



## Short communication

# A case of low success of blind vaccination campaigns against myxomatosis and rabbit haemorrhagic disease on survival of adult European wild rabbits



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## ABSTRACT

Vaccination campaigns against myxomatosis and rabbit haemorrhagic disease (RHD) are commonly used in translocation programs conducted for the purpose of recovering wild European rabbit populations in Iberian Mediterranean ecosystems. In most cases rabbits are vaccinated 'blind' (i.e. without assessing their prior immunological status) for economic and logistic reasons. However, there is conflicting evidence on the effectiveness of such an approach. We tested whether blind vaccination against myxomatosis and rabbit haemorrhagic disease improved rabbit survival in a rabbit translocation program where wild rabbits were kept in semi-natural conditions in three enclosures. We conducted nine capture sessions over two years (2008–2010) and used the information collected to compare the survival of vaccinated ( $n = 511$ ) versus unvaccinated ( $n = 161$ ) adult wild rabbits using capture-mark-recapture analysis. Average monthly survival was no different for vaccinated versus unvaccinated individuals, both in the period between release and first capture (short-term) and after the first capture onward (long-term). Rabbit survival was lower in the short term than in the long term regardless of whether rabbits were vaccinated or not. Lower survival in the short-term could be due to the stress induced by the translocation process itself (e.g. handling stress). However, we did not find any overall effect of vaccination on survival which could be explained by two non-exclusive reasons. First, interference of the vaccine with the natural antibodies in the donor population. Due to donor populations have high density of rabbits with, likely, high prevalence of antibodies as a result of previous natural exposure to these diseases. Second, the lack of severe outbreaks during the study period. Based on our findings we argue that blind vaccination of adult rabbits in translocation programs may be often mostly ineffective and unnecessarily costly. In particular, since outbreaks are hard to predict and vaccination of rabbits with natural antibodies is ineffective, it is crucial to assess the immunological status of the donor population before translocating adult rabbits.

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## 1. Introduction

In Iberian Mediterranean ecosystems, the European rabbit (*Oryctolagus cuniculus*) is a keystone species that has declined dramatically, with profound implications for conservation and management (Delibes-Mateos et al., 2008). The appearance of myxomatosis in the 1950s and the arrival of rabbit haemorrhagic

disease (RHD) at the end of the 1980s caused substantial reductions in rabbit population density (Calvete et al., 2002), and the extinction of many local wild populations (Villafuerte et al., 1995; Delibes-Mateos et al., 2009). Myxomatosis and RHD are caused by a leporipoxvirus and a calicivirus respectively, with both diseases now endemic in the Iberian Peninsula. Resulting rabbit population declines are ongoing; for example a new variant of RHD (i.e. RHDV2) caused a considerable decrease in wild rabbit numbers in France (2010), Spain (2011) and Portugal (2012) (Delibes-Mateos et al., 2014; Le Gall-Reculé et al., 2013).

Considerable effort has been made in recent decades to reduce mortality from these diseases by translocating wild rabbits into areas where wild populations are low or extinct and implementing vaccination campaigns just before release. Indeed, these measures

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are among the most frequent management actions made to stimulate the recovery of wild rabbit populations in this region (Angulo and Villafuerte, 2003). In most translocation programs, for supposed economic and logistic reasons rabbits are vaccinated without previously assessing their immunological status, and additional information on sex, health status or age of the individuals for example, are rarely collected (i.e. 'blind' vaccination; Cabezas et al., 2006). For the purposes of this paper, blind vaccination campaigns are defined as vaccinating animals without prior knowledge of their immunological status.

The effectiveness of blind vaccination against myxomatosis and RHD has been questioned by hunters, conservationists and wildlife managers. Few studies have assessed its effectiveness in improving rabbit survival and population recovery (Cabezas et al., 2006; Calvete et al., 2004a,b), and the results from those are conflicting. Improvements in survival seem to depend on a variety of factors such as handling stress, individual physical condition, previous immunological status, and population and disease dynamics (Calvete et al., 2004b; Ferreira et al., 2014).

The aim of this case study was to evaluate the effectiveness of a blind vaccination campaign against myxomatosis and RHD in improving both short and long-term survival of wild adult rabbits kept in semi-natural conditions as part of translocations conducted for an endangered predators' conservation program in southwest Spain.

## 2. Material and methods

### 2.1. Ethics statement

Manipulations of all animals reported in this study were in accordance with Spanish and European regulations (Law 32/2007, R.D. 1201/2005 and Council Directive 2010/63/EU).

### 2.2. Study site, vaccination and data collection

The study took place in the southwestern Iberian Peninsula (Hornachuelos Natural Park; 37°49'N, 5°15'W; 100–700 m elevational range), where the climate is Mediterranean with hot, dry summers and cool, wet winters. We analysed capture-recapture data collected during ten capture sessions over two years in three enclosures (E1, E2, E3; about 4 ha each) built as rabbit breeding zones. The enclosures were between 2 and 4 km apart. Each enclosure was surrounded by a 2.5-m high chain-link fence to exclude terrestrial predators (Rouco et al., 2008) and contained 30 regularly distributed artificial warrens. Water and pellet food was supplied *ad libitum*, along with sown grass to increase the availability of fresh food. Each warren was built with a capture device consisting of a wire net fence with metal cage-traps attached to holes in the fence (see Santoro et al. (2014) for a complete description of the study area).

We conducted a standard vaccination campaign for rabbit translocations. Rabbits were captured from wild donor populations by trapping or ferreting. Rabbits released in E1 (in March 2008) and E2 (in April 2008) were from two populations in the municipality of Córdoba (one donor population per enclosure), while those released in E3 (in May 2008) were from a population in Cádiz. Both donor population areas are located in southwestern Spain, about 70 and 160 km from the study area respectively. Individuals were released into enclosures without quarantine periods, but were confined inside warren pens for their first 6 nights (a practice that improves survival; Rouco et al., 2010). All rabbits released within an enclosure were captured using the same methodology and were handled and released under similar conditions.

Randomly selected rabbits were injected subcutaneously just before release into each enclosure with commercial vaccines against myxomatosis (live Shope fibroma virus Mixohipra-FSA, Hipra Laboratory, Gerona, Spain) and RHD (ARVILAP, Ovejero Laboratory, León, Spain), at the doses recommended for domestic rabbits. Only adult rabbits were translocated. Ninety-four males ( $n_{\text{vaccinated}} = 71$ ) and 159 females ( $n_{\text{vaccinated}} = 121$ ) were released into E1; 56 males ( $n_{\text{vaccinated}} = 44$ ) and 81 females ( $n_{\text{vaccinated}} = 71$ ) into E2; 103 males ( $n_{\text{vaccinated}} = 74$ ) and 179 females ( $n_{\text{vaccinated}} = 130$ ) into E3 (Table 1S, see Supplementary material).

Capture-recapture data was collected from 9 live-trapping recapture sessions following the release sessions, from March–June 2008 to April 2010. Captures took place on one night only and involved activation of the capture devices at midday, when the rabbits were less active and mostly underground. The following morning, the rabbits trapped inside the cages were counted and handled (i.e. all animals were weighed, sexed and ear-marked with numbered metal tags (Presadom n°3, France). Previous studies have shown that live-trapping in our study area can capture a large proportion of the rabbit population (i.e. 50–60% of the population) in only one night (see Santoro et al., 2014). All individuals were released at their capture location. Time intervals between capture sessions were unequal (Table 1S, see Supplementary material), but this was specifically accounted for in capture-recapture analyses conducted.

Additionally, from August 2008 to November 2009, in order to determine any outbreak of disease, we inspected on a monthly basis each enclosure by walking inside along 20–30 min looking for rabbit carcasses. When possible, causes of death were determined by post-mortem examination of rabbit carcasses. Predation was assigned to raptors when evidence including feathers, characteristic tufts of torn-out fur, or remains of long bones were found. However, rabbits assigned to predation could also be scavenged. Diseases was assigned to those rabbits with clear lesions due to myxomatosis and/or RHD. Deaths included in the "other causes" category included those assigned to handling stress or aggression associated with social interactions (Calvete and Estrada 2004; Moreno et al., 2004).

### 2.3. Goodness of fit

Animals born during the study period were excluded from analyses. To estimate survival, and identify its determinants, we performed a capture-recapture analysis which provides accurate estimates even when not all alive marked individuals are recaptured (Lebreton et al., 1992). Before this analysis we used U-CARE 2.3.2 (Choquet et al., 2005) to test the goodness of fit of the Cormack–Jolly–Seber (CJS) model (a fully parameterized model allowing for time variation in both survival and individual capture probabilities). The fit of the CJS model is routinely assessed prior to full analysis since an adequate fit indicates that the data is also valid for the set of candidate models (with less parameters) used for hypotheses-testing. U-CARE also allows testing for specific causes of lack of fit such as trap-dependence (i.e. individual capture probability depending on whether it was captured or not in the previous session). A goodness of fit analysis was performed separately for each population (E1–E3).

### 2.4. Capture-recapture analyses

At all trapping sessions, a number of randomly selected individuals ( $N_{\text{total}}$ : E1 = 24, E2 = 32 and E3 = 27) were removed just after being captured and translocated into nearby areas as a part of the ongoing translocation program. Removals were coded in the data sets and do not affect estimation of parameters nor should they

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