Original investigation

Recent negative trends of wild rabbit populations in southern Spain after the arrival of the new variant of the rabbit hemorrhagic disease virus RHDV2

José Guerrero-Casado [a,b,*], Antonio J. Carpio [a], Francisco S. Tortosa [a,c]

[a] Departamento de Zoología, Universidad de Córdoba, Edifico Charles Darwin, Campus de Rabanales, 14071 Córdoba, Spain
[b] Universidad Técnica de Manabí, Portoviejo, Manabí, Ecuador
[c] Escuela Superior Politécnica Agropecuaria de Manabí (ESPAM), Calceta, Manabí, Ecuador

ARTICLE INFO

ABSTRACT

The arrival of a new variant of rabbit haemorrhagic disease virus, known as RHDV2, has recently taken place in the native range of the European rabbit (Oryctolagus cuniculus), a keystone species which has undergone a sharp decline over the last sixty years as a consequence of certain harmful factors. Several works have noted the presence of this new variant in wild rabbit populations, and have in some cases recorded high mortality rates. However, little is known about the response to the arrival of this new virus variant at the population level. The goal of this work is therefore to show recent trends in 26 wild rabbit populations between 2010 (before the outbreak of the disease) and 2014 (after its onset) in two different ecosystems (woodland and agricultural areas), in order to test how their abundances changed over this period, which coincided with the spread of the RHDV2. Overall, our results showed that rabbit abundance was much lower in 2014 than in 2010, and that only 11.5% of the populations monitored proved to have a positive trend, that is, a higher abundance in 2014 than 2010. A positive correlation between rabbit abundance in 2010 and rabbit population trends was obtained, thus suggesting that the impact of the new variant on rabbit abundance is less evident in high density populations. Our results suggest that smaller rabbit populations are those most vulnerable to the outbreak of RHDV 2 and are therefore likely to decline sharply or even become extinct.

© 2016 Deutsche Gesellschaft für Säugetierkunde. Published by Elsevier GmbH. All rights reserved.

Introduction

The abundance of wild rabbits has, historically speaking, been extremely high in their native range. However, these populations have undergone a sharp decline, mainly as a consequence of optimal habitat loss and fragmentation, and the outbreak of two viral diseases (Ward, 2005): myxomatosis in the 1950s and rabbit haemorrhagic disease virus (RHDV) at the end of the 1980s. RHDV is a member of the Lagovirus genus, of the Caliciviridae family. It arrived in Spain in 1988 and devastated Iberian wild rabbit populations, causing mortality rates of 55–75% (Villafuerte et al., 1995). Nevertheless, a new variant of the RHDV, known as RHDV2 was discovered in rabbitries in north-western France in 2010 (Le Gall-Reculé et al., 2011). This new variant has also been reported in wild populations in France, reaching unusual mortalities of up to 90%, which are similar to those resulting from the initial outbreak caused by the classical RHDV at the end of the eighties (Le Gall-Reculé et al., 2011). The reason for this high mortality rate is that this new strain kills young rabbits, whereas the former RHDV infection was not previously known to be deadly in rabbits of this age (Dalton et al., 2012), and it can also kill rabbits that have previously been vaccinated against RHDV (Dalton et al., 2014). Outside the rabbit's native range, RHDV2 has also been reported in Australia (Hall et al., 2015), Italy (Camarda et al., 2014) and in Great Britain (Westcott and Choudhury, 2014).

In Iberia, this new variant was first found on rabbit farms in 2011 (Dalton et al., 2012), and was later reported in several wild populations in Spain and Portugal in the following years (Abrantes et al., 2013; Dalton et al., 2014), suggesting that the new variant has spread rapidly throughout the Iberian Peninsula (Delibes-Mateos

et al., 2014). Indeed, the presence of the new strain was confirmed in only 3 provinces in north-eastern Spain in 2011 (Dalton et al., 2012), whereas in 2012 RHDV2 was already isolated in 10 additional provinces distributed throughout mainland Spain, which proves the ability of this new strain to spread (Dalton et al., 2014). It is therefore imperative to assess the effect of the spread of this new variant on wild rabbit populations in order to establish the conservation and sanitary measures needed to prevent or reduce the ecological, economic and sociological impact related to a great decrease in rabbit numbers.

In this respect, monitoring wild rabbit populations could shed light on the fluctuations in rabbit abundance during the outbreak and the spread of RHDV2. For instance, an abrupt decrease in rabbit numbers has recently been recorded in the two areas still inhabited by the Iberian lynx (*Lynx pardinus*) an extremely specialist rabbit predator that is highly threatened: in Doñana National Park the decline was greater than 80% during the period 2012–2013, while in Andújar Natural Park it was 75% between 2010 and 2013 (Delibes-Mateos et al., 2014). However, little is known about the recent trends of wild rabbit populations and their response to the arrival of RHDV2 in relation to the initial density before the outbreak. According to Calvete (2006), a major impact of classical RHDV strains is expected to occur in low rabbit density populations located in poor habitats, and we therefore hypothesized that the decrease in rabbit abundance would be more abrupt in those smaller populations after the arrival of RHDV2. Here we show data from 26 populations in southern Spain in which a wide range of rabbit abundance was monitored between 2010 and 2014 coinciding with the spread of the RHDV2, in order to assess the plausible effect of the arrival of this new strain.

Material and methods

Rabbit abundance was measured between 2010 and 2014 in 26 rabbit populations located in Córdoba province (Andalusia, southern Spain) in two different biotopes: Guadalquivir Valley and the Sierra Morena mountain chain, both characterised by a Mediterranean climate with hot dry summers and mild winters. Guadalquivir valley (37°33′N, 4°37′W) is an intensive agricultural area with scarce natural vegetation in which the principal crops are olives (*Olea europea*), grapes (*Vitis vinifera*), wheat (*Triticum* sp.) and sunflowers (*Helianthus annuus*), and in which rabbits can produce crop damage (Guerrero-Casado et al., 2015). Sierra Morena (38°5′N, 5°16′W) is a mountain forest area (altitude ranges from 400 to 800 m.a.s.l) dominated by holm oak (*Quercus ilex*), cork oak (*Quercus suber*) and pine reforestations (*Pinus* spp.), together with Mediterranean sclerophyll scrubland composed of *Cystus* spp., *Pistacia* spp. and *Rosmarinus* spp., and pasture areas occupied by oak savannah (dehesa) (Guerrero-Casado et al., 2013). The presence of RHDV2 has been reported in both areas from 2013 onwards (Gómez-Guillamón, 2015).

Rabbit abundance was estimated by counting latrines in walking transects of 2 km in length (mean ± S.E = 2.21 ± 0.07). A latrine was defined as any accumulation of pellets containing at least 20 pellets over a surface area of 20 cm × 30 cm (Virgós et al., 2003). The latrine counts were always performed by the authors together with L. Ruiz-Aizpurua and L. Martin-Prada (see Acknowledgements), all of whom have wide experience in the use of this methodology. All these transects were GPS referenced, thus allowing the process to be repeated in the following years.

Two different models were used to analyse rabbit abundance (RA). First, in order to test the differences in RA in the years studied, a generalised linear mixed model was created using RA in each year as a response variable, which fits a Poisson distribution with a log-function. The year and the zone (agricultural and woodland)

Table 1
Fixed effects of the explanatory variables on rabbit abundance. d.f. refers to degree of freedom of the numerator.

Term	Chi-square	df	*p*-Value
Year	115.60	4	<0.0001
Zone	15.75	1	0.0001
Year × zone	71.97	4	<0.0001

in that model were included as explanatory variables, while the transect was included as a random factor. The interaction between year and zone was also included, and a post hoc test within the mixed analysis was developed to check for differences among the level of categorical variables. In the second model, a rabbit trend index (RTI) was created by applying the formula: RTI = (RA 2014–RA 2010)/RA 2010 (Delibes-Mateos et al., 2008). Positive RTI values indicate a positive trend (population increase), whereas negative values signify a decrease in the population. This index was used as a response variable in the linear model, whereas RA in 2010 and the zone and their interaction were considered as explanatory variables. The assumptions of normality, homogeneity and independence of residuals were confirmed in this second model. The entire statistical analysis was performed using *InfoStat* software.

Results

Overall, RA decreased between 2010 (mean ± S.E. = 29.04 ± 8.53) and 2014 (mean ± S.E. = 12.54 ± 4.00). The first model (pseudo-R^2 = 0.42) showed that the RA was different in the period and zones studied (Table 1), with higher values recorded for the transects located in agricultural areas (mean ± S.E. = 47.23 ± 5.56) than those in woodland areas (mean ± S.E. = 7.59 ± 0.96). The Post hoc test showed that rabbit abundance in agricultural areas significantly decreased between 2011 (mean ± S.E. = 57.86 ± 13.20) and 2012 (mean ± S.E. = 39.71 ± 7.89), with no significant differences in the subsequent years (Fig. 1). In contrast, in woodland areas, although RA increased slightly between 2011 (mean ± S.E. = 7.01 ± 1.65) and 2012 (mean ± S.E. = 12.55 ± 1.98), it decreased in the following year (mean ± S.E. = 6.11 ± 1.98), with the lowest value being obtained in 2014 (mean ± S.E. = 4.75 ± 1.71).

With regard to the second model, only 3 populations had a positive trend (RTI > 0), whereas the remaining populations decreased from 2010 to 2014 (mean ± S.E. = −0.57 ± 0.08). This model (R^2 = 0.56) evidenced a positive effect of RA in 2010 (Table 2, Fig. 2), and a significant difference among places (Table 2), with
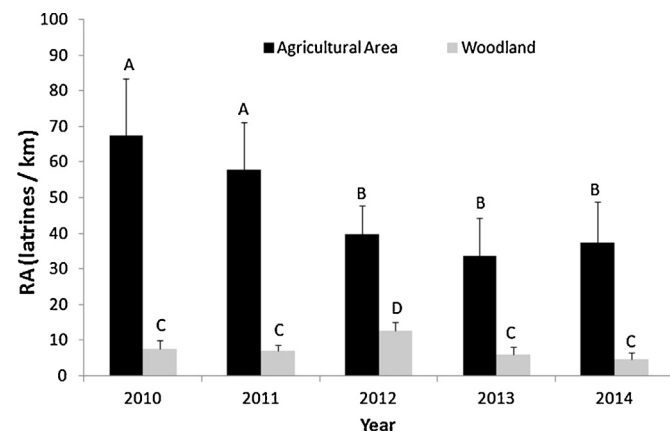

Fig. 1. Rabbit abundance (RA) expressed as the number of latrines per kilometre during the study period in agricultural (Guadalquivir Valley, black) and woodland (Sierra Morena mountain chain, grey) areas. Error bars represent the standard error. Different uppercase letters indicate significant differences among areas and years according to the post-hoc tests (*p*-value < 0.05).