Effects of vaccination against viral haemorrhagic disease and myxomatosis on long-term mortality rates of European wild rabbits

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The effects of vaccination against myxomatosis and viral haemorrhagic disease (VHD) on long-term mortality rates in European rabbits (*Oryctolagus cuniculus*) were studied from 1993 to 1996 by radiotracking a free-living population of wild rabbits. During the three months after immunisation, unvaccinated young rabbits weighing between 180 and 600 g were 13·6 times more likely to die than vaccinated young rabbits. In adult rabbits, vaccination did not significantly decrease mortality, mainly owing to the high proportion of rabbits which had previously been exposed to the antigens of both diseases. Compared with adult rabbits with natural antibodies to VHD, rabbits without these antibodies were 5·2 times more likely to die of VHD during annual outbreaks.

THE European wild rabbit (*Oryctolagus cuniculus*) is one of the most important vertebrate prey species in Spanish Mediterranean ecosystems, and several predator species threatened with extinction, including the Iberian lynx (*Lynx pardina*) and the imperial eagle (*Aquila adalberti*), depend on high-density populations of rabbits (Delibes and Hiraldo 1981). Myxomatosis, which first appeared in Spanish rabbit populations in the 1950s, caused a substantial reduction in their population density and significant changes in their distribution throughout Spain (Muñoz 1960). Further reductions in the abundance of rabbits were observed after viral haemorrhagic disease (VHD) arrived in Spain in 1988 (Argüello and others 1988), and when this disease became enzootic in wild populations (Calvete and others 2002) many of the populations continued to decrease, some to the point of extinction (Villafuerte and others 1995).

Since both myxomatosis and VHD have had a substantial impact on wild rabbit populations (Arthur and Louzis 1988, Villafuerte and others 1994, Cooke 1996, Marchandeau and Boucraut 1999, Calvete and others 2002), efforts have been made to revive these populations by vaccinating rabbits against these viral diseases. This procedure has been authorised by veterinary authorities, and the vaccination campaigns have required the capture of large numbers of wild rabbits every year throughout Spain. Traditionally, rabbits are captured by trapping or ferreting, vaccinated with commercial vaccines against both diseases and then released at the site where they were captured. However, the success of these vaccination campaigns is thought to be negligible. In an effort to enhance the effectiveness of vaccination, a new generation of recombinant vaccines has been developed (Bertagnoli and others 1996, Castañón and others 1999, Fernández-Fernández and others 2001, Torres and others 2001); some of the recombinant viruses are designed to be transmissible, and it is hoped that in future they can be used in the wild. However, the long-term effects of vaccination on the survival of wild rabbits have not been examined, and there are few data about the efficacy of previous vaccination campaigns.

In response to the decline in wild rabbit populations in Spain since the arrival of VHD, a three-year field study of the epidemiology of myxomatosis and VHD was conducted by using radiotelemetry (Calvete and others 2002). At the same time, the effects of the vaccination campaigns against myxomatosis and VHD, carried out using the protocol traditionally applied in Spain, were investigated. This paper describes the effects of vaccination against both diseases, in a natural population of European wild rabbits, on their long-term mortality rates.

MATERIALS AND METHODS

Study area

The study was performed in the Central Ebro Valley, Zaragoza Province, north-east Spain, an area characterised by a temperate continental Mediterranean climate with little rain (yearly average 350 mm). The wild rabbit population was located in a 250 ha area representative of the Mediterranean ecosystem of the valley. The landscape consisted of low hillocks interspersed with small fields of wheat and barley. The natural vegetation was sparse steppe scrub with species such as *Genista scorpius* (scorpion's thorn), *Rosmarinus officinalis* (rosemary) and *Thymus* species (thyme), which were restricted to the hillocky regions where rabbit warrens were abundant.

Data collection

Between November 1992 and June 1996, wild rabbits were live-trapped to monitor the populations' pattern of mortality (Calvete and others 2002). The captured rabbits were sexed and weighed; each was placed inside a cloth bag, and a 1·5 ml blood sample was obtained from an incision of the auricular marginal vein and stored in glass tubes without anticoagulant (Calvete and others 2002). The blood samples were left to coagulate at room temperature, centrifuged, and the sera were frozen at –20°C and sent to a laboratory (Hipra SA) to determine the antibody concentrations against VHD and myxomatosis by ELISA (Pagés and others 1991). The antibody concentration was expressed in terms of a relative index of immunity (RI), with values ranging from 1 to 10, and sera with an RI of 2 or more were scored as positive. Blood samples were not taken from rabbits weighing less than 300 g (aged up to approximately eight weeks) to avoid possible interference from maternal antibodies and the possible risk to their survival.

Randomly selected rabbits were injected subcutaneously with commercial vaccines against myxomatosis (POX-LAP; Ovejero) and VHD (CYLAP-VHD; Sobrino-Cyanamid) at the doses recommended for domestic rabbits.

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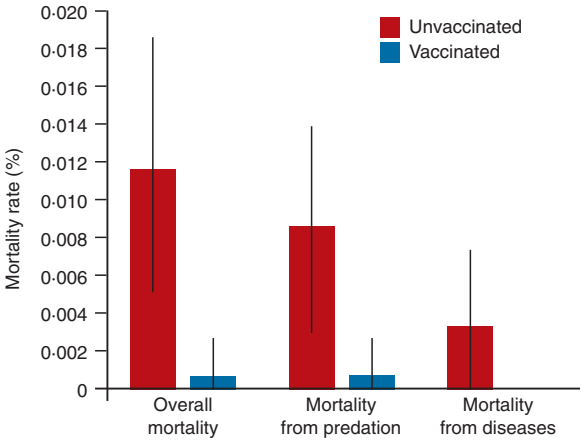

FIG 1: Estimated daily mortality rates (mean proportion of young rabbits dead in a day) for young unvaccinated and vaccinated rabbits. Confidence intervals were set at 95 per cent

Rabbits found dead were examined postmortem, primarily for lesions due to VHD or myxomatosis. When possible, a direct haemagglutination (HA) test was applied to detect VHD antigens in liver tissues. The tests were carried out by a micromethod by the same laboratory that conducted the antibody tests.

Radiotracking

Each rabbit was marked with a transmitter equipped with an activity sensor (Biotrack) attached to 20 g radiocollars, except for those weighing less than 600 g, which were tagged with 5 g radiotags on an ear. Each rabbit was located once a day during the first eight days after its capture and release, and at least once every three days throughout the remaining period of the survey. Each localisation consisted of determining whether the rabbit was alive or dead and, if dead, the cause of death. The possible outcomes were mortality by predation or disease. When rabbits died inside a warren, and it was not possible to recover the carcase, disease was presumed to be the cause. Data obtained during the first week after the rabbits were tagged were excluded from the analysis because there may have been a temporary increase in mortality due to handling and adapting to the radiotags (Berteaux and others 1994, Cypher 1997, Gil 1999).

Data analysis

The rabbits were classified into two age groups; young rabbits were those born in the same year and having a bodyweight between 180 and 600 g, and adult rabbits were those born in previous years.

Young rabbits were trapped and radiotagged from February to the end of April each year, during the breeding season, except in 1996, when no young rabbits were tagged. Because all these rabbits were tagged with radiotags on their ears and the lifespan of these light transmitters was approximately four months, the radio data were collected for only 90 days from the start of the survey period. In all, data from 34 young rabbits (16 female and 18 males) were included in the analysis. Nineteen of the rabbits (nine females and 10 males) were vaccinated against both myxomatosis and VHD, but the other 15 rabbits (seven females and eight males) were not vaccinated. Owing to the low bodyweight of most of these young rabbits blood samples were not taken, and their antibody titres are therefore unknown.

For the young rabbits, a Cox's proportional hazard regression model for censored data was used to estimate the association between the risk of dying and various independent variables over the 90-day survey period (Hougaard 2000). Bodyweight, vaccination and sex were included in the initial set of independent variables as fixed effects. To control for variations between years, this variable was introduced into the model as a random factor (Therneau and Grambsch 2001). The final model was derived by means of a backward selection procedure based on the likelihood ratio test (LRT).

Adult rabbits were assumed to have a high prevalence of antibodies to myxomatosis. As a result of the annual mortality due to VHD experienced by adult rabbits, the analysis was focused on the efficacy of vaccination against the disease, and only data obtained during annual periods of mortality due to VHD were considered. In most years, they occurred during January and February, except for 1996, when the period of mortality was during April and May (Calvete and others 2002). In each period, the time that elapsed between the first and last radiotagged adult rabbit found dead of VHD ranged from five to 30 days. To test the efficacy of vaccination, the annual period during which there was a risk of death due to VHD was defined conservatively as the 60-day interval centred on the middle day of the annual period of mortality. Data for tagged rabbits found dead between the annual periods at risk of VHD and data for adult rabbits tagged within the annual periods of risk were not included in the analysis. For rabbits that remained alive through several annual periods of mortality due to VHD, only data from the first period after tagging were considered.

Data for the adult rabbits were analysed by fitting a Cox's proportional hazard regression model, following the same selection procedure as for the young rabbits. Year was controlled as a random factor, and sex, vaccination, the prevalence of antibodies to VHD and myxomatosis, and the interval in days between vaccination and the start of the annual risk period (ranging from seven to 386 days) were included in the initial set of independent variables as fixed effects. Second-grade interactions between vaccination and the prevalence of antibodies, and between vaccination and the interval between vaccination and the start of the annual risk period, were also included.

RESULTS

Young rabbits

Of the 19 unvaccinated young rabbits, eight died from predation and three from disease. Of the latter, two died inside their warrens and could not be recovered; the third had gross lesions compatible with VHD, although a certain laboratory diagnosis could not be made owing to the poor condition of the carcase. Among the 15 vaccinated rabbits, no disease-related mortality was observed and only one died from predation. The mortality due to both disease and predation were higher in the unvaccinated than in the vaccinated young rabbits (Fig 1). The Cox's regression model fitted to the data from these animals (R^2=0·29; LRT=11·8; P<0·001) showed that the unvaccinated rabbits were 13·6 (se 2·6) times more likely to have died (P<0·013) during the 90-day period of the survey. The rabbits' sex and bodyweight were not associated with the risk of death.

Adult rabbits

In all, data for 84 adult rabbits (48 females and 36 males) were obtained, 32 vaccinated and 52 unvaccinated. Of the 32 vaccinated rabbits, 31 (96·9 per cent) had serum antibodies to myxomatosis and 17 (53·1 per cent) had serum antibodies to VHD at the time of capture. Of the 52 unvaccinated rabbits, 48 (92·3 per cent) were seropositive against myxomatosis and 25 (48·1 per cent) were seropositive against VHD.

Among the 52 unvaccinated adult rabbits, three died from predation, and disease was considered to have been the cause of death of 10; of the latter, four died inside their warrens and only one of their carcases could be recovered, because of the size and complexity of the warrens; the other three were assumed to have died of disease. Postmortem examination of the seven carcases recovered revealed obvious lesions com-

patible with VHD in all of them. Two had antibodies to VHD, with RIs of 4 and 6, when they were tagged five and six months, respectively, before they died. VHD antigen was detected by the HA test in three of the four carcases found in good condition.

Of the 32 vaccinated adult rabbits, four died from predation and only one died from disease; when it was tagged, 40 days before it died, this rabbit was seropositive against VHD with an RI of 3. At postmortem examination, it had gross lesions compatible with VHD and VHD antigen was detected by the HA test.

In both the vaccinated and unvaccinated rabbits, mortality was higher among those which were seronegative for VHD when they were tagged than among those which were seropositive. However, vaccination slightly reduced the mortality among the VHD-seronegative rabbits (Fig 2). The Cox's regression model (R^2=0·11; LRT=9·86; P=0·006) showed that the seronegative rabbits were 5·2 (1·9) times more likely to have died during the annual periods of peak VHD mortality (χ^2=6·73; P=0·009) than the seropositive rabbits, but there was no statistically significant association between the risk of death and vaccination or the interval since vaccination. There was also no association between the risk of death and the prevalence of antibodies to myxomatosis.

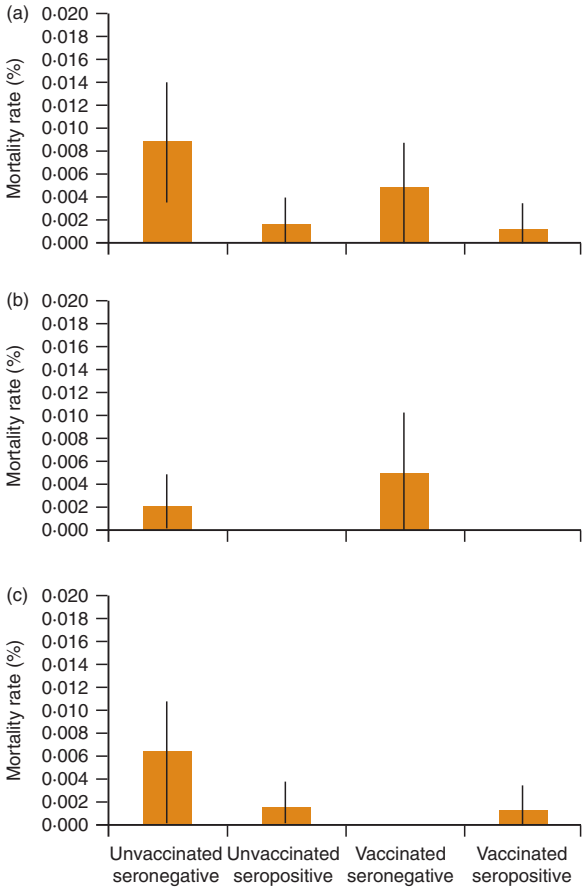

FIG 2: Estimated daily mortality rates for adult rabbits (mean proportion of adult rabbits dead in a day). (a) Overall mortality, (b) mortality from predation, and (c) mortality from diseases. Confidence intervals were set at 95 per cent. Data for four years have been pooled

DISCUSSION

The traditional protocol used in vaccination campaigns consists of vaccinating animals whose immunological status is unknown. Wild populations of rabbits are characterised by age-dependent increases in the prevalence of antibodies to myxomatosis and VHD (Arthur and Louzis 1988, Cooke and others 2000, Calvete and others 2002). In the study area, both myxomatosis and VHD were endemic diseases, with annual outbreaks associated with the breeding season and new infections of young rabbits. The epidemiological pattern of myxomatosis was characterised by a high prevalence of antibodies in the adult rabbits, and a rapid increase in the prevalence of antibodies in young rabbits as a result of the annual outbreak. The epidemiological pattern of VHD was similar, except that in the adult rabbits the prevalence of antibodies to VHD was lower than the prevalence of antibodies to myxomatosis. Annual periods of mortality of adult rabbits due to VHD have been detected at the start of every breeding season (Calvete and others 2002).

In the adult rabbits, vaccination was associated with a slight but statistically non-significant reduction in the mortality of VHD-seronegative rabbits, suggesting that the high prevalence of natural antibodies to myxomatosis (94 per cent) and VHD (50 per cent), presumably due to past exposure to the two viruses, overlapped the effects of vaccination. Moreover, adult rabbits exposed in the past to VHD antigen (VHD-seropositive rabbits) suffered a lower mortality from this disease during the risk periods than seronegative rabbits, although three VHD-seropositive rabbits, one of which was vaccinated, died from VHD. However, it is not known whether the resistance to VHD observed in the seropositive rabbits was due to the presence of serum antibodies or to the development of short-term immunity to the virus arising from a recent infection, as has been suggested for other caliciviruses (Matsui and Greenberg 2000).

Although the young rabbits were not tested for the presence of antibodies to myxomatosis and VHD, and it was therefore not known whether they had been exposed to these viruses, it was found that vaccination significantly reduced their risk of death. It is likely that the reduction in mortality observed in the vaccinated young rabbits may have been due to the lower proportion of young rabbits previously exposed to the viruses, and to the higher incidence of both diseases among them. However, although it is not known whether young rabbits which were classified as predated could have been sick rabbits which died and were eaten as carrion, or rabbits with chronic disease that were predated. The higher mortality by predation suffered by the unvaccinated animals suggests that, in the wild, there may be a compensatory relationship between mortality by disease (especially chronic myxomatosis) and predation, as has been suggested in other surveys (Trout and others 1992, Villafuerte and Viñuela 1999, Calvete and others 2002). As a result of this compensatory relationship, predators may actively select substandard prey, that is, sick rabbits (Temple 1987), and the intensity of selection may depend on the response of predators, which is directly related to the relative abundance of easy prey (Pech and others 1995). The vaccination of young rabbits may therefore increase their rate of survival, not only by reducing their incidence of disease but also by decreasing their rate of predation. However, this decrease in the predation rate will not be constant but will depend on the response of the predators, and will therefore depend on the proportion of vaccinated rabbits in the population. This suggests that the higher the proportion of vaccinated rabbits, the lower the reduction in their mortality due to vaccination will be, because the reduction of their probability of being predated will also have decreased.

Although the effects of vaccination on the survival of young rabbits may have been overestimated because the proportion of vaccinated rabbits in the overall population was low, the results suggest that, to date, vaccination campaigns in Spain have been focused appropriately, because rabbits born in the same year are those most targeted for vaccination. However, the results only estimated the effects of vaccination at the level of individual rabbits. A true assessment of the efficacy of a vaccination campaign should take into account its other effects, both on individual rabbits and on

the rabbit population as a whole. For example, the trapping and handling of young rabbits may increase their mortality rates in the days immediately after trapping (Cypher 1997, Calvete and Estrada 2000). In addition, the efficacy of immunisation is dependent on the vaccine used and the physiological status of the animal. The proportion of the population that can be captured to be vaccinated and the population dynamics of wild rabbits are also important in determining the efficacy of a vaccination campaign. For example, since the overall mortality rates are notably higher among young rabbits than adults (Parer 1977, Wood 1980, Gibb 1993, Kunkele and Von Holst 1996), the apparently higher efficacy of vaccination of young rabbits may actually be lower, owing to the high natural mortality rates among young rabbits. In addition, there may be a compensatory relationship between the effects of diseases and the effects of predation, as discussed above.

The negative impact of VHD on rabbit populations may also be directly associated with the mean age at which the animals are infected. Rabbits less than two months old have been shown to be resistant to the disease, and a lower mean age of infection that includes this age group will therefore result in a lower overall mortality from the disease (Calvete and Estrada 2000, Cooke 2002). This suggests that in any population the vaccination of very young rabbits could decrease the force of VHD infection among them (Anderson and May 1982) and raise the mean age of infection, that is, the vaccination of a proportion of young rabbits could reduce the transmission of the VHD virus among them. Thus, paradoxically, the possible benefits of vaccination against this disease to individual rabbits may result in a decrease of morbidity but in an increase in the mean mortality due to the virus, because unvaccinated young animals may be infected when they are older and less resistant to the disease.

Vaccination campaigns against myxomatosis and VHD have been conducted frequently in Spain, being authorised by the veterinary services of local governments for both game and wildlife conservation goals. To be effective, a large number of rabbits needs to be vaccinated every year, but owing to the low density of rabbit populations, they are captured by ferreting, and the procedure is expensive. Moreover, commercial vaccines, which were designed for domestic rabbits, are used, although a new generation of recombinant and transmissible vaccines, specifically designed to be used in wild populations, has recently been developed (Torres and others 2001). Despite the cost and effort of conducting traditional vaccination campaigns or developing a new generation of vaccines, the true efficacy of vaccination campaigns in populations of wild rabbits remains unknown. The present results show that vaccination is effective in individual rabbits, but that other unmeasured factors can modulate the efficacy of a vaccination campaign; they suggest that there is a need for further theoretical modelling of the factors affecting both individual rabbits and populations of rabbits. Controlled field experiments to test these models should then be undertaken, and only after this research process should the decision to authorise vaccination campaigns against myxomatosis and VHD be taken.

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