CSIRO PUBLISHING
www.publish.csiro.au/journals/wr

Review
Wildlife Research, 2008, **35**, 633–650

The future biological control of pest populations of European rabbits, *Oryctolagus cuniculus*

Robert P. Henzell[A,B,D], *Brian D. Cooke*[B,C] *and Gregory J. Mutze*[A,B]

[A]Department of Water, Land and Biodiversity Conservation, GPO Box 2834, Adelaide, SA 5001, Australia.
[B]Invasive Animals Cooperative Research Centre, University of Canberra, Bruce, ACT 2601, Australia.
[C]University of Canberra, Bruce, ACT 2601, Australia.
[D]Corresponding author. Email: henzell.bob@saugov.sa.gov.au

Abstract. European rabbits are exotic pests in Australia, New Zealand, parts of South America and Europe, and on many islands. Their abundance, and the damage they cause, might be reduced by the release of naturally occurring or genetically modified organisms (GMOs) that act as biological control agents (BCAs). Some promising pathogens and parasites of European rabbits and other lagomorphs are discussed, with special reference to those absent from Australia as an example of the range of necessary considerations in any given case. The possibility of introducing these already-known BCAs into areas where rabbits are pests warrants further investigation. The most cost-effective method for finding potentially useful but as-yet undiscovered BCAs would be to maintain a global watch on new diseases and pathologies in domestic rabbits. The absence of wild European rabbits from climatically suitable parts of North and South America and southern Africa may indicate the presence there of useful BCAs, although other explanations for their absence are possible. Until the non-target risks of deploying disseminating GMOs to control rabbits have been satisfactorily minimised, efforts to introduce BCAs into exotic rabbit populations should focus on naturally occurring organisms. The development of safe disseminating GMOs remains an important long-term goal, with the possible use of homing endonuclease genes warranting further investigation.

Introduction

European rabbits, *Oryctolagus cuniculus* (L.), are exotic pests in many countries and islands outside their natural range on the Iberian Peninsula, especially in Australasia, South America and Europe (Flux *et al*. 1990; Flux 1993; Myers *et al*. 1994; Rogers *et al*. 1994; Thompson 1994; Williams *et al*. 1995; Jaksic 1998; White and Newton-Cross 2000; Long 2003). Their high reproductive rate and physiological adaptations for occupying Mediterranean-type environments can frustrate most efforts to substantially reduce their numbers and the damage they cause. Since 1950 the numbers of wild rabbits in Australia have been reduced by the intentional introduction of four biological control agents (BCAs): myxoma virus (MV), rabbit haemorrhagic disease virus (RHDV), and two disease vectors, European and Spanish rabbit fleas (Fenner and Fantini 1999). (Note that improvements in techniques for the control of rabbits by warren destruction, poisoning and fumigation contributed to this reduction: Williams *et al*. 1995.) MV has also been intentionally introduced into Europe and southern South America, and RHDV into New Zealand (Fenner and Fantini 1999). RHDV may have been accidentally introduced into Europe from China, but this is uncertain because the origins of RHDV are unclear: the disease was first noticed in domestic rabbits that had been imported into China from Germany a few days earlier, and present controversial research suggests that RHDV may have existed in Europe for a very long time (Forrester *et al*. 2006). Attempts to introduce MV into New Zealand failed (Gibb and Williams 1994). Hoddle (1999) has reviewed the use of biological agents to control vertebrate pests.

Despite the post-1950 reduction in rabbit numbers in Australia, rabbits continue to cause serious damage to biodiversity and agricultural production in Australia, and further reductions in their numbers are warranted. Production losses in the sheep, cattle and cropping industries caused by rabbits were valued by McLeod (2004) at AU$88 million annually in Australia. Costs of managing rabbits and conducting research on their control increased this to AU $113 million. The value of losses to other industries (such as horticulture) and to biodiversity were not evaluated. A BCA that produced even a small reduction in these losses would more than pay for itself.

For the purposes of this paper, we have adopted the broad definition of biological control proposed by Beirne (1963, p. 240): 'the use of living organisms to restrain, reduce, or eliminate the harm caused by living organisms to man and his property', although we extend it to include harm caused to the environment. Note that under this definition, disease vectors are BCAs. Our main focus is on Australasia, where there is most interest in the release of further BCAs to control rabbits. Attitudes towards the rabbit in its naturalised range in Europe are mixed because in many countries rabbits are valued as game animals or for their role in the maintenance of anthropogenic landscapes (Rogers *et al*. 1994; Thompson 1994). Furthermore, given the experience resulting from the spread of myxomatosis through Europe, the high probability that a BCA released in the rabbit's exotic range in Europe would spread to and affect rabbits in their natural range in Spain and Portugal would almost certainly preclude any intentional release in Europe. Wild European rabbits

10.1071/WR06164 1035-3712/08/070633

are regarded as a pest on many islands (Flux 1993; Long 2003) and in South America (Jaksic 1998; Bonino and Soriguer 2004), but their parasites and diseases appear to be little studied.

The rabbit BCAs present in Australia and New Zealand are generally similar, with several notable exceptions. Most significant among these are the absence from New Zealand of MV and two of its host-specific vectors, European and Spanish rabbit fleas (Gibb and Williams 1994). The failure of attempts during the 1950s to introduce MV into New Zealand was attributed to a lack of competent vectors, but subsequent proposals to introduce MV and European rabbit fleas were not supported by Government (Gibb and Williams 1994). The suites of avian and mammalian predators in Australia and New Zealand differ substantially (for example, red foxes and dingoes are present only in Australia, and mustelids only in New Zealand; both countries have feral cats) but, as mentioned below, we consider predators to be unsuitable for introduction, and they will not be considered in detail. The species of *Eimeria* (coccidia) present in New Zealand and Australia show several differences (cf. lists in Gibb and Williams 1994; Myers *et al*. 1994; Williams *et al*. 1995; Norbury and Reddiex 2005), but the identification of some species is listed as uncertain in these publications or has been thrown into doubt (Hobbs and Twigg 1998, and below). The mite *Psoroptes cuniculi* is present in New Zealand (Norbury and Reddiex 2005) but published accounts differ regarding its presence in Australia: Strong and Halliday (1992) report its presence but Mykytowycz (1957, 1958), Williams (1972), Myers *et al*. (1994) and Williams *et al*. (1995) do not mention it. The reasons for this difference are unclear and may warrant further investigation, but we accept that the mite is present in Australia. It appears that many of the candidate BCAs we consider below are absent from Australia and New Zealand and could be considered for introduction into both. They could also be considered for release in other places where they are absent and rabbits are a pest.

This paper briefly reviews the previous biological control of rabbits in Australia, and considers the prospects for the further biological control of rabbits by means of naturally occurring or genetically modified organisms (GMOs). Some pathogens and parasites found in rabbits overseas are absent from Australia and New Zealand, and their possible use as BCAs is discussed. The possible use against rabbits of a disseminating immunocontraceptive GMO developed in Australia, and of 'selfish genes', is considered.

The release of naturally occurring BCAs or of a disseminating immunocontraceptive GMO to control rabbits is most unlikely to lead to eradication other than in localised, marginal habitats. This point is discussed further below, along with the likely short- or long-term nature of any benefits resulting from the release of further BCAs to control rabbits.

Each country has its own legislation governing the introduction of BCAs; in Australia it is the Commonwealth *Biological Control Act 1984* (see http://www.austlii.edu.au/au/legis/cth/consol_act/bca1984186/ and http://www.weeds.gov.au/government/legislation.html), together with parallel Acts in the States. The legislation establishes procedures to ensure that: (a) there is a need to control the target species; (b) the BCA is likely to mitigate the damage caused by the pest; and (c) the release of the BCA would not cause significant non-target harm or would cause less harm than would result from doing nothing or using alternative means of controlling the pest. In part, (c) entails an evaluation of the host-specificity of the BCA. The procedure is exemplified by the importation of RHDV, as described in Fenner and Fantini (1999). For more information on the protocol for BCA introductions in Australia, see http://www.daff.gov.au/ba/about/plant/protocol-biological.

Animal welfare considerations are becoming increasingly important in vertebrate pest control (Olsen 1998; White and Newton-Cross 2000; HVPCWG 2004). For example, a proposal to introduce MV into Australia today might be rejected in part on animal welfare grounds, as happened recently in New Zealand (PCE 1998; Norbury 2001). Many of the BCAs we consider below raise animal welfare concerns, but in most cases insufficient data are available for these to be considered in detail. Any BCA that kills rabbits or reduces their capacity to grow or reproduce is likely to cause some degree of discomfort or suffering. This harm needs to be weighed against the economic, environmental and other benefits to be gained by reducing the damage caused by rabbits, and compared with the suffering caused by other feasible means of mitigating damage, before a judgement can be made regarding the merits of introducing the BCA (HVPCWG 2004). This utilitarian approach seeks to maximise expected utility by adopting the course of action that does the most expected good, where good and harm are summed across all the expected consequences of our actions (Baron 2006). These consequences are direct in the case of rabbits affected by the BCA, and indirect in the case of the biodiversity and natural resources that benefit from the reduced numbers of rabbits. A utilitarian assessment of animal welfare issues according to the principles outlined in HVPCWG (2004) should therefore form part of a more detailed case for the introduction of any particular one of these BCAs.

Biological control agents previously introduced into Australian rabbits

MV was released into wild rabbits in Australia in 1950 and initially produced extremely high reductions in rabbit numbers in areas where competent vectors were present. Although rabbit populations partially recovered within a few years as a result of host–pathogen coevolution, MV remains an effective BCA (Williams *et al*. 1995, p. 46; Fenner and Fantini 1999).

In drier parts of Australia vectors were present in large numbers only after exceptionally heavy rainfall, and MV had, at best, an intermittent effect until competent vectors were introduced. The release in 1968 of European rabbit fleas, *Spilopsyllus cuniculi*, as a vector for MV resulted in reductions in rabbit numbers in some areas where vectors had previously been scarce, notably semiarid areas receiving more than 200–250 mm annual rainfall (Cooke 1983; Williams *et al*. 1995).

European rabbit fleas cannot persist in arid areas, and rabbits remained a major problem (Fenner and Fantini 1999). To overcome this difficulty, the more arid-adapted Spanish rabbit flea, *Xenopsylla cunicularis*, was introduced in 1993 as a vector for MV (Fenner and Fantini 1999). Rabbit haemorrhagic disease virus (RHDV) was introduced in 1995, just as Spanish rabbit fleas were becoming widely established, and as a result of this

coincidence it was not possible to determine the capacity of the fleas in their own right to improve transmission of MV and help reduce rabbit numbers in arid areas.

The rabbit trypanosome *Trypanosoma nabiasi* has recently been found in European rabbits in Australia (Hamilton *et al*. 2005). As European rabbit fleas are their only known vector, Hamilton *et al*. (2005) suggested that *T. nabiasi* may have been inadvertently introduced into Australia together with the fleas in the 1960s. They further argued that although trypanosomes of the group to which *T. nabiasi* belongs are generally regarded as non-pathogenic, the evidence relating specifically to *T. nabiasi* is equivocal, and that some of the reduction in rabbit numbers attributed to the enhanced transmission of MV by *S. cuniculi* may, in fact, have been due to the separate effects of *T. nabiasi*. Reglero *et al*. (2007) report that in adult rabbits the abundance of trypanosomes is highest in rabbits in poor condition, and suggest that adults unable to eliminate the parasites may lose weight as a result of persistent infection. Weight loss would be expected to reduce their capacity for reproduction and hence their fitness. Furthermore, the parasites 'are able to depress the humoral immune response and during this period the animals are at risk of exacerbation of concomitant infections'.

The rapid and intensively studied spread of RHDV following its introduction in 1995 (Kovaliski 1998) allowed its effects to be distinguished from those of the more slowly moving Spanish rabbit fleas. RHDV resulted in initial reductions of rabbit numbers that ranged from major in arid areas (~85%, approaching 100% in some areas), to small or non-existent in cool, moist areas (Cooke 1999*a*; Fenner and Fantini 1999; Cooke and Fenner 2002; Cooke *et al*. 2002; Edwards *et al*. 2002; Henzell *et al*. 2002). Following its initial success, subsequent RHD outbreaks usually occurred in the presence of the acquired immunity of recovered rabbits, often at different times of the year from the first outbreak, and in rabbit populations that had been reduced in numbers by earlier RHDV outbreaks. These factors may have resulted in later outbreaks being less effective, and as a result some populations have recovered slightly. The current situation with respect to RHDV is summarised in White and Newton-Cross (2000), Cooke (2002) and Mutze *et al*. (2008).

Two unforeseen features of the epidemiology of RHD in Australia were the degree of protection against RHD apparently provided to rabbits by immunity to one or more pre-existing benign caliciviruses, especially in cool, moist areas (Cooke 2002; Robinson *et al*. 2002), and the important role played by insects in the transmission of the disease (Cooke 2002, p. 351). The latter feature is likely to have led to the unexpected escape of RHDV from quarantine on Wardang Island in 1995.

In some cool, moist areas, such as the Mt Lofty Ranges in South Australia, landholders report higher rabbit numbers after the arrival of RHDV than before, but these reports are unsupported by accurate measurements of population size. If correct, the reports indicate that the limited mortality caused by RHD in these areas has been more than offset by a reduction in the effectiveness of other factors controlling rabbit numbers. For example, the effectiveness of myxomatosis may have been reduced by a shift in the timing of outbreaks to warmer times of the year when the disease is less effective (Fenner and Fantini 1999, pp. 107–108 and 199–200; Mutze *et al*. 2002).

There is little evidence relating to direct interference between myxomatosis and RHD. Fenner and Fantini (1999, p. 265) note briefly that 'both diseases may be active at the same time, apparently without diminishing the lethality of either disease'. It is unclear whether this statement holds true in those cool, moist parts of Australia where rabbit numbers may have increased since the arrival of RHD. In France, rabbits that were seropositive to myxomatosis were more likely to be seropositive to RHD and *vice versa* (Marchandeau *et al*. 2004). However, their serology did not distinguish between RHDV and benign caliciviruses, and the authors commented that 'the meaning of this link remains unknown'.

RHDV appears to have maintained its virulence for longer than MV, but recent unpublished observations suggest that in some areas its virulence may be decreasing.

Potential for the wider dissemination of biological control agents already present in Australia

Numerous introductions of rabbits into Australia were made in the past, independently of the main 1859 introduction at Barwon Park in Victoria (Rolls 1969; Stodart and Parer 1988). Different suites of pathogens, parasites and disease vectors may have accompanied these separate introductions, and it is possible that BCAs introduced into some areas persisted locally but have yet to spread throughout the range of the wild rabbit in Australia. Early introductions of rabbits were made in Tasmania, Melbourne in Victoria, Sydney and Armidale in New South Wales, Port Lincoln and Kapunda in South Australia, and Geraldton, Cheynes Beach and the Darling Ranges in Western Australia (Rolls 1969; Stodart and Parer 1988; Abbott 2008), and there were undoubtedly other, undocumented, introductions. The introductions to Tasmania, Melbourne, Port Lincoln, Armidale, and some offshore islands in Western Australia predated the Barwon Park introduction (Rolls 1969; Stodart and Parer 1988; Abbott 2008) and are especially promising because they must have been sourced independently and may therefore have been accompanied by different suites of BCAs. The Sydney introduction also predated that at Barwon Park, but the nematode and protozoan parasites of rabbits from Sydney and eastern Australia are now the same, and it may be concluded that any differences in their parasite burdens that once existed have now disappeared as a result of spread (Phillips *et al*. 2002). In the remaining cases it is unclear whether the rabbits were fresh importations or were descendents of Barwon Park animals. However, at the three Western Australian localities listed above the rabbits became established but failed to spread (Stodart and Parer 1988), suggesting that they were of domestic origin and not derived from the more invasive wild-type rabbits released at Barwon Park. Future studies of the population genetics and DNA and RNA cladistics of rabbits and their pathogens and symbionts may allow the provenance of the Barwon Park and other rabbit introductions, and the invasion history of rabbits in Australia, to be further resolved. Zenger *et al*. (2003) and Ferrand and Branco (2007) have taken initial steps in this direction.

In support of our argument for examining sources of BCAs within Australia it emerges that two pathogenic forms of intestinal coccidiosis (*Eimeria intestinalis* and *E. flavescens*) have been

recorded in western but not eastern Australia (Myers *et al*. 1994; Williams *et al*. 1995; Hobbs *et al*. 1999). However, Hobbs and Twigg (1998) suggest that their reported absence from eastern Australia may be due to misidentification, and this suggestion needs to be tested. If the absence of these two species from eastern Australia is confirmed, it has in all probability been maintained by the arid environment that separates the wetter eastern and western segments of the rabbit's distribution in Australia. This arid area may impede the movement of these parasites: in eastern Australia, Stodart (1968) reported that *Eimeria* infections were less intense, less frequent and caused by fewer species at a semiarid site than at wetter sites. Hobbs *et al*. (1999) concluded that none of the *Eimeria* spp. present in Western Australia caused severe mortality. Nevertheless, given the caveats mentioned in Hobbs *et al*. (1999), their evidence on this point was not clear cut and the possible benefits of releasing *E. intestinalis* and *E. flavescens* in eastern Australia should be investigated.

Such a release would be a useful test of the utility of a within-country translocation of a well established pathogen or parasite as a vertebrate pest BCA. *Eimeria* spp. generally have a narrow host range (Duszynski and Upton 2001), and species parasitising rabbits therefore may have originated in the Iberian Peninsula and could be expected to persist in climatically similar areas in eastern Australia. Although there appear to be few documented precedents for the likely efficacy of this approach for biocontrol purposes, it is likely to be successful: the dangers of spreading pathogens by translocating animals for conservation or hunting purposes are well recognised (see e.g. Griffith *et al*. 1993; Viggers *et al*. 1993; Cunningham 1996; Daszak *et al*. 2001; for rabbits, see Calvete *et al*. 2005).

The microsporidian protozoan parasite *Encephalitozoon cuniculi* has been reported from wild rabbits in Western Australia but not Victoria or New Zealand (Cox *et al*. 1980; Thomas *et al*. 1997). Although infection is usually subclinical, growth rates and feed conversion may be reduced and heavy infestations can cause nervous system disease and death (Wilber 1999; Percy and Barthold 2007). The parasite has a wide host range, including rodents and humans (Baker 1998; Percy and Barthold 2007). Its apparent absence from wild rabbits in Victoria and New Zealand is puzzling, especially as it is present in laboratory rabbit colonies in these areas (Cox *et al*. 1980). Whatever the explanation for this absence, in view of the parasite's wide host range it is unsuitable for introduction into wild rabbits in eastern Australia or New Zealand.

Potential for the introduction of biological control agents not yet present in Australia

The control of rabbits in certain situations by predators, in Australia and elsewhere, is well documented (Wood 1980; Trout and Tittensor 1989; Williams *et al*. 1995; Pech and Hood 1998; Reddiex *et al*. 2002). Compared with Spain, Australia has a smaller complement of predators for which rabbits comprise a major proportion of the diet (Myers *et al*. 1994), especially predators that prey on young rabbits in their warrens. Presumably, the introduction of additional exotic predators such as polecats, weasels, stoats, ferrets, lynx and falcons into the wild in Australia (see Jaksic and Soriguer 1981 for Spanish and Chilean candidates) might further reduce rabbit numbers, but the predators are likely to become pests themselves and are therefore unsuitable for introduction. Similarly, competitors of rabbits introduced from overseas could also become pests. For this reason, rabbit competitors and predators will not be seriously considered here.

Potentially suitable BCAs include disease vectors, parasites and pathogenic microorganisms not yet present in Australia, and also ecotypes of BCAs already present. They should have a narrow host range and affect rabbits but not humans, Australian wildlife or domestic species other than the rabbit. They could be sought in the following situations (note that for rabbits useful precedents could only be found for the first three categories):

(1) In domestic rabbits kept in laboratory animal houses or commercial rabbitries or as pets anywhere in the world. Most of the BCAs considered below belong in this category. Novel pathogens are more likely to be noticed in intensively managed captive rabbits than wild ones. Domestic rabbits can be regarded as sentinel animals in this context. The two rabbit BCAs that have proven most useful in Australia were discovered in this way: Brazilian MV in Uruguay and RHDV in China (Fenner and Fantini 1999). MV occurs naturally in *Sylvilagus* in the Americas, including Brazil, but not in Uruguay, where wild *Sylvilagus* are absent (Chapman and Ceballos 1990). MV was discovered when it caused mortality in domestic European rabbits imported into Uruguay from Brazil (Fenner and Fantini 1999, p. 67). The origins of RHDV are less clear (see the Introduction), but the virus was discovered when it caused disease in domestic European rabbits in China.
(2) In wild rabbits in their natural range in Europe. This approach is likely to reveal BCAs not brought to Australia when European rabbits were originally introduced, as was the case for Spanish rabbit fleas (Fenner and Fantini 1999). It might also reveal local strains of BCAs better adapted to particular areas in Australia than the strains already present.
(3) In wild European rabbits in areas overseas where they have been introduced. The role of European rabbit fleas in the transmission of MV was revealed in European rabbits in their naturalised range in Europe (Fenner and Fantini 1999). This approach is also likely to reveal BCAs occurring naturally in other species that can spread to, and adversely affect, European rabbits. BCAs with a narrow host range are most likely to be discovered in areas where other wild lagomorphs are also present.
(4) Where species of lagomorphs have been introduced into areas still occupied by wild European rabbits, as for example where European hunting reserves were stocked with *Sylvilagus* to replace myxomatosis-depleted European rabbit populations.
(5) In areas overseas that are climatically suitable for wild European rabbits but where rabbits are uncommon or absent, especially areas such as North America and South Africa where deliberate large-scale releases are known to have failed.
(6) In other species of lagomorphs anywhere, especially those that are closely related and live in areas climatically suitable for European rabbits. The genera most closely related to the monotypic *Oryctolagus* are *Caprolagus*, *Bunolagus*,

Pentalagus, *Sylvilagus*, *Brachylagus*, and *Lepus* (Matthee *et al*. 2004). Rabbit BCAs discovered in this way are more likely to have a narrow host range than those originating in less closely related mammals. MV, for example, occurs naturally in *Sylvilagus* and is only known to affect species of the closely related genera *Sylvilagus*, *Oryctolagus* and *Lepus*. Although climatic similarity may increase the likelihood of finding suitable BCAs it is not necessary and may not be as important as the presence of other lagomorphs – the Brazilian strain of MV introduced into Australia came from a species that does not have a very similar climatic range (see above).

Potential BCAs need not cause high mortality to be useful (Hoddle 1999). Moderate reductions in population fitness resulting from low levels of mortality, delayed breeding or reduced reproductive success might reduce rabbit populations and result in significant reductions in rabbit damage. In this respect it should be noted that Dunsmore (1966*a*, 1966*b*, 1981) carried out field studies to describe the seasonal patterns of prevalence of the nematodes *Graphidium strigosum* and *Trichostrongylus retortaeformis* commonly found in Australian rabbits and undertook a series of careful experiments to verify the factors that promoted high infections of those parasites. Dunsmore (1981) also showed that rabbits experimentally infected with *T. retortaeformis* raised 17% fewer young to weaning, and the young from infected females were 15% lighter at weaning simply because the infected rabbits produced less milk than uninfected ones.

Agents present overseas in European rabbits

The main pathogens known to affect captive European rabbits are summarised in Weisbroth *et al*. (1974), Baker (1998), Wilber (1999), Fenner and Fantini (1999), Suckow *et al*. (2002) and Percy and Barthold (2007). The suitability for introduction of these agents is difficult to assess accurately on the basis of published information, and further work will be required for more reliable assessments. Most uncertainty relates to the likely transmissibility and persistence of the BCAs in wild rabbits, and a BCA's behaviour when introduced into wild rabbits may differ from that observed in captive rabbits. For example, pessimistic prior beliefs about the transmissibility of MV and RHDV led to the escape of both viruses from quarantined field trials in Australia, in 1950 and 1995 respectively (Fenner and Fantini 1999). In the case of MV, the escape led to '. . . one of the most remarkable events in the history of infectious diseases, the development of an epizootic that for scale and speed of spread is probably without parallel' (Fenner and Fantini 1999, p. 138). For these reasons we do not consider a potential BCA to be unpromising solely on the grounds of incomplete knowledge regarding its transmissibility. The main pathogens affecting wild rabbits are reviewed in Rogers *et al*. (1994) for continental Europe, Thompson (1994) for Britain, Myers *et al*. (1994) and Williams *et al*. (1995) for Australia, and Norbury and Reddiex (2005) for New Zealand. We consider the most promising below. Others, considered less promising, are listed in Appendix 1, such as those that are highly pathogenic but unlikely to be permitted to be introduced owing to their lack of host specificity or potential to cause suffering.

Viruses

Herpesviruses

Herpesvirus infections resulted in mortality without clinical signs being observed in two Canadian rabbitries in 1990, in north-eastern Alberta and northern British Columbia (Swan *et al*. 1991; Onderka *et al*. 1992). These two outbreaks may have had a common origin. The mode of transmission and natural host of this virus (and the Alaskan virus mentioned below) are unknown, and Suckow *et al*. (2002) write that the 'virus has not been well documented'. The properties of this virus differ from the two herpesviruses previously described in lagomorphs (Onderka *et al*. 1992; Hudson 1994). The virus could have originated as a lethal mutant of *Herpesvirus cuniculi*, or been a previously undescribed virus whose natural host is a species other than *Oryctolagus cuniculus*. If the former, it might no longer exist, as the viral isolate was not kept (John Wu, pers. comm.; Alberta, 2005). If the latter, the natural host is most likely to be a *Lepus* sp. occurring naturally in the same area as the herpesvirus infections. Less likely hosts are Nuttall's cottontail, *Sylvilagus nuttallii*, or the more distantly related American pika, *Ochotona collaris*, which both occur in Alberta and British Columbia (Chapman and Ceballos 1990; Smith *et al*. 1990) but not in the same areas as the herpesvirus infections. Another lethal putative herpesvirus outbreak in an Alaskan rabbitry was described very recently (Jin *et al*. 2008). Although Jin *et al*. (2008) suggest that this virus is the same as the Canadian herpesvirus mentioned above, it, unlike the Canadian virus, produces gross clinical signs. This is an important difference for two reasons: (1) it has significant animal welfare implications; and (2) it may suggest that the viruses are in fact different species, or at least different strains of the same species.

Rabbit vesivirus

This cultivable calicivirus was recently isolated from dead or diseased young rabbits with diarrhoea from a rabbitry in Oregon in the United States (Martín-Alonso *et al*. 2005; Percy and Barthold 2007). Percy and Barthold (2007) commented that 'The importance of this isolate as a pathogen in commercial rabbitries is yet to be determined' (see also Martín-Alonso *et al*. (2005) on this point). The virus's origin, host specificity, mode of transmission and possible utility as a BCA are also unclear, but warrant further investigation.

Malignant rabbit fibroma virus (MRFV)

MRFV is a naturally occurring recombinant between MV (natural host *Sylvilagus bachmani*) and Shope's fibroma virus (SFV, natural host *S. floridanus*), which kills all infected rabbits in ~14 days (Block *et al*. 1985; Fenner and Fantini 1999). It was discovered in laboratory rabbits, and it is unclear whether it occurs in the wild. MRFV possibly originated in a laboratory rabbit simultaneously infected with MV and SFV – the distributions of the two parent viruses do not overlap in the field (Fenner and Fantini 1999). Its potential as a BCA is unclear. This is especially the case if MRFV were to be introduced into an area where MV already occurred: genetic recombination between the two viruses could occur and change the properties of both, and its potential consequences should be explored before release. Compared with MV, the much lower amount of MRFV in the skin overlying

lesions could reduce MRFV's transmissibility by insect vectors. However, this disadvantage may be offset by the much lower dose required to infect rabbits (Strayer *et al*. 1983). In addition, artificial selection followed by natural selection may increase MRFV's transmissibility, as suggested in Appendix 1 for cottontail rabbit papillomavirus. However, like MV, infection with MRFV produces extreme clinical signs (Strayer *et al*. 1983), and animal welfare concerns may preclude its use.

Endoparasites

Coccidia

The protozoan parasites *Eimeria coecicola*, *E. matsubayashi*, *E. nagpurensis*, *E. vejdovskyi* and *E. roobroucki* have been recorded in European rabbits overseas (Levine and Ivens 1972; Pakandl 1988; Grès *et al*. 2002; de Almeida *et al*. 2006), but not Australia or New Zealand (Myers *et al*. 1994; Hobbs and Twigg 1998; Norbury and Reddiex 2005). *E. vejdovskyi* and *E. roobroucki* were described only recently, and their absence from Australasia is unconfirmed. *E. coecicola* is highly pathogenic and is present in Britain in farmed rabbits (Catchpole and Norton 1979; Duszynski and Upton 2001), but apparently was not brought to Australia or New Zealand in rabbits imported from that country. Although the species of *Eimeria* present in Australian rabbits nearly all occur in cottontails (Duszynski and Marquardt 1969; Levine and Ivens 1972), the reverse is not the case and species absent from Australia should be investigated for their potential utility as BCAs. *E. neoleporis*, whose natural host is *S. floridanus*, has been recorded from domestic European rabbits (Levine and Ivens 1972). The pathogenicity in wild rabbits of most *Eimeria* spp. is unknown. The coccidian *Isospora gigantmicropyle* has been found in European rabbits in China (Fu 1984); there are no records of its occurrence elsewhere.

Rabbit epizootic enteropathy (REE)

REE is a new clinical syndrome of unknown origin occurring in rabbitries in continental Europe, where it causes heavy mortality (Marlier *et al*. 2003; Licois *et al*. 2005). It has not been reported from wild rabbits. Similar in some respects to mucoid enteropathy (which is present in the United Kingdom and the United States), a direct link between the two is considered plausible but unlikely. Mucoid enteropathy was uncommon before the use of high-energy, low-fibre diets in commercial rabbitries (Percy and Barthold 2007). If a similar change in feeding practices resulted in the emergence of REE in Europe, this would not only explain its absence from wild rabbits, but would also suggest that the causative agent is unlikely to be useful as a BCA. However, if REE emerged as the result of a cross-species transfer of a pathogen that has yet to invade wild rabbits, it may be useful as a BCA, depending on its potential host range and transmissibility between wild rabbits. Until causative agents for these diseases are identified and further characterised, it is unclear whether they might be useful as BCAs in Australasia.

Obeliscoides cuniculi

This nematode was recently retrieved from introduced cottontail rabbits, *S. floridanus*, in Italy (Tizzani *et al*. 2002). It is generally considered to have originated in North America but has previously been detected in *Oryctolagus* (Anderson 2000). Baker (1998) reported declines in the general condition and breeding performance of laboratory rabbits coinfected with *Passalurus ambiguus* and *O. cuniculi* (note that unlike *O. cuniculi*, *P. ambiguus* already occurs in Australia: Dunsmore 1966*c*). The development of these parasites in laboratory rabbits has been documented and they appear to infect lagomorphs and woodchucks more readily than laboratory mice and hamsters (Measures and Anderson 1983); further host-specificity testing would therefore be required before they could be considered seriously for introduction into Australia. From an Australian perspective, the presence of wild cottontails in Europe provides a chance to study the impact of their parasites on wild European rabbit populations and non-target species before considering them as candidates for possible use in Australia.

Other endoparasites

Several endoparasites present in rabbits overseas are absent from Australia, including four cestodes and the rabbit venereal spirochaete *Treponema paraluis-cuniculi* (Myers *et al*. 1994; Williams *et al*. 1995). Although Johnson (1977) reports that *T. paraluis-cuniculi* appears to cause only mild venereal disease in rabbits, its effect on fertility is unknown and, in addition, active infections may increase the susceptibility of rabbits to other pathogens (Percy and Barthold 2007).

Disease vectors

Rogers *et al*. (1994) list genera of ectoparasites present in Spain but not Australia, some of which could act as vectors for BCAs. It is unclear whether the introduction of additional vectors would improve the effectiveness of MV and RHDV in Australia. A greater knowledge of the distribution, effectiveness and rate of spread of the Spanish rabbit flea, *Xenopsylla cunicularis*, following its release in Australia in 1993 would allow this question to be considered further. Another rabbit flea, *Caenopsylla laptevi*, was investigated at the same time as the Spanish flea, and although the colony failed because mechanisms for breaking pre-pupal diapause were never devised (Cooke 1999*b*), it may warrant further consideration.

Better-adapted ecotypes or variants of BCAs already present in Australia

Better-adapted ecotypes or variants of BCAs already present in Australia probably exist overseas. Coccidiosis, especially hepatic coccidiosis due to *Eimeria stiedae*, sometimes produces substantial mortality in rabbits in the high-rainfall areas of eastern Australia (Myers *et al*. 1994; Williams *et al*. 1995). Arid-adapted ecotypes of these species sourced from dry parts of the rabbit's range in Spain could prove to be effective BCAs in arid areas in Australia and elsewhere.

RHDVa, a new variant of RHDV, has been spreading overseas (Capucci *et al*. 1998; McIntosh *et al*. 2007; Lavazza and Capucci 2008), and accumulating information may help to assess its potential role in Australia.

Where rabbits aren't: do undiscovered BCAs prevent rabbit invasions overseas?

Where introductions of rabbits failed: North America and southern Africa

Potential BCAs could be discovered overseas in areas that are climatically suitable for wild European rabbits but where they remain uncommon or absent despite attempts to introduce them. For example, wild European rabbits do not occur in continental North America or southern Africa, despite vast areas with cool-temperate or Mediterranean climates similar to those that have proved eminently suitable for rabbits in Europe, Australia and New Zealand. Many factors could contribute to the failure of releases, such as the suitability of the breed of rabbit introduced, the number of animals released (which would affect the genetic diversity of the founding stock and the likelihood of extinction due to stochastic effects), the level of husbandry practised during the critical period immediately following release, the suitability of the habitat, and the presence of natural enemies (predators, competitors, parasites and pathogens). The absence of rabbits from apparently suitable areas therefore may indicate, but would not prove, the presence there of BCAs.

European rabbits are established on some North American islands but extensive efforts to translocate them to the mainland all failed (Long 2003). Failure of large-scale releases in Indiana during the 1950s, involving more than 6000 rabbits in at least 50 counties, was attributed primarily to predation and lack of suitable habitat (Kirkpatrick 1960). However, a possible role of potentially useful pathogenic BCAs cannot be ruled out given the European rabbit's wide habitat tolerance, the variety of release habitats documented, including 'lush grasslands, and dense stands of weeds, brush and timber' (Kirkpatrick 1959*a*) and 'barnyards, pastures, croplands and timbered areas' (Kirkpatrick 1960), attempted predator control that accompanied at least some of the introductions, and the fact that rabbits appear to have been selectively released in areas used for hunting endemic populations of *Sylvilagus* whose populations had declined (Kirkpatrick 1959*b*). The genetic stock for the Indiana releases may have contributed to the lack of success: the rabbits came from wild populations that had been established on the San Juan Islands more than 50 years earlier, and were considered by museum authorities to be a domestic variety that had reverted somewhat towards the original wild stock (Kirkpatrick 1959*b*; see also Couch 1929). Rabbits were introduced to islands along the coast of California in the 1940s (Long 2003). Although acclimatisation societies were less active in North America than in Australia in the second half of the nineteenth century (Dunlap 1997), and we have found no documented evidence of attempts to introduce rabbits to continental California (see also Lidicker 1991), it is likely that such attempts were made given the widespread attempts in eastern and midwestern USA in the 1950s. Jin *et al*. (2008) report that feral domestic rabbits were present near a rabbitry in Alaska, but no other information about the status of these animals is available. The absence of wild European rabbits from the western mainland of North America in areas where *Sylvilagus bachmani* occurs may be due partly or entirely to the presence of MV, but other BCAs or other factors may also be involved.

The rabbit's absence from the wild in continental South Africa may be due in part to the early Dutch settlers' policy prohibiting the introduction of rabbits onto mainland South Africa (Lever 1985; Bigalke and Pepler 1991; Long 2003). However, Froggatt (1906) discussed reports indicating that rabbits could be produced very successfully on the South African mainland in hutches, but that numerous releases of 'rabbits of all kinds' into fenced enclosures or the wild eventually failed, including one large release of 700. Observations reported by Froggatt (1906) attributed their absence not to legislation but to various BCAs, including subterranean meat ants (which reportedly consumed young rabbits in their burrows) and native mammalian predators (including jackals and polecats). FitzSimons (1906, p. 151) described one such release: 'Some years ago I let loose a large number of rabbits on my land, which began to breed very rapidly in the sod fences and prickly pear hedges until a few of these polecats took up their quarters in the vicinity. The rabbits then rapidly diminished in numbers. One of these bunnies was a great pet, and on the slightest alarm would always make for home at full speed and seek 'sanctuary' under my bed'. Froggatt (1906) concluded that the lack of host specificity of these apparently generalist BCAs rendered them unsuitable for use in Australia to reduce rabbit numbers. Brooke *et al*. (1986) concluded that in South Africa 'a great suite of indigenous species making use of its resources, including many ... predators, not to mention [unspecified] pathogens ... seems to make it very difficult for aliens to establish themselves' outside man-modified areas. They highlighted the contrasting success of European rabbits on Robben Island where there are few predators. Although the accounts mentioned above attributed the absence of wild rabbits from South Africa primarily to predators (which we consider not suitable for use as BCAs), it is possible that other, undiscovered, pathogens contributed to the observations reported by Froggatt (1906).

Chlamydophila abortus may limit production in intensive domestic rabbitries in the Western Cape of South Africa (Zumpt 1976). This bacterium, formerly part of *Chlamydia psittaci*, is now regarded as a separate species and different from the two *Chlamydophila* spp. present in Australian koalas (Everett *et al*. 1999). *C. abortus* causes enzootic abortion of ewes, and has not been reported from Australia or New Zealand (McCauley *et al*. 2007). In the Western Cape the disease was first diagnosed in sheep in 1972 and in domestic rabbits and other mammals shortly thereafter, and it is therefore unlikely to have contributed to the absence of wild European rabbits in South Africa before then. See Appendix 1 for further comments.

Several genera of lagomorphs other than *Oryctolagus* occur naturally in North America and southern Africa, and may harbour rabbit BCAs suitable for introduction into Australia. However, exploratory work in wild animals in North America or southern Africa might be expected to be less cost-effective than waiting for agents to reveal themselves free of charge in captive European rabbits that are farmed or kept as laboratory animals or pets in these areas or elsewhere.

The anomalous biogeography of European rabbits in South America

European rabbits have successfully established in the wild in South America only in central Chile (from where they are now slowly expanding their distribution across the Andes into

Mendoza province in Argentina), and Tierra del Fuego and a small part of the adjacent mainland (Flux *et al*. 1990; Flux 1994; Bonino and Soriguer 2004). These areas lack native lagomorphs. However, rabbits are absent from much larger areas also free of native lagomorphs, including most of Argentina, a situation which sets South America apart from North America and southern Africa, where native lagomorphs are widespread. In the latter areas, native lagomorphs and their associated predators and pathogens may prevent European rabbits establishing in the wild, but this cannot be the case in parts of South America lacking native lagomorphs.

The anomalous situation in South America may have arisen as a result of several factors:

(1) The presence of MV in *Sylvilagus brasiliensis* north of ~30°S latitude (Fenner and Ratcliffe 1965) may have prevented small founder populations of European rabbits establishing in the wild in that area. The areas where rabbits did naturalise lacked MV until well after rabbits had become firmly established; MV was introduced in Tierra del Fuego in 1953/54 and central Chile/adjacent Argentina in 1971 and 1972 (Flux *et al*. 1990, p. 151; Flux 1994, p. 13; Fenner and Fantini 1999). The initial absence of MV would have made it easier for rabbits to establish in Argentina and Chile compared with northern South America, where MV is present. The situation in northern South America therefore resembles that in California, where MV is present and wild European rabbits are also absent. Note that Jaksic and Fuentes (1991) reported MV to be 'nonexistent' in central Chile and it may have died out in that area, but they presented no serological or other evidence to support their seemingly unlikely observation. It is possible that MV is still present but that as a result of host–pathogen coevolution its effect on rabbits has diminished and disease is no longer obvious. Such a reduction in effectiveness would mirror similar changes in Australia that followed the introduction of MV in 1950 (Fenner and Fantini 1999).

(2) The high probability that wild rabbits in South America were of domestic origin (Ferrand and Branco 2007). Attempts to establish domestic rabbits in the wild in Australia met with only modest success. Feral populations of domestic rabbits flourished locally but did not irrupt dramatically; this occurred only when wild-type rabbits were introduced (Coman 1999; Fenner and Fantini 1999; Long 2003).

(3) Possible competition between rabbits and South America's diverse caviomorph rodent fauna, which includes species broadly similar to rabbits in size, dietary preferences and burrowing habits. A deficiency in competitive herbivorous mammals in lowland Iberia may have contributed to the original restriction of the rabbit to that area and its absence elsewhere in Europe (Corbet 1994).

(4) The presence in South America of a suite of native predators adapted to catching native caviomorph rodents may have deterred an invasion by rabbits. However, Jaksic (1998) points out that rabbits have a different escape response from caviomorph rodents, which may have reduced the effectiveness of those predators in capturing rabbits.

(5) The high prevalence of grasses employing the C_4 photosynthetic pathway in much of South America. European rabbits originated in Mediterranean Europe, an area where C_3 grasses predominate (Sage *et al*. 1999). Compared with C_3 plants, many C_4 plants have characteristics that would disadvantage a herbivorous small mammalian *r*-strategist like the rabbit: a high fibre content, low leaf nitrogen and phosphorus concentrations, low digestibility, and an internal architecture (Kranz anatomy) that impedes access by herbivores to some internal nutrients (Wilson and Hattersley 1989; Heckathorn *et al*. 1999). The success of rabbits as colonisers of Australia is believed to result in part from the elimination by domestic stock of low-quality 'tropical' grasses (which were C_4) and their replacement with high-quality annual grasses and forbs (which are C_3) (Stodart and Parer 1988; Myers *et al*. 1994). It may be no coincidence that the areas where rabbits first became established in the wild in South America were Tierra del Fuego (cold climate) and central Chile (Mediterranean climate). Both areas have cool growing seasons where C_3 photosynthesis predominates (Cerling *et al*. 1993; Sage *et al*. 1999, p. 329; Epstein *et al*. 2002). The slowness of the invasion of Argentina's Mendoza province by rabbits (Bonino and Soriguer 2004) may result from the prevalence of C_4 grasses in the lower-lying, unoccupied areas: the province receives predominantly summer rainfall and below ~500 m the grasses are mostly C_4 (Cavagnaro 1988; Ojeda *et al*. 1998; Sage *et al*. 1999).

(6) Possible competition with European hares, which are widespread in southern South America (see below).

(7) The possibility that the releases were small and were not husbanded well enough until they were established.

(8) The presence in South America of as-yet undiscovered BCAs in non-lagomorphs that can cross the species barrier into European rabbits. For example, a new species of coccidian, *Besnoitia oryctofelisi*, was recently described in domestic rabbits in Argentina (Venturini *et al*. 2002; Dubey *et al*. 2003). However, its pathogenicity for rabbits is unknown.

An explanation in terms of Factors 1–7 above may be sufficient to explain the relatively poor success of European rabbits in South America compared with Australia, without the need to invoke the presence in South America of BCAs (Factor 8). Nevertheless, such BCAs may exist. South America may be more representative than Australia of the overall invasiveness of European rabbits: Flux (1994) points out that the rabbit's reputation for rapid spread relies mainly on its exceptional performance in Australia (reviewed in Stodart and Parer 1988).

Two anomalies in the comparative biogeography of lagomorphs in South America that may indirectly indicate the presence there of BCAs are considered below.

European rabbits and European hares in Argentina compared

Both European rabbits and European hares occur wild in Argentina, but hares have a much wider distribution and occupy nearly all of Argentina (Flux and Angermann 1990; Flux *et al*. 1990).

It was suggested above that the high prevalence of C_4 grasses in parts of Argentina might impede its invasion by rabbits. Hares

might be better adapted than rabbits to exploit C_4 grasses because, of the two species, they possess the digestive system better able to cope with low-quality vegetation (Kuijper *et al*. 2004). The evolutionary origin of this difference in digestive strategy is unclear because the grasses are predominantly C_3 in the natural ranges of both the hare and the rabbit in Europe and Asia (see distribution maps in Flux and Angermann 1990; Gibb 1990; Sage *et al*. 1999). Kuijper *et al*. (2004) also showed that wild rabbits selected a diet higher in nitrogen and lower in fibre than hares in the same area. These differences in feeding and digestive strategy between rabbits and hares would be expected to give hares a greater capacity than rabbits to utilise C_4 forage, and possibly provide hares with a greater capacity to invade C_4 grasslands. It might also give hares the capacity to exclude rabbits from these areas, although the nature of interactions between rabbits and hares are unclear (Flux 2008). This explanation does not exclude a role for BCAs, but it may be sufficient to explain the differential success of European rabbits and hares in Argentina.

Biogeography of Sylvilagus *in South America*

Three species of lagomorph (*Sylvilagus brasiliensis*, *S. floridanus* and *S. varynaensis*) occur naturally in South America, but only in northern areas (Hershkovitz 1972; Chapman and Ceballos 1990; Durant and Guevara 2001). *Sylvilagus* first arrived in South America during the Great American Interchange, after the establishment of a land connection to North America during the Pliocene, about three million years ago (Simpson 1980; Webb and Marshall 1982). The failure of *Sylvilagus* to occupy southern South America may result from their relatively recent arrival and their adaptation to more tropical climates, together with some of the factors proposed above to explain the absence of European rabbits from southern South America. The role of C_4 grasses is unclear: little is known of the digestive capacity of South American *Sylvilagus* spp. or how this compares with that of European hares, which have colonised much of southern South America (see above).

Conclusions from biogeographical discussion

The anomalies in the distributions of European rabbits, European hares, and native *Sylvilagus* species in the wild in southern South America are explicable without the need to invoke the presence there of BCAs. In contrast, the absence of wild European rabbits in continental North America, continental southern Africa, and possibly northern South America seems more likely to be due, in part, to the presence of BCAs in other lagomorphs, and these places would therefore appear to be more likely sources of useful BCAs than southern South America.

The possible use of genetically modified organisms as biological control agents

In addition to naturally occurring BCAs, GMOs could possibly be employed to control rabbit numbers in parts of the introduced range (Tyndale-Biscoe 1994; Barlow 2000). However, research to develop a disseminating immunocontraceptive GM MV in Australia has recently been suspended because levels of infertility induced in the laboratory by the GMO were insufficient to be likely to produce a major reduction in rabbit damage in the field (Twigg *et al*. 2000; Hardy *et al*. 2006; McLeod and Twigg 2006; van Leeuwen and Kerr 2007). The effectiveness of the transgenes might be improved by the use of other viruses or organisms as vectors, especially if they established a longer-lasting infection than MV (van Leeuwen and Kerr 2007).

The deployment of a disseminating GMO carries a significant risk of harm to non-target organisms (Angulo and Cooke 2002; Henzell 2007; Angulo and Gilna 2008). Non-target organisms at risk include European rabbits that may be exposed to the GMO as a result of the transboundary (transjurisdictional) movement of the GMO (especially movement to the rabbit's natural range in Europe), and species other than European rabbits that are susceptible to the effects of the GMO. In the case of a GM MV, several non-target species are at risk in addition to MV's main hosts, *Sylvilagus bachmani* and *S. brasiliensis*. Antibodies to MV have been found in wild *S. mansuetus* and *Lepus insularis* in Mexico (Licon Luna 2000) and in wild cottontails, *S. floridanus*, in Europe (Tizzani *et al*. 2002); natural infection of mountain hares, *L. timidus*, and European hares, *L. europaeus*, with MV has been observed in the wild (Anon. 1955; Fenner and Fantini 1999); and several *Sylvilagus* spp. develop tumours in the laboratory in response to injections of the Brazilian strain of MV (Regnery 1971). These and possibly other lagomorph species could be exposed to the transgenes if the GM MV were to be released illegally in areas they inhabit, and the possible effects of the GMO on them should be considered before any release. In addition, transboundary spread followed by genetic recombination could introduce the transgenes into closely related poxviruses with host ranges different from that of MV.

In Australasia, exotic European hares are the only species other than European rabbits likely to be susceptible to MV (Fenner and Fantini 1999). However, only occasional hares are observed with clinical signs of myxomatosis, and unless the presence of the transgenes increases the likelihood of infection, populations of hares in Australasia are unlikely to be affected by a GM MV. The risk of natural spread to non-target animals outside Australasia is also probably low. Unauthorised transboundary movement of the GMO by humans is the most likely means of entry into non-target populations, as occurred with the introduction of MV into France in 1952, of RHDV into New Zealand in 1997, and possibly of MV into England in 1953 (Fenner and Fantini 1999; Henzell 2007). International agreements and quarantine procedures appear unlikely to prevent such deliberate spread of a GM MV, especially because MV can remain viable when dried, in which form it is easy to transport and conceal. Also, it would be virtually impossible to eliminate the immunocontraceptive GM MV if it became established in the wild in another country: no mechanism has been developed to recall it or reverse its effects. In view of the historical precedents with unauthorised releases of MV and RHDV, safer GMOs, i.e. those that pose a lower threat to non-target organisms and whose effects can be reversed, should be developed.

Homing endonuclease genes (HEGs) are selfish or parasitic genes that can spread through populations of fungi, plants, bacteria and bacteriophages (Burt and Trivers 2006). Apart from some sea anemones (Beagley *et al*. 1996; Goddard *et al*. 2006), they are unknown from multicellular animals. HEGs encode an enzyme that recognises (homes) and cleaves a

20–30 base pair sequence found on chromosomes not containing a copy of the HEG (Burt 2003). The HEG itself is inserted in the middle of its own recognition sequence, so that chromosomes carrying the HEG are protected from being cut. The cell will typically repair the cleaved chromosome by using the intact homologous chromosome containing the HEG as a template. After repair, both chromosomes will contain a copy of the HEG, and a heterozygote will have been converted into a homozygote. The target gene is chosen such that the knockout mutation has little phenotypic effect in the heterozygous state, but is severely deleterious when homozygous (i.e. the knockout is recessive). A HEG used for pest control would be under the control of a meiosis-specific promoter, so that heterozygous zygotes would develop normally, but transmit the HEG to a disproportionate fraction of their gametes (Burt 2003). Zygotes homozygous for the HEG would die.

Because HEGs can propagate within the genome, '. . . they can be used to manipulate natural populations, even if the number of individuals released is a small fraction of the entire population. For example, a genetic load sufficient to eradicate a population can be imposed in fewer than 20 generations . . .' (Burt 2003). In addition, in theory their effects can be reversed (Burt 2003). For both these reasons, HEGs targeting essential genes may eventually prove to be a preferable alternative to disseminating immunocontraceptive GMOs and naturally occurring BCAs for controlling European rabbits.

However, the apparent absence of naturally occurring HEGs from vertebrates suggests that they may not work or be able to persist in these animals. Even if this apparent barrier to their use against rabbits could be overcome, the wisdom of doing so would be questionable because this achievement may then extend the host range of HEGs to include all vertebrate taxa. This and other potentially significant safety concerns regarding HEGs must be allayed before they could be used (Henzell 2007). Developing a means to reverse the effects of the HEG should it affect other species, or rabbits in their native range, would go part of the way to realising this, but in the case of spread to other species it is possible that a HEG that was able to spread rapidly would simply overwhelm these counter measures.

Other selfish genetic elements, such as transposons, may be able to be developed to control rabbits (Burt 2003; Grigliatti *et al*. 2007). Transposons are mobile genetic elements that can move and integrate into different locations within the genome; some can insert copies of themselves elsewhere in the genome through replicative transposition. Transposons that can multiply within the genome and that carry genetically engineered, conditionally expressed pest-incapacitating genes may be able to be used as BCAs. As with HEGs, safety is a key issue that in practice may only be resolved through attempts to apply biotechnology to controlling human diseases such as malaria (Gould *et al*. 2006). Current indications are that HEGs may be less likely to transfer horizontally to non-target species of vertebrates than transposons (Goddard *et al*. 2006; Grigliatti *et al*. 2007), and Burt (2003) argues that HEGs also offer several other advantages.

In the long term, it seems likely that a safe GMO for controlling rabbits in Australia will be developed and deployed. When perfected, GM technology offers the prospect of releasing an endlessly varied sequence of safe GM BCAs into Australian rabbits, such that as rabbits develop a resistance to one, a successor can be released, while – in the case of HEGs – possibly providing a capacity to reverse the effects of a misbehaving GMO. If safe HEGs can be developed, eradication of rabbits from areas where they are pests might be possible. However, given that the risks of non-target effects of disseminating GMOs need to be substantially reduced before they can be considered safe, these enticing prospects are a long way from being realised.

How long will a new BCA remain effective, and is the eradication of rabbits possible?

Populations of an organism targeted by a BCA may, in time, recover from its effects as a result of natural selection for genetic resistance in the host, genetic avirulence in the pathogen or merely from the accumulation of immune survivors after the initial impact of introducing the BCA into a naive host population. However, recovery may not be complete and is not inevitable. For example, 50 years after its release in Australia, MV remains a useful BCA even though its virulence and pathogenicity declined significantly within a few years of its release as a result of host–parasite coevolution (Williams *et al*. 1995, p. 46; Fenner and Fantini 1999). The evolution of avirulence cannot be taken for granted, especially if the BCA depends for its transmission on its generation within the host of a large number of propagules (see Weiss 2002 for review). Other factors, such as the spatial patchiness of host populations, may also affect the outcome (Holt *et al*. 1999). A wide range of evolutionary outcomes can therefore be expected.

A clear distinction can be drawn between BCAs originating from rabbits in their natural range and pathogens that have recently crossed the species barrier from other hosts into European rabbits (GMOs can be placed in the latter category because they are a novel combination of genetic material and they are designed to produce a novel effect in their target). In the former case the host in its natural range has coexisted with the BCA for a long time, but in its exotic range may, if the BCA was not introduced at the same time, have lost some of its genetic resistance to the BCA; in this case, some reduction in the effectiveness of the BCA following its introduction can be expected as genetic resistance is regained. All of the BCAs originating in rabbits in their natural range are therefore likely to reduce rabbit numbers but not result in eradication other than possibly at a local scale in marginal habitats. Examples of the apparent eradication of rabbits by introduced BCAs are provided by Flux (1993) for islands and by Katona *et al*. (2004) for an area in Hungary where outbreaks of myxomatosis and RHD were followed by a very cold, snowy winter. In the case of GMOs and BCAs crossing the species barrier into rabbits, the outcome is much less predictable. For viruses, cross-species transfer can be associated with either higher or lower virulence in the new host and an uncertain evolutionary outcome (Weiss 2002). Eradication of the host is nevertheless unlikely: the BCA must spread from rabbit to rabbit before the death of its host, and as the rabbit density decreases this will become increasingly unlikely. A BCA crossing the species barrier will take some time to evolve mechanisms to ensure its transmission in its new host, by which time the host may have evolved genetic resistance. The development of GMOs for the management of vertebrates is very recent and none has been

released into the wild; there are therefore no precedents for their likely long-term effects on target and non-target animals.

Appropriately designed selfish genes (such as HEGs) offer a possible route to avoid the barrier to eradication because they may be able to be engineered to propagate within the genome of the host, and to be transmitted when the pest reproduces. Rabbits, being mortal, must reproduce to persist, thereby providing selfish genes with opportunities both to reduce the number of offspring and to ensure their transmission to new hosts. The capacity of selfish genes to propagate within the genome would introduce a novel attribute into vertebrate pest control, one that could lead to eradication (Burt 2003). However, depending on the extent of gene flow between low-density populations in areas only patchily suitable for rabbits, the selfish genes may not reach all parts of their host's range before they extinguish themselves and the populations of their host they have invaded, and further releases of rabbits carrying selfish genes may be needed (cf. discussions in Holt *et al*. 1999 and Wilson *et al*. 2002). Australia-wide eradication may be possible under these circumstances, but would require a substantial commitment to detect and eliminate all remaining low-density and isolated populations.

Discussion

Several candidate BCAs are already known to exist in Australia or overseas, and others may be discovered in future. In addition, BCAs already present in some, but not all, parts of the rabbit's range in Australia could be translocated to new areas, offering the prospect of rapid – albeit probably small – reductions in rabbit numbers with little cost or effort. Larger reductions in rabbit numbers may result from the introduction of BCAs from overseas. The possibility of introducing one or more already-known BCAs should be investigated. Other useful BCAs may exist but be as-yet undiscovered, and are most likely to be found overseas in domestic European rabbits kept in areas where wild lagomorphs are present. The most cost-effective method for discovering new BCAs would be to maintain a global watch on new pathologies in domestic rabbits.

We suggest that initially several avenues be explored simultaneously to investigate potential BCAs to determine their suitability:

(1) Further characterise promising BCAs present in rabbits overseas. These include BCAs absent from Australia and better-adapted strains of BCAs already present (see list below). Further studies of the *Eimeria* spp. already present in Australia (and New Zealand) are needed to clarify the situation in those countries. Work overseas should proceed when collaborative opportunities with established research groups permit.
(2) Establish a global watch to collect and evaluate information on new and emerging diseases in rabbits, especially domestic rabbits. Contacts should be established with veterinary personnel and the farmed rabbit industry overseas to start this process.
(3) BCAs present in only part of the rabbit's range in Australia should be evaluated for translocation to other suitable areas. *Eimeria intestinalis* and *E. flavescens* are examples (subject to confirmation of their absence from eastern Australia), but other candidates may exist in areas where rabbits were introduced independently of the 1859 Barwon Park release. The possible presence of *Paraspidodera uncinata* in guinea-pigs in Australia should be investigated (see Appendix 1).

Of the candidate BCAs discussed above, the most promising would appear to be:

(1) The Canadian herpesvirus (subject to its reisolation, and to its transmission between rabbits by means other than direct contact). The virus is less likely to be useful if it is transmitted only by direct contact, but could still be useful as a biocide. The Alaskan putative herpesvirus causes extreme clinical signs and might be ruled out on animal-welfare grounds.
(2) Rabbit vesivirus, to assess its host-specificity, transmissibility, and pathogenicity.
(3) Malignant rabbit fibroma virus, subject to its being transmissible between rabbits by means other than direct contact. However, like MV, infection with MRFV produces extreme clinical signs (Strayer *et al*. 1983), and unless a substantial resurgence in rabbit numbers warranted its use, it might be ruled out on animal welfare grounds.
(4) *Eimeria* spp. Initial work should clarify the status of *Eimeria* spp. in Australia and New Zealand, with a view to possibly introducing pathogenic species not already present and translocating *E. intestinalis* and *E. flavescens* from western to eastern Australia.
(5) Rabbit epizootic enteropathy, to determine the pathogen responsible, its host specificity, and its likely transmissibility between rabbits in the wild.
(6) In the very long term: disseminating GMOs, subject to improvements in their safety and efficacy.

Most of these potential BCAs could be considered for use in any country from which they are currently absent and where rabbits are a pest. In Chile and Argentina, where RHDV and possibly MV are absent, and on islands where rabbits are a pest and MV and/or RHDV are absent, the missing viruses and possibly their vectors could be added to the above list. MV is absent from New Zealand, but that country rejected a relatively recent proposal to introduce MV and European rabbit fleas (Gibb and Williams 1994).

Further evaluation of these potential BCAs is required before their release to establish their suitability and utility with respect to several criteria, including:

(1) their likely efficacy in reducing rabbit numbers in the field;
(2) the likely benefits of their release for primary production, biodiversity conservation and landscape stability;
(3) their host specificity and the likelihood of undesirable non-target effects (on other species, or on wild European rabbits in their natural range); and
(4) their potential to cause pain, distress or suffering.

Until the risks of deploying disseminating GM BCAs to control wild vertebrates have been satisfactorily minimised, it would be prudent for efforts to introduce new BCAs into exotic rabbit populations to focus on non-GM BCAs. It would also be prudent for countries where rabbits are regarded as pests and where MV and/or RHDV are present to attempt to protect the

utility of these BCAs by minimising the risk of entry of the disseminating GMO developed in Spain (but not yet released from quarantine) to immunise rabbits against MV and RHDV (Torres *et al*. 2001; Angulo and Bárcena 2007). Although this GMO appears to possess a limited capacity for horizontal transmission, this might not remain the case in the wild if genetic recombination introduces the transgenes into a more transmissible strain of MV. As with the Australian immunocontraceptive GMO, no mechanism exists to recall the Spanish GMO, and improving the safety of disseminating GMOs remains a crucial goal.

Acknowledgements

We are grateful to the many people on the IUCN's Aliens list and elsewhere who provided information on rabbits in South Africa and North America. Four anonymous referees made helpful and constructive comments on an earlier version of the manuscript.

References

Abbott, I. (2008). Historical perspectives of the ecology of some conspicuous vertebrate species in south-west Western Australia. *Conservation Science Western Australia* **6**, 1–214.

Anderson, R. C. (2000). 'Nematode Parasites of Vertebrates: Their Development and Transmission.' 2nd edn. (CABI Publishing: Wallingford, UK.)

Angulo, E., and Bárcena, J. (2007). Towards a unique and transmissible vaccine against myxomatosis and rabbit hemorrhagic disease for rabbit populations. *Wildlife Research* **34**, 567–577. doi: 10.1071/WR06160

Angulo, E., and Cooke, B. (2002). First synthesize new viruses then regulate their release? The case of the wild rabbit. *Molecular Ecology* **11**, 2703–2709. doi: 10.1046/j.1365-294X.2002.01635.x

Angulo, E., and Gilna, B. (2008). When biotech crosses borders. *Nature Biotechnology* **26**, 277–282. doi: 10.1038/nbt0308-277

Anon. (1955). Myxomatosis in hares. *The Veterinary Record* **67**, 455.

Baker, D. G. (1998). Natural pathogens of laboratory mice, rats, and rabbits and their effects on research. *Clinical Microbiology Reviews* **11**, 231–266.

Barlow, N. D. (2000). The ecological challenge of immunocontraception: editor's introduction. *Journal of Applied Ecology* **37**, 897–902. doi: 10.1046/j.1365-2664.2000.00591.x

Baron, J. (2006). 'Against Bioethics.' (MIT Press: Cambridge, MA.)

Beagley, C. T., Okada, N. A., and Wolstenholme, D. R. (1996). Two mitochondrial group I introns in a metazoan, the sea anemone *Metridium senile*: one intron contains genes for subunits 1 and 3 of NADH dehydrogenase. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 5619–5623. doi: 10.1073/pnas.93.11.5619

Beirne, B. P. (1963). The meaning and definition of 'biological control'. *Entomophaga* **8**, 237–243. doi: 10.1007/BF02377529

Bigalke, R. C., and Pepler, D. (1991). Mammals introduced to the mediterranean region of South Africa. In 'Biogeography of Mediterranean Invasions'. (Eds R. H. Groves and F. di Castri.) pp. 285–292. (Cambridge University Press: Cambridge.)

Block, W., Upton, C., and Mcfadden, G. (1985). Tumorigenic poxviruses: genomic organization of malignant rabbit virus, a recombinant between Shope fibroma virus and myxoma virus. *Virology* **140**, 113–124. doi: 10.1016/0042-6822(85)90450-7

Bonino, N., and Soriguer, R. C. (2004). Distribución actual y dispersión del conejo europeo (*Oryctolagus cuniculus*) en Mendoza (Argentina). *Mastozoología Neotropical* **11**, 237–241.

Boucher, S., Gracia, E., Villa, A., Fernandez, A., Nouaille, L., Briffaud, M. A., Albizu, I., and Baselga, R. (2001). Pathogens in the reproductive tract of farm rabbits. *The Veterinary Record* **149**, 677–678.

Brooke, R. K., Lloyd, P. H., and de Villiers, A. L. (1986). Alien and translocated terrestrial vertebrates in South Africa. In 'The Ecology and Management of Biological Invasions in Southern Africa'. (Eds I. A. W. Macdonald, F. J. Kruger and A. A. Ferrar.) pp. 63–74. (Oxford University Press: Cape Town.)

Burt, A. (2003). Site-specific selfish genes as tools for the control and genetic engineering of natural populations. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **270**, 921–928. doi: 10.1098/rspb.2002.2319

Burt, A., and Trivers, R. (2006). 'Genes in Conflict: the Biology of Selfish Genetic Elements.' (Harvard University Press: Cambridge.)

Calvete, C., Angulo, E., Estrada, R., Moreno, S., and Villafuerte, R. (2005). Quarantine length and survival of translocated European wild rabbits. *Journal of Wildlife Management* **69**, 1063–1072. doi: 10.2193/0022-541X(2005)069[1063:QLASOT]2.0.CO;2

Capucci, L., Fallacara, F., Grazioli, S., Lavazza, A., Pacciarini, M. L., and Brocchi, E. (1998). A further step in the evolution of rabbit haemorrhagic disease virus: the appearance of the first consistent antigenic variant. *Virus Research* **58**, 115–126. doi: 10.1016/S0168-1702(98)00106-3

Catchpole, J., and Norton, C. C. (1979). The species of *Eimeria* in rabbits for meat production in Britain. *Parasitology* **79**, 249–257.

Cavagnaro, J. B. (1988). Distribution of C_3 and C_4 grasses at different altitudes in a temperate arid region of Argentina. *Oecologia* **76**, 273–277. doi: 10.1007/BF00379962

Cerling, T. E., Wang, Y., and Quade, J. (1993). Expansion of C_4 ecosystems as an indicator of global ecological change in the late Miocene. *Nature* **361**, 344–345. doi: 10.1038/361344a0

Chapman, J. A., and Ceballos, G. (1990). The cottontails. In 'Rabbits, Hares and Pikas: Status Survey and Conservation Action Plan'. (Eds J. A. Chapman and J. E. C. Flux.) pp. 95–110. (IUCN: Gland, Switzerland.)

Charrel, R. N., Attoui, H., Butenko, A. M., Clegg, J. C., Deubel, V., *et al*. (2004). Tick-borne virus diseases of human interest in Europe. *Clinical Microbiology and Infection* **10**, 1040–1055. doi: 10.1111/j.1469-0691.2004.01022.x

Chastel, C. (1998). Erve and Eyach: two viruses isolated in France, neuropathogenic for man and widely distributed in Western Europe. *Bulletin de L'Académie Nationale de Médecine* **182**, 801–810.

Coman, B. (1999). 'Tooth & Nail: the Story of the Rabbit in Australia.' (Text Publishing: Melbourne.)

Cooke, B. D. (1983). Changes in the age-structure and size of populations of wild rabbits in South Australia, following the introduction of European rabbit fleas, *Spilopsyllus cuniculi* (Dale), as vectors of myxomatosis. *Australian Wildlife Research* **10**, 105–120. doi: 10.1071/WR9830105

Cooke, B. D. (1999*a*). Rabbit Calicivirus Disease Program Report 2: epidemiology, spread and release in wild rabbit populations in Australia. A report of research conducted by participants in the Rabbit Calicivirus Disease Monitoring and Surveillance Program and Epidemiology Research Program. Prepared for the RCD Management Group. Bureau of Rural Sciences, Canberra.

Cooke, B. D. (1999*b*). Notes on the life-history of the rabbit flea *Caenopsylla laptevi ibera* Beaucournu & Marquez, 1987 (Siphonaptera: Ceratophyllidae) in eastern Spain. *Parasite (Paris, France)* **6**, 347–354.

Cooke, B. D. (2002). Rabbit haemorrhagic disease: field epidemiology and the management of wild rabbit populations. *Review scientifique et technique de l' Office international des Epizooties* **21**, 347–358.

Cooke, B. D., and Fenner, F. (2002). Rabbit haemorrhagic disease and the biological control of wild rabbits, *Oryctolagus cuniculus*, in Australia and New Zealand. *Wildlife Research* **29**, 689–706. doi: 10.1071/WR02010

Cooke, B. D., McPhee, S., Robinson, A. J., and Capucci, L. (2002). Rabbit haemorrhagic disease: does a pre-existing RHDV-like virus reduce the effectiveness of RHD as a biological control in Australia? *Wildlife Research* **29**, 673–682. doi: 10.1071/WR00092

Corbet, G. B. (1994). Taxonomy and origins. In 'The European Rabbit: the History and Biology of a Successful Colonizer'. (Eds H. V. Thompson and C. M. King.) pp. 1–7. (Oxford University Press: Oxford.)

Couch, L. K. (1929). Introduced European rabbits in the San Juan Islands, Washington. *Journal of Mammalogy* **10**, 334–336. doi: 10.2307/1374122

Cox, J. C., Pye, D., Edmonds, J. W., and Shepherd, R. (1980). An investigation of *Encephalitozoon cuniculi* in the wild rabbit *Oryctolagus cuniculus* in Victoria, Australia. *Journal of Hygiene* **84**, 295–300.

Cunningham, A. A. (1996). Disease risks of wildlife translocations. *Conservation Biology* **10**, 349–353. doi: 10.1046/j.1523-1739.1996.10020349.x

Daszak, P., Cunningham, A. A., and Hyatt, A. D. (2001). Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica* **78**, 103–116. doi: 10.1016/S0001-706X(00)00179-0

de Almeida, A. J., Mayen, F. L., and de Oliveira, F. C. R. (2006). Espécies do gênero *Eimeria* observadas em fezes de coelhos domésticos (*Oryctolagus cuniculus*) criados no município de Campos dos Goytacazes, estado do Rio de Janeiro, Brasil. *Revista Brasileira de Parasitologia Veterinaria = Brazilian Journal of Veterinary Parasitology; Orgao Oficial Do Colegio Brasileiro de Parasitologia Veterinaria* **15**, 163–166.

De Leener, K., Rahman, M., Matthijnssens, J., Van Hoovels, L., Goegebuer, T., van der Donck, I., and Van Ranst, M. (2004). Human infection with a P[14], G3 lapine rotavirus. *Virology* **325**, 11–17. doi: 10.1016/j.virol.2004.04.020

Dubey, J. P., Sreekumar, C., Lindsay, D. S., Hill, D., Rosenthal, B. M., Venturini, L., Venturini, M. C., and Greiner, E. C. (2003). *Besnoitia oryctofelisi* n. sp. (Protozoa: Apicomplexa) from domestic rabbits. *Parasitology* **126**, 521–539.

Dunlap, T. R. (1997). Remaking the land: the acclimatization movement and Anglo ideas of nature. *Journal of World History* **8**, 303–319. doi: 10.1353/jwh.2005.0062

Dunsmore, J. D. (1966*a*). Nematode parasites of free-living rabbits, *Oryctolagus cuniculus* (L.), in eastern Australia. I. Variations in the number of *Trichostrongylus retortaeformis* (Zeder). *Australian Journal of Zoology* **14**, 185–199. doi: 10.1071/ZO9660185

Dunsmore, J. D. (1966*b*). Nematode parasites of free-living rabbits, *Oryctolagus cuniculus* (L.), in eastern Australia. II. Variations in the numbers of *Graphidium strigosum* (Dujardin) Railliet and Henry. *Australian Journal of Zoology* **14**, 625–634. doi: 10.1071/ZO9660625

Dunsmore, J. D. (1966*c*). Nematode parasites of free-living rabbits, *Oryctolagus cuniculus* (L.), in eastern Australia. III. Variations in the numbers of *Passalurus ambiguus* (Rudolphi). *Australian Journal of Zoology* **14**, 635–645. doi: 10.1071/ZO9660635

Dunsmore, J. D. (1981). The role of parasites in population regulation of the European rabbit (*Oryctolagus cuniculus*) in Australia. In 'Worldwide Furbearer Conference Proceedings'. (Eds J. A. Chapman and D. Pursley.) pp. 654–669. (University of Maryland: Frostburg, MD.)

Durant, P., and Guevara, M. A. (2001). A new rabbit species (*sylvilagus*, Mammalia: Leporidae) from the lowlands of Venezuela. *Revista de Biología Tropical* [online] **49**, 369–381. [sighted 18 December 2007] Available from World Wide Web: <http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S0034-77442001000100036&lng=en&nrm=iso>

Duszynski, D. W., and Marquardt, W. C. (1969). *Eimeria* (Protozoa: Eimeriidae) of the cottontail rabbit *Sylvilagus audubonii* in northeastern Colorado, with descriptions of three new species. *Journal of Protozoology* **16**, 128–137.

Duszynski, D. W., and Upton, S. J. (2001). *Cyclospora*, *Eimeria*, *Isospora* and *Cryptosporidium* spp. In 'Parasitic Diseases of Wild Mammals'. (Eds W. M. Samuel, M. J. Pybus and A. A. Kocan.) pp. 416–459. (Iowa State University Press: Ames, IA.)

Edwards, G. P., Dobbie, W., and Berman, McK, D. (2002). Population trends in European rabbits and other wildlife of central Australia in the wake of rabbit haemorrhagic disease. *Wildlife Research* **29**, 557–565. doi: 10.1071/WR00097

Epstein, H. E., Gill, R. A., Paruelo, J. M., Lauenroth, W. K., Jia, G. J., and Burke, I. C. (2002). The relative abundance of three plant functional types in temperate grasslands and shrublands of North and South America: effects of projected climate change. *Journal of Biogeography* **29**, 875–888. doi: 10.1046/j.1365-2699.2002.00701.x

Everett, K. D. E., Bush, R. M., and Andersen, A. A. (1999). Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. *International Journal of Systematic Bacteriology* **49**, 415–440.

Fenner, F. (1994). Rabbitpox virus. In 'Virus Infections of Rodents and Lagomorphs'. (Ed. A. D. M. E. Osterhaus.) pp. 51–57. (Elsevier: Amsterdam.)

Fenner, F., and Fantini, B. (1999). 'Biological Control of Vertebrate Pests: The History of Myxomatosis – an Experiment in Evolution.' (CABI Publishing: Wallingford, UK.)

Fenner, F., and Ratcliffe, F. N. (1965). 'Myxomatosis.' (Cambridge University Press: Cambridge.)

Ferrand, N., and Branco, M. (2007). The evolutionary history of the European rabbit (*Oryctolagus cuniculus*): major patterns of population differentiation and geographic expansion inferred from protein polymorphism. In 'Phylogeography of Southern European Refugia'. (Eds S. Weiss and N. Ferrand.) pp. 207–235. (Springer: Berlin/Heidelberg/New York.)

FitzSimons, F. W. (1906). The wild animals of South Africa. *Natal Agricultural Journal* **9**, 142–153.

Flux, J. E. C. (1993). Relative effect of cats, myxomatosis, traditional control, or competitors in removing rabbits from islands. *New Zealand Journal of Zoology* **20**, 13–18.

Flux, J. E. C. (1994). World distribution. In 'The European Rabbit: the History and Biology of a Successful Colonizer'. (Eds H. V. Thompson and C. M. King.) pp. 8–21. (Oxford University Press: Oxford.)

Flux, J. E. C. (2008). A review of competition between rabbits (*Oryctolagus cuniculus*) and hares (*Lepus europaeus*). In 'Lagomorph Biology: Evolution, Ecology and Conservation'. (Eds P. C. Alves, N. Ferrand and K. Hackländer.) pp. 241–249. (Springer: Berlin/Heidelberg.)

Flux, J. E. C., and Angermann, R. (1990). The hares and jackrabbits. In 'Rabbits, Hares and Pikas: Status Survey and Conservation Action Plan'. (Eds J. A. Chapman and J. E. C. Flux.) pp. 61–94. (IUCN: Gland, Switzerland.)

Flux, J. E. C., Duthie, A. G., Robinson, T. J., and Chapman, J. A. (1990). Exotic populations. In 'Rabbits, Hares and Pikas: Status Survey and Conservation Action Plan'. (Eds J. A. Chapman and J. E. C. Flux.) pp. 147–153. (IUCN: Gland, Switzerland.)

Forrester, N. L., Trout, R. C., Turner, S. L., Kelly, D., Boag, B., Moss, S., and Gould, E. A. (2006). Unravelling the paradox of rabbit haemorrhagic disease virus emergence, using phylogenetic analysis; possible implications for rabbit conservation strategies. *Biological Conservation* **131**, 296–306. doi: 10.1016/j.biocon.2006.05.005

Froggatt, W. W. (1906). Rabbits and ants. *Agricultural Gazette of New South Wales* **17**, 1113–1119.

Fu, A. Q. (1984). The coccidian species of rabbit and their distribution in China. *Chinese Journal of Rabbit Farming* **1**, 34–37.

Gibb, J. A. (1990). The European rabbit *Oryctolagus cuniculus*. In 'Rabbits, Hares and Pikas: Status Survey and Conservation Action Plan'. (Eds J. A. Chapman and J. E. C. Flux.) pp. 116–120. (IUCN: Gland, Switzerland.)

Gibb, J. A., and Williams, J. M. (1994). The rabbit in New Zealand. In 'The European Rabbit: the History and Biology of a Successful Colonizer'. (Eds H. V. Thompson and C. M. King.) pp. 158–204. (Oxford University Press: Oxford.)

Goddard, M. R., Leigh, J., Roger, A. J., and Pemberton, A. J. (2006). Invasion and persistence of a selfish gene in the Cnidaria. *PLoS ONE* **1**(1), e3. doi: 10.1371/journal.pone.0000003

Gould, F., Magori, K., and Huang, Y. (2006). Genetic strategies for controlling mosquito-borne diseases. *American Scientist* **94**, 238–246.

Grès, V., Marchandeau, S., and Landau, I. (2002). Description d'une nouvelle espèce d'*Eimeria* (Coccidia, Eimeridea) chez le lapin de garenne *Oryctolagus cuniculus* en France. *Zoosystema* **24**, 203–207.

Griffith, B., Scott, J. M., Carpenter, J. W., and Reed, C. (1993). Animal translocations and potential disease transmission. *Journal of Zoo and Wildlife Medicine* **24**, 231–236.

Grigliatti, T. A., Meister, G., and Pfeifer, T. A. (2007). TAC–TICS: Transposon-based biological pest management systems. In 'Novel Biotechnologies for Biocontrol Agent Enhancement and Management'. (Eds M. Vurro and J. Gressel.) pp. 327–351. (Springer: Dordrecht.)

Hamilton, P. B., Stevens, J. R., Holz, P., Boag, B., Cooke, B., and Gibson, W. C. (2005). The inadvertent introduction into Australia of *Trypanosoma nabiasi*, the trypanosome of the European rabbit (*Oryctolagus cuniculus*), and its potential for biocontrol. *Molecular Ecology* **14**, 3167–3175. doi: 10.1111/j.1365-294X.2005.02602.x

Hardy, C. M., Hinds, L. A., Kerr, P. J., Lloyd, M. L., Redwood, A. J., Shellam, G. R., and Strive, T. (2006). Biological control of vertebrate pests using virally vectored immunocontraception. *Journal of Reproductive Immunology* **71**, 102–111. doi: 10.1016/j.jri.2006.04.006

Heckathorn, S. A., McNaughton, S. J., and Coleman, J. S. (1999). C_4 plants and herbivory. In 'C_4 Plant Biology'. (Eds R. F. Sage and R. K. Monson.) pp. 285–312. (Academic Press: San Diego.)

Henzell, R. (2007). Genomic age series. Part 5: Prospects for managing vertebrate pests in Australia with GMO technologies. *Agricultural Science* **20**, 41–45.

Henzell, R. P., Cunningham, R. B., and Neave, H. M. (2002). Factors affecting the survival of Australian wild rabbits exposed to rabbit haemorrhagic disease. *Wildlife Research* **29**, 523–542. doi: 10.1071/WR00083

Hershkovitz, P. (1972). The recent mammals of the Neotropical region: a zoogeographic and ecological review. In 'Evolution, Mammals and Southern Continents'. (Eds A. Keast, F. C. Erk and B. Glass.) pp. 311–431. (State University of New York: Albany, NY.)

Hobbs, R. P., and Twigg, L. E. (1998). Coccidia (*Eimeria* spp.) of wild rabbits in southwestern Australia. *Australian Veterinary Journal* **76**, 209–210. doi: 10.1111/j.1751-0813.1998.tb10131.x

Hobbs, R. P., Twigg, L. E., Elliot, A. D., and Wheeler, A. G. (1999). Evaluation of the association of parasitism with mortality of wild European rabbits *Oryctolagus cuniculus* (L.) in southwestern Australia. *Journal of Parasitology* **85**, 803–808. doi: 10.2307/3285814

Hoddle, M. S. (1999). Biological control of vertebrate pests. In 'Handbook of Biological Control'. (Eds T. S. Bellows and T. W. Fisher.) pp. 955–974. (Academic Press: San Diego, CA.)

Holt, R. D., Hochberg, M. E., and Barfield, M. (1999). Population dynamics and the evolutionary stability of biological control. In 'Theoretical Approaches to Biological Control'. (Eds B. A. Hawkins and H. V. Cornell.) pp. 219–230. (Cambridge University Press: Cambridge.)

Hornick, R. (2001). Tularemia revisited. *The New England Journal of Medicine* **345**, 1637–1639. doi: 10.1056/NEJM200111293452211

Hudson, J. B. (1994). Herpesviridae. In 'Virus Infections of Rodents and Lagomorphs'. (Ed. A. D. M. E. Osterhaus.) pp. 81–84. (Elsevier: Amsterdam.)

HVPCWG (Humane Vertebrate Pest Control Working Group) (2004). A national approach towards humane vertebrate pest control. Discussion paper arising from the proceedings of an RSPCA Australia/AWC/VPC joint workshop, August 4–5, Melbourne. RSPCA Australia, Canberra.

Jaksic, F. M. (1998). Vertebrate invaders and their ecological impacts in Chile. *Biodiversity and Conservation* **7**, 1427–1445. doi: 10.1023/A:1008825802448

Jaksic, F. M., and Fuentes, E. R. (1991). Ecology of a successful invader: the European rabbit in central Chile. In 'Biogeography of Mediterranean Invasions'. (Eds R. H. Groves and F. di Castri.) pp. 273–283. (Cambridge University Press: Cambridge.)

Jaksic, F. M., and Soriguer, R. C. (1981). Predation upon the European rabbit (*Oryctolagus cuniculus*) in Mediterranean habitats of Chile and Spain: a comparative analysis. *Journal of Animal Ecology* **50**, 269–281. doi: 10.2307/4044

Jin, L., Valentine, B. A., Baker, R. J., Löhr, C. V., Gerlach, R. F., Bildfell, R. J., and Moerdyk-Schauwecker, M. (2008). An outbreak of fatal herpesvirus infection in domestic rabbits in Alaska. *Veterinary Pathology* **45**, 369–374. doi: 10.1354/vp.45-3-369

Johnson, R. C. (1977). The spirochetes. *Annual Review of Microbiology* **31**, 89–106. doi: 10.1146/annurev.mi.31.100177.000513

Katona, K., Bíró, Z., Hahn, I., Kertész, M., and Altbäcker, V. (2004). Competition between European hare and European rabbit in a lowland area, Hungary: a long-term ecological study in the period of rabbit extinction. *Folia Zoologica* **53**, 255–268.

Kirkpatrick, R. D. (1959*a*). San Juan rabbit in Indiana. *Outdoor Indiana* **3**, 1–2.

Kirkpatrick, R. D. (1959*b*). San Juan rabbit investigation. Final Report. Indiana Department of Conservation, Indianapolis.

Kirkpatrick, R. D. (1960). The introduction of the San Juan rabbit (*Oryctolagus cuniculus*) in Indiana. *Proceedings of the Indiana Academy of Sciences* **69**, 320–324.

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.

Kuijper, D. P. J., van Wieren, S. E., and Bakker, J. P. (2004). Digestive strategies in two sympatrically occurring lagomorphs. *Journal of Zoology* **264**, 171–178. doi: 10.1017/S0952836904005722

Labudovic, A., Perkins, H., van Leeuwen, B., and Kerr, P. (2004). Sequence mapping of the Californian MSW strain of *Myxoma virus. Archives of Virology* **149**, 553–570. doi: 10.1007/s00705-003-0222-6

Lavazza, A., and Capucci, L. (2008). How many caliciviruses are there in rabbits? A review on RHDV and correlated viruses. In 'Lagomorph Biology: Evolution, Ecology and Conservation'. (Eds P. C. Alves, N. Ferrand and K. Hackländer.) pp. 263–278. (Springer: Berlin/Heidelberg.)

Lever, C. (1985). 'Naturalized Mammals of the World.' (Longman: Harlow.)

Levine, N. D., and Ivens, V. (1972). Coccidia of the Leporidae. *Journal of Eukaryotic Microbiology* **19**, 572–581. doi: 10.1111/j.1550-7408.1972.tb03531.x

Licois, D., Wyers, M., and Coudert, P. (2005). Epizootic rabbit enteropathy: experimental transmission and clinical characterization. *Veterinary Research* **36**, 601–613. doi: 10.1051/vetres:2005021

Licon Luna, R. M. (2000). First report of myxomatosis in Mexico. *Journal of Wildlife Diseases* **36**, 580–583.

Lidicker, W. Z. (1991). Introduced mammals in California. In 'Biogeography of Mediterranean Invasions'. (Eds R. H. Groves and F. di Castri.) pp. 263–271. (Cambridge University Press: Cambridge.)

Long, J. L. (2003). 'Introduced Mammals of the World: their History, Distribution and Influence.' (CSIRO Publishing/CABI: Melbourne.)

Marchandeau, S., Bertagnoli, S., Peralta, B., Boucraut-Baralon, C., Letty, J., and Reitz, F. (2004). Possible interaction between myxomatosis and calicivirosis related to rabbit haemorrhagic disease affecting the European rabbit. *The Veterinary Record* **155**, 589–592.

Marlier, D., Dewrée, R., Delleur, V., Licois, D., Lassence, C., Poulipoulis, A., and Vindevogel, H. (2003). Description des principales étiologies des maladies digestives chez le lapin européen (*Oryctolagus cuniculus*). *Annales de Médecine Vétérinaire* **147**, 385–392.

Martín-Alonso, J. M., Skilling, D. E., González-Molleda, L., del Barrio, G., Machín, Á., Keefer, N. K., Matson, D. O., Iversen, P. L., Smith, A. W., and Parra, F. (2005). Isolation and characterization of a new *Vesivirus* from rabbits. *Virology* **337**, 373–383. doi: 10.1016/j.virol.2005.04.018

Matthee, C. A., van Vuuren, B. J., Bell, D., and Robinson, T. J. (2004). A molecular supermatrix of the rabbits and hares (Leporidae) allows for the identification of five intercontinental exchanges during the Miocene. *Systematic Biology* **53**, 433–447. doi: 10.1080/10635150490445715

Matthijnssens, J., Rahman, M., Martella, V., Xuelei, Y., De Vos, S., De Leener, K., Ciarlet, M., Buonavoglia, C., and Van Ranst, M. (2006). Full genomic analysis of human rotavirus strain B4106 and lapine rotavirus strain 30/96 provides evidence for interspecies transmission. *Journal of Virology* **80**, 3801–3810. doi: 10.1128/JVI.80.8.3801-3810.2006

Mbuthia, P. G., Gathumbi, P. K., Bwangamoi, O., and Wasike, P. N. (1993). Natural besnoitiosis in a rabbit. *Veterinary Parasitology* **45**, 191–198. doi: 10.1016/0304-4017(93)90074-W

McCauley, L. M. E., Lancaster, M. J., Young, P., Butler, K. L., and Ainsworth, C. G. V. (2007). Comparison of ELISA and CFT assays for *Chlamydophila abortus* antibodies in ovine sera. *Australian Veterinary Journal* **85**, 325–328. doi: 10.1111/j.1751-0813.2007.00189.x

McIntosh, M. T., Behan, S. C., Mohammed, F. M., Lu, Z., Moran, K. E., *et al*. (2007). A pandemic strain of calicivirus threatens rabbit industries in the Americas. *Virology Journal* **4**: 96. doi: 10.1186/1743-422X-4-96

McLeod, R. (2004). 'Counting the Cost: Impact of Invasive Animals in Australia, 2004.' (Cooperative Research Centre for Pest Animal Control: Canberra.)

McLeod, S. R., and Twigg, L. E. (2006). Predicting the efficacy of virally-vectored immunocontraception for managing rabbits. *New Zealand Journal of Ecology* **30**, 103–120.

Measures, L. N., and Anderson, R. C. (1983). Development of the stomach worm *Obeliscoides cuniculi* (Graybill) in lagomorphs, woodchucks and small rodents. *Journal of Wildlife Diseases* **19**, 225–233.

Mutze, G., Bird, P., Kovaliski, J., Peacock, D., Jennings, S., and Cooke, B. (2002). Emerging epidemiological patterns in rabbit haemorrhagic disease, its interaction with myxomatosis, and their effects on rabbit populations in South Australia. *Wildlife Research* **29**, 577–590. doi: 10.1071/WR00100

Mutze, G., Bird, P., Cooke, B., and Henzell, R. (2008). Geographic and seasonal variation in the impact of rabbit haemorrhagic disease on wild rabbits, *Oryctolagus cuniculus*, and rabbit damage in Australia. In 'Lagomorph Biology: Evolution, Ecology and Conservation'. (Eds P. C. Alves, N. Ferrand and K. Hackländer.) pp. 279–293. (Springer: Berlin/Heidelberg.)

Myers, K., Parer, I., Wood, D., and Cooke, B. D. (1994). The rabbit in Australia. In 'The European Rabbit: the History and Biology of a Successful Colonizer'. (Eds H. V. Thompson and C. M. King.) pp. 108–157. (Oxford University Press: Oxford.)

Mykytowycz, R. (1957). Ectoparasites of the wild rabbit, *Oryctolagus cuniculus* (L.), in Australia. *CSIRO Wildlife Research* **2**, 63–65.

Mykytowycz, R. (1958). Contact transmission of infectious myxomatosis of the rabbit, *Oryctolagus cuniculus* (L.). *CSIRO Wildlife Research* **3**, 1–6.

Norbury, G. (2001). Advances in New Zealand mammalogy, 1990–2000: lagomorphs. *Journal of the Royal Society of New Zealand* **31**, 83–97.

Norbury, G., and Reddiex, B. (2005). European rabbit. In 'The Handbook of New Zealand Mammals'. (Ed. C. M. King.) pp. 131–150. (Oxford University Press: Melbourne.)

Ojeda, R. A., Campos, C. M., Gonnet, J. M., Borghi, C. E., and Roig, V. G. (1998). The MaB Reserve of Ñacuñán, Argentina: its role in understanding the Monte Desert biome. *Journal of Arid Environments* **39**, 299–313. doi: 10.1006/jare.1998.0398

Olsen, P. (1998). 'Australia's Pest Animals: New Solutions to Old Problems.' (Bureau of Rural Sciences: Canberra, & Kangaroo Press: Sydney.)

Onderka, D. K., Papp-Vid, G., and Perry, A. W. (1992). Fatal herpesvirus infection in commercial rabbits. *The Canadian Veterinary Journal. La Revue Veterinaire Canadienne* **33**, 539–543.

Pakandl, M. (1988). Description of *Eimeria vejdovskyi* sp.n. and redescription of *Eimeria media* Kessel, 1929 from the rabbit. *Folia Parasitologica* **35**, 1–9.

PCE (Parliamentary Commissioner for the Environment) (1998). 'The Rabbit Calicivirus Disease Saga: a Biosecurity/Biocontrol Fiasco.' (PCE: Wellington.)

Pech, R. P., and Hood, G. M. (1998). Foxes, rabbits, alternative prey and rabbit calicivirus disease: consequences of a new biological control agent for an outbreaking species in Australia. *Journal of Applied Ecology* **35**, 434–453. doi: 10.1046/j.1365-2664.1998.00318.x

Percy, D. H., and Barthold, S. W. (2007). 'Pathology of Laboratory Rodents and Rabbits.' 3rd edn. (Blackwell: Ames, IA.)

Petersen, J. M., and Schriefer, M. E. (2005). Tularemia: emergence/re-emergence. *Veterinary Research* **36**, 455–467. doi: 10.1051/vetres:2005006

Phillips, S., Zenger, K. R., and Richardson, B. J. (2002). Are Sydney rabbits different? *Australian Zoologist* **32**, 49–55.

Pinto, R. M., Gomes, D. C., Menezes, R. C., Gomes, C. T., and Noronha, D. (2004). Helminths of rabbits (Lagomorpha, Leporidae) deposited in the helminthological collection of the Oswaldo Cruz Institute. *Revista Brasileira de Zoologia* **21**, 599–604.

Reddiex, B., Hickling, G. J., Norbury, G. L., and Frampton, C. M. (2002). Effects of predation and rabbit haemorrhagic disease on population dynamics of rabbits (*Oryctolagus cuniculus*) in North Canterbury, New Zealand. *Wildlife Research* **29**, 627–633. doi: 10.1071/WR00088

Reglero, M., Vicente, J., Rouco, C., Villafuerte, R., and Gortazar, C. (2007). *Trypanosoma* spp. infection in wild rabbits (*Oryctolagus cuniculus*) during a restocking program in southern Spain. *Veterinary Parasitology* **149**, 178–184. doi: 10.1016/j.vetpar.2007.08.001

Regnery, D. C. (1971). The epidemic potential of Brazilian myxoma virus (Lausanne strain) for three species of North American cottontails. *American Journal of Epidemiology* **94**, 514–519.

Robinson, A. J., Kirkland, P. D., Forrester, R. I., Capucci, L., Cooke, B. D., and Philbey, A. W. (2002). Serological evidence for the presence of a calicivirus in Australian wild rabbits, *Oryctolagus cuniculus*, before the introduction of rabbit haemorrhagic disease (RHDV): its potential influence on the specificity of a competitive ELISA for RHDV. *Wildlife Research* **29**, 655–662. doi: 10.1071/WR00096

Rogers, P. M., Arthur, C. P., and Soriguer, R. C. (1994). The rabbit in continental Europe. In 'The European Rabbit: the History and Biology of a Successful Colonizer'. (Eds H. V. Thompson and C. M. King.) pp. 22–63. (Oxford University Press: Oxford.)

Rolls, E. (1969). 'They All Ran Wild: the Story of Pests on the Land in Australia.' (Angus and Robertson: Sydney.)

Sage, R. F., Wedlin, D. A., and Li, M. (1999). The biogeography of C_4 photosynthesis: patterns and controlling factors. In 'C_4 Plant Biology'. (Eds R. F. Sage and R. K. Monson.) pp. 313–373. (Academic Press: San Diego.)

Silvers, L., Inglis, B., Labudovic, A., Janssens, P. A., van Leeuwen, B. H., and Kerr, P. J. (2006). Virulence and pathogenesis of the MSW and MSD strains of Californian myxoma virus in European rabbits with genetic resistance to myxomatosis compared to rabbits with no genetic resistance. *Virology* **348**, 72–83. doi: 10.1016/j.virol.2005.12.007

Simpson, G. G. (1980). 'Splendid Isolation: the Curious History of South American Mammals.' (Yale University Press: New Haven, CT.)

Smith, A. T., Formozov, N. A., Hoffmann, R. S., Changlin, Z., and Erbajeva, M. A. (1990). The pikas. In 'Rabbits, Hares and Pikas: Status Survey and Conservation Action Plan'. (Eds J. A. Chapman and J. E. C. Flux.) pp. 14–60. (IUCN: Gland, Switzerland.)

Sorvillo, F., Ash, L. R., Berlin, O. G. W., Yatabe, J., Degiorgio, C., and Morse, S. A. (2002). *Baylisascaris procyonis*: an emerging helminthic zoonosis. *Emerging Infectious Diseases* **8**, 355–359.

Stodart, E. (1968). Coccidiosis in wild rabbits, *Oryctolagus cuniculus* (L.), at four sites in different climatic regions in eastern Australia. II. The relationship of oocyst output to climate and to some aspects of the rabbit's physiology. *Australian Journal of Zoology* **16**, 619–628. doi: 10.1071/ZO9680619

Stodart, E., and Parer, I. (1988). Colonisation of Australia by the rabbit, *Oryctolagus cuniculus* (L.). CSIRO Division of Wildlife and Ecology Project Report No. 6. CSIRO, Australia.

Strayer, D. S., Cabirac, G., Sell, S., and Leibowitz, J. L. (1983). Malignant rabbit fibroma virus: observations on the culture and histopathologic characteristics of a new virus-induced rabbit tumor. *Journal of the National Cancer Institute* **71**, 91–104.

Strong, K. L., and Halliday, R. B. (1992). Biology and host specificity of the genus *Psoroptes* Gervais (Acarina: Psoroptidae), with reference to its occurrence in Australia. *Experimental & Applied Acarology* **15**, 153–169. doi: 10.1007/BF01195788

Suckow, M. A., Brammer, D. W., Rush, H. G., and Crisp, C. E. (2002). Biology and diseases of rabbits. In 'Laboratory Animal Medicine'. (Eds J. G. Fox, L. C. Anderson, F. M. Loew and F. W. Quimby.) pp. 329–364. (Academic Press: London.)

Swan, C., Perry, A., and Papp-Vid, G. (1991). Herpesvirus-like viral infection in a rabbit. *The Canadian Veterinary Journal* **32**, 627–628.

Thomas, C., Finn, M., Twigg, L., Deplazes, P., and Thompson, R. C. A. (1997). Microsporidia (*Encephalitozoon cuniculi*) in wild rabbits in Australia. *Australian Veterinary Journal* **75**, 808–810. doi: 10.1111/j.1751-0813.1997.tb15658.x

Thompson, H. V. (1994). The rabbit in Britain. In 'The European Rabbit: the History and Biology of a Successful Colonizer'. (Eds H. V. Thompson and C. M. King.) pp. 64–107. (Oxford University Press: Oxford.)

Tizzani, P., Lavazza, A., Capucci, L., and Meneguz, P. G. (2002). Presence of infectious agents and parasites in wild population of cottontail (*Sylvilagus floridanus*) and consideration on its role in the diffusion of pathogens infecting hares. European Association of Zoo- and Wildlife Veterinarians (EAZWV) 4th scientific meeting, joint with the annual meeting of the European Wildlife Disease Association (EWDA) May 8–12, 2002, Heidelberg, Germany.

Torres, J. M., Sanchez, C., Ramirez, M. A., Morales, M., Bárcena, J., Ferrer, J., Espuna, E., Pages-Mante, A., and Sanchez-Vizcaino, J. M. (2001). First field trial of a transmissible recombinant vaccine against myxomatosis and rabbit hemorrhagic disease. *Vaccine* **19**, 4536–4543. doi: 10.1016/S0264-410X(01)00184-0

Trout, R. C., and Tittensor, A. M. (1989). Can predators regulate wild rabbit *Oryctolagus cuniculus* population density in England and Wales? *Mammal Review* **19**, 153–173. doi: 10.1111/j.1365-2907.1989.tb00409.x

Twigg, L. E., Lowe, T. J., Martin, G. R., Wheeler, A. G., Gray, G. S., Griffin, S. L., O'Reilly, C. M., Robinson, D. J., and Hubach, P. H. (2000). Effects of surgically imposed sterility on free-ranging rabbit populations. *Journal of Applied Ecology* **37**, 16–39. doi: 10.1046/j.1365-2664.2000.00471.x

Tyndale-Biscoe, C. H. (1994). Virus-vectored immunocontraception of feral mammals. *Reproduction, Fertility and Development* **6**, 281–287. doi: 10.1071/RD9940281

van Leeuwen, B. H., and Kerr, P. J. (2007). Prospects for fertility control in the European rabbit (*Oryctolagus cuniculus*) using myxoma virus-vectored immunocontraception. *Wildlife Research* **34**, 511–522. doi: 10.1071/WR06167

Venturini, L., Petrucelli, M., Piscopo, M., Ungaza, J. M., Venturini, M. C., Bacigalupe, D., Basso, W., and Dubey, J. P. (2002). Natural *Besnoitia* sp. infection in rabbits from Argentina. *Veterinary Parasitology* **107**, 273–278. doi: 10.1016/S0304-4017(02)00156-5

Viggers, K. L., Lindenmayer, D. B., and Spratt, D. M. (1993). The importance of disease in reintroduction programmes. *Wildlife Research* **20**, 687–698. doi: 10.1071/WR9930687

Webb, S. D., and Marshall, L. G. (1982). Historical biogeography of recent South American land mammals. In 'Mammalian Biology in South America'. (Eds M. A. Mares and H. H. Genoways.) pp. 39–52. (University of Pittsburgh: Linesville, PA.)

Weisbroth, S. H., Flatt, R. E., and Kraus, A. L. (Eds) (1974). 'The Biology of the Laboratory Rabbit.' (Academic Press: New York.)

Weiss, R. A. (2002). Virulence and pathogenesis. *Trends in Microbiology* **10**, 314–317. doi: 10.1016/S0966-842X(02)02391-0

Whipp, M. J., Davis, J. M., Lum, G., de Boer, J., Zhou, Y., Bearden, S. W., Petersen, J. M., Chu, M. C., and Hogg, G. G. (2003). Characterisation of a *novicida*-like subspecies of *Francisella tularensis* isolated in Australia. *Journal of Medical Microbiology* **52**, 839–842. doi: 10.1099/jmm.0.05245-0

White, P. C. L., and Newton-Cross, G. (2000). An introduced disease in an invasive host: the ecology and economics of rabbit calicivirus disease (RCD) in rabbits in Australia. In 'The Economics of Biological Invasions'. (Eds C. Perrings, M. Williamson and S. Dalmazzone.) pp. 117–137. (Edward Elgar: Cheltenham, UK.)

Wilber, J. L. (1999). 'Pathology of the Rabbit.' Available at: http://morfz.com/patho_rabbit.pdf [Accessed 8 November 2007.]

Williams, C. K., Parer, I., Coman, B. J., Burley, J., and Braysher, M. L. (1995). 'Managing Vertebrate Pests: Rabbits.' Bureau of Resource Sciences/CSIRO Division of Wildlife and Ecology. (Australian Government Publishing Service: Canberra.)

Williams, R. T. (1972). The distribution and abundance of the ectoparasites of the wild rabbit, *Oryctolagus cuniculus* (L.), in New South Wales, Australia. *Parasitology* **64**, 321–330.

Wilson, J. C., Fuller, S. J., and Mather, P. B. (2002). Formation and maintenance of discrete wild rabbit (*Oryctolagus cuniculus*) population systems in arid Australia: habitat heterogeneity and management implications. *Austral Ecology* **27**, 183–191. doi: 10.1046/j.1442-9993.2002.01169.x

Wilson, J. R., and Hattersley, P. W. (1989). Anatomical characters and digestibility of leaves of *Panicum* and other grass genera with C_3 and different types of C_4 photosynthetic pathway. *Australian Journal of Agricultural Research* **40**, 125–136. doi: 10.1071/AR9890125

Wood, D. H. (1980). The demography of a rabbit population in an arid region of New South Wales Australia. *Journal of Animal Ecology* **49**, 55–79. doi: 10.2307/4277

Zenger, K. R., Richardson, B. J., and Vachot-Griffin, A.-M. (2003). A rapid population expansion retains genetic diversity within European rabbits in Australia. *Molecular Ecology* **12**, 789–794. doi: 10.1046/j.1365-294X.2003.01759.x

Zumpt, I. F. (1976). Some diseases of domestic rabbits encountered in the western Cape. *Journal of the South African Veterinary Association* **47**, 117–122.

Manuscript received 1 December 2006, accepted 10 June 2008

Appendix 1. Biological control agents considered less suitable for introduction

Californian strain of myxoma virus (MV)

The Californian strain of MV (natural host: *Sylvilagus bachmani*), like the Brazilian strain, is highly pathogenic to European rabbits (Fenner and Fantini 1999; Silvers *et al*. 2006). It causes high mortality in captive wild European rabbits held in quarantine in Australia, despite the host–pathogen coevolution that has already occurred with the Brazilian strain of MV (Silvers *et al*. 2006). It is not known whether it would interact adversely with the Brazilian strain of MV, or with RHDV, in the field, or how it would coevolve with wild European rabbits in the presence of RHDV and the Brazilian strain of MV. It is likely that genetic recombination could occur between the two strains of MV: Californian MV is related more closely to Brazilian MV than to another leporipoxvirus, Shopes fibroma virus (SFV), and it is known that recombination can occur between MV (strain unknown) and SFV (Block *et al*. 1985; Labudovic *et al*. 2004). The competitive ability and pathogenicity of such a recombinant are impossible to predict. However, even if it was likely to prove a useful BCA in Australia, importation of Californian MV might be refused on animal welfare grounds.

Cottontail rabbit papillomavirus

This virus is common in *Sylvilagus* of the mid-western and western United States but uncommon in laboratory *Oryctolagus* (Weisbroth *et al*. 1974; Baker 1998). It is transmitted by arthropod vectors, and transmission to domestic rabbits probably occurs exclusively from wild *Sylvilagus* because the virus is rarely observed in lesions of *Oryctolagus*. In laboratory rabbits the virus frequently produces squamous cell carcinomas that commonly metastasise to regional lymph nodes and lungs. It is unclear how useful it might be as a BCA in wild rabbits. While the lack of virus in lesions of laboratory *Oryctolagus* might be expected to reduce transmissibility, it is possible that greater quantities of virus would be produced in wild *Oryctolagus* in Australia. In any event, it might be possible to artificially select for viral genotypes that produced sufficient virus in lesions of laboratory *Oryctolagus* for a weakly self-transmitting strain to be developed. Once such a strain existed, natural selection – either in the laboratory or in the wild in Australia – might be expected to increase transmissibility (cf. stabilising selection for virulence in MV: Fenner and Fantini 1999).

Rabbitpox

The highly lethal and extremely contagious rabbitpox virus causes rare disease outbreaks in laboratory rabbits in the United States and the Netherlands (Fenner 1994). It appears to be an artefact as it is known only from laboratory rabbits. Spread appears to be by aerosol but since insects are usually well controlled in laboratory animal houses, this does not exclude the possibility that vectors could spread the virus. Both described strains have a wide host range and are highly pathogenic for mice as well as rabbits. Mice are also a pest in Australia, but the virus's wide host range precludes its introduction into Australia.

Lapine rotaviruses

Lapine rotaviruses can cause diarrhoea in rabbits, but recent evidence suggests that they may be able to infect humans and other animals (De Leener *et al*. 2004; Matthijnssens *et al*. 2006). Unless strains with a narrower host range can be found, they are unsuitable for use as BCAs.

Eyach virus (EYAV)

EYAV is present in mainland Europe in European rabbits, which are thought to be the reservoir host (Chastel 1998; Charrel *et al*. 2004). Its pathogenicity in rabbits is unknown. However, EYAV causes disease in humans (Chastel 1998), and is therefore unsuitable for use as a BCA for rabbits.

Paraspidodera uncinata*, and other nematodes*

The nematode *Paraspidodera uncinata* commonly infects guinea-pigs and octodontid rodents in Brazil, where it has also been recorded in European rabbits (Pinto *et al*. 2004). It is not known whether it causes disease in rabbits, or if it is present in guinea-pigs in Australia. If it is present in Australian guinea-pigs, its introduction into rabbits in Australia should be investigated. Its potential effects on Australian native rodents would also need to be investigated. The exotic lungworm *Protostrongylus rufescens cuniculorum* occurs in rabbits overseas, but experiments to assess its potential as a BCA were terminated when it was found that it could also develop in sheep (Williams *et al*. 1995). The zoonotic raccoon roundworm *Baylisascaris procyonis* causes fatal cerebrospinal disease in rabbits, but its capacity to infect humans (Sorvillo *et al*. 2002) precludes its use as a BCA.

Besnoitia *spp.*

Naturally occurring besnoitiosis, caused by protozoan parasites of the genus *Besnoitia*, occurs in domestic rabbits in Kenya and Argentina (Mbuthia *et al*. 1993; Venturini *et al*. 2002; Dubey *et al*. 2003), but not Australia. Little is known about the unnamed Kenyan species other than it was present in a rabbit submitted for necropsy after sudden death. The pathogenicity of the Argentinean *Besnoitia oryctofelisi* is unknown. *B. oryctofelisi* causes illness in its definitive host (domestic cats), mortality in at least one non-target species (gerbils, *Meriones unguiculatus*), and infection in laboratory mice, and is unlikely to be suitable for introduction into Australia.

Tularemia

Tularemia is a zoonosis caused by the bacterium *Francisella tularensis* (Petersen and Schriefer 2005). *F. tularensis tularensis*, the North American Type A subspecies, is the most pathogenic for European rabbits (Hornick 2001). However, tularemia's pathogenicity for humans and lack of host specificity preclude the introduction of any exotic subspecies into Australia.

A *novicida*-like subspecies of *F. tularensis* was recently discovered in a human in northern Australia, in an area where wild rabbits do not occur (Whipp. *et al*. 2003; M. J. Whipp. pers. comm. Melbourne, 2006). Its host range, geographical distribution, and pathogenicity for rabbits are unknown. Tularemia is not known to occur in rabbits in Australia, but given the risk to humans its deliberate use for biocontrol purposes is most unlikely. It could only be contemplated if: (1) the causative organism was already widespread in areas where rabbits occur but had not spilled over into them; (2) it was shown to be pathogenic in rabbits and capable of persisting in them; (3) it was likely to significantly mitigate the damage caused by rabbits; and (4) its introduction into rabbits would not increase the risk to non-target organisms (particularly humans).

Chlamydophila abortus

Enzootic abortion of ewes, caused by the bacterium *Chlamydophila abortus*, has not been reported from Australia or New Zealand (McCauley *et al*. 2007). *C. abortus* was formerly part of *Chlamydia psittaci* but is now regarded as a separate species (Everett *et al*. 1999). It is important in domestic rabbits under intensive conditions in the Western Cape of South Africa (Zumpt 1976). The bacterial strain involved in South Africa may be more pathogenic to rabbits than strains occurring elsewhere (e.g. in France: see Boucher *et al*. 2001), but it is unsuitable for use as a BCA because it is not host specific and has zoonotic potential (Everett *et al*. 1999).