Chapter 6
Options for the Control of Disease 1: Targeting the Infectious or Parasitic Agent

Jean Blancou, Marc Artois, Emmanuelle Gilot-Fromont, Volker Kaden, Sophie Rossi, Graham C. Smith, Michael R. Hutchings, Mark A. Chambers, Steve Houghton, and Richard J. Delahay

6.1 Introduction

There are three basic approaches to managing diseases: directly reduce the reproductive rate of the pathogen, reduce host (or infected host) density, or manipulate the environment to reduce contact between diseased and susceptible animals. In this chapter we will look at the first of these approaches. Since disease transmission results from direct or indirect contact between infectious and susceptible individuals,

J. Blancou
11, rue Descombes 75 017 Paris, France

M. Artois
Université J. Fourier, Laboratoire TIMC-IMAG, Unité Environnement et Prévision de la Santé des Populations F-38000 Grenoble; Ecole Nationale Vétérinaire de Lyon, France

E. Gilot-Fromont
Université de Lyon; Université Lyon 1; CNRS; UMR5558, Laboratoire de Biométrie et Biologie Evolutive, 43 Boulevard du 11 Novembre 1918, Villeurbanne F-69622, France

V. Kaden
Friedrich-Loeffler-Institut, Bundesforschungsinstitut für Tiergesundheit, Institut für Infektionsmedizin, 17493 Greifswald-Insel Riems, Germany

S. Rossi
Unité Sanitaire de la Faune, Micropolis, la Bérardie, Belle Aureille, 05000 GAP, France

G.C. Smith and R.J. Delahay
Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK

M.R. Hutchings
SAC, West Mains Road, Edinburgh, EH9 3JG, UK

M.A. Chambers
TB Research Group, Department of Statutory and Exotic Bacterial Diseases, Veterinary Laboratories Agency, Weybridge, New Haw, Addlestone, Surrey, KT15 3NB, UK

S. Houghton
Veterinary Vaccines Consultancy, Coles Cottage, Plum Park Lane, Paulerspury, Northants, NN12 7NN, UK

R.J. Delahay et al. (eds.), *Management of Disease in Wild Mammals,*
DOI:10.1007/978-4-431-77134-0_6, © Springer 2009

there are two ways to target an infectious agent: either limit the number of susceptible individuals by vaccinating them, or treat infected individuals in order to reduce the duration or intensity of the infectious period and the number of infectious individuals present at any given time. The overall aim of this chapter is to consider the conditions under which vaccination and treatment may make a valuable contribution to the control of infectious diseases in wild mammal populations. Both field research and mathematical modelling approaches have been used to address this question. For vaccination, early mathematical models of infectious disease dynamics suggested a simple answer: vaccination is useful as soon as the rate of control ensures that a sufficient proportion of the population is immune for a sufficient period of time (Bailey 1957). At the individual level, this herd immunity means that any given infectious individual has a low probability of encountering a susceptible animal. If the disease is introduced into a vaccinated population, the mean number of secondary infections caused by each infected case will be lower than unity, thus preventing further outbreaks from occurring ($R < 1$: see Chapter 3). However, this generalised scenario may be considered overly simplistic, as the practicalities of vaccination campaigns often complicate matters. For example, modelling studies often include assumptions about perfect vaccine efficacy, and the efficiency of delivering the vaccine to a population that may or may not reflect the situation in the field.

Red fox (*Vulpes vulpes*) rabies in Europe provided the earliest example of a disease of wildlife where vaccination appeared a realistic possibility, thanks to the pioneering work of Frantz Steck, Alexander Wandeler and their co-workers (see Section 6.2). Owing to the inadequacy of fox culling as a method of rabies control (see Chapter 7), European countries pursued the development of oral vaccination. As soon as appropriate baits for oral vaccination and safe vaccines were available for use on a large scale, the relative merits of vaccination and culling were investigated. The ensuing studies showed that vaccination of foxes was more efficient at halting epizootics than culling, it was less costly in the long-term, and importantly it could be rapidly deployed in response to the re-emergence of disease (Aubert 2003). Vaccination also had the added benefit that unlike culling it did not destabilise the social structure of fox populations, and so avoided the potential for perturbation to enhance transmission rates (Macdonald 1995) (see Chapter 2). The eradication of rabies from Western Europe at the end of the 20th century, following a period of intensive oral vaccination of foxes, finally paved the way for other vaccination strategies to combat wildlife diseases (Pastoret and Brochier 1999).

Contrary to the assumptions of most early models of wildlife disease dynamics, wild mammal populations are not homogeneous. Under the assumption of population homogeneity, all determinants of disease propagation are identical over space, and control measures are not expected to affect the spatial and social behaviour of individuals. These are clearly unrealistic expectations given the potential social complexity of mammal populations and the profound influence this can exert on disease dynamics and management efforts (see Chapter 2).

Hence, vaccination programmes may require heterogeneous effort over space and time (May and Anderson 1984) in order to optimally deploy resources for disease control. Vaccination is predicted to be the most efficient method in populations where rates of host birth and death, and disease propagation are relatively low. Elsewhere, culling or combined strategies may be more efficient (Barlow 1996). Nevertheless, the appropriate approach may vary widely between different mammal species, depending on their particular ecological and behavioural characteristics and the epidemiology of infection in the target population. Therefore, obtaining basic information on host population processes and disease dynamics at the appropriate spatial scale is an essential first step in determining the most appropriate control plan.

Financial cost is clearly a consideration when developing vaccination strategies (see Chapter 5) and will vary widely depending on how long vaccination is predicted to be necessary in order to achieve eradication or some other stated aim. The potential occurrence of multiple disease outbreaks or failure of early confinement may have dramatic effects on limited resources, and so contingencies for such events should be built into any vaccination campaign. Moreover, vaccinating or treating may have other indirect costs. Vaccinating a reservoir population may potentially lead to an increase in host density, and so enhance the risk of transmission of other diseases or amplify any other problems associated with the host (e.g. damage to crops or livestock). Human population growth in parts of Africa has been accompanied by a dramatic rise in domestic dog (*Canis lupus familiaris*) populations, which act as reservoirs of rabies and distemper viruses with spillover into wild canid populations. Vaccination of domestic dogs offers the potential to benefit endangered wild canids by reducing the risks of spillover, but could also have the potentially undesirable effect of increasing dog populations further (Cleaveland et al. 2002). Vaccination and direct medication of wild mammals may also have undesirable evolutionary consequences. For example, the long-term use of vaccines will impede any selection in the host population for resistance, or could lead to the selection of non-vaccinal strains of the pathogen, depending on the mode of action of vaccine induced immunity. Similarly, the inappropriate application of direct medication (e.g. antibiotics) may give rise to the emergence of antibiotic resistance in the pathogen.

Whether vaccination or treatment are useful options in any given situation will be guided in part by an evaluation of the population status of the host and the role of the pathogen in host dynamics. The extent to which pathogens have significant long-term effects on host population dynamics remains unclear. When the host is perceived to be ‘over-abundant’, the initial response is often to cull. In contrast, in small populations of endangered species, the value of each animal takes on a greater significance, such that treating individuals may become a viable option (see Chapter 11). In the future vaccination and treatment strategies may become more attractive as disease control options in a wider range of circumstances, particularly as more candidate vaccines are developed for use in wildlife, and if practical methods of deployment can be improved.

6.2 Early History

6.2.1 Medication

Large-scale use of direct medication has rarely been contemplated for wild mammals except in exceptional circumstances where there has been a serious threat to human health, or to highly valued wildlife. Although there are a few examples of the direct medication of wild mammals on a limited scale (see Section 6.3), this approach has remained largely restricted to those individual cases treated in veterinary surgeries, and during rehabilitation (e.g. at marine mammal centres) and translocation exercises. This is understandable, given the substantial practical difficulties in handling wild mammals, and the cost of veterinary drugs.

6.2.2 Vaccination

Vaccination is a term based on Edward Jenner's use of cowpox virus administered to humans to protect them against smallpox (*vacca* means cow in Latin). This practice worked because the immune response to cowpox protected individuals from the more virulent smallpox. Early vaccines were produced by one of two methods. Firstly, the repeated passage of the microorganism through rabbits or mice can create a weakened strain, which when injected into a patient may protect them from the virulent strain. Alternatively, microorganisms may be killed using chemicals or heat treatment and then used as a vaccine. In both instances the vaccine contains antigens associated with the pathogen and so invokes an immune response in the immunised host, but without causing disease.

In 1971 foxes were first immunised against rabies in the USA by the oral administration of the SAD ("Street Alabama Dufferin") vaccine, which is a live attenuated strain of the rabies virus (Baer et al. 1971). The research programme was prematurely terminated following the accidental infection of one of the scientists. However, work continued in Western Europe, and the first field trials using SAD virus in chicken-head baits were conducted in an Alpine valley in Switzerland by Frantz Steck, who unfortunately died when his helicopter crashed during the distribution of vaccine baits in the mountains. In 1983, trials using the SAD-B19 strain in chicken-head baits were conducted in Germany, followed in 1985 by the same strain in manufactured baits, and two years later by the use of the SAD-Bern strain in a field trial in southern Ontario (Canada). The first national vaccination campaigns using the SAD-B19 strain began in France and Luxembourg in 1986.

A new approach to rabies vaccination was developed in the 1980s, involving the delivery of the rabies virus surface antigen (glycoprotein) in a genetically modified vaccinia (pox) virus (VRG, or Vaccinia-rabies glycoprotein recombinant virus), which replicates within the vaccinated host (Thomas et al. 1990). The VRG vaccine was first used in Belgium in 1987 (Pastoret et al. 1988). A large-scale field trial of raccoon

(*Procyon lotor*) vaccination commenced in New England, USA in 1992 using VRG in oral baits distributed by hand and helicopter over 559 km^2. From 1995 to 2000, successful vaccination campaigns continued in Western Europe using the SAG2 strain of live attenuated virus and VRG, until the eradication of the disease. During this period large scale VRG vaccination campaigns were also conducted in coyotes (*Canis latrans*) and gray foxes (*Urocyon cinereoargenteus*) in Texas, USA (Black and Lawson 1980; Blancou et al. 1991; Brochier et al. 1996; Fearneyhough et al. 1998; Hanlon et al. 2002). An investigation of the relative cost-effectiveness of oral vaccination versus fox culling concluded that the former became economically beneficial after four years, and that culling had only ever resulted in a transient lull in the occurrence of the disease, while oral vaccination resulted in elimination (Aubert 1999).

Oral vaccination against classical swine fever (CSF) has been investigated in Europe since the 1960s, both under laboratory conditions and in the field. Initially, the vaccination of wild boar (*Sus scrofa*) was carried out in the former Soviet Union by adding liquid or freeze-dried CSF vaccine to cereal-based feed in heaps or troughs. Vaccination was performed in response to CSF epidemics in what are now parts of Russia, Byelorussia, Moldavia and Ukraine during the periods 1975–1976 and 1990–1991. The efficacy of vaccination varied depending, amongst other things, on the course of the epidemic and the size of the area affected. During the 1990s a freeze-dried vaccine was developed in Russia and was administered to boar in food provided exclusively in winter (Kolomitsev et al. 1998). In the mid-1980s in Romania, oral vaccination of wild boar against CSF was investigated by feeding them the hind legs of rabbits (*Oryctolagus cuniculu*s) used for the production of rabbit-passaged CSF vaccine, or with hen's eggs that had been inoculated with live virus vaccine immediately before distribution. At the same time, oral immunisation of wild boar was carried out under laboratory conditions in the former German Democratic Republic using parts of rabbit carcasses derived from animals prepared for the production of rabbit-passaged C-strain (China-strain) vaccine. In Italy (Rutili et al. 1987) and in France (Chenut et al. 1999), oral vaccination of wild boar was also investigated experimentally, using rabbit-passaged Chinese CSF virus. The results of these studies were generally encouraging, and subsequent work led to the development of a commercial CSF bait vaccine in Germany (Kaden et al. 2000), and culminated in the introduction of oral immunisation of wild boar to control CSF in several parts of Europe.

In 1990, oral immunisation of wild boar against CSF was again studied in Germany under laboratory conditions using the live attenuated C-strain virus, in baits similar to those previously used to deliver rabies vaccine to foxes. From 1993 to 1995 a field study was carried out in an area of approximately 270 km^2 in Lower Saxony. The C-strain virus used for oral vaccination was incorporated into a cereal-based bait matrix. Vaccination campaigns took place in spring and autumn, and each consisted of two bouts of vaccine deployment 14 days apart. In a subsequent study the prevalence of CSF antibodies in boar varied from 49% to 60% in the vaccinated population, with over 50% of the young animals failing to ingest bait and become immunised. Consequently, intensive hunting of the young animals was deemed necessary as an adjunct to oral vaccination, and after the third immunisation campaign, no virus was detected in the treated areas (Kaden et al. 2000). The subsequent deployment of vaccine

baits in other parts of Germany included the addition of a campaign in the summer (also consisting of two parts) and an extension of the interval between bouts of vaccine deployment to 4 weeks. In Baden-Württemberg, where this procedure was first applied, the prevalence of CSF antibodies in wild boar continued to increase until the third immunisation campaign when it peaked at 72%. The addition of a third campaign also succeeded in achieving higher levels of immunisation amongst young wild boar compared to previous campaigns (Kaden et al. 2005b) (see Box 6.3).

Although routine vaccination against CSF is prohibited in domestic pigs within the European Union, emergency vaccination is permitted (by oral immunisation) if, in its absence, the extensive spread of virus is considered to be likely. The vaccination procedure for wild boar, which has been used in several German Federal States, France (since 2004), Luxembourg (from 2003 to 2004), the Slovak Republic (since 2005) and Bulgaria (since 2006), has comprised of three vaccination campaigns in spring, summer and autumn (Kaden et al. 2006; Rossi et al. 2006).

6.3 Wildlife Medication

6.3.1 Basic Principles

Direct medication of free-ranging wild mammals is most likely to be seriously considered when there is no other way to control a disease that affects individuals of an endangered or valuable (e.g. as hunted game) species. Direct medication has however, also been used in some cases where the targeted disease threatened public health, or represented a threat to livestock or game animal production, or to international trade of animals and derived products. Nevertheless, the prohibitive costs and substantial practical difficulties of administering medication to wild mammals means that such examples are rare (see Chapter 11). In contrast direct medication is common practice in some countries during the rehabilitation of wild mammals, and is advisable during translocations that may incur a risk of spreading pathogens that are zoonotic or of significant potential economic or conservation concern.

6.3.2 Advantages and Disadvantages

Direct medication of wild mammals may allow the control of pathogens when the isolation or culling of hosts is not practical or acceptable, and is a more ethically attractive option. As individual animals may need to be captured or restrained, then this may provide an opportunity to carry out health checks, which may be particularly valuable in the case of endangered species. Also, where direct medication is aimed at specific individuals it is likely to incur negligible risks to non-target species.

Direct medication requires the use of regulated drugs, and official authorisation would be necessary for their use in wild animals. Such drugs may be expensive and

may need to be deployed by qualified professionals, potentially causing costs to grow substantially if the disease is not rapidly eradicated. Undesirable potential side effects of direct medication include the persistence of harmful residues of veterinary drugs in the environment and non-target species (Green et al. 2006). Also, evolutionary effects such as the emergence of drug-resistant disease strains and inhibition of selection for resistant hosts, could potentially lead to more extreme epizootics in the future.

The effects of direct medication in wild mammal populations can be difficult to predict. Baits containing the anthelmintic (de-worming) drug praziquantel (Droncit®) have been deployed for the control of the cestode *Echinococcus multilocularis* in European red foxes (see below). However, since the drug is not an ovicide, it has been suggested that uptake by foxes could result in a mass release of *E. multilocularis* eggs into the environment, which could in turn increase the probability of host exposure to the parasite (Petavy 2008). Concern has also been expressed amongst some game managers over the use of salt stones (mineral licks) as bait for the distribution of anthelmintics (or other drugs) to free ranging deer, as this could potentially encourage aggregation and so facilitate the spread of contagious pathogens. Experimental use of an acaricide in rabbit burrows dramatically reduced the numbers of rabbit fleas, the vector of myxomatosis, and resulted in a two- to three-fold increase in rabbit density (Trout et al. 1992). This clearly demonstrated how much the disease was suppressing the population. Nevertheless, the ecological consequences of direct medication as a tool for controlling infectious disease in wild mammals remain poorly understood.

6.3.3 Approaches

An important and successful example of treating wild mammals was achieved in the National Wildlife Research Centre of Taif (Saudi Arabia), although this involved a prolonged period of captivity. Following an outbreak of bovine tuberculosis (caused by *Mycobacterium bovis*) in a herd of Arabian oryx (*Oryx leucoryx*), individuals were captured and treated in captivity with antimycobacterial combination therapy. This was successful in producing tuberculosis-free oryx for release into the wild (Greth et al. 1994). Similarly, capture followed by treatment was used to eradicate sarcoptic or notoedric mange in free-ranging Spanish ibex (*Capra ibex*), cheetah (*Acinonyx jubatus*) and other endangered or genetically compromised populations (Pence and Ueckermann 2002 and Section 11.2). Game species have also benefited from direct medication for the control of helminth infections. Successful examples include anthelmintic treatments in bighorn sheep (*Ovis canadensis*) (Schmidt et al. 1979), snowshoe hares (*Lepus americanus*) (Murray et al. 1996) and white-tailed deer (*Odocoileus virginianus*) (Qureshi et al. 1994). In these instances the anthelminthic drugs were administered orally, being mixed with food or salts in areas where the target animals were known to feed.

Finally, as mentioned previously, direct medication was employed to protect public health by controlling *E. multilocularis* in populations of wild red foxes in Europe through the delivery of a bait containing praziquantel (Droncit®). After six bait deployment campaigns the average prevalence of infected foxes had declined from 32% to 4%. However, re-infection is likely to occur, since the infection does not produce a strong immune response. This approach is therefore still under evaluation in southern and northern Germany (Eckert et al. 2001) (see Box 6.1).

6.4 Wildlife Vaccination

6.4.1 Basic Principles

The goal of vaccination in wild mammals may be to eliminate a disease (e.g. wildlife rabies), and therefore to remove the threat to human health or susceptible domestic species, to reduce the prevalence of a disease to an acceptable level, or to prevent the extinction of a valued population or species. The best known and most successful example of the application of vaccination to manage disease in wildlife is the immunisation of wild mammals against rabies. Targeting the European red fox has resulted in the near complete elimination of rabies from West and Central Europe. Similar strategies have subsequently been used to control rabies in other species, including raccoon dogs (*Nyctereutes procyonoides*) in Europe and coyotes, striped skunks (*Mephitis mephitis*) raccoons and arctic foxes (*Alopex lagopus*) in the USA and Canada. The oral vaccination of wild boar against CSF (see Section 6.2.2) has also met with some success. This approach achieved the elimination of CSF infection in wild boar in several German Landër (states) within one or two years (Kaden et al. 2003; Von Rüden et al. 2008), and subsequent successes have been anecdotally reported in other European countries.

Following these two success stories, vaccines have become more widely considered as potential options for the control of disease in wild mammals. Particular interest has focused on the potential vaccination of wild mammals against bovine tuberculosis caused by *Mycobacterium bovis*. Currently, the only tuberculosis vaccine available to investigate in wildlife is BCG (Bacille Calmette Guérin), which is a live attenuated strain of *M. bovis* used extensively in humans. BCG has been tested experimentally in Eurasian badgers (*Meles meles*) in the Republic of Ireland (Southey et al. 2001), in wild boar (*Sus scrofa*) in Spain (Ballesteros et al. 2007) in African buffalo (*Syncerus caffer*) in South-Africa, in white-tailed deer in the United States (Waters et al. 2004; Palmer et al. 2007), and in red deer (*Cervus elaphus*) (de Lisle et al. 2002), ferrets (*Mustela putorius furo*) (Qureshi et al. 1999), and brushtail possums (*Trichosurus vulpecula*) in New Zealand (Corner et al. 2002; Wedlock et al. 2005). These studies have usually involved administration by injection as proof of principle, but some work has also explored alternative means of delivery, such as by nasal, conjunctival

Box 6.1 Echinococcus treatment in foxes

Recent studies in Europe, Asia and North America have revealed that the zoonotic tapeworm, *Echinococcus multilocularis*, has a far wider geographic distribution in carnivores (predominantly foxes) than previously thought. In Europe, growing red fox *(Vulpes vulpes)* populations and their increasing colonisation of urban areas, may potentially represent an emerging hazard to public health. Therefore, the development and implementation of effective methods of disease control and prevention are required. *E. multilocularis* is typically perpetuated in a wildlife host community, which includes foxes (genera *Vulpes* and *Alopex*) as definitive hosts and various rodent species as intermediate hosts. Humans can accidentally ingest the eggs, which hatch, and the larval stages (metacestodes) then usually enter the liver but can spread to other organs, and can lead to potentially fatal alveolar disease. Risk factors for alveolar echinococcosis may include occupational and behavioural activities. Areas of eastern France with high water vole (*Arvicola terrestris*) densities yielded a ten-fold higher risk of human alveolar echinococcosis compared to those with low densities of this important intermediate host (Viel et al. 1999). In an area where *E. multilocularis* was endemic, as many as 39% of water voles and 7% of domestic dogs with free access to rodents were infected (Gottstein et al. 2001). Red foxes are likely to be the most important definitive hosts in many regions. In the past two decades, foxes have started to colonise cities around the world, and evidence is growing of a perpetual parasite life cycle in urban areas.

Few field studies focus on anthelmintic treatment of definitive hosts. In rural areas of Germany and Japan, baits laced with praziquantel (an anthelmintic) lowered the prevalence of *E. multilocularis* in foxes, although rapid recovery of the disease was also observed (Hansen et al. 2003), suggesting that prolonged repeated treatment may be necessary (Tsukada et al. 2002). Until recently no attempt had been made to evaluate the treatment of foxes in urban areas. In an experimental field study in Zurich, Switzerland, the effects of anthelmintic baits were investigated in urban areas where the organism was endemic (Hegglin et al. 2003). Over a 19-month period, 50 baits containing praziquantel were distributed per km^2 every month in six 1 km^2 areas and one 6 km^2 area. By the end of the trial, the proportion of fox faecal samples that were antibody positive to *E. multilocularis* had decreased significantly in all the baited areas. *E. multilocularis* prevalence in the intermediate host (water vole) also decreased significantly in treated areas. This experimentally controlled study suggests that a pronounced reduction in *E. multilocularis* egg contamination is achievable by treating foxes in urban areas where the organism is endemic.

(Corner et al. 2002; Corner and Buddle 2005) or oral (Aldwell et al. 1995b; Qureshi et al. 1999; Aldwell et al. 2003b; Wedlock et al. 2005; Buddle et al. 2006b) routes.

Vaccination of bison (*Bison bison*) and elk (*Cervus elaphus*) against brucellosis (*Brucella melitensis*) has been considered in the USA, using vaccines (either the S19 or the RB51 strains) administered by hand or contained in ballistic capsules. However, the S19 strain was not as effective in bison as it was in domestic cattle, and the RB51 strain caused inflammation of the placenta and spontaneous abortions. The release of live *Brucella* vaccine strains in wildlife is therefore of concern as it could lead to environmental contamination and infection of other wild species (Godfroid 2002; Olsen et al. 2002; Olsen et al. 2006).

Parenterally administered (injected) vaccines have been tested on a number of wild mammal species. These were either experimental studies or interventions with a follow up investigation, or actions performed for conservation reasons without any subsequent monitoring of individuals. Examples where useful data on the effects of vaccination were recorded include studies on anthrax in cheetahs and black rhinoceros (*Diceros bicornis*), (Turnbull et al. 2004), pasteurellosis in bighorn sheep (Kraabel et al. 1998), rabies in the Ethiopian wolf (*Canis simensis*) (Haydon et al. 2006) and myxomatosis and rabbit haemorrhagic disease (RHD) in European rabbits (Calvete et al. 2004b). Beneficial effects were reported in all these studies. In contrast, the control of anthrax (injection by hand, or by dart from an aircraft) in buffalo, black and white (*Ceratotherium simum*) rhinoceros, roan antelope (*Hippotragus equinus*) and hippopotamus (*Hippopotamus amphibius*) (Clegg et al. 2007), of morbillivirus infection in seals (*Phoca vitulina* and *Monachus monachus*), and of distemper in the endangered black-footed ferret (*Mustela nigriceps*) in the USA (Moutou, 1995) took place in the interests of conservation, but with no follow up of individuals.

Experimental oral vaccination by direct dosing has been demonstrated to protect black-tailed prairie dogs (*Cynomys ludovicianus*) against sylvatic plague (*Pasteurella pestis*) (Creekmore et al. 2002; Mencher et al. 2004; Morton et al. 2004) and wild rodent reservoirs of *Borrelia burgdorferi* against Lyme disease (Tsao et al. 2004; Gomes-Solecki et al. 2006; Scheckelhoff et al. 2006). Oral baits have been successfully employed in the experimental vaccination of feral pigs and wild boar against pseudorabies using a live recombinant vaccine. Recent work has indicated that abrasive agents in bait may enhance uptake of live vaccines by allowing them to penetrate the tissues of the buccal cavity (Edmonds et al. 2001).

The current body of evidence demonstrates the potential for vaccination to make significant contributions to the future management of disease in wild mammals. Although approaches involving the capture or darting of individuals may always be limited by the high levels of effort and costs involved, the delivery of vaccines in oral baits lends itself to larger-scale deployment. It is likely that in the near future vaccination will play an increasing role in the management of wildlife diseases other than rabies and CSF, with bovine tuberculosis looking to be at the top of that list.

6.4.2 Advantages and Disadvantages

If the practical challenges of delivering a vaccine to wild mammals can be overcome (see Section 6.4.4), and successful immunisation of the required number of animals is achieved, then this approach may offer a viable alternative to culling hosts. For this reason vaccination strategies are frequently advocated by conservationists, animal welfare groups and the general public. However, other considerations that need to be met include demonstration that the vaccine is either safe in, or unavailable to, non-target species (including humans) and is environmentally benign.

When evaluating candidate vaccines to be considered for use in wildlife, it is live vaccines that pose the most questions. Reversion to virulence has to be addressed as part of the licensing procedure by sequential passage in the target species (but see Section 6.4.3.1). Exposure of non-target species also needs to be considered. However, since experimental studies may be difficult to conduct it is important to understand the nature of the attenuation to inform an assessment of the likelihood of the vaccine strain posing any safety risk. Given the limitations of the analyses required to achieve marketing authorisation for a vaccine, the risks associated with its use in the field can only ever be minimised, not removed.

Vaccination of a wild host population may have significant long-term ecological consequences. By reducing the rate of disease-induced mortality for example, vaccination may have the effect of increasing host population size, and altering demographic structure and processes. This could have potential 'knock-on' effects for the wider ecological community, including for instance predator, prey and vegetation communities. A further concern regarding the long-term use of vaccines in wildlife populations is that protecting hosts from the selective pressure of infection, may remove selection for natural resistance to diseases (Woodroffe 2001). As a consequence, vaccinated populations could potentially become more susceptible to infection in the future, particularly after the vaccination campaign stops. Nevertheless, highly virulent infections such as rabies induce very low levels of natural immunity in most host species, and so in this instance the costs of vaccination in terms of loss of selection pressure, would be relatively small. On the other hand, these costs could be significant in the case of less virulent infections, which induce higher levels of natural immunity.

The financial costs of vaccination may increase substantially in the long term if the disease is not rapidly eradicated, although this would need to be weighed up against any benefits accruing from the level of disease control achieved (see Chapter 5). Rising costs may be a particular issue for chronic diseases such as tuberculosis, which may take many years of vaccination before they are eliminated. The sustainability of the long-term use of both medication and vaccines is therefore an important consideration that requires careful evaluation before any programme is implemented, particularly in the case of pathogens of low virulence.

The use of vaccines in wild mammal populations has not been without controversy. This has been most frequent where vaccination has required that animals are

handled, which is known to incur risks of stress-induced mortality (Arnemo et al. 2006). After the disappearance of African wild dogs (*Lycaon pictus*) from the Serengeti National Park, it was argued that the stress associated with handling during anti-rabies vaccinations may have reactivated quiescent disease and caused increased mortality (Burrows 1992). However, subsequent field data and a review of the available evidence suggested that this was unlikely to have been a contributory factor (De Villiers et al. 1995; Woodroffe 2001). The handling of rabbits during vaccination campaigns against myxomatosis was also suggested to be detrimental, as young and sub-adult vaccinates exhibited enhanced rates of mortality during the first week after handling (Calvete et al. 2004a). Hence, the potential impact of capture-related stress and myopathy should be fully considered for any proposed vaccination campaign in which it is necessary to trap, restrain or handle the wild host.

6.4.3 Characteristics of Vaccines

The required properties of a vaccine will vary according to the characteristics of the pathogen and host. In the case of rabies**,** the vaccine must be delivered as a live modified virus or a live vector (e.g. vaccinia virus) because the immune reaction can only develop if the vaccine strain multiplies in the oral mucosa. In addition, the characteristics of the vaccine will be required to comply with prevailing legislation and guidelines for best practice. In Europe, vaccines intended for wildlife must fulfil all the requirements of the European Pharmacopoeia, which is a list of pharmaceutical substances and associated quality standards expected by the European Directorate for the Quality of Medicines (EDQM 2008). Recommendations on vaccine safety are also published by the World Health Organization (WHO 2008a).

To date, only three diseases have been targeted by vaccines that were either developed or adapted specifically for use in wild animal populations, the best known example being rabies. A number of different oral rabies vaccines, attenuated by repeated passage, have been produced. These may have one or more mutations that affect their virulence and pathogenicity. In general, the more mutations in a strain the less likely it is to revert to being pathogenic. These vaccines have been used in foxes and raccoon dogs in France, Belgium and Switzerland since 1985, with no major problems reported (Brochier et al. 1996; Aubert 2003; Cliquet et al. 2006). Since 1985 VRG has been used to vaccinate foxes and other carnivores against rabies in France, Belgium, Canada and the USA also without any problems (Blancou et al. 1986; Blancou et al. 1988; Blancou et al. 1992; Aubert 2003) (but see Section 6.4.3.1).

Classical Swine Fever vaccines consist of live attenuated strains of either CSF or another virus (e.g. bovine viral diarrhoea virus or adenovirus) that has been genetically engineered to carry the main immunogen (E2) of the CSF virus. Most conventional live attenuated CSF vaccines, including that contained within the German vaccine bait, are based on the C-strain. Recently, a chimeric Pestivirus (CP7_E2alf) has been developed and is being studied for oral vaccination against

CSF. Although no specific requirements have been defined for oral CSF vaccines in Europe, they must fulfil the general requirements of the European Pharmacopoeia regarding safety and efficacy, and of the relevant European Directives (European Directive 2001/82 as amended by Directive 2004/28).

The BCG vaccine against tuberculosis is a live attenuated bacterium that needs to be delivered to the host in a viable state in order to generate effective protection (Buddle et al. 1997; Skinner et al. 2005). This poses a substantial challenge for oral delivery in particular, as it requires that the immunising bacilli remain viable during formulation, storage and deployment in bait, as well as retaining viability in the host up to the point of immune induction, and ensuring that the consequent immune response is sufficient to confer protection (Cross et al. 2007b). If BCG is incorporated into a lipid matrix, it can be stored in a live state for weeks to allow distribution in the field (Aldwell et al. 2006). However, this matrix may require some modification to transform it into an attractive and palatable bait for the target species, and the time period needed for BCG stability has to take into account the time taken for batch testing and distribution. The steps being taken to evaluate the use of BCG in badgers in the UK and the Republic of Ireland are described in Box 6.2.

Other vaccines (e.g. anthrax, brucellosis, distemper, myxomatosis, pasteurellosis, RVHD) have been licensed for use in domestic animals and can be obtained from commercial sources, although their safety and efficacy in wild mammals cannot be guaranteed until the necessary studies have been performed in the target species. Novel types of vaccine that are considered to have potential for use in wildlife disease control include modified live bacterial vectors, plant-derived vaccines and DNA expressing protective antigens (Cross et al. 2007a). However, despite encouraging results obtained *in vitro* or in laboratory animals, none have been tested in wild mammals, and they are unlikely to be available in the near future.

6.4.3.1 Safety

In general the safety of candidate vaccines for use in wildlife is first assessed in laboratory animals and then in the target species in captivity, before being evaluated in the field. Further investigations may involve wild or domestic species that are likely to be exposed to the vaccine, particularly when it is to be delivered in bait. Safety studies will be required in order to obtain marketing authorisation for use of the vaccine. Establishing the safety of candidate vaccines in both target and non-target species is an essential early stage in the development of a vaccination strategy for wild mammals. Experimental studies on the safety of anti-brucellae vaccines in wild bison demonstrated viral shedding, chronic infection and vaccine-induced abortions (Godfroid 2002), which may indicate either that the correct dose is critical for protection, or that the vaccine is not suitable in bison. However, the licensing authority will take into consideration the risk-to-benefit ratio on a case-by-case basis when determining whether to grant a marketing authorisation. Consequently they may grant a licence subject to certain conditions and restrictions, and may

Box 6.2 Development of a BCG vaccine for badgers

The Eurasian badger (*Meles meles*) represents a wildlife source of recurrent *Mycobacterium bovis* infection to cattle in Great Britain (GB) and the Republic of Ireland and its vaccination against bovine tuberculosis (bTB) with BCG (Bacille Calmette-Guérin) is an attractive disease control option in both countries. BCG has the advantage of a long history of safety and efficacy in a variety of animal species (Murphy et al. 2008).

Safety of BCG (the Danish 1331 strain) was first demonstrated in captive badgers in a GB study (Lesellier et al. 2006a). Badgers were vaccinated with two consecutive doses of BCG via either the subcutaneous or intramuscular routes. The first dose was high ($16–22 \times 10^7$ colony forming units (CFU)), representing between 20 and 1,100 fold the actual target dose, and was followed 15 weeks later by a lower dose (of $4–7 \times 10^5$ CFU). The vaccine was tolerated well, with the only observed effect being localised swelling at the site of BCG injection, which disappeared 48 days after intramuscular vaccination but persisted at least three times longer in those vaccinated subcutaneously. Strong cellular immune responses were observed 13 days after the first vaccination, which persisted for at least 76 days. The lower dose induced a weaker and shorter-lived response.

There are active R&D programmes in both GB and the Republic of Ireland aimed at obtaining marketing authorisation for the use of BCG in badgers. As a starting point to both programmes, the Danish 1331 strain of BCG is being used as it is manufactured in an EU Good Medical Practice (GMP) facility, and is already licensed for use in humans. As such, essential quality and analytical data are already available for inclusion in a marketing authorisation.

Having been demonstrated as safe when administered to captive badgers, work in GB has progressed to evaluation of the vaccine in a small-scale (55 km^2) field study. Permission to conduct the study was granted by the Veterinary Medicines Directorate (the UK veterinary medicines licensing body) in the form of an Animal Test Certificate, following submission of a summary of the quality data, a report on the GLP (Good Laboratory Practice) safety study and a detailed study protocol. The study started in 2006 and should be concluded by 2010. It is conducted according to the principles of Good Clinical Practice (GCP) (EMEA 2000), and has two specific aims: (a) to confirm the safety of BCG Danish 1331 previously demonstrated in the GLP safety study, when given intramuscularly to wild badgers at a dose of $2–8 \times 10^6$ CFU; and b) to investigate the immunogenicity and efficacy of BCG in wild badgers. These data will indicate the potential for investigating the likely benefits of widespread badger vaccination with BCG.

In parallel with the GB studies, protocols for the experimental infection of captive badgers by endobronchial instillation of *M. bovis* were developed in Ireland (Corner et al. 2007). These have been used to demonstrate the efficacy of BCG vaccine delivered via a number of routes, including subcutaneous, nasal/conjunctival, and oral (Buddle et al. 2006a; Lesellier et al. 2006b). Equivalent studies are underway in GB using either the intramuscular or oral

routes of administration, in order to generate definitive efficacy data and define the lowest efficacious dose that might be used, thereby keeping the cost of the vaccine to a minimum.

A combination of the safety and efficacy data derived from studies with both captive and wild badgers, together with quality and analytical data on the vaccine, will form the bulk of the application to obtain a Marketing Authorisation for the intramuscular administration of BCG to badgers. Possible applications for the use of the injectable vaccine in the UK and Ireland are being considered by the respective Governments. However, it is broadly recognised that the application of an injectable vaccine will be significantly restricted by the cost and practicalities associated with its delivery in the field. Nonetheless, data obtained with an injectable form of BCG in badgers would build confidence in the possible performance of a future oral bait form of the vaccine.

Delivery of BCG in oral bait holds the best prospect for vaccinating badgers over a wide geographical area. However, as a live replicating vaccine, BCG has the limitation of little to no efficacy if delivered orally in a non-viable state (Skinner et al. 2005). This is exacerbated in the case of oral delivery, by inactivation in the low pH environment of the stomach (Aldwell et al. 1995a; Buddle et al. 1997; Skinner et al. 2005). Recent advances in the formulation of BCG for oral vaccination of possums *(Trichosurus vulpecula)* in New Zealand (Aldwell et al. 2003b) are being exploited for the vaccination of badgers. These studies are at an early stage but encouraging results are being obtained with BCG delivered in a lipid matrix (Lesellier et al. 2006b). An additional challenge remains in identifying a suitable bait that is compatible with the BCG formulation, and that has the optimal properties of attractiveness, palatability, and stability in the field, whilst complying with all pertinent legislation (see Section 6.4.4). Assuming all these criteria can be met, the method of delivery in the field may have an even greater impact on the success of any oral vaccination campaign than the choice of the bait itself (Cagnacci et al. 2007).

Alongside the vaccine development studies, supporting work in the UK and Ireland has resulted in a range of immunological tests for the badger (Goodger et al. 1994; Dalley et al. 1999; Southey et al. 2002; Greenwald et al. 2003; Kämpfer et al. 2003; Sawyer et al. 2007; Dalley et al. 2008), some of which are being used to monitor the responses of captive and wild badgers to vaccination and challenge. Whilst not strictly necessary for the monitoring of vaccine success, either during the development or implementation phases of a vaccine programme, a sensitive, non-invasive test (Dalley et al. 2008) has been instrumental in establishing the TB-free status of badgers brought from the wild into captivity. As well as the health and safety benefits associated with this screening, experimental efficacy data must be obtained from animals initially free of the disease of interest. A lack of suitable immunological or other tests for determining disease status of the target species may significantly hamper efforts to develop and license vaccines.

subsequently ask for more data to be generated in particular species, or may refuse the application altogether.

During the development of rabies vaccines for wildlife**,** safety was assessed in laboratory animals, and in both target and non-target wild species. In laboratory studies, the live attenuated SAD-B19 strain rabies vaccine was harmless in all but a few rodent species (Vos et al. 1999), which suffered from residual pathogenicity but no viral excretion. Safety in the field was first tested in small mammals on an island and subsequently in an isolated valley in Switzerland, without any evidence of rabies-induced cases or of uncontrolled spread of the attenuated virus. Since 1985, this and the related SAD-P5/88 strain have been used in several Western European countries with no reported adverse effects. Other strains (SAG1 and SAG2) have subsequently been derived from the SAD-Bern strain and their safety has been demonstrated in laboratory mice, wild rodents and monkeys, before deployment in the field (Coulon et al. 1992). In raccoon dogs direct instillation or delivery in oral bait of at least ten times the field dose of the SAG2 vaccine strain resulted in seroconversion and all animals remained healthy (Cliquet et al. 2006). However, the use of attenuated live rabies vaccines has in some instances resulted in disease in some vaccinates (e.g. Fehlner-Gardiner et al. 2008), and although there is no evidence that attenuated vaccines have reverted to a virulent strain and subsequently spread, this may remain a possibility.

The safety of VRG has been tested in laboratory animals, wild rodents and a wide variety of non-target species. Safety in wild non-target species was demonstrated in trials where baits containing the vaccine were deployed in fenced enclosures of varying sizes. Although VRG is now widely recognised as presenting no hazard to humans or non-target species, a reported instance of a mild pox infection in a pregnant woman after contact with VRG bait (Rupprecht et al. 2001) demonstrates that there will always be some, albeit small, residual risk.

In the case of CSF vaccines**,** the safety of the live attenuated C-strain virus used in bait, was experimentally assessed in the laboratory in mice, rabbits, foxes, domestic pigs, goats and cattle (Kaden and Lange 2008). Safety in wild boar was assessed in both laboratory and field studies (Kaden et al. 2003). A ten-fold vaccine dose was administered in safety tests that were carried out before the release of vaccine batches. The safety of the vaccine candidate CP7_E2alf has been experimentally evaluated in cattle, sheep and goats. As this vaccine candidate represents a genetically modified pathogen, substantial further safety studies are likely to be necessary in order to obtain a marketing authorisation for use in wildlife.

6.4.3.2 Efficacy

Demonstration of the efficacy (i.e. effectiveness in protecting against infection and/or the consequences of infection) of a vaccine destined for use in wild animals is a required element of the application for any marketing authorisation. The data are most frequently generated from studies using captive animals that are vaccinated and then subsequently challenged with the pathogen. The results of such studies may be

supplemented with experimental data from field trials, and both form the basis for the claims made for the vaccine in the summary of product characteristics, the wording of which may be restricted and prescribed by licensing authorities. Unlike safety studies which must address the safety of an overdose of the vaccine (typically two times the field dose for non-living vaccines, and ten times for live vaccines), the efficacy of the vaccine should normally be demonstrated for the lowest possible dose, taking into account the potency or titre of the vaccine at the end of its shelf-life.

The immunogenicity and efficacy of rabies vaccines have been tested by antibody titration in target species (e.g. foxes, raccoons, raccoon dogs) and by the direct challenge of vaccinated animals and controls. In all instances the vaccinated animals resisted the challenge several months after vaccination (Brochier et al. 1996; Cliquet et al. 2006). Rabies vaccines have also been tested in susceptible non-target species, and shown to be less effective (e.g. VRG in badgers and striped skunks) (Brochier et al. 1989; Grosenbaugh et al. 2007).

The efficacy of CSF (C-strain) vaccine baits was investigated by challenging vaccinated domestic pigs and wild boar of different ages, and unvaccinated control animals. The studies demonstrated that animals that had received one dose of vaccine, whether in bait or by injection, were fully protected and did not develop clinical signs, viraemia (presence of virus in the bloodstream) or excrete virus (Kaden and Lange 2001). Oral vaccination of wild boar does not induce chronic infection, after either challenge of vaccinated pregnant sows, or infection of vaccinated non-pregnant animals. Efficacy of vaccination against CSF has been evaluated in relation to the prevalence of both antibodies and virus. Following the application of vaccination in a wild boar population, an increase in the proportion of antibody-positive animals (i.e. seroprevalence) in the hunting bag and a decrease in virus prevalence would be expected. However, the observed seroprevalence will not only depend on the performance of the vaccine but will also vary in relation to the composition of the hunting bag (see Box 6.3).

6.4.4 Vaccine Delivery

A variety of approaches have been considered for the delivery of vaccines to wild mammals. The most suitable mode of vaccine delivery will depend on the characteristics of the vaccine, the target species and the environment where it will be deployed. The two principle routes of vaccine administration are by injection (parenteral) and oral ingestion. Although rabies vaccines have primarily been delivered in oral bait, injection by hand has been used for vaccination of skunks and raccoons for a focal rabies outbreak in Canada (Rosatte et al. 1992). Similarly, intramuscular injection of BCG is likely to be the route of administration for the first licensed badger tuberculosis vaccine in the UK. As mentioned above (Section 6.4.1), there are now many examples of both parenteral and oral delivery of vaccines for species of conservation concern. During the early years of wildlife vaccination in the USA automatic injection devices were trialled, but have not been developed further

(Baer 1991). Dart guns have also been used to deliver vaccines to wild mammals in disposable darts, and in compressed pellets known as 'bio-bullets'. In the USA, vaccine darts have been used to immunise elk against brucellosis as they congregated on their feeding grounds (Wobeser 2002) and to deliver a vaccine against pasteurellosis to both bighorn sheep and elk (Cassirer et al. 2002). In Southern Africa, vaccine darts were delivered to antelopes from helicopter, to immunise them against anthrax (De Vos et al. 1973; Clegg et al. 2007) and in Canada bio-bullets were successfully employed for the vaccination of bison against brucellosis (Olsen et al. 2006).

Administration of vaccine to wild mammals via the oral route is usually achieved with an ingestible bait. The most successful examples are the rabies and CSF oral vaccine baits. Oral bait consists of two main components; the bait matrix, which is comprised of an attractive food, and the vaccine, which may be encapsulated within a protective capsule or substance. The bait matrix must obviously be attractive to the target species, and so a variety of imaginative formulations have been proposed as vehicles for vaccines, including eggs, meat, chocolate, polyurethane sponge and fishmeal. Flavourings and scented attractants can be used to enhance bait appeal. For example, synthetic fermented egg (the smell of rotting meat) appears to increase the rate of bait uptake by wild carnivores (Hunt et al. 2007). The ideal choice of bait matrix is usually determined by carrying out palatability studies, perhaps in captive animals initially, but should always be tested on the wild target species. For the main commercial rabies baits, there is little to choose between them in terms of bait acceptance (Smith and Woods 2007). Perhaps the most technically challenging aspect of bait formulation however, is ensuring that the vaccine remains stable during processing, storage and in the environment, and survives passage to its destination within the target animal. This may require encapsulation in some protective substance or structure. For example, large-scale rabies vaccination programmes have delivered liquid vaccine enclosed within a plastic capsule in either chicken heads, commercially produced tablets of ground meat (Blancou et al. 1991), or in blister packs. Rabies vaccine is believed to target the buccal cavity during mastication of the bait, and thus does not need to survive passage through the stomach.

A lipid-based formulation was developed in New Zealand for the oral delivery of BCG vaccine to brushtail possums, which permits survival of the vaccine through the stomach to the delivery site in the intestines. Use of the lipid-matrix allowed BCG to be retained in a viable, but static state for at least several weeks at ambient temperature (Aldwell et al. 2003a). In rodent models and brushtail possums, oral delivery of lipid formulations containing live BCG was shown to establish populations of viable, replicating BCG in the alimentary tract lymphatic system (Aldwell et al. 2005b; Wedlock et al. 2005), which in mice persisted for at least seven months post-vaccination (Aldwell et al. 2006). Voluntary uptake of the vaccine (which could be readily induced following flavouring of the lipid matrix) was shown to confer protection against virulent *M. bovis* or *M. tuberculosis* aerosol challenge in mice (Aldwell et al. 2003a; Aldwell et al. 2005a; Aldwell et al. 2006), and in possums and cattle against challenge to the respiratory tract with virulent *M. bovis* (Aldwell et al. 2003b; Buddle et al. 2005). The duration of protection after

oral vaccination was maintained for at least seven months in mice and 12 months in possums (Aldwell et al. 2006; Buddle et al. 2006b).

Baits containing rabies vaccine were first distributed in the field by hand in the 1980's in Europe, and this is still the case for vaccination campaigns targeting specific populations, such as during the initial stages of an outbreak. Distribution of vaccine baits by hand is also the method of choice for CSF vaccination of wild boar. Rabies vaccine baits are distributed at an average rate of 15 baits per km^2. For CSF vaccine distribution, 20 to 40 baits per km^2 (Kaden et al. 2005a and Box 6.3). Vaccines can also be delivered from aircraft, as has been the case for most broad-scale vaccination campaigns against rabies in France, Belgium, Switzerland and Germany. Aerial distribution of baits may also be considered for vaccination of wild boar against CSF if distribution by hand is impractical or uneconomic, such as in challenging habitats like extensive coastal reed beds. The overall objective here should be to deliver the minimum number of baits per unit area, but to still achieve the objectives of disease management. Delivery systems are now increasingly subjected to economic evaluation to identify the most cost-effective solution (see Box 5.1).

One important consideration in the development of baits for use in wildlife is the potential for legal restrictions on the deployment of certain substances in the environment. This is especially likely to be a factor where exposure to non-target livestock cannot be ruled out. For example in the UK, current legislation relating to disease risks from animal by-products, significantly restricts the nature of the materials that can incorporated into any bait that will be deployed in an environment where livestock are present.

6.4.5 Monitoring Success

There are three distinctly different but complementary approaches that can be used to monitor the success of a vaccination campaign. These are quantification of the rate of bait uptake, quantification of the rate of vaccine-related immune response in the target population, and evaluation of the epidemiological consequences of vaccination.

At its simplest, the evaluation of vaccine bait uptake may involve observation of the rate of bait disappearance. However, this is generally not a sufficiently rigorous method for monitoring the success of a vaccination campaign, since many baits may be removed by a single animal, or by non-target species. More robust information on uptake may be gleaned by impregnating baits with a biomarker of some kind, and subsequently sampling the target population for its occurrence. Examples include the antibiotic tetracycline, which is detectable in bones and teeth, rhodamine dye, which can be detected in hair and whiskers, and analogues of iophenoxic acid, which are detectable in blood. Tetracycline biomarkers proved useful during early rabies vaccine trials, in which they demonstrated widespread acceptance of bait amongst target (and sometimes non-target) species. In European studies it was estimated that

on average between 70% and 80% of baits were taken by red foxes (Blancou et al. 1988). A similar approach was taken to monitor bait uptake during the first field trial of oral baits for vaccinating wild boar against CSF in Germany. About 85% of baits disappeared within five days of deployment, and examination of the bones of shot wild boar identified the biomarker oxytetracycline (OTC) in 52 to 68% of individuals and indicated that uptake was high in areas where baits were buried but was low amongst juvenile boar (Kaden et al. 2000) (see Box 6.3).

Bait uptake rates may vary in response to external factors such as the availability of natural food or crops, and weather conditions. Also, in some populations it may take time for animals to become accustomed to taking a novel food source, so uptake rates may improve over time. The potential influence of such factors can be evaluated from appropriately designed field experiments using biomarkers.

The second way to evaluate the success of a vaccination campaign is to monitor the rate of immune response (e.g. presence of antibodies) resulting from vaccination. This is likely to require the collection of blood (or other body fluids) from a subsample of individuals, so that appropriate diagnostic tests can be performed to assess their immunological status. Following rabies vaccination campaigns, this approach identified rates of seroconversion in red foxes of 60% to 70% (Blancou et al. 1988). The deployment of CSF vaccine in bait in Germany was followed by the testing of all wild boar that had been shot, found dead or involved in road traffic accidents in the area for virus (by fluorescent antibody test, ELISA or Real Time-PCR) and antibodies (virus neutralising test or ELISA). This showed that rates of seroconversion differed considerably both spatially and between different age classes.

For some diseases, such as tuberculosis, the predominant immunological response is cellular rather than humoral, and thus, monitoring a serological antibody response is likely to miss a large proportion of vaccinated or infected animals. Although assays exist for measuring cellular immunity (Dalley et al. 2008), they are often considerably more time-consuming and expensive than antibody tests, which potentially limits their application for monitoring wildlife vaccination campaigns.

Immune responses induced by vaccines may potentially confound interpretation of the epidemiological situation, if no appropriate diagnostic tool is available with which to discriminate infected from vaccinated animals. So called DIVA (Differentiating Infected from Vaccinated Animals) methods have been successfully applied in the control and local eradication of Aujeszky's disease, infectious bovine rhinotracheitis, CSF, foot and mouth disease and avian influenza (Vannie et al. 2007). Such tests could potentially be applied more generally to the vaccination of wild mammals if sufficient information is known about the host immune response to vaccination and infection. DIVA tests however, are not required for all vaccination campaigns. For terrestrial rabies, animals generally only produce antibodies in the days immediately prior to death, so naturally seropositive animals are very rare in the population.

Finally, and perhaps most importantly, the success of any vaccination campaign can be assessed on the basis of its epidemiological consequences, and in particular the extent to which it reduces the incidence of disease in the target population. This requires that disease surveillance data is collected before, during and after vaccina-

tion campaigns, as was the case for red fox rabies in Europe. In 1989 the number of registered cases of fox rabies recorded in France peaked at 4,213. From the spring and autumn of 1992 onward, vaccine baits were distributed throughout the entire affected area (over 192,418 km^2) and as a result, the incidence of rabies diminished by about 60% each year until 1997, when it was finally eliminated (Aubert 2003). Similar figures were reported in other European countries following oral vaccination campaigns (Brochier et al. 1996).

As with any management intervention, monitoring is a vital component of a vaccination campaign. It not only provides hard evidence of success (or otherwise), but also permits a greater understanding of the epidemiology and logistics of disease control (see Box 6.3).

6.5 Conclusions

The effectiveness of any programme to vaccinate wild mammals will be a product of the proportion of animals that receive the vaccine and the proportion that become immunised. Hence, not only must a vaccine for wildlife be efficacious at the indi-

Box 6.3 CSF vaccination in wild boar

In Baden-Württemberg, Germany, vaccination of wild boar (*Sus scrofa*) against CSF began in 1999. The programme involved two deployments of baits containing vaccine every spring, summer and autumn until 2001. Hunting bags indicated that seroprevalence rates in wild boar were higher after these three seasonal campaigns than after vaccination in only spring and autumn. However, the hunting bags also revealed age-dependent variation in seroprevalence. During the first five seasonal vaccination campaigns, between 50% and 83% of adults (i.e. boar over 1 year old) were seropositive, compared to an average of only 45% of juveniles (i.e. those less than 1 year old) which decreased to approximately 30% thereafter. Further investigation showed that the proportion of antibody-positive young boar was less than 40% in the 3–5 month age group suggesting that these animals were largely responsible for the lower prevalence amongst juveniles (Kaden et al. 2005b). In further vaccination campaigns in Germany (e.g. in Saarland and Rhineland-Palatinate) and in France, post-vaccination seroprevalence was also lower amongst juvenile boar (Rossi et al. 2006).

Following three vaccination campaigns in France, CSF was still present in wild boar in the treated areas. This failure may have been related to poor uptake of vaccine baits, particularly amongst young animals (Rossi et al. 2006). Infected boar also remained following vaccination in North Rhine-Westphalia, although there was no indication of virus persistence in vaccinated individuals. One possible explanation was the vertical transmission of infection from sows to their offspring, although this phenomenon was not

(continued)

Box 6.3 (continued)

observed in a separate area with moderate infection pressure (North-Western Pomerania). Laboratory studies in which pregnant sows received a single oral vaccination, failed to demonstrate the transmission of virus to the foetus following experimental challenge. This indicates that transplacental virus transmission does not play a crucial role in the perpetuation of CSF virus in wild boar (Kaden et al. 2008). Rather, individual young wild boar that survive infection with moderately virulent virus, or partially protected piglets (e.g. animals with low maternal antibody titres), might exhibit a transient infection. The infrequent occurrence of persistently infected wild boar after post-natal infection, and the absence of infected foetuses in an experimental field study suggest that these are unlikely to be important routes of transmission. Rather, it seems likely that the high proportion of susceptible juvenile wild boar and population density are the crucial determinants of virus persistence.

The effectiveness of CSF vaccination and successful eradication of the disease in wild boar populations depend on several factors. Of principal importance is ensuring adequate provision of vaccine baits. Consistent results have been achieved employing 0.5–1 bait stations km^{-2}, each of which contained between 20 to 40 individual baits. This has been combined with population reduction achieved by hunting throughout the year, which is targeted at juveniles (i.e. <6 months old). To maximise the likelihood of local eradication of CSF in wild boar populations, vaccination should continue for at least one, if not two years after detection of the last CSF virus positive animal. During this period, all animals found dead, involved in road traffic accidents or shot should be the subject of virological and serological monitoring. Thereafter, surveillance for individuals at an early stage of infection should be carried out in wild boar populations.

vidual level, but it must also be delivered to a sufficient number of animals to impact on disease prevalence at the population level. This requires a clear understanding of the practical constraints that may be imposed by ecological factors. In addition, the vaccine must be safe for use in the host and in any non-target species that may be exposed to it. These issues represent considerable challenges to the development of effective vaccination programmes for wildlife, but the dramatic reduction in rabies incidence in Western Europe illustrates what is possible.

The most likely reason for the failure of an efficacious vaccine in wildlife is likely to be that it is not delivered to a sufficient proportion of the target population. For instance it has been suggested that an insufficient level of immunisation of the fox population against rabies allowed the infection to persist for longer in comparison with non-vaccinated areas (Smith and Harris 1989; Suppo et al. 2000), and may explain the resurgence of disease in suburban areas of Germany (Thulke et al. 2000). Sub-optimal vaccine coverage may arise if public or financial support for the campaign is inadequate, or if the treatment is not applied to a sufficiently large area.

It can also occur if the delivery of the vaccine fails to account for important aspects of host behaviour. For example, the social organisation and density of red foxes appears to have a key effect on the success of rabies control strategies involving culling, vaccination or fertility control (Smith and Wilkinson 2003). Thus optimum strategies can involve focal culling with ring vaccination in some circumstances (see Box 4.3). In order that mistakes are not perpetuated, and to enable vaccination strategies to be adapted when necessary, some form of monitoring is crucial during interventions. This should also be sufficient to identify the pre-determined conditions that will indicate that the objective of disease control or eradication has been achieved, and the campaign can end (see Chapter 9).

Whilst vaccination is often seen as one of the most attractive wildlife disease control options, it is not without its potentially undesirable side effects. Vaccines, bait compounds and methods of deployment can be potentially harmful to target or non-target species. Attenuated "live" vaccines can induce infection in species for which the vaccine has not been developed; LEP (low egg passage) rabies vaccine is known to induce rabies in several non-canids, and similarly live canine distemper vaccines can be problematic for highly susceptible mammal species (Griot et al. 2003). Consequently, although their use for the protection of small populations of African canids has been considered, these two vaccines should probably be disregarded (Laurenson et al. 2004). It is essential that the potentially negative effects of direct medication and vaccination are always thoroughly and systematically evaluated prior to their deployment in free ranging wildlife. In this regard there is no substitute for rigorous scientific investigation and economic evaluation (see Chapter 5) of any vaccine and proposed programme of deployment.

Intervention targeting the pathogen in the host population is aimed at achieving the ultimate goal of preventing inter-individual transmission, such that the pathogen eventually dies out. Basically, that goal is achieved when R (the effective reproduction number) is reduced below unity (see Chapter 3). There is evidence that R is influenced by risk factors related to host ecology, behaviour, density, phenotype (mass immunity), and host and parasite genotypes (Woolhouse et al. 2005). Hence we may speculate on the potential ecological and evolutionary consequences of reducing R below unity through the application of vaccines.

Parasites and pathogens can influence ecosystem structure and processes, and as a consequence, the control of pathogens in natural systems can have far-reaching consequences. For example, where a pathogen limits host abundance, then vaccination may lead to an increase in host population size which will in turn impact on other components of the ecological community (see also Section 7.3.2). It has been suggested that oral vaccination of foxes against rabies in Europe may have facilitated the spread of echinococcosis, although the role of rabies in limiting fox populations is not proven (Chautan et al. 2000). It is thought that some pathogens may effectively mediate competition between species (Hudson and Greenman 1998), in which case vaccination might theoretically enable a previously suppressed host species to become dominant.

Recent years have seen growing research interest in a new generation of vaccines. Genetic engineering techniques offer increasing opportunities to develop live vac-

cines with specific characteristics that can spread between hosts and so enhance coverage. However, these opportunities bring with them significant risks: transmissible vaccines could spread into non-target species or populations, with unexpected results. In Australia, an engineered myxomavirus has been proposed to control the fertility rate of introduced rabbits, but in parts of Europe rabbits are considered as desirable for hunting and biodiversity. Consequently, in Spain, hunters consider that rabbits should be immunised against RHD, and so a myxomavirus modified to express RHD genes has been developed as a vaccine (Angulo and Cooke 2002). Such opposing uses of the same technology demonstrate the potential risks of translocation of one such vaccine outside of its intended range. Enthusiasm for the development and application of such vaccines for the control of diseases in free-ranging animals should be tempered with a critical appraisal of the associated risks.

In conclusion it seems unlikely that direct medication of wild mammals will be an appropriate approach for the management of disease outbreaks in wild mammals in all but a minority of specific circumstances. Nevertheless, it will continue to be an important routine tool in the rehabilitation and translocation of mammals. Vaccination on the other hand, has already demonstrated its ability to eliminate disease from wild mammal populations over extensive areas. This is largely the result of the highly successful application of oral rabies vaccines. The results of current attempts to manage CSF in wild boar and the ongoing development of a TB vaccine for badgers will demonstrate over the coming years whether similar success can be achieved with other diseases of wild mammals.