

SHORT-TERM NEGATIVE EFFECTS OF VACCINATION CAMPAIGNS AGAINST MYXOMATOSIS AND VIRAL HEMORRHAGIC DISEASE (VHD) ON THE SURVIVAL OF EUROPEAN WILD RABBITS

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Abstract: The capture and handling of live European wild rabbits (*Oryctolagus cuniculus*) are unavoidable initial steps for immunization by vaccination against viral hemorrhagic disease (VHD) and myxomatosis as a management tool aimed at enhancing wild rabbit populations. We investigated the short-term effects of vaccination campaigns against VHD and myxomatosis on the survival of 206 European wild rabbits in a Spanish population. While no effect was observed on the survival of adult rabbits, young and subadult rabbits ($P < 0.001$) had higher mortality rates during the first week after handling than in the subsequent 3 weeks. The hazard rate of death from disease was inversely correlated with body condition ($P < 0.001$) during the first week. Vaccination increased the hazard rate of death due to disease during the first 7 days post-handling, although this detrimental effect was higher in young rabbits ($P = 0.012$) and modulated by body condition ($P = 0.004$). The hazard rate of dying from predation during the first week was higher for males than females ($P = 0.023$) and in subadults compared to young rabbits ($P = 0.004$). Body condition was inversely related to the predation hazard rate ($P = 0.002$). Our results suggest that vaccination campaigns had a short-term negative impact caused by the stress of handling in addition to the detrimental effects of vaccination against VHD and myxomatosis. Future studies should consider this negative impact to assess the true efficacy of vaccination campaigns in wild populations.

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European wild rabbit is the primary small-game species in sport hunting and also is among the most important vertebrate species in Spanish Mediterranean ecosystems. The biodiversity of these ecosystems usually is associated with large numbers of rabbits. Several predators that are threatened with extinction, such as the Iberian lynx (*Lynx pardina*) and the imperial eagle (*Aquila adalberti*), depend on rabbit population abundance (Delibes and Hiraldo 1981). However, viral diseases like myxomatosis and VHD have a strong detrimental impact on wild rabbit populations (Villafuerte et al. 1994, Marchandau and Boucraut 1999, Calvete et al. 2002). Consequently, for several decades, efforts have been made to increase rabbit populations through vaccination campaigns against myxomatosis and VHD autho-

rized by the local governments. This management, carried out with hunting and conservation goals, requires the capture of unknown but high numbers of wild rabbits every year across Spain. Rabbits are captured by trapping or ferreting, vaccinated using commercial vaccines against both diseases, and then released at the same site where they were captured. The effectiveness of these vaccination campaigns has been debated by hunters, conservationists, and wildlife managers since the success of these procedures has been generally negligible. Rabbits born in the same year are the age class most frequently targeted in vaccination campaigns. Rabbits usually are tagged at the time of capture and vaccination, but the recuperation rate of tagged, vaccinated rabbits during shooting seasons is low. In an effort to enhance the effectiveness of vaccination campaigns, a new generation of recombinant vaccines has been developed (Castañón et al. 1999, Fernández et al. 2001, Torres et al. 2001). Some of these recombinant viruses are designed to be transmissible, and these

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can be used in the future in the wild (Angulo and Cooke 2002). Nevertheless, the effects of vaccination campaigns on the survival of wild rabbits have not been examined to date.

The assessment of the true efficacy and the optimal design of vaccination campaigns should be based, in a first step, on theoretical modeling that should comprise disease epidemiology, rabbit population dynamic, efficacy of immunization by vaccination, and other determinant factors derived from vaccination campaign procedures. However, knowledge is lacking on these factors. For this reason, simultaneously to a 3-year field study aimed at examining mortality causes of wild rabbits, we conducted a study to investigate the short-term effects of vaccination campaigns against myxomatosis and VHD. We followed the same vaccination protocol that traditionally has been used in Spain. Our hypothesis was that the success of vaccination campaigns was partially reduced by the short-term detrimental effects of the vaccination procedure. We analyzed the effects of handling and vaccination associated with vaccination campaign procedures on short-term mortality rates and causes of mortality in a natural population of European wild rabbits.

STUDY AREA

We performed our study in the Central Ebro Valley, Zaragoza Province, northeast Spain. The area is characterized by a temperate, continental Mediterranean climate with little rain (yearly average = 350 mm). The landscape was comprised of low hillocks alternated with small fields of wheat and barley. The natural vegetation was sparse steppe scrub and included representative species, such as rosemary (*Rosmarinus officinalis*), thyme (*Thymus* sp.), and furze (*Genista scorpius*), whose distribution was restricted to the surface of the hillocks where rabbit warrens were abundant.

METHODS

From 1993 to 1996, we live-trapped wild rabbits in their warrens with cage traps located in the warren entrances. We identified rabbits with numbered ear tags (Presadom No. 3, Brives, France) and equipped rabbits with transmitters containing activity sensors (Biotrack, Wareham, United Kingdom). Rabbits weighing >600 g were equipped with a 20-g radiocollar while lighter rabbits were marked with a 5-g radio-eartag. We recorded sex, body mass, cubit length, and radiotransmitter mass to body-mass ratio of each rabbit. The apophyseal line of the tibia was used as an age

indicator of captured rabbits (Watson and Tyndale-Biscoe 1953). We identified 2 age-related classes: adult rabbits without a palpable apophyseal gap and rabbits less than 7–9 months in age with a gap. For analytical purposes, we classified rabbits into 3 age groups: young (180–600 g; marked with 5 g radio-eartags), subadults (600–1,360 g), and adults (1,080–1,660 g). Young and subadult rabbits displayed a palpable apophyseal gap.

To determine whether vaccination against myxomatosis and VHD affected short-term survival, we vaccinated approximately half of the radio-marked rabbits against both diseases simultaneously but separately by subcutaneous injection. Our goal was to test the combined effects of injection and of vaccines per se, not separately. For this reason, we did not include control animals injected with placebo. Commercial vaccines against myxomatosis (POX-LAP, Ovejero Laboratory, León, Spain) and VHD (CYLAP-VHD, Sobrino-Cyanamid Laboratory, Madrid, Spain) were used at doses recommended for domestic rabbits. After handling, we released trapped rabbits into their respective warrens.

Because physiological conditions can influence survival after trapping and handling, we estimated a body-condition index for each rabbit by calculating body-mass to cubit-cube ratio. This index is a measure of relative body-mass corrected for differences in structural body size (Johnson et al. 1985, Blem 1990). However, this morphometric variable was dependent on sex and age due to the broad range of ages of rabbit samples. Therefore, we performed a multiple regression analysis with body-mass to cubit-cube ratio as dependent variable and sex, rabbit group, and cubit length as independent variables, and we used standardized residuals as body-condition index, independent of sex and age.

We used data from 206 trapped rabbits, including 44 young (19 females, 25 males), 97 subadult (59 females, 38 males), and 65 adult (35 females, 30 males) animals. We injected 20 young, 44 subadult, and 22 adult rabbits with vaccines against myxomatosis and VHD.

We located all rabbits once a day during the first 8 days after capture, and at least once every 3 days during the following 3 weeks. Localization consisted of checking whether each rabbit was alive or dead and, if dead, determining the cause of death. We employed a Cox's proportional hazard regression model for censored data with the forward stepwise procedure to estimate the association between the dying hazard rate and inde-

pendent variables. This regression technique is appropriate to handle survival data and allows obtaining direct estimation of the relative risk of dying for each categorical level determined by the independent variables (Hougaard 2000). An initial analysis was conducted to test differences in overall dying hazard rate throughout our survey period. On the basis of the evident change exhibited by the slope of cumulative survival rates of young and subadult rabbits after first week, we chose weekly intervals for analytical purposes. To test differences among weekly intervals controlling for other factors, the analysis was performed with the overall dying hazard rate as the dependent variable, and weekly interval, age group, sex, body-condition index, vaccination, and their interactions of second grade as independent variables. In this analysis, we compared the hazard rate calculated at weekly intervals against the overall mean hazard rate at 4-week intervals after capture and handling. In a second analysis, we examined the overall dying hazard rate and the hazard rate due to disease and predation every week against sex, vaccination, age group, body-condition index, and all second-grade interactions as independent variables. Due to the different pattern of mortality showed by adult rabbits with respect to young and subadults, data from adult rabbits were analyzed separately.

RESULTS

Over the 4 weeks, 52 radiomarked rabbits died, including 38 (73%) in the first week. The main causes of death were predation and disease. Specifically, 37 rabbits were killed by predators over the 4 weeks, of which 24 (65%) were killed in the first week. Raptors targeted young and subadults rabbits only (11), while red foxes (*Vulpes vulpes*) killed 12 rabbits belonging to the 3 age groups over the 4-week study period. We were unable to identify the predator of the other 14 rabbits because only radiocollars with blood and rabbit fur were found. Disease was established as the cause of death in 15 rabbits, 14 of which (93%) died in the first week. Six animals displayed gross lesions compatible with VHD at necropsy, 1 died from myxomatosis, and 2 others died from intestinal acute coccidiosis. Six other rabbits died in warrens, but the big size and complexity of the warrens hindered excavation; only 1 rabbit carcass with compatible VHD lesions was ultimately recovered. Accordingly, we assumed that disease was the cause of death for the 5 other rabbits dead in their warrens.

Estimated radiotransmitter mass to body-mass ratio was relatively low: 1.5% in young rabbits (SE = 0.06, range = 0.78–2.78), 2% in subadults (SE = 0.04, range = 1.47–3.23), and 1.5% in adults (SE = 0.02, range = 1.2–1.85).

Mortality patterns across the study period were different from adult to young and subadult rabbits (Fig. 1). Young and subadult rabbits displayed higher mortality rates, especially during the first week after capture. The initial regression analysis performed with young and subadult rabbit data ($-2LL = 517.72$, $\chi^2 = 51.05$, $df = 5$, $P < 0.001$) revealed that hazard rates varied significantly across weekly intervals ($P < 0.001$), since rabbits displayed higher dying hazard rates during the first week following capture ($B = 1.18 \pm 0.25$, $P < 0.001$). The risk of dying during the first week was 3.3 times higher than the risk estimated for the overall survey period. Males displayed a higher hazard rate (risk of dying 2.1 times higher) than females ($B = 0.75 \pm 0.3$, $P = 0.014$), and body-condition index was inversely related to dying hazard rate ($B = -0.48 \pm 0.13$, $P < 0.001$). No statistically significant difference ($P > 0.05$) was found between both age groups or between vaccinated and nonvaccinated rabbits. The analysis of adult rabbit data did not reveal an association of any independent variable with dying hazard rate of this age group.

In the second regression analysis performed at weekly intervals (Fig. 2), the overall dying hazard rate of young and subadult rabbits during the first week was associated with independent variables ($-2LL = 294.6$, $\chi^2 = 20.88$, $df = 4$, $P < 0.001$). Young rabbits displayed a risk of dying 2.3 times lower than subadults ($B = -0.85 \pm 0.42$, $P = 0.043$). Males had a higher death hazard rate (risk of dying 2.7 times higher) than females ($B = 0.99 \pm 0.37$, $P = 0.008$). Body-condition index was inversely associated with the dying hazard rate during the first week ($B = -1.21 \pm 0.27$, $P < 0.001$). Vaccination was not retained in the final regression model; however, the interaction term between body condition and vaccination was additionally related to the hazard rate ($B = 0.77 \pm 0.34$, $P = 0.023$). In this case, vaccinated rabbits had a higher probability of dying during the first week. However, this effect was modulated by body condition. The lower survival rate of rabbits with poor body condition overlapped the detrimental effect of vaccination, which was only detectable in rabbits with medium-to-good body condition (Fig. 3).

The relative risk of dying due to the short-term effects of vaccination was estimated forcing to

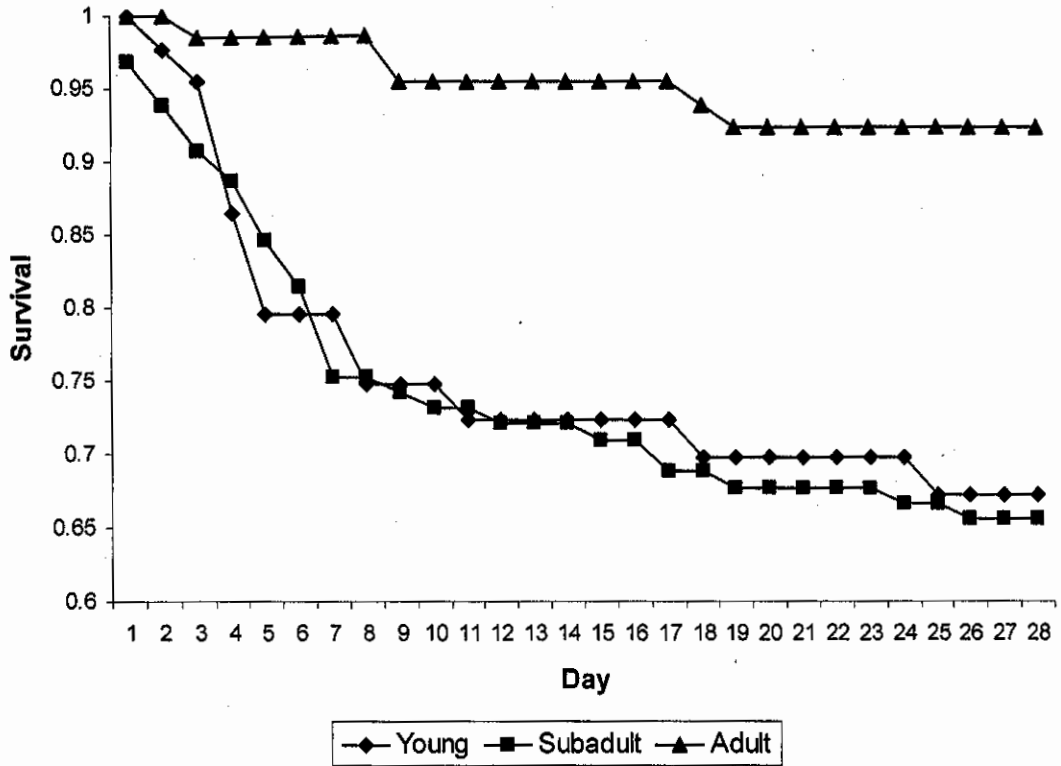


Fig. 1. Cumulative survival rates of young, subadult, and adult European wild rabbits during the first 4 weeks after capture and vaccination in the central Ebro Valley of Spain, 1993–1996.

enter in the final regression model the independent variable "Vaccination" and removing its interaction with body-condition index. This way, the risk of dying for vaccinated young and subadult rabbits was 1.1 times higher for nonvaccinated rabbits. However, as the detrimental effect of vaccination was revealed in rabbits with medium-high body-condition index values, body-condition index was replaced in the former regression model by a dummy categorical variable with 2 levels: rabbits with body-condition index less than or equal to the mean, and rabbits with value of the index above the mean. The relative risk estimation obtained showed that vaccinated rabbits with body-condition index above the mean displayed a risk of dying 3 times higher than their nonvaccinated counterparts during the first week after capture and vaccination.

We found no statistically significant association between overall mortality and independent variables for adult rabbits during the first week.

Among rabbits born in the year, the death hazard rate due to predation in the first week was

dependent on sex, age, and body condition ($-2LL = 202.3$, $\chi^2 = 16.34$, $df = 4$, $P = 0.001$). Males had a higher hazard rate of dying from predation than females (risk 2.7 times higher; $B = 0.99 \pm 0.44$, $P = 0.023$). Moreover, age was associated with hazard rate. Young rabbits had a risk of dying from predation 9.6 times lower than subadult rabbits ($B = -2.27 \pm 0.8$, $P = 0.004$). Body condition was inversely related to the predation rate ($B = -0.76 \pm 0.24$, $P = 0.002$), but in this case, we observed no interaction with vaccination.

The dying hazard rate due to disease during the first week was statistically associated with independent variables ($-2LL = 79.04$, $\chi^2 = 15.7$, $df = 3$, $P = 0.001$). The hazard rate of dying from disease was inversely associated with the body-condition index ($B = -1.95 \pm 0.53$, $P < 0.001$). Vaccination increased the hazard rate, although this effect was modulated by rabbit age and body condition. Vaccination had a greater detrimental effect on the survival of young rabbits than on survival of subadults ($B = 2.18 \pm 0.87$, $P = 0.012$), since vaccinated young rabbits displayed a risk of

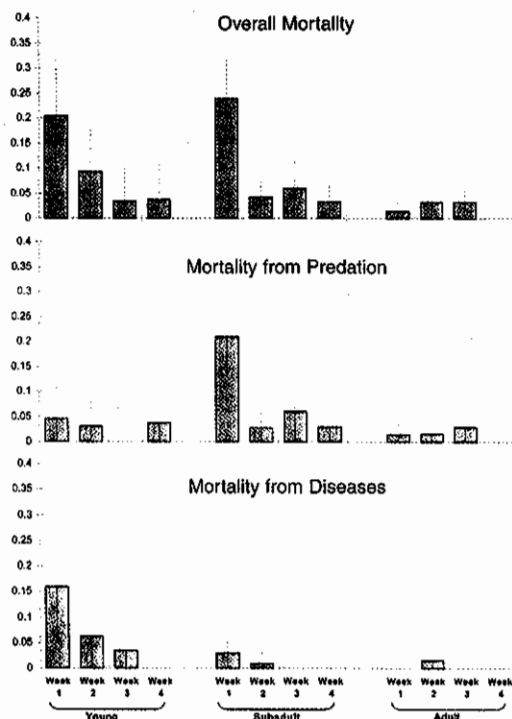


Fig. 2. Weekly accumulated rates (and 95% CI) of overall mortality and death due to disease and predation estimated for young, subadult, and adult European wild rabbits during the first 4 weeks after capture and vaccination in the central Ebro Valley of Spain, 1993–1996.

dying from diseases 1.4 times higher than non-vaccinated. Similarly to the case of overall mortality, the increase in hazard rate of dying from disease due to vaccination was less evident in rabbits with lower body condition for both age classes ($B = 1.76 \pm 0.61$, $P = 0.004$).

Again, no association was found between independent variables and hazard rate of dying from disease or predation for adult rabbits. Similarly, analyses performed in the second, third, and fourth weeks revealed no association between independent variables and hazard rates of dying from disease, predation, or overall mortality for young, subadult, or adult rabbits.

DISCUSSION

We have shown that across our entire study period, rabbit mortality was inversely correlated with age, a finding in agreement with those of earlier studies on the population dynamics of wild rabbits (Tyndale-Biscoe and Williams 1955, Wheeler and King 1985, Gibb 1993). A statistically

significant increase in mortality, however, was noted during the first week after the start of vaccination campaign procedures.

Radiomarking has been used frequently in the study of wild animals. To our knowledge, however, this method has not been utilized in studies of vaccination campaigns. Radiomarking can have detrimental effects on the body mass or survival rates of birds and mammals due to the uncomfortable or inappropriate design of the attached radiotransmitter (Gilmer et al. 1974, Gil 1999, Swenson et al. 1999) or to the excessive ratio of radiotransmitter mass to animal mass (Cypher 1997). In our study, this mass ratio was low. Moreover, in our 3-year survey of the epidemiology of myxomatosis and VHD in rabbits, suppurative dermatitis caused by radiocollars and external otitis caused by radio-ear tags were observed in only 3 of 25 rabbits recaptured several months after radiomarking. While radiotransmitters may affect the long-term physiological condition of some animals, our findings suggest that they were not likely the cause of the high mortality observed during the first week of our study.

Research has suggested that the stresses of being trapped, handled, and tagged, coupled with the initial discomfort, comprise the primary causes of decreased body condition or survival rates immediately after tagging (MacArthur and Geist 1986, Berteaux et al. 1994, Cypher 1997). Our observations agree with this conclusion. Our finding of a high correlation between body-condition index at the moment of capture and the hazard rate of dying suggests that the stress caused by trapping, handling, and possibly the post-tagging acclimation period may be the main cause of mortality increase, especially in rabbits born during that year and in those with the poorest body condition. Unfortunately, the lack of control animals in our study does not allow us to differentiate among the true effects of trapping, handling, and marking.

Our results showed that current vaccination campaign procedures had short-term, negative effects on rabbit survival. Short-term adult survival rates did not seem to be affected by handling or vaccination, since their hazard rates did not alter significantly throughout the 4 weeks of the survey. Nevertheless, a major increase in mortality during the first week was evident in rabbits born during that year, a pattern resulting from the combination of disease and predation. During this first week, the relative incidence of mortality due to either disease or predation differed

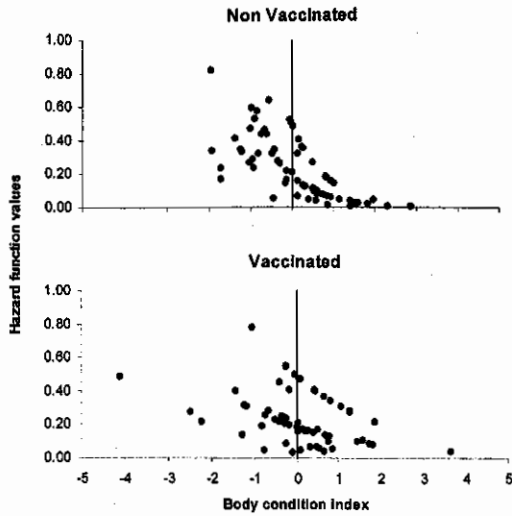


Fig. 3. Graphical representation of effects of vaccination on dying hazard of young and subadult European wild rabbits in relation to their body-condition index. Hazard values were estimated from the Cox's regression model performed with data obtained during the first week after capture and vaccination in the central Ebro Valley of Spain, 1993–1996. Vertical line is located in the mean value of body-condition index. Vaccination increased the dying hazard of rabbits with medium–high body-condition index values (dots at the right of the vertical line).

between young and subadult rabbits. Moreover, each was individually associated with various factors, including sex, vaccination, and body condition. In contrast, we observed no correlation between any factor and mortality due to disease or predation during weeks 2–4 of the survey.

During the first week, mortality due to disease mainly affected young rabbits, regardless of sex. Myxomatosis and VHD were the primary diseases within the study area (Calvete et al. 2002), and their incidence has been shown to decrease with age due to an increase in acquired immunity (Arthur and Louzis 1988, Marchandeu and Boucraut 1999, Cooke et al. 2000). Although differences between the rates of mortality due to disease among age groups may originate from variations in the prevalence of acquired immunity against these diseases, epidemiological patterns were not consistent with the higher mortality rates estimated during the first week compared to the remaining 3 weeks of the study period. These findings indicate that the natural incidence of both diseases was not sufficient to explain the pattern of mortality. Body condition was inversely associated with the hazard rate of dying from disease, suggesting that the detri-

mental effects associated with stress due to handling aggravated any preexisting subclinical disease or facilitated the development of novel or nonspecific diseases in rabbits. Unfortunately, due to the death of many young rabbits in warrens, the only mortality factor other than VHD and myxomatosis that could be successfully identified was coccidiosis.

The specific sensitivity of young rabbits to mortality by disease was especially evident from the negative impact of vaccination against VHD and myxomatosis. Both vaccines are broadly used in domestic and wild rabbits despite the described moderate adverse effects of vaccination on physiology (Arguello 1986, Peeters et al. 1995, Twigg et al. 1997). Moreover, both vaccines have relatively high long-term efficacy and are considered safe in domestic rabbits. Nevertheless, we have shown that the use of these vaccines in young wild rabbits had a detrimental effect on survival rates, resulting in increased hazard rates of dying from disease during the first week after handling and vaccination. This negative effect was modulated by body condition, suggesting that the secondary effects of vaccination on the physiological condition of these rabbits was additive to the detrimental effects caused by stress. This was supported by our finding that, for several days after vaccination, vaccinated rabbits displayed a higher hazard rate of dying from disease than nonvaccinated animals.

In subadult rabbits, however, predation was the primary mortality factor during the first week, and the hazard rate of death by predation was higher among subadult males than females. Although this might be due to physiological differences between the sexes, more likely is that handling procedures increased inherent dispersal behavior, which is especially pronounced in subadult male rabbits (Webb 1993, Kunkle and Von Holst 1996). This dispersal behavior thus increased mortality due to predation in subadult males during the days following handling. We also found, however, that body condition was inversely related to the predation hazard rate. Therefore, although rabbits with the best body condition had a higher probability of surviving, the true impact of disease may have been underestimated in subadult rabbits. That is, predation of sick, subadult rabbits may have been facilitated by their higher activity rate outside the warrens, compared to young rabbits. Conversely, animals that died from disease may have been consumed as carrion and thus wrongly classified.

MANAGEMENT IMPLICATIONS

When mass immunization programs of wild populations are used to control diseases that affect human health (Winkler et al. 1975, Rosatte et al. 1992) or to prevent economic losses in stockbreeding (Kaden et al. 2000), the effectiveness of vaccination campaigns mainly are evaluated on the basis of disease control or the safeguarding of the health of the public and/or the domestic species. Our findings suggest, however, that when mass immunization programs are carried out with conservation goals, the possible negative effects of vaccination campaign procedures on species being immunized should be considered, especially if the species targeted is prone to detrimental effects from handling.

In vaccinating European wild rabbits, our results have shown that current vaccination campaign procedures—comprising capture, handling, and vaccination with commercial vaccines—have short-term, negative effects on rabbit survival, modulated primarily by the body condition and age of rabbits. These negative effects, previously unidentified, reduce the objectives of these vaccination campaigns (i.e., the hypothetical increase in rabbit survival due to immunization against both VHD and myxomatosis).

Previous theoretical approaches suggested that the effectiveness of vaccination campaigns in rabbits is highly dependent on the targeted age group (Calvete and Estrada 2000). For this reason, future theoretical studies should include the negative effects reported in our work, especially the varying negative impacts of handling and vaccination among age classes, to determine the rabbit age class that should be the primary vaccination target. Studies also could evaluate the use of the new generation of transmissible recombinant vaccines, instead of the current commercial vaccines now in use. These new theoretical and empirical studies should evaluate whether vaccination campaigns are effective in any instance and, if appropriate, optimize their design to obtain the best results.

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