transfer (Regnery and Marshall 1971). A more extensive assessment of this would be required to develop probabilities of a recombinant strain becoming established. Laboratory testing of susceptibility to infection and transmission would determine whether the risk is real for these other species and so contribute to assessment of their magnitude. In other parts of the world, where leporipoxviruses do not occur, the susceptibility of rare leporids might have to be determined.

Develop Means To Contain Its Spread, Should an Outbreak Occur.—From the foregoing, the probability of an outbreak spreading in a population of rabbits that are already exposed to leporipoxvirus is low. However, in a naive population the myxoma virus can spread rapidly, as occurred in Europe in 1952 (Fenner and Ross 1994). If other leporids are shown to be susceptible to the virus and there was deemed to be a risk of transfer of the immunosterilizing virus, contingency plans would have to be considered. In Australia, contingency plans have been developed for containing an outbreak of major diseases of domestic stock, such as foot-and-mouth disease virus in feral pigs (Pech and McIlroy 1990), and similar models could be developed for myxoma virus. The important factor is to recognize the outbreak at an early stage and to reduce the proportion of susceptible animals rapidly, either by destroying them or by immunizing them against the pathogen. In the case of myxoma virus, there are now several isolates of the virus that are attenuated but immunogenic. P. J. Kerr (pers. comm) is currently investigating the genetic basis of pathogenesis and virulence using these isolates. These studies may provide strains of the virus that could be used to deliver broad-scale immunization to contain an unwanted outbreak. In California and in France, highly attenuated strains of myxoma virus have been developed as vaccines to protect domestic and wild *O. cuniculus,* but attempts to produce an inactivated myxoma virus vaccine have been unsuccessful (Fenner and Ross 1994).

Conclusion

Effective control of pest mammals is immensely difficult. While current methods using specific disease organisms or poisons may have some benefit, it is widely acknowledged that none has long-term promise. So far as poisons are concerned, there is doubt even of their medium-term efficacy. A new approach to pest animal control is urgent; the concept of a viral-vectored immunosterilant is such an approach. Because it is novel, the outcome is uncertain, and the risks are considerable. However, if the risks can be reduced to an acceptable level and this methodology is effective in controlling rabbits, the benefits will be very great. Furthermore, because the concept is generic, if it is effective in this species, it has the potential to be applied to other species as well. Three species are already being investigated—the European red fox, *Vulpes vulpes* (Bradley, this volume), and the brushtail possum, *Trichosurus vulpecula* (Jolly, this volume), in New Zealand; and the wild house mouse, *Mus domesticus* (Shellam 1994), in Australia.

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