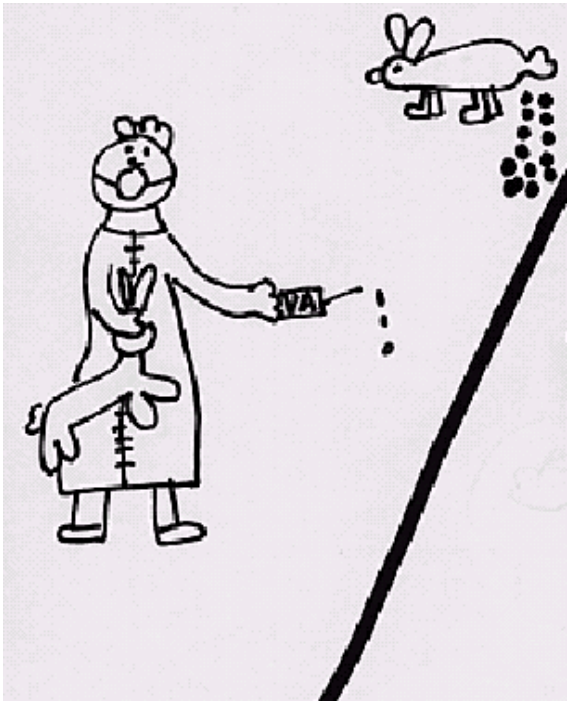

CAPÍTULO 4

Las enfermedades del conejo. Las nuevas herramientas de gestión a través del uso de virus modificados genéticamente

CAPÍTULO 4

Las enfermedades del conejo. Las nuevas herramientas de gestión a través del uso de virus modificados genéticamente

> *"*Diversity among practitioners in science yields diversity of approaches and perspectives, which cancel the bias inherent in each individual approach.*"*
> Pickett S.T.A. 1999. The culture of synthesis: habits of mind in novel ecological integration. Oikos 87:479-487.

Resumen

En este capítulo se discuten las técnicas moleculares que se están desarrollando actualmente para la gestión del conejo a escala mundial. Estas técnicas se basan en la modificación genética del virus de la mixomatosis, que sería posteriormente liberado en las poblaciones de conejo silvestre. Dos líneas de ecología aplicada son la base del desarrollo de estos virus recombinantes: por un lado, la investigación en la lucha contra las enfermedades del conejo para la conservación de sus poblaciones en el Sudoeste de Europa, y por otro lado, la investigación sobre la lucha biológica contra los propios conejos en los lugares donde son una especie invasora.

Teniendo en cuenta ambas líneas de investigación aplicada, totalmente opuestas en sus objetivos finales, y considerando que los resultados de ambas investigaciones proponen la suelta de un virus genéticamente modificado en las poblaciones de conejo silvestre, el objetivo básico de este trabajo es profundizar sobre el posible impacto de dicha suelta.

En la primera publicación que se adjunta se realiza una previa exposición breve del problema. En la segunda publicación, se aborda el mismo a través de la información recopilada sobre la problemática de las poblaciones de conejo a nivel mundial, las bases moleculares de ambas líneas de investigación, el posible impacto de los virus recombinantes resultantes (basados en la historia previa de las enfermedades del conejo), y las normas legales que existen hasta la fecha (tanto nacionales como internacionales) que podrían regular su uso.

Durante el siglo XX aparecieron las dos enfermedades del conejo, la mixomatosis y la enfermedad hemorrágica (EHV). Para entender adecuadamente el potencial de los virus recombinantes (a liberar en poblaciones silvestres de conejo) es básico conocer la historia de estas enfermedades. La mixomatosis se descubrió en 1896 en Montevideo, donde unos conejos europeos de granja se infectaron con un virus que afectaba de manera leve a algunas

especies de conejo americano. Dada la alta mortalidad que produjo, el virus se ensayó como método de control biológico para las poblaciones de conejo, tanto en Europa como en Australia. La primera liberación exitosa se produjo en Australia en 1950; no tardó en liberarse en Francia en 1952, foco de su expansión natural al resto de Europa. También se produjeron liberaciones ilegales en otros continentes. Las mixomatosis produjo mortalidades de hasta un 99% en las poblaciones de conejo. La rápida capacidad de cambio del virus ha permitido la recuperación en diferentes grados de muchas poblaciones.

La EHV apareció en 1984 en granjas de conejo doméstico en China; en 1987 llegó a las granjas italianas, y posteriormente se detectó en el campo. La EHV cruzó de forma natural a Gran Bretaña en 1992; en 1995 se escapó de una isla experimental cruzando los 5 km. que le separaban del continente australiano; por último, fue introducida ilegalmente en Nueva Zelanda. La EHV supuso mortalidades variables en campo, pero siempre mayores al 50%. Aún no se conoce el origen de este virus; una de las hipótesis más aceptadas es que procede de la mutación de un virus inocuo, que existiría de forma natural en algunas poblaciones silvestres como en Gran Bretaña.

En la lucha contra estas enfermedades, un grupo de investigación español, ha desarrollado un virus vacunal contra las dos enfermedades a través de la modificación genética del virus de la mixomatosis. El gen de la proteína viral del virus de la EHV se inserta en el genoma del virus de la mixomatosis. Al expresarse dicho gen dentro de los conejos, produce inmunidad contra la EHV, mientras que al escoger una cepa muy atenuada de mixomatosis el virus recombinante actúa como vacuna contra la misma. Por otro lado, en la lucha contra el conejo como especie invasora, un equipo australiano de investigación ha modificado el virus de la mixomatosis insertándole un gen que produce una proteína esencial para la unión del óvulo con el gameto. Una vez en el conejo, el animal produce anticuerpos contra esa proteína, que interfieren con la reproducción, produciendo infertilidad en las hembras. Este proceso se denomina inmunocontracepción mediada por virus y se está investigando también para otras especies en distintos centros australianos.

El desarrollo exponencial de las técnicas moleculares de modificación genética (surgidas en los años 1970) no ha ido paralelo al de una legislación que regule su uso, algo que sería muy necesario. En Europa, la Directiva 219 CEE de 1990 regulaba las liberaciones de organismos modificados. En la Directiva actual, 18 CE de 2001, todos los países deben estar de acuerdo en la decisión que ha tomado uno de ellos (la fecha para transponer esta Directiva en España era octubre de 2002). En Australia, no ha habido una normativa legal hasta 2001. En esta ley, el virus recombinante fue clasificado en la categoría de “organismo confinado no destinado a la liberación en el campo”. Respecto a las normas internacionales, la mayoría de organismos emiten recomendaciones que no son útiles como normas legales.

correspondence

Silicon philanthropists follow a great tradition

Sir — What is being called 'venture philanthropy' in your News feature 'Biomedical philanthropy, Silicon Valley style' (*Nature* **410,** 140–143; 2001) is hardly a new concept — it is an example of what has traditionally characterized 'thoughtful philanthropy'. In fact, it is very like the 'old' strategic philanthropy envisioned and practised by many private foundations established at the beginning of the twentieth century.

Robert Kohler's book *Partners in Science* (Univ. Chicago Press, 1991) provides many examples of the essential role private funders played in the development of modern molecular biology, genetics, public health and other biomedical research fields. The Silicon Valley entrepreneurs interviewed in your feature are following a tradition long established by private funders who viewed their money as providing venture capital for the common good and who long held the view that philanthropy should invest in the acquisition of new knowledge and in its responsible application. Further, the staff of the new foundations are following in the footsteps of a profession first defined by the Rockefeller Foundation 'circuit riders' — programme officers knowledgeable in their fields, actively seeking out promising research projects needing support.

I do not intend to detract from the laudable efforts of these new philanthropists to pursue thoughtful giving that takes risks and invests in new ideas with a minimum of hassle and red tape. But I do not see why they need to be flattered into thinking they are inventing something new — or that, as some of them seem to believe, they have a new model that needs to be emulated by all funders. Just as there is a certain dishonesty in the communication between researchers and government funders (the 'proposal' describing work already completed), there is a growing risk of dishonest dialogue between scientists and some of the philanthropists identified by the anecdotes Trisha Gura relates ('No one but you has the keen insight to recognize my brilliant idea').

The number of projects and researchers supported by private philanthropy will remain small compared with those receiving government support, and private funding relies on its partnership with public dollars. Peer review may not be perfect, but neither is investing in whoever grabs someone's attention or ear.

The true richness of private philanthropy is found in its diversity of approaches and its distributed decision-making processes that allow many different points of view — and many different grant-making approaches — to flourish.

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When DNA research menaces diversity

Sir — The Nature Science Update "Synthetic viruses just around the corner"[1] reported a discussion about scientists' imminent ability to synthesize new viruses for producing better vaccines or for devising deadlier biological weapons. I would like to give an example where genetic engineering of viruses can undermine careful management of natural resources.

Two new rabbit virus strains are being developed for opposing reasons. On the one hand, a group in Australia is modifying rabbit myxoma virus to transmit reproductive sterility and so reduce numbers in a region where rabbits constitute a pest[2]. On the other, a European group is modifying myxoma virus to express rabbit haemorrhagic disease virus capsid protein[3] to protect rabbits against both diseases and encourage the recovery of wild populations within the species' original distribution area.

Each goal is logical within its regional context. However, the history of rabbit viruses shows that outcomes can conflict with initial goals. First recorded in Montevideo in 1896, myxoma virus was successfully released in Australia in 1950 to control rabbit populations, but an illegal release in 1952 in France led to the virus spreading throughout Europe. Similarly, rabbit haemorrhagic disease, which spread naturally from Chinese rabbitries throughout Europe in 1987, spread in Australia after an accidental release from a trial island and in New Zealand after an illegal release in 1997.

The new modified viruses could spread worldwide as easily as myxomatosis and rabbit haemorrhagic disease initially spread. The establishment of modified myxoma virus into inappropriate regions could have disastrous effects on biodiversity. The preservation of Australasian ecosystems, as well as the conservation of endangered predators in Europe, depends on the same species: the wild rabbit, the target of both modified viruses.

It is therefore essential that modified viruses are very carefully and appropriately used. Biotechnology policies on the release of modified organisms rely on national authorities. But rabbits are distributed throughout the world, so it is essential to guide the development, release and regulation of rabbit virus biotechnology, and to enforce international controls to prevent accidental spread of genetically modified viruses.

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1. Whitfield, J. Nature Science Update. http://www.nature.com/nsu/010222/010222-17.html (accessed 21 February 2001).
2. Kerr, P. J. *et al. Biol. Reprod.* 61, 606–613 (1999).
3. Bárcena, J. *et al. J. Virol.* 74, 1114–1123 (2000).

Gulf syndrome research has passed peer review

Sir — Your News report (*Nature* **410,** 135; 2001) mentioned the negative comments made by a former US Department of Defense (DoD) employee, Bernard Rostker, and by Philip Landrigan, a Clinton committee appointee, about our published research on Gulf War syndrome. Because your story stated that our work was supported by a private foundation and non-peer-reviewed grant funds, and involved small patient samples, readers might be left with the impression that our findings are invalid.

This is not true. Over the past four years we have published 12 papers in prominent scientific journals establishing that there is a new syndrome with three variants in Gulf War veterans of a naval reserve battalion (see, for example, ref. 1). Our study of 63 cases and 186 controls identified strong associations with risk factors for exposure to sarin nerve gas and related chemicals. We have also identified affected brain regions and a genetic predisposition using a variety of techniques. Our design and sample sizes are equivalent to those used by the Centers for Disease Control and Prevention in such classic epidemic investigations as toxic shock syndrome, Four-Corners hantavirus pneumonia and AIDS.

The funding proposals for all our studies were rejected by the DoD's peer-review system but were funded by a private foundation or after appeal to higher government levels. Our results later passed rigorous peer review before publication in respected scientific journals. A consistent publishing record is a better indicator of scientific merit than the sources of funding, particularly in the politically charged environment of Gulf War research.

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1. Haley, R. W. *et al. Arch. Neurol.* 57, 1280–1285 (2000).

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COMMENTARY

First synthesize new viruses then regulate their release? The case of the wild rabbit

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Abstract

European wild rabbits originated in southwestern Europe but have been introduced into many other countries world-wide, becoming serious pests in many instances. As a consequence of rabbits being regarded so differently, applied research for their management often has opposing goals, namely their conservation or their control. Furthermore, modern gene technology has led to the concept of using genetically modified myxoma viruses for rabbit management, again with quite contrary aims in mind. In this paper we explain the possible ecological and economic consequences of using these genetically modified viruses inappropriately and we consider whether national and international regulations are sufficient to prevent improper use. If international regulations are inadequate, molecular biologists and ecologists must consider the consequences of their research and advice beyond their own country to avoid unwanted impacts.

Keywords: conservation, GMO, myxomatosis, *Oryctolagus cuniculus*, rabbit haemorrhagic disease, wild rabbit

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Introduction

Organisms modified using gene technology are commonly referred to as genetically modified organisms (GMOs). They are now widely used in industry, agriculture, health care, and biological control, usually involving releases into the environment.

Nevertheless, developments in this area have often progressed faster than the legislation that provides for their safe use and Tiedje *et al.* (1989) have recommended that research should be carried out within a framework of science-based regulation that encourages innovation without compromising environmental values. As GMOs may be spread beyond political boundaries, it is essential to achieve international co-ordination in developing such regulations.

Here we present the case of genetically modified rabbit viruses, one developed to help conserve rabbits, the other developed for their control, in which the lack of effective international co-ordination and control could compromise the management of rabbit populations in countries other than those in which the viruses were developed (Angulo 2001).

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Current distribution and ecological problems concerning wild rabbits

The European wild rabbit, *Oryctolagus cuniculus*, originated in southwestern Europe on the Iberian peninsula (Fig. 1). It is a prolific species and has always supported a diverse predator community. In Spain it is the staple prey of two endangered predators, the imperial eagle (*Aquila adalberti*) and the Iberian lynx (*Lynx pardinus*) (Delibes & Hiraldo 1981). Humans have also taken advantage of rabbit abundance: over one million hunters generate an estimated US$ 1.2×10^9 annually in Spain (Villafuerte *et al.* 1998). However, in the last 50 years wild rabbit populations have undergone a sharp decline caused mainly by the appearance of two viral diseases, myxomatosis and rabbit haemorrhagic disease (RHD) (Queney *et al.* 2000). Hunters and conservationists alike are concerned.

Fig. 1 Current distribution of European wild rabbits. Arrows indicate small areas where rabbits have been introduced. Natural populations marked in grey and introduced populations in black.

Besides spreading naturally into other European countries, rabbits have been distributed world-wide by man for food and hunting (Fig. 1). In many areas rabbits have become a real pest, multiplying 'like rabbits' in an optimal environment and with the lack of effective predators (Holland 1999). In Australia, rabbits cause erosion, land degradation and loss of native plants (Fenner & Fantini 1999) and rabbit control and agricultural losses cost US$ 310 million annually (Robinson *et al.* 1997). In Britain, crop damage is estimated at over US$ 170 million annually (R. C. Trout, personal communication).

New rabbit management programmes: Australian vs. Spanish GM viruses

Given the two distinct lines of research for the management of wild rabbits, conservation and pest control, it is intriguing that, for each, a solution is being sought through the genetic manipulation of the myxoma virus (MV) originally derived from cottontail rabbits (*Sylvilagus* spp.) in the Americas.

In trying to deal with diseases in wild rabbits in south-west Europe hunters and conservationists have increasingly turned to molecular technology. Immunization of rabbits against myxomatosis has long been possible using cell culture-attenuated MV strains. However, during the last few years, researchers have explored ways of developing recombinant vaccines that express the RHD virus (RHDV) capsid protein. These include the use of baculovirus (Laurent *et al.* 1994), poxivirus (Fischer *et al.* 1997), plant viruses such as potyvirus (Fernández-Fernández *et al.* 2001), or plants (Castañón *et al.* 1999). Most importantly, Bertagnoli *et al.* (1996) produced a recombinant vaccine based on an attenuated MV that expressed RHDV capsid protein to protect simultaneously against both diseases. Most of the systems listed rely on direct inoculation of individual rabbits, and consequently are not suited for large-scale wild rabbit vaccination. However, Spanish scientists have recently developed an alternative GM virus, based on an attenuated but transmissible field strain of MV, genetically modified to provide protection against RHD as well. It is capable of horizontal transmission by contact between rabbits; thus, only a few rabbits need to be initially vaccinated to achieve immunization of the greater population (Bárcena *et al.* 2000). The Spanish National Committee of Biosafety authorized the experimental test release of this recombinant on a Mediterranean island, Isla del Aire, to assess its potency and safety. Infected rabbits produced antibodies against both viruses, and horizontal transmission to about 50% of uninoculated rabbits in the field was observed during the short trial period (Torres *et al.* 2001). Scientists are hopeful of widespread release soon.

The same concept of natural spread of virus to affect rabbit populations on a wide scale is also being considered to control rabbits. One initiative of the Pest Animal Control Cooperative Research Centre (PAC-CRC) in Australia is the use of GM MV to reduce rabbit fertility through transmissible (virally vectored) immunocontraception. This concept was proposed at the Conference on Fertility Control in Wildlife held in Melbourne in 1990. The idea was to develop recombinant viral vectors that can transmit immunogens to induce a specific immune response in the target animal against reproductive proteins. Specific and contagious viruses, in this case MV, could disseminate the contraceptive agent into the population (Tyndale-Biscoe 1991). The recombinant MV produces the rabbit zona pellucida glycoprotein B and initial experiments have induced temporary infertility in 25% of female rabbits (Kerr *et al.* 1999).

Impact of GM rabbit viruses: the world-wide spread of MV and RHD

While both GM viruses could be valuable in managing rabbits in the countries where they are being developed, the problem is that they may cause an entirely unwanted effect in another country, and the history of rabbit viruses shows clearly that they are well suited to global spread.

Myxomatosis was first recorded in Montevideo, in 1896 (Fig. 2a) when it was spread from the native South American cottontail rabbit, *Sylvilagus brasiliensis* to European rabbits. Soon after its discovery, MV was suggested as a possible tool for the control of rabbits in Australia. During the 1920s–1940s, there was great debate over the use of MV to control rabbits, but it was nevertheless legally released in Australia in 1950 (Ratcliffe *et al.* 1952). The success of myxomatosis in Australia led a French landowner to release the virus illegally in 1952, and subsequently myxomatosis spread naturally through the rest of Europe (Muñoz 1960; Sellers 1987). Myxomatosis was illegally

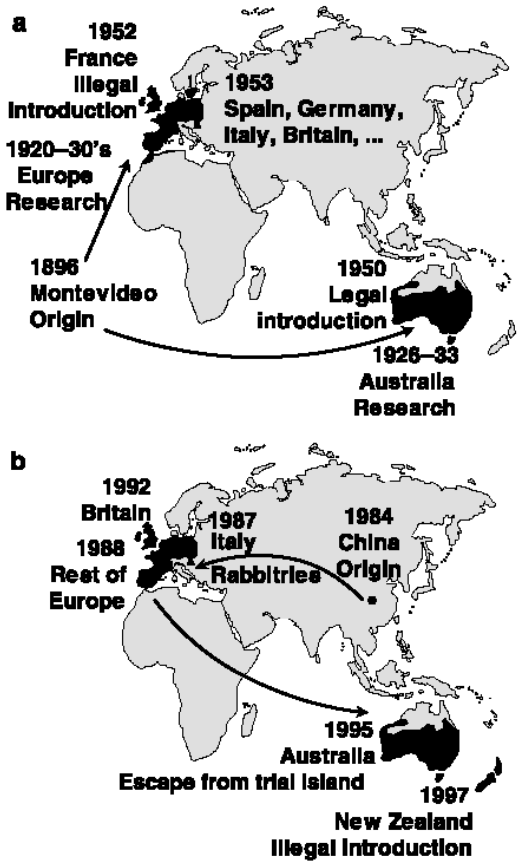

Fig. 2 (a) Origin and expansion of myxoma virus. (b) Origin and expansion of rabbit haemorrhagic disease. (Virus spread in rabbitries outside the wild rabbit distribution has not been shown.)

used by Argentinean landowners to control the spread of wild rabbits and was illegally distributed in Britain (Fenner & Fantini 1999).

Myxomatosis initially reduced British wild rabbit populations by 99% (Flowerdew *et al.* 1992). In Australia MV was also highly effective at first but attenuated into less virulent strains and rabbits developed genetic resistance to the disease so that today there is a dynamic balance between virulence and host resistance in which myxomatosis kills between 40 and 60% of infected susceptible rabbits (Kerr & Best 1998). This also explains why rabbits were relatively rare in Britain for about 25 years (Lloyd 1981) and why MV continues to regulate their populations today (Trout *et al.* 1992).

MV has caused major declines in native wild rabbit populations of southwest Europe. In Spain, it resulted in a reduction of hunting activity (Muñoz 1960), and negatively affected endangered predators (Delibes & Hiraldo 1981). MV also had negative environmental (Flowerdew *et al.* 1992) and economic impacts (Fenner & Fantini 1999).

In 1984, a new disease, RHD [also known as rabbit calicivirus disease (RCD) in Australasia], appeared in rabbitries in China (Fig. 2b). In 1987 it appeared in Italy and broke out simultaneously in several other European countries, transmitted largely through trade in domestic rabbits. It quickly expanded into wild rabbit populations, even crossing the English Channel into Britain by 1992 (Chasey & Trout 1995). In 1995, before it was fully evaluated as a new rabbit control agent in Australia, RHD escaped from an experimental trial on a quarantined island and crossed 5 km of sea to mainland Australia where it soon became established (Kovaliski 1998). In 1997 it was illegally introduced in New Zealand (O'Keefe *et al.* 1999).

In Australia, the initial effectiveness of RHD was variable, with the highest levels in arid and semiarid areas where mortality reached 95%, leading to the collapse of rabbit commerce (Fenner & Fantini 1999). Meanwhile, RHD had sharply reduced native wild rabbit populations in southwest Europe. The first RHD epizootics caused mortality rates between 70 and 90% in domestic rabbits, and between 50 and 60% in wild rabbits (Villafuerte *et al.* 1994), although Marchandeau *et al.* (1998) detected mortality rates up to 80% in wild rabbits. In Spain, few populations have recovered to prior levels, directly affecting hunting activity and endangered predators (Fernández 1993; Villafuerte *et al.* 1998; Martínez & Calvo 2001).

A single pathogenic RHDV serotype seems to exist to date (Asgari *et al.* 1999). But a nonpathogenic rabbit calicivirus related to RHDV has been described in domestic rabbits (Capucci *et al.* 1996). Besides, seropositive rabbits, apparently carrying antibodies raised against a related nonvirulent calicivirus and protected from severe RHD, have been found in Europe (Trout *et al.* 1997), Australia (Nagesha *et al.* 2000) and New Zealand (O'Keefe *et al.* 1999). Mutation of an avirulent form of the calicivirus is a possible explanation for the origin of RHD (Rodak *et al.* 1990).

Can viruses be contained within target populations or distinct geographical areas?

For both the Spanish GM virus, which vaccinates rabbits against myxomatosis and RHD, and the Australian virus, aimed at reducing the fertility of rabbits, it is envisaged that active viruses that retain their capacity to spread would be most useful. This is important because it would not be necessary to vaccinate every rabbit. A naturally spreading vaccine could be introduced into some rabbits then spread to a greater part of the population. However, it is precisely this characteristic that would make them so difficult to contain.

The ready spread of both MV and RHDV raises many questions about our ability to contain such viruses. Clearly landholders interested in reducing rabbit problems deliberately spread MV and RHDV. There is also a risk, as happened with RHDV in Australia, of underestimating the role of insect vectors in transmitting the virus over very long distances. Sea birds have also been implicated in the spread of both MV and RHDV. It is therefore quite conceivable that recombinant MV could be used in areas where such risks were not fully considered. Indeed, the most recent trials with a GM MV were carried out on Isla del Aire, only 1 km offshore from one of the larger Balearic Islands and where there is a seagull colony and regular hunting activity.

Other issues also need to be fully understood. These include the potential for interaction between GM viruses and field strains of MV (Tyndale-Biscoe 1994) including genetic exchange between GM viruses and wild viruses which may have different virulence or greater ability to compete. It is also necessary to understand and counter any potential impact of GM viruses on *Sylvilagus* spp., the original hosts of MV.

Although such questions are being considered with the idea of developing safeguards in the GM viruses, the idea of using actively spreading viruses remains problematic. As we have seen, MV and RHDV are difficult to contain within distinct geographical areas. It is essential to ask whether it would be possible to prevent the potential for spread of GM rabbit viruses into inappropriate regions through currently available mechanisms such as international controls and regulations.

What regulations cover research and release of GMOs at national levels?

During the 1970s many countries launched biotechnology policies and management plans. Most distinguish between contained GMO work and deliberate releases into the environment with separate legislation. A national authority generally regulates approval for release following risk assessment that may include scientific and ethical considerations as well as public consultation.

For example, in 1990, the European Union allowed for releases of GMOs through Directive 90/219/EEC (EEC 1990). Within that framework a national authority could evaluate risks. This directive resolved the problems on a national level but created a problem on the European level, as other Member States could not discuss the decision. In April 2001 a new Directive 2001/18/EC was adopted (EC 2001), whereby the release of a GMO in any country needs the agreement of the European Commission and the rest of the member states. The final date for Member States to comply with this Directive is October 2002 (although it has not yet been adopted in Spain). Until this date, GMO applications (i.e. recombinant vaccine MV) may be subject to the Directive 90/220/EEC.

In New Zealand, the Hazardous Substances and New Organisms Act covers the importation, development, field-testing and the intentional release of GMOs into the environment (http://www.hsno.govt.nz/). For GM viruses, an assessment would obviously be made in terms of their capacity to cause disease. But, it is not clear whether international risks or consequences are considered by this legislation.

Gene technology was subject to voluntary assessment in Australia from 1975 until June 2001. Responsibilities were held by different committees, but their recommendations were not enforced. In 1997 Australia began preparing new legislation to tighten assessment. Called the Gene Technology Act 2000, it commenced operation in June 2001 (Radke 2001). For the release of GMO into the environment, the Gene Technology Regulator may consult international experts. The Gene Technology Regulator can impose conditions to limit the spread or persistence of the GMO in the environment. However, the release may be approved, claiming isolation distances or physical barriers to other continents. Currently, research on modified MV done by the Pest Animal Control CRC and Australian National University is licensed as a dealing not involving intentional release into the environment.

International agreements on research and release of GMOs

International organizations such as the Organization for Economic Cooperation and Development (OECD), World Trade Organization (WTO), the World Organization for Animal Health (OIE), the World Health Organization, or the Convention on Biological Diversity, try to unify national regulations. However, international organizations only develop recommendations and guidelines, and these may or may not necessarily be adopted by individual countries.

The OECD seeks to ensure safety, develop effective regulatory oversight and facilitate trade in biotechnology products between the 29 member countries. The OECD has organized international meetings on GMOs, mainly on modified food and crops. Similarly, the WTO has developed the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) to protect animals and plants from pests and diseases and GMOs were discussed during SPS Committee meetings in November 2001. The OIE informs countries of the occurrence of animal diseases, harmonizes regulations for trade in animals or animal products, and develops recommendations to prevent disease spread.

The Convention on Biological Diversity adopted an agreement known as the Cartagena Protocol of Biosafety in

January 2000, to protect biological diversity from potential risks posed by GMOs. It established a procedure specifically focusing on cross-border movement of GMOs in which risks are assessed by national authorities but final decisions regarding the importation or release of GMOs must be communicated to the Convention. By November 2001 only eight countries had ratified the Cartagena Protocol, but neither countries of the European Community nor Australia and New Zealand were signatories.

We conclude that, while there are some regulations focused on the research and release of GMOs there appear to be few agreements that specifically address safe research, handling and release of GMOs at an international level. Moreover, regulations are very general, or focus on safety issues regarding GM food trade and importation, and their effectiveness is weak, as shown by the discovery of GM crops growing in areas where permission had not been granted (Dalton 2001; Jayaraman 2001). Regulations for nontradable GMOs or GM viruses seem to be even less well considered.

Conclusion

Applied research for the management of wild rabbits in different parts of the world has opposing goals. This may lead to the creation and release of antagonistic GM viruses, one aimed at conservation, the other aimed at rabbit control. The use of virally vectored immunocontraception to control pests is currently being investigated for a number of different species (Tyndale-Biscoe 1991), including possums (Sutherland *et al*. 1996), foxes (Holland 1999), cats (Courchamp & Cornell 2000) and rodents (Ylönen 2001). Bearing in mind the facility with which viruses spread or can be intentionally spread and the difficulty of virus control in the field (Fenner & Fantini 1999) mere legislation is not enough. To avoid unexpected effects of the introduction of new GMOs for wildlife management, it is essential to get international agreement and co-ordination in the development and use of such strategies.

It is essential that research on rabbit control and conservation try to achieve realistic management goals where risks are minimized (Angulo 2001). Certainly, Australian and Spanish scientists follow the guidelines established in their respective countries (Robinson *et al*. 1997; Bárcena *et al*. 2000), but a greater effort should be made to promote the international communication between scientists and national and international authorities (Tyndale-Biscoe 1994). Evaluating the possible impact of release of GMOs into the environment requires expertise in many scientific disciplines. Between them, molecular biologists, veterinarians and ecologists must consider the consequences of their decisions, beyond their own country.

This paper takes a step in that direction by pointing out some potential impacts of GMOs being developed for managing wild rabbit populations. Past studies have focused on general ecological and evolutionary aspects (Tiedje *et al*. 1989) or particular legal and ethical issues (Tyndale-Biscoe 1994), but none has provided a thorough assessment of the risks. We make no specific recommendations about a course of action that can be taken other than to list some questions that might be raised in international scientific or regulatory meetings. These include asking: (i) whether accidental or illegal spread could be prevented by existing international controls or conventions that regulate cross-border GMO movements; (ii) what international scientific structures should be established to enable the rational development of GMOs for wildlife management; (iii) how can international regulations on GMO releases be designed to be acceptable to and implemented within individual countries?

In essence, there is a need for scientific and regulatory structures that guide the development and release of GMOs by (i) evaluating their potential to escape and establish abroad; (ii) assessing whether or not risks are internationally acceptable at scientific, economic and environmental levels; and (iii) developing specific regulation of their use.

In the meantime, ecologists, veterinarians and molecular biologists must keep an international perspective on their work and devise measures to reduce the risk of unwanted ecological and economic impacts, of the kind illustrated here for viruses being designed to manage wild rabbits.

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References

Angulo E (2001) When DNA research menaces diversity. *Nature*, **410**, 739.

Asgari S, Hardy JRE, Cooke BD (1999) Sequence analysis of rabbit haemorrahic disease virus (RHDV) in Australia: alterations after its release. *Archives of Virology*, **144**, 135–145.

Bárcena J, Morales M, Vázquez B *et al*. (2000) Horizontal transmissible protection against myxomatosis and rabbit hemorrhagic disease by using a recombinant myxoma virus. *Journal of Virology*, **74**, 1114–1123.

Bertagnoli S, Le Gall G, Boilletot E *et al*. (1996) Protection against myxomatosis and rabbit viral hemorrhagic disease with recombinant myxoma viruses expressing rabbit hemorrhagic disease virus capsid protein. *Journal of Virology*, **70**, 5061–5066.

Capucci L, Fusi P, Lavazza A, Pacciarini ML, Rossi C (1996) Detection and preliminary characterization of a new rabbit calicivirus

related to rabbit hemorrhagic disease virus but nonpathogenic. *Journal of Virology*, **70**, 8614–8623.

Castañón S, Marín MS, Martín-Alonso JM *et al.* (1999) Immunization with potato plants expressing VP60 protein protects against rabbit hemorrhagic disease virus. *Journal of Virology*, **73**, 4452–4455.

Chasey D, Trout RC (1995) Rabbit haemorrhagic disease in Britain. *Mammalia*, **59**, 599–603.

Courchamp F, Cornell SJ (2000) Virus-vectored immunocontraception to control feral cats on islands: a mathematical model. *Journal of Applied Ecology*, **37**, 903–913.

Dalton R (2001) Transgenic corn found growing in Mexico. *Nature*, **413**, 337.

Delibes M, Hiraldo F (1981) The rabbit as prey in the Iberian Mediterranean ecosystem. In: *Proceedings of the World Lagomorph Conference* (eds Myers K, MacInnes CD), pp. 614–622. University of Guelph, Ontario, Canada.

EC (2001) Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. *Official Journal*, **L106**, 1–39.

EEC (1990) Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. *Official Journal*, **L117**, 15–17.

Fenner F, Fantini B (1999) *Biological Control of Vertebrate Pests: the History of Myxomatosis; An Experiment in Evolution*. CABI Publishing, Oxford.

Fernández C (1993) Effect of the viral haemorrhagic pneumonia of the wild rabbit on the diet and breeding success of the golden eagle *Aquila chrysaëtos* (L.). *Revue D'Ecologie*, **48**, 323–329.

Fernández-Fernández MR, Mouriño M, Rivera J *et al.* (2001) Protection of rabbits against rabbit hemorrhagic disease virus by immunization with the VP60 protein expressed in plants with a potyvirus-based vector. *Virology*, **89**, 283–291.

Fischer L, LeGros FX, Mason PW, Paoletti E (1997) A recombinant canarypox virus protects rabbits against a lethal rabbit hemorrhagic disease virus (RHDV) challenge. *Vaccine*, **15**, 90–96.

Flowerdew JR, Trout RC, Ross J (1992) Myxomatosis: population dynamics of rabbits (*Oryctolagus cuniculus* Linnaeus, 1758) and ecological effects in the United Kingdom. *Revue Scientifique et Technique*, **11**, 1109–1113.

Holland MK (1999) Fertility control in wild populations of animals. *Journal of Andrology*, **20**, 579–585.

Jayaraman KS (2001) Illicit GM cotton sparks corporate fury. *Nature*, **413**, 555.

Kerr PJ, Best SM (1998) Myxoma virus in rabbits. *Revue Scientifique et Technique*, **17**, 256–268.

Kerr PJ, Jackson RJ, Robinson AJ *et al.* (1999) Infertility in female rabbits (*Oryctolagus cuniculus*) alloimmunized with the rabbit zona pellucida protein ZPB either as a purified recombinant protein or expressed by recombinant myxoma virus. *Biology of Reproduction*, **61**, 606–613.

Kovaliski J (1998) Monitoring the spread of rabbit hemorrhagic disease virus as a new biological agent for control of wild European rabbits in Australia. *Journal of Wildlife Diseases*, **34**, 421–428.

Laurent S, Gelfi J, Le Gall G *et al.* (1994) Recombinant rabbit hemorrhagic disease virus capsid protein expressed in baculovirus self-assembles into viruslike particles and induces protection. *Journal of Virology*, **68**, 6794–5066.

Lloyd HG (1981) Biological observations on post-myxomatosis wild rabbit populations in Britain 1955–79. In: *Proceedings of the World Lagomorph Conference* (eds Myers K, McInnes CD), pp. 623–628. University of Guelp, Ontario, Canada.

Marchandeau S, Ricci JC, Chantal J (1998) Taux de prévalence sérologique du virus de la maladie virale hémorragique (VHD) du lapin de garenne (*Oryctolagus cuniculus*) et de ses formes apparentées au sein de différentes populations sauvages en France. *Mammalia*, **62**, 95–103.

Martínez JE, Calvo JF (2001) Diet and breeding success of eagle owl in southeastern Spain: effect of rabbit haemorrhagic disease. *Journal of Raptor Research*, **34**, 259–262.

Muñoz G (1960) *Anverso Y Reverso de la Mixomatosis*. Servicio Nacional de Pesca Fluvial y Caza Ed., Madrid.

Nagesha HS, McColl KA, Collins BJ *et al.* (2000) The presence of cross-reactive antibodies to rabbit haemorragic disease virus in Australian wild rabbits prior to the escape of virus from quarantine. *Archives of Virology*, **145**, 749–757.

O'Keefe JS, Tempero JE, Motha MXJ, Hansen MF, Atkinsona PH (1999) Serology of rabbit haemorrhagic disease virus in wild rabbits before and after release of the virus in New Zealand. *Veterinary Microbiology*, **66**, 29–40.

Queney G, Ferrand N, Marchandeau S *et al.* (2000) Absence of a genetic bottleneck in a wild rabbit (*Oryctolagus cuniculus*) population exposed to a severe viral epizootic. *Molecular Ecology*, **9**, 1253–1264.

Radke S (2001) Biotechnology gets big backing in Australia. *Nature*, **412**, 765.

Ratcliffe FN, Myers K, Fennessy BV, Calaby JH (1952) Myxomatosis in Australia. A step forwards the biological control of the rabbit. *Nature*, **170**, 1–13.

Robinson AJ, Jackson R, Kerr P *et al.* (1997) Progress towards using recombinant myxoma virus as a vector for fertility control in rabbits. *Reproduction, Fertility and Development*, **9**, 77–83.

Rodak L, Smid B, Valícek T *et al.* (1990) Enzyme-linked immunosorbent assay of antibodies to rabbit haemorrhagic disease virus and determination of its major structural proteins. *Journal of General Virology*, **71**, 1075–1080.

Sellers RF (1987) Possible windborne spread of myxomatosis to England in 1953. *Epidemiology and Infection*, **98**, 119–125.

Sutherland ORW, Cowan PH, Orwin J (1996) Biological control of possums *Trichosurus vulpecula* and rabbits *Oryctolagus cuniculus* in New Zealand. *Wildlife Biology*, **2–3**, 165–170.

Tiedje JM, Colwell RK, Grossman YL *et al.* (1989) The planned introduction of genetically engineered organisms: ecological considerations and recommendations. *Ecology*, **70**, 298–315.

Torres JM, Sánchez C, Ramírez MA *et al.* (2001) First field trial of a transmissible recombinant vaccine against myxomatosis and rabbit hemorrhagic disease. *Vaccine*, **19**, 4536–4543.

Trout RC, Ross J, Tittensor AM, Fox AP (1992) The effect on a British wild rabbit population (*Oryctolagus cuniculus*) of manipulating myxomatosis. *Journal of Applied Ecology*, **29**, 679–686.

Trout RC, Chasey D, Sharp G (1997) Seroepidemiology of rabbit haemorzhagic disease (RHD) in wild rabbits (Oryctolagus *cuniculus*) in the United Kingdom. *Journal of Zoology*, **243**, 846–853.

Tyndale-Biscoe CH (1991) Fertility control in wildlife. *Reproduction, Fertility and Development*, **3**, 339–343.

Tyndale-Biscoe CH (1994) Virus-vectored inmunocontraception of feral mammals. *Reproduction, Fertility and Development*, **6**, 281–287.

Villafuerte R, Calvete C, Gortázar C, Moreno S (1994) First epizootic of rabbit hemorrhagic disease in free living populations of *Oryctolagus cuniculus* at Doñana National Park, Spain. *Journal of Wildlife Diseases*, **30**, 176–179.

Villafuerte R, Viñuela J, Blanco JC (1998) Extensive predator persecution caused by population crash in a game species: the case of red kites and rabbits in Spain. *Biological Conservation*, **84**, 181–188.

Ylönen H (2001) Rodent plagues, immunocontraception and the mousepox virus. *Trends in Ecology and Evolution*, **16**, 418–420.

This review stems from Elena Angulo's PhD thesis on the ecological factors influencing abundance and distribution of wild rabbit populations in Spain. This work showed that diseases were among the most important limiting factors. Brian Cooke is a Principal Research Scientist with CSIRO. He has been involved with research on the biological control of wild rabbits in Australia for 35 years, working mainly with rabbit haemorrhagic disease over the last 14 years.