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Field experimental vaccination campaigns against myxomatosis and their effectiveness in the wild

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ABSTRACT

We conducted a field experiment in SW Spain to test the efficacy of a myxomatosis vaccine, a viral disease strongly affecting wild rabbit populations, by assessing individual survival and antibody seroprevalence of monthly live-trapped, vaccinated ($N = 466$) and unvaccinated ($N = 558$) juvenile wild rabbits, between April and October 2007. Eight percent of all juveniles caught from April to June showed maternal antibodies against myxomatosis, whereas all animals were seropositive to the disease after the outbreak. Juveniles vaccinated before the outbreak showed 17% higher survival (31% vs. 14%) and an increased mortality probability of 8% after the outbreak. Results suggest that only a costly and systematic vaccination performed before the annual myxomatosis outbreak, would improve the survival of juvenile rabbits, a premise not always accomplished that compromises its efficacy in the field.

1. Introduction

The European wild rabbit (*Oryctolagus cuniculus*) is a keystone species and an ecosystem engineer of the Mediterranean Basin [11] that has declined in the Iberian Peninsula over the last 50 years, mainly due to the strong detrimental impact of two viral diseases, myxomatosis and rabbit haemorrhagic disease [RHD, 29]. Despite efforts to reduce their effects, periodic outbreaks of both diseases still occur and cause high rabbit mortality [5]. In particular the virulence of myxomatosis has been considered one of the most important factors explaining wild rabbit distribution [e.g. 1], and is held as one of the most dangerous diseases in rabbit husbandry [23].

In the wild, the epidemiological pattern of myxomatosis is characterised by a rapid increase of antibodies in young rabbits just after the outbreak, resulting in a high prevalence of antibodies in adult rabbits [6]. Although juvenile rabbits (<1 month old) could present maternal conferred immunity against the disease [14] most of them remain seronegative to myxomatosis until their first contact with the virus. This is why myxomatosis outbreaks in the wild occur annually shortly after the emergence of juveniles which typically peak in numbers in June or July [5].

Vaccination against myxomatosis of juvenile captive rabbits is considered almost completely effective [23], which is why this management tool is one of the most popular among game managers and conservationists to boost wild rabbit numbers [1]. In the field, vaccines are typically administrated to rabbits of any age class, either captured at the site in their warrens (employing ferrets, *Mustela putorius furo*), or brought from a different area, when associated with restocking operations [1]. Although vaccination may occur throughout the year, game managers usually capture rabbits after the breeding season (late summer), to avoid killing of kittens by ferrets or to reduce the number of rabbits to be bought (and hence released). Whether because of economic and/or logistic constrains, ultimately rabbits are vaccinated only once, regardless of their age or serological status, in a date that can vary greatly between regions and/or years [30], and they are seldom revaccinated (or recaptured). Such vaccination programmes are called "blind" vaccinations and little is known about their efficacy [4,5].

All the previously mentioned factors, together with others as the capture system employed (different in the proportions of animals captured, stress induced, etc.) have the potential to strongly determine the effectiveness of wild rabbit vaccination campaigns. Perhaps this is why studies carried out to date have provided only inconclusive results regarding rabbits' immune response elicited by vaccination [4,8,28]. On one hand, Calvete et al. [6] and Guitton et al. [16] suggested that monthly vaccinations of free-living, naïve, wild juvenile rabbits against myxomatosis improved

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individual survival. However, short-term negative effects of vaccinations on juveniles have been described [7] whereas the intensity of the immune response induced by vaccination has also been shown to be affected by body condition [4]. Acquired immunity and the length of the breeding season can play a crucial role in the differential impact of myxomatosis on wild rabbit populations [14]. Therefore, even under controlled conditions it is not completely clear which factors should be manipulated to enhance the effectiveness of this management tool. In the field, the performance of "blind", non-systematic, vaccinations in low density rabbit populations may actually make them more vulnerable to extinction due to a multitude of factors [9]. For example, one feature that could compromise their success is the time when the outbreak occurs, which is seldom considered by managers and often neglected in experimental studies. The end of the breeding season, when several cohorts of juvenile rabbits naïve to myxomatosis begin to coexist, could be an appropriate occasion to perform a single vaccination campaign, but this would imply the existence of rigorous population monitoring schemes which are absent most of the times. Systematic vaccinations throughout the pre-, during and post-phases of the disease incidence could be another alternative with the advantage of potentially reversing the short-term negative effects associated with vaccination [7]. The development of recombinant vaccines that can be transmitted horizontally in the wild is a more recent instrument designed to overcome the need for systematic vaccinations, although with yet limited transmissibility [2], which turns the problem of protecting wild rabbit populations against myxomatosis one of the chimeras of rabbit's management and conservation with few easy and visible short-term solutions.

The purpose of this study was to determine the effectiveness of a systematic vaccination scheme against myxomatosis in the wild. We vaccinated a known proportion of juvenile wild rabbits in a high-density, closed population, located in Southern Spain, over several months. We assessed individual survival and immunological status of vaccinated and unvaccinated juveniles captured during monthly, live-trapping sessions and determined the factors affecting the success of vaccination campaigns before, during and after the myxomatosis outbreak. Our final goal was to provide evidence for managers to decide under which conditions and circumstances, vaccination campaigns would provide a useful management tool.

2. Materials and methods

2.1. Study site and data collection

This study was carried out in Los Melonares, located in the south of the Sierra Norte Natural Park, Seville province, SW Spain. Details of the study area can be found in Rouco et al. [25]. Two 200 m × 200 m nuclei were built, approximately 2-km from each other. These were fenced around their perimeter to prevent the entrance of mammalian predators, but did not impede avian raptor predation. In each nucleus, 18 artificial warrens were constructed on four parallel lines of four or five warrens separated by approximately 40 m. Each warren was surrounded with a wire net (approx. 1 m high, 0.5 m underground, 1.5 cm diameter), with gaps where live-traps were placed so rabbits could only leave or enter warrens by passing through the inactivated traps. This particular capture system presented the advantage of providing high capture rates (approximately 50% [24]). Food and water were provided *ad libitum* next to each warren (see Rouco et al. [25] for more details).

Myxomatosis was known to be present in the population with annual outbreaks occurring mainly between late-spring and the end of summer [24]. Rabbits were live-trapped in all warrens every month from April to October 2007. Throughout these sampling months, at their first capture, animals were marked with individually numbered ear tags and measured (sex, weight, tarsus and ear length). Additionally, a blood sample was obtained on two occasions (see following section). Rabbits were considered juveniles when their weight was below 900 g and adults when their weight was equal or above 900 g. In each nucleus, half of the warrens were randomly allocated to one of two treatments: control or vaccination against myxomatosis. Vaccination against myxomatosis involved injecting all juvenile rabbits of the corresponding warrens with 0.5 ml of a homologous vaccine from Sanarelli virus (lineage León-162; POX-LAP, Ovejero Laboratories), which are the doses recommended for domestic rabbits (VACCINATED). All juvenile rabbits of the same size captured on the rest of the warrens in each nucleus were injected with 0.5 ml placebo (commercial physiological saline solution, UNVACCINATED). Therefore, all rabbits were administrated, at their first capture, with either the vaccine or the placebo, according to the treatment they belonged to. Evidence of the incidence of myxomatosis on rabbits was based on the presence of myxomas and lesions (usually in eyes, ears and anus [18,26]). All animals were treated in compliance with guidelines outlined by the animal ethics committee in Spain.

2.2. Seroprevalence analysis

Blood sample collection was performed in two occasions only, one at the beginning and the other at the end of the experiment (April and October, pre-myxomatosis and post-outbreak periods, respectively). In April, the blood sample was taken before the administration of any of the vaccine or placebo treatments. In both months, the blood sample was obtained from all captured animals from an incision of the marginal ear vein and stored in Eppendorf tubes without anticoagulant [5]. The blood samples were left to coagulate at room temperature, centrifuged and the sera were frozen at −20 °C. To determine the antibody presence/absence against Myxoma virus by indirect ELISA we used the commercial kit "CIVTEST Cuni Mixomatosis" (HIPRA S.A., Girona, Spain) following the manufacturer recommendations.

2.3. Survival analysis

During the experiment a total of 78 juvenile rabbits (weight from 600 to 900 g.) were radio-collared (Biotrack, Wareham, UK) and tracked twice a day to check for survival ($N_{VACCINATED} = 36$; $N_{UNVACCINATED} = 44$). When possible, the cause of death was assessed by examining the carcass, by the location of the remains and by other signs, such as feathers [20]. Those rabbits that died below ground were considered most likely to have suffered from an undetermined disease. Radio-collars were removed from those animals still alive at the end of the experiment. Survival rates estimates were made using MICROMORT 1.3 [17].

3. Results

From April to October 2007 a total of 2158 rabbits were live-trapped and measured. Of these, 1024 (47%) were juveniles (544 males, 462 females and 18 undetermined). According to the type of warren where first captured, a total of 466 young rabbits were vaccinated against myxomatosis while 558 were injected with placebo (unvaccinated; Table 1). Differences between the number of animals captured in both treatments throughout the experiment were not significant ($\chi^2 = 4.73$; d.f. = 6; $p > 0.05$).

The proportion of juvenile rabbits captured varied between months, being highest in April (28%; Fig. 1). The proportion of new juvenile rabbits emerging in the population decreased steadily from June onwards until it reached a minimum of 3% in September, when most of the animals captured were adults. The proportion of recaptured individuals also decreased throughout the study (Fig. 1).

Table 1
Numbers of juvenile rabbits first captured between April and October 2007 in Los Melonares, Seville, belonging to vaccinated (against myxomatosis) and unvaccinated treatments.

Month of first capture	Vaccinated	Unvaccinated
April	146	151
May	106	137
June	100	138
July	55	61
August	31	36
September	11	19
October	17	16
Total	466	558

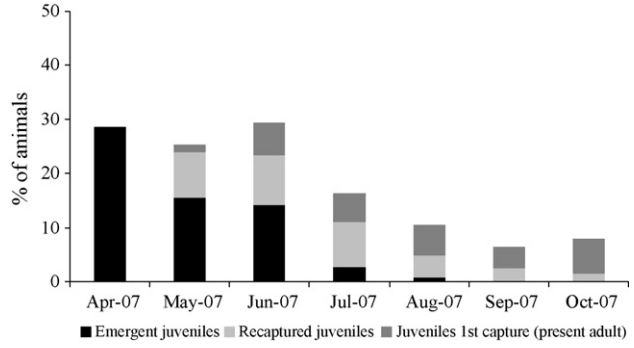

Fig. 1. Proportion of emergent, recaptured juvenile rabbits and juveniles at 1st capture (in relation to the total number of juveniles caught during the whole experiment) captured between April and October 2007 in Los Melonares, Seville.

None of the adults captured showed myxomatosis symptoms, and there were virtually no juveniles showing symptoms of myxomatosis between April and June 2007 (Fig. 2). However, the proportion of juvenile rabbits showing symptoms peaked in July and then tailed off. Considering that there is a virus incubation period of at least 10 days before the manifestation of the symptoms, the incidence of the disease would be possible from days 80 to 140. The most severe symptoms of myxomatosis were exhibited by young rabbits captured in July, when half of the animals showed severe lesions of the anogenital region, very swollen eyelids and severe congestion. There were no differences between the monthly proportion of vaccinated and unvaccinated juvenile rabbits showing myxomatosis symptoms during the study ($\chi^2 = 6.81$; d.f. = 6; $p > 0.05$). In spite of this, vaccinated juvenile rabbits showed 1.9-fold higher overall survival rate than unvaccinated ones, respectively 0.39 (±0.08SE) and 0.21 (±0.08SE), although these differences were not quite statistically significant (one-tailed Z-test, $Z = 1.59$, $p = 0.056$). This means that overall unvaccinated juvenile rabbits presented

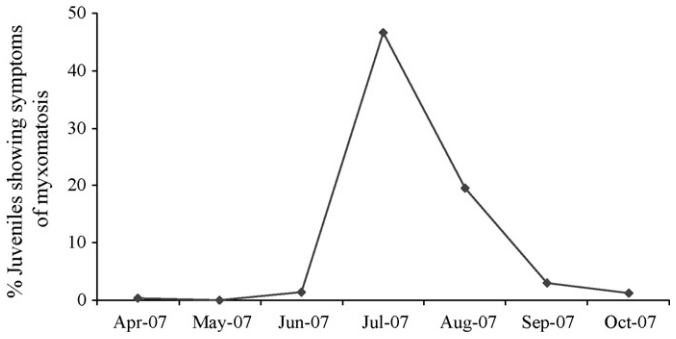

Fig. 2. Proportion of juvenile rabbits showing symptoms of myxomatosis between April and October 2007 in Los Melonares, Seville.

Table 2
Proportion of juvenile rabbits seropositive and seronegative to myxomatosis in April and October 2007 in Los Melonares, Seville, belonging to vaccinated (against myxomatosis) and unvaccinated treatments.

Blood sample	April 2007		October 2007	
	NEG[a]	POS[a]	NEG[a]	POS[a]
UNVACC	83%	11%	0%	100%
VACC	89%	5%	0%	100%

[a] Seroprevalence.

an increased mortality rate of 18% comparing to vaccinated juveniles.

Blood samples of 157 juveniles in April and 74 juveniles in October were taken and analysed overall. In April, only an average of 8% of juvenile rabbits showed natural antibodies against the disease (Table 2). By October, however, all animals were seropositive to myxomatosis regardless of whether they had been vaccinated or not against the disease prior to the outbreak. Radio-tracking data suggested that juvenile rabbits that were vaccinated before the outbreak showed higher survival (31%) compared to unvaccinated individuals (14%; one-tailed Z-test, $Z = 0.56$, $p = 0.212$; Fig. 3). In contrast, individuals vaccinated during the myxomatosis outbreak showed lower survival (8%) compared to unvaccinated juveniles (16%; one-tailed Z-test, $Z = 0.77$, $p = 0.279$). Although the differences are not significant, these results may suggest that the impact of vaccination varies depending on the time when it is administered.

4. Discussion

Myxomatosis is still an important regulation factor of wild rabbit populations. Our results suggest that individual survival of juvenile rabbits could be improved through systematic vaccinations, as found in other studies [e.g. 15], especially when performed before the disease outbreak. However, care has to be taken when making general considerations about the effectiveness of this tool, since the circumstances under which it is usually undertaken in the field by game managers and/or conservationist are quite different from experiments carried out by scientists. Rabbits' physiological stress levels can affect the vaccine's power [4], which implies

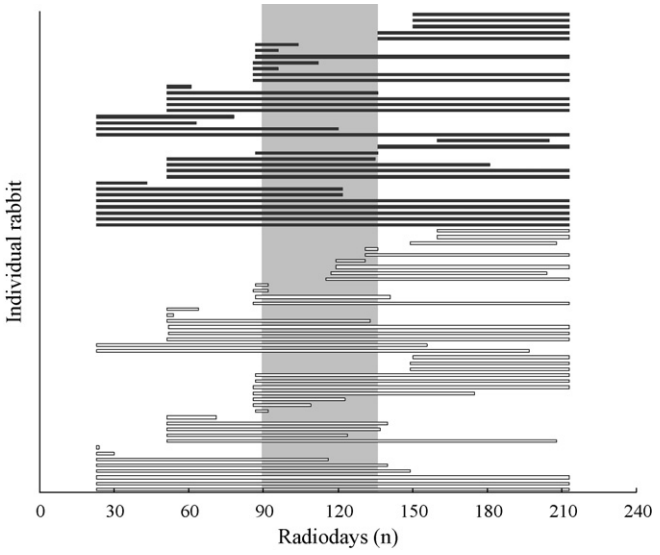

Fig. 3. Fate of post-emergent radio-collared unvaccinated (white lines) and vaccinated (black lines) juvenile rabbits between April and October 2007 in Los Melonares, Seville. Each line represents the period between collaring of the rabbit (start of line) and its subsequent mortality or removal of the radio-collar (end of line). The shaded area represents the period when myxomatosis was active on the study area.

that the capture and manipulation procedures could highly influence the efficacy of the immunization campaigns. Moreover, some of the commercial vaccines against myxomatosis (such as POX-LAP) need to be prepared immediately before their administration to rabbits usually loosing efficacy within 15–20 min after their first application. Vaccination campaigns in the field can additionally be influenced by the highly variable spatial-temporal pattern exhibited by the virus [30], which is a function of a panoply of factors such as the virulence of circulating strains or population density [3], providing paradoxical effects at the individual level.

In Mediterranean ecosystems wild rabbit populations reach their reproductive peak between April and June [e.g. 15] and so by the end of June a greater proportion of susceptible individuals is available to ensure a high circulation rate of the myxoma virus. In 2007 the myxomatosis outbreak was observed in our study plots between July to mid-August, the season of greater juvenile availability. Since passive immunity conferred by the maternal transfer of antibodies could provide protection to the young for approximately 1-month [13] symptoms of the disease would only be detectable in a relative proportion of the young rabbits during summer, as was observed in our study. Our results suggest that an average of 8% of young rabbits were naturally protected against myxomatosis before the epizootic event, through acquired immunity from these maternal antibodies, which corresponds to a residual proportion of the population. This implies that before the arrival of myxomatosis, nearly 90% of juvenile individuals were susceptible to the disease. Moreover, the fact that captures (and vaccinations) were performed systematically in our study over several months guaranteed that immunization was provided to the largest proportion of juveniles, as opposed to a single vaccination campaign. The latter, had it been performed in late summer, when game managers usually do it, would only have protected a residual (Fig. 1) and already seroconverted fraction of the rabbit population (Table 2). In this case, the typical blind vaccination would have compromised any well-intentioned attempt to recover this particular rabbit population.

In our study, young rabbits vaccinated against myxomatosis also presented higher survival rates and lived longer than unvaccinated individuals (at least when vaccinated before the disease outbreak). Nevertheless, the fact that all juveniles were seropositive to myxomatosis at the end of the experiment (October), regardless of the treatment, implies that these animals would have developed antibodies against the disease anyway after being exposed to it. This means that every year, after the disease outbreak, virtually every individual in the population (that does not succumb to the disease) will be seropositive to myxomatosis (hence probably immune to following infections by the disease) and the only new susceptible will be the juveniles emerging in the next breeding season, which is in accordance with the known disease infectious pattern [6]. On the other hand, juveniles vaccinated during the myxomatosis outbreak survived for less time than unvaccinated individuals. This could be indicative of an immunosuppressant effect due to vaccination, as already suggested by Marlier et al. [19], or of a lack of efficiency of the vaccine when administrated during the outbreak. If one considers the additional array of potential undesirable effects caused by the administration of the vaccine, such as fever, the formation of secondary myxomas and anaphylactic reactions [22], the data suggest that a single vaccination campaign, that neglects the time when the outbreak occurs, has potentially negative management implications.

Finally, an aspect of our experiment that could have favoured the vaccine treatment was our capture system that provided high capture rates (approximately 50% [24]). This contrasts with the efficacy of the traditional capture method used by hunters (ferreting) which allows the capture of a low proportion of the rabbit population (about 36%, [10]). On the other hand, our study plots were closed, high-density systems, with terrestrial predator exclusion, whose characteristics allowed for the capture of many rabbits. In low density populations, even with a good capture system, the number of rabbits captured would not probably reach the proportion of the population necessary to assure antibody protection from the vaccine by a horizontal transmission [27]. Therefore, under low density conditions, juvenile immunization against myxomatosis due to vaccination would be, to say the least, impractical.

Wild rabbit populations are still declining in the Iberian Peninsula [e.g. 12,21] although the species seems to be recovering in some specific areas [31]. Still, under a declining scenario, vaccination campaigns against myxomatosis and RHD are frequently employed management tools on the assumption of their usefulness to increase rabbit numbers [4]. The evidence of their effectiveness is only negligible [6] and overall there seems to be no relevant relation of this tool with rabbit population change after the arrival of RHD to the Iberian Peninsula [12]. This could be associated

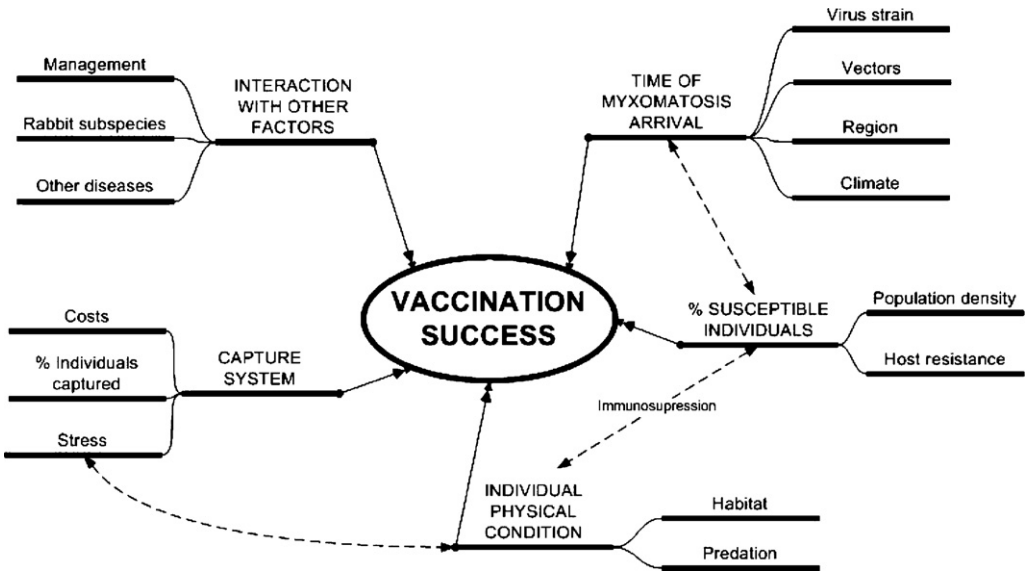

Fig. 4. Factors affecting the success of wild rabbit vaccination against myxomatosis.

with the multitude of factors affecting the success of vaccination campaigns in the field (Fig. 4), like the general low density of wild rabbit populations, the cost of capturing animals [12], the individual physiological condition [4,7], the presence and density of vectors [22] or the time the immunization takes place [9]. In the light of our results, we believe that vaccination campaigns against myxomatosis, as they are currently performed in the field, are generally not functional tools to conserve wild rabbit populations. We consider that game managers should reflect on the relationship costs vs. benefits to assess whether the technique would actually improve the status of the local rabbit population. We argue that this measure should be avoided, and instead efforts should be made to understand the immunological status of the population and other crucial parameters such as body condition [4,7].

Further research should focus on the following topics: investigate the success of systematic vaccination campaigns using vaccines against both myxomatosis and RHD; understand if the immunity provided by the vaccine is similar to the one conferred by maternal antibodies (are animals as effectively protected against the disease artificially as with innate or acquired immunity); if the vaccine dosage currently administrated (the same recommended for domestic rabbits) is adequate for wild specimens; what is the minimum number of rabbits to be vaccinated to ensure protection of a significant part of the population; how the increased survival in vaccinated individuals prior to the outbreak affects population productivity, and if vaccination against myxomatosis is effective in low density rabbit populations.

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References

[1] Angulo E. Factores que afectan a la distribución y abundancia del conejo en Andalucía. PhD in Biological Sciences thesis, Complutense University, Madrid, Spain, 2003.
[2] Angulo E, Bárcena J. Towards a unique and transmissible vaccine against myxomatosis and rabbit haemorrhagic disease for rabbit populations. Wildl Res 2007;34:567–77.
[3] Arthur CP, Louzis C. La myxomatose du lapin en France: une revue. Rev Sci Tech Off Int Epiz 1988;7:937–57.
[4] Cabezas S, Calvete C, Moreno S. Vaccination success and body condition in the European wild rabbit: applications for conservation strategies. J Wildl Manage 2006;70(4):1125–31.
[5] Calvete C, Estrada R, Villafuerte R, Osacar JJ, Lucientes J. Epidemiology of viral haemorrhagic disease (VHD) and myxomatosis in free-living population of wild rabbits. Vet Rec 2002;150:776–82.
[6] Calvete C, Estrada R, Lucientes J, Osacar JJ, Villafuerte R. Effects of vaccination against viral haemorrhagic disease (VHD) and myxomatosis on long-term mortality rates of European wild rabbits. Vet Rec 2004;155:388–92.
[7] Calvete C, Estrada R, Lucientes J, Osacar JJ, Villafuerte R. Short-term negative effects of vaccination campaigns against myxomatosis and viral haemorrhagic disease (VHD) on the survival of European wild rabbits. J Wildl Manage 2004;68:198–205.
[8] Calvete C, Angulo E, Estrada R, Moreno S, Villafuerte R. Quarantine length and survival of translocated European wild rabbits. J Wildl Manage 2005;69:1063–72.
[9] Calvete C. The use of immunization programs in wild populations: modelling effectiveness of vaccination campaigns against rabbit hemorrhagic disease. Biol Conserv 2006;130:290–300.
[10] Cowan DP. The use of ferrets (*Mustela furo*) in the study and management of the European wild rabbit (*Oryctolagus cuniculus*). J Zool Lond 1984;204:570–4.
[11] Delibes-Mateos M, Delibes M, Ferreras P, Villafuerte R. Key role of European rabbits in the conservation of the Western Mediterranean Basin Hotspot. Conserv Biol 2008;22(5):1106–17.
[12] Delibes-Mateos M, Ferreras P, Villafuerte R. Rabbit populations and game management: the situation after 15 years of rabbit haemorrhagic disease in central-southern Spain. Biodiv Conserv 2008;17:559–74.
[13] Fenner F, Marshall ID. Passive immunity in myxomatosis of the European rabbit (*Oryctolagus cuniculus*): the protection conferred on kittens born by immune does. J Hyg 1954;52:321–36.
[14] Fouchet D, Marchandeau S, Langlais M, Pontier D. Waning of maternal immunity and the impact of diseases: the example of myxomatosis in natural rabbit populations. J Theor Biol 2006;242:81–9.
[15] Gonçalves H, Alves PC, Rocha A. Seasonal variation in the reproductive activity of the wild rabbits (*Oryctolagus cuniculus algirus*) in a Mediterranean ecosystem. Wildl Res 2002;29:165–73.
[16] Guitton JS, Devillard S, Guénézan M, Fouchet D, Pontier D, Marchandeau S. Vaccination of free-living juvenile wild rabbits (*Oryctolagus cuniculus*) against myxomatosis improved their survival. Prev Vet Med 2008;84:1–10.
[17] Heisey DM, Fuller TK. Evaluation of survival and cause-specific mortality rates using telemetry data. J Wildl Manage 1985;49(3):668–74.
[18] Jeklova E, Leva L, Matiasovic J, Kovercik K, Kudlackova H, Nevorankova Z, et al. Characterisation of immunosuppression in rabbits after infection with myxoma virus. Vet Mic 2008;129(1–2):117–30.
[19] Marlier D, Mainil J, Boucraut-Baralon C, Linden A, Vindevogel H. The efficacy of two vaccination schemes against experimental infection with a virulent amyxomatous or a virulent nodular myxoma virus strain. J Comp Pathol 2000;122:115–22.
[20] Moreno S, Villafuerte R, Cabezas S, Lombardi L. Wild rabbit restocking for predator conservation in Spain. Biol Conserv 2004;118:183–93.
[21] Moreno S, Beltrán JF, Cotilla I, Kuffner B, Laffite R, Jordán G, et al. Long-term decline of the European wild rabbit (*Oryctolagus cuniculus*) in south-western Spain. Wildl Res 2007;34:652–8.
[22] Rosell JM, Arguëllo JL, Badiola JI, Cuervo L. Enfermedades víricas: Mixomatosis. In: Rosell JM, editor. Enfermedades del conejo, Tomo II: Enfermedades. Barcelona, Spain: Ediciones Mundi-Prensa; 2000. p. 301–31.
[23] Rosell JM. Health status of commercial rabbitries in the Iberian Peninsula. A practitioner's study. Technical Note. World Rabb Sci 2003;11:157–69.
[24] Rouco C. Restauración de las poblaciones de conejo de monte y mejora de la gestión para su conservación. PhD in Biology and Technology of game resources thesis, Instituto de Investigación en Recursos Cinegéticos, Castilla-La Mancha, Spain, 2008.
[25] Rouco C, Ferreras P, Castro F, Villafuerte R. The effect of exclusion of terrestrial predators on short-term survival of translocated European wild rabbits. Wildl Res 2008;35:625–32.
[26] Stanford MM, Werden SJ, McFadden G. Myxoma virus in the European rabbit: interactions between the virus and its susceptible host. Vet Res 2007;38:08.
[27] Torres JM, Sánchez C, Ramírez MA, Morales M, Bárcena J, Ferrer J, et al. First field trial of a transmissible recombinant vaccine against myxomatosis and rabbit hemorrhagic disease. Vaccine 2001;19:4536–43.
[28] Twigg LE, Wheeler AG, Parkinson J. Adverse reactions in wild, free-ranging European rabbits vaccinated against rabbit haemorrhagic virus. Aust Vet J 1997;75:448–9.
[29] Villafuerte R, Calvete C, Gortázar C, Moreno S. First epizootic of rabbit haemorrhagic disease in free living populations of *Oryctolagus cuniculus* at Doñana National park. Spain J Wildl Dis 1994;30:176–9.
[30] Villafuerte R, Jordán G, Angulo E. Biología y factores de riesgo en el conejo silvestre. In: Rosell JM, editor. Enfermedades del conejo, Tomo II: Enfermedades. Barcelona, Spain: Ediciones Mundi-Prensa; 2000. p. 174–88.
[31] Williams D, Acevedo P, Gortázar C, Escudero MA, Labarta JL, Villafuerte R. Hunting for answers: rabbit (*Oryctolagus cuniculus*) population trends in north-eastern Spain. Eur J Wildl Res 2007;53:19–28.