# Establishing a serological surveillance protocol for rabbit hemorrhagic disease by combining mathematical models and field data: implication for rabbit conservation

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ORIGINAL PAPER

# Establishing a serological surveillance protocol for rabbit hemorrhagic disease by combining mathematical models and field data: implication for rabbit conservation

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**Abstract** Rabbit hemorrhagic disease (RHD) became endemic in wild rabbit (*Oryctolagus cuniculus*) populations in the Iberian Peninsula after its first arrival in 1988. This had significant implications for both the economy and environmental conservation because rabbits are one of the main game species in the Iberian Peninsula and a keystone species in the Mediterranean ecosystems. As a consequence, it is planned to include RHD surveillance in the Spanish Wildlife Disease Surveillance Strategy. Nevertheless, there is no practical methodology included in this program to help conservationists and gamekeepers understand the impact of disease on wild rabbit populations. Results from sera collected during the hunting season from 11 rabbit populations of Central and South Spain, which differed in their population abundance and trends, allowed us to use mathematical models to interpret the serological results gathered and determine the best strategy for finalizing a plan of RHD surveillance. Put simply, we focused our field surveys within the hunting season (October-January), and those times when the rabbit populations are at their highest (June or July). Field results showed that both rabbit abundance and population trend are closely related to the prevalence of RHD antibodies when rabbit abundance was at its annual low point (usually October-November). Rabbit population trends were positive only if antibody prevalence was high (>40%), and always negative if prevalence was low. Moreover, rabbit populations where abundance was low always showed low antibody prevalence. Since our models predicted a low variability in the prevalence obtained during the hunting season, it is suggested that future serological surveys should be carried out within this period to avoid problems related to the low sample size in low density rabbit populations.

**Keywords** RHD-*Oryctolagus cuniculus* · Spain-wildlife epidemiology-population trend-abundance

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## Introduction

Rabbit hemorrhagic disease (RHD) has caused substantial economic and ecological disruption in the Iberian Peninsula since it appeared at the end of the 1980s (Argüello et al. 1988). Economically, RHD reduced Spanish rabbit (*Oryctolagus cuniculus*) production by more than 20 % in a year and lead to the disappearance of many rabbit farms (Rosell et al. 2000; Rosell 2003). Similarly, RHD heavily reduced hunting activity in Spain and caused associated severe economic losses to the hunting industry (e.g., Villafuerte et al. 1998; Angulo and Villafuerte 2003). Although difficult to evaluate economically, it is also likely that there has been an even greater negative impact on biodiversity conservation since rabbits are regarded as a keystone species in Iberian Mediterranean ecosystems (Delibes-Mateos et al. 2007). Due to its multiple roles as an ecosystem engineer, the decline in

rabbits has affected many aspects of biodiversity (Delibes-Mateos et al. 2008a). In spite of the generalized reduction in abundance, in some agricultural areas of Spain where food is abundant and few predators are present, rabbits have been able to maintain high abundance and even become pests in some localities (Villafuerte and Delibes-Mateos 2008; Delibes-Mateos et al. 2009a).

Different management strategies are being employed with the aim of reducing or increasing rabbit abundance in the Iberian Peninsula by farmers, gamekeepers, hunters, and conservationists (e.g., Delibes-Mateos et al 2009b; Ferreira and Alves 2009). Management involves high costs, both economic and physical, especially when managers are ill-prepared to respond in a timely way to sharp increases or declines in rabbit abundance. For this reason, it would be very useful if each local manager was aware of the rabbit population trends and be aware of the most appropriate management programs to implement (e.g., protecting crops, feeding artificially endangered predators, etc.). It is surprising that currently there is no established plan to monitor rabbit abundance or trends in Spain (Delibes-Mateos et al. 2009b), nor any RHD surveillance, in spite of the disease being responsible for most of the above mentioned changes in abundance, and a notifiable disease for the World Organization for Animal Health (www.oie.int).

A priori, the uniqueness of the disease and the biological characteristics of the rabbit discourage the use of protocols of RHD surveillance based only on passive surveys for several reasons. RHD is now endemic in every wild population (Villafuerte et al. 1995), so this type of sampling, although it may be appropriate for determining the periods of greatest loss, is not useful to determine its incidence because predators can remove sick or dead animals very quickly (e.g., Villafuerte et al. 1994). In addition, many rabbits die in their burrows, making it even more difficult to obtain reliable epidemiological information. For example, Rouco (2008) found that two thirds of rabbits dying from RHD were found in their burrows. Finally, and no less important, even in those occasions when dying or dead animals are found, the identification of the cause of death might entail some difficulty, requiring complex analysis or diagnosis.

In turn, protocols for monitoring the RHD based on active sampling also involve some difficulties. On the basis of published field studies, it is assumed that the effect of RHD is lower in areas of high density because the rabbit population has been reduced proportionately less (Blanco and Villafuerte 1993; Delibes-Mateos 2006). Furthermore, mathematical models predict an average annual high prevalence of animals with antibodies in high-density populations (Calvete et al. 2002; Calvete 2006). Therefore, to correctly interpret the results of sampling both population abundance and serology are important explanatory variables. Moreover, the date selected for sampling is also a particularly important parameter. The marked seasonality of rabbit reproduction is highly dependent on the change of food quality (e.g., Villafuerte et al. 1997) and can profoundly affect the impact of RHD (Mutze et al. 2008). Finally, the greatest difficulty in establishing a protocol for monitoring is the collection of samples in the field. However, in the case of the rabbit, this could be facilitated with the collaboration of hunters.

In this study, our main goal is to set the groundwork for a future plan for the epidemiological surveillance of RHD in the Iberian Peninsula. Specifically, we address the following partial objectives: (1) to verify the relationship between population abundance and the prevalence of RHD antibodies in rabbits based on samples collected during the hunting season. (2) To establish an epidemiological model with the aim of determining the validity of sampling campaigns that could be performed during the general hunting season, which is from October to January. Finally, (3) to check if sampling carried out during the period of maximum abundance of rabbits (usually in June or July) is appropriate for low density areas or for certain areas in which rabbits are hunted only during this period.

## Material and methods

### Rabbit hemorrhagic disease

RHD is caused by a calicivirus (Öhlinger et al. 1990; Parra and Prieto 1990; Öhlinger and Thiel 1991) that spreads very quickly by direct or indirect contact (Xu and Chen 1989). Given the rapid development of the disease, rabbits are usually in good condition at time of death (Lavazza and Capucci 2008). The incubation period varies between 1 and 3 days, and death usually occurs between 12 and 32 h after the onset of fever (>40°C). During an epidemic, a few individuals (5-10%) have a chronic or subclinical disease, and death usually occurs within 1 or 2 weeks (Lavazza and Capucci 2008). However, there are animals that survive the disease, showing a strong seroconversion that is easily detected 4-6 days after infection (Lavazza and Capucci 2008). This is the case particularly for juveniles, whose mechanism of resistance to disease is related both to the pathogenesis of infection and age-related physiological susceptibility (see review in Cooke 2002).

Although several studies have suggested the existence of one or more types of non-pathogenic virus (Rodak et al. 1990; Capucci et al. 1991; Trout et al. 1997; Marchandeau et al. 1998a, b; Lavazza and Capucci 2008), such viruses have not yet been isolated from wild rabbit populations in Europe (Cooke and Fenner 2002; Calvete 2006). Moreover, White et al. (2002, 2004) have suggested that the same

RHD virus might have both pathogenic and non-pathogenic modes of spread, causing (1) an acute illness that, although very lethal, would allow the few survivors to produce antibodies, (2) a chronic infection, not lethal, which would promote an less vigorous immune response (i.e. Mutze et al. 2008). However, recently, Strive et al. (2009) have isolated and partially characterized a non-pathogenic virus related to RHDV from wild rabbits in Australia.

It is possible that such non-pathogenic viruses is present in the wild rabbit populations in Spain; but in the absence of further information, we simply assume that in most cases the RHDV antibodies seen in Spanish wild rabbits largely reflect activity of virulent RHDV.

## Rabbit abundance and trend

We performed two identical surveys in 1993 and 2002 in the same 11 areas to determine rabbit population change in central-southern Spain (see for details, Delibes-Mateos et al. 2008b). Along each transect, two observers walked 4 km obtaining a variety of information to quantify rabbit abundance (e g., latrines, scrapes, warrens, etc.) to derive a relative density index (Villafuerte et al. 1998; Delibes-Mateos et al. 2008b). The rabbit population change between dates was obtained according to Delibes-Mateos et al. (2008b), as:

$$\mathrm{RATE} = \left(\mathrm{IDR}_{2002} - \mathrm{IDR}_{1993}\right) / \mathrm{IDR}_{1993.}$$

To simplify the methodology for estimates of abundance that could be done by non-technical staff, the number of rabbit pellets within a 0.5 $m^2$ circular plot was also counted at 100-m intervals along the transect, avoiding counts on or near a latrine. The number of pellets counted in the 40 plots along each transect provide a pellet abundance index (pellets/$m^2$; PAI; Delibes-Mateos et al. 2007).

## Collection of rabbit samples and serological analysis

During the 2003 hunting season, we collected rabbit samples from the 11 locations where rabbit abundances had been previously surveyed (see above). Before the beginning of the hunting season, we contacted managers of the hunting estates to finalize a date (ideally one) when rabbit samples could be collected: an eye for age determination and blood for serological study. We aimed to collect samples from 15 to 20 shot rabbits at each locality. To reach this goal, we sometimes had to make several visits to those places where the abundance of rabbits was low. Piorno (2006) found that during the first days of the hunting season the hunters kill more rabbits; hence, our sampling campaigns took place in November. 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 RHD virus by indirect ELISA we used the commercial kit "INGEZIM RHDV" (INGENASA S.A., Madrid, Spain) following the manufacturer's recommendations. The antibody concentration is expressed in terms of a relative index on immunity (RI) ranging from 1 to 10. Sera with an RI>2 were considered positive because the antibody concentration is high enough for rabbit to survive the disease (for details, see Calvete et al. 2002).

## Rabbit model

We used an individual-based model to simulate the consequences of the interaction of members of a population. We modeled a population of rabbits which move around a number of 10 m×10 m grid squares in an area of 4 ha, resembling the model used by Fa et al. (2001). The population was classified into three age classes: newborns, juveniles, and adults. Newborns remain in the nest for 20-21 days and are largely dependent on their mother for survival. Survival of new born rabbits has been estimated as being close to 0.9 (Villafuerte 1994; Smith 1997). Juvenile rabbits (1-4 months old) are the age class that cannot breed and suffer high mortality, with approximately 70% dying due to diseases (mainly myxomatosis) and predation before reaching adulthood (Angulo and Villafuerte 2003). Both males and females become sexually mature in their fifth month of age, and fecundity is age dependant (Trout and Smith 1995). Yearly mortality rate of adults is approximately 60% (Angulo and Villafuerte 2003). Month-specific proportions of breeding females were obtained from Trout and Smith (1995), while month-specific litter size and survival rates were obtained from Smith (1997) and Villafuerte (1994). For further details, see Angulo and Villafuerte (2003).

The number of newborn rabbits is a function of the initial number and fecundity of adult females. As rabbits exhibit post-partum oestrous, females become available for mating immediately after giving birth. The model included a 30-day pregnancy period for females, with the probability of pregnancy being determined by the reproductive rate. Seasonality in breeding is user-defined by setting different probabilities of pregnancy for each month.

Rabbits undergo age-specific mortality, which is expressed as the probability of survival over a given time period for each age class. Mortality factors for rabbits include exposure to diseases and predators. Only the monthly mortality rates are available from the literature, so we transformed these data into a daily mortality parameter as described by Fa et al. (2001). Mortality due to RHD varies from 0 to 0.90 depending on the age of the affected individual and the presence of maternal antibodies.

The stochastic components were based on the natural variance of field data collected by Trout and Smith (1995).

We did not assume density-dependent survival or fecundity due to a lack of data to support this assumption (Smith and Trout 1994; Angulo and Villafuerte 2003).

We set the beginning of the study in the month 0 (January) and the end in the month 11 (December), and simulated death and birth processes for each individual at daily time steps.

### Epidemiology model

The population has four different rabbit classes according to their RHD status: susceptible, infected, chronically infected, and recovered individuals (Calvete 2006). Newborn age class was subdivided into two maternal RHD-antibody levels based on the absence or presence of maternal antibodies, but they are not included in the RHD dynamics because of they do not reproduce and do not interact socially with other rabbits (Robinson et al. 2002; Calvete 2006).

When a rabbit is infected, its mean survival time is 2 days, but the mortality rate depends on the age class and the absence or presence of maternal antibodies (Robinson et al. 2002). If an infected individual survives acute infection, this develops a chronic, nonlethal form of RHD and is recruited into chronic class. This class lasted for 20 days during which the individuals shed infective virus and after which the survivors became immune for life and were recruited into the recovered class. A proportion of immune individuals act as reservoirs of the virus and the value is chosen arbitrarily. For more detail, see Calvete (2006).

We modeled RHD epidemiology by running the rabbit model ten times for each rabbit population density and introducing the disease during the third year of the 8-year simulation. At the end of the simulation, we calculated the likely prevalence of antibodies in different age groups of rabbits to see if predictions were in line with expectations and field data. The specific dates for the model prevalence values were: (1) November, because this is the month in which biological samples in field were collected (2) in July, because this is the month of maximum rabbit abundance, and (3) all the 15 days periods comprised from October 15 to January 15 in order to assess how the variation of this parameter would result in samples that were undertaken throughout the period general hunting. The prevalence ($P$) was calculated as follows:

$$P = \frac{n^{\circ}\ \text{chronic individuals} + n^{\circ}\ \text{immune individuals}}{n^{\circ}\ \text{total individuals}}$$

We only took into account the individuals of >60 days age because these are the individuals susceptible to capture or shooting (Angulo and Villafuerte 2003).

## Results

Due to the low abundance of rabbits in three areas (<2 pellets/$m^2$) it was not possible to obtain the number of rabbits we originally planned to sample. In all these populations, we had to make more than one visit; e.g., at one site three visits. In total, we analyzed 150 samples of hunted rabbits in the 11 areas of study.

Seroprevalence ranged from 0% to 80% with an average of 37.33%. After analyzing the ages of the hunted rabbits, we realized that most of them were adults (>5 months old), representing the 82.76% of the hunted rabbits. Similarly, the seroprevalence in each age class differed noticeably. Almost half of adults (49.17%) were carriers of antibodies to RHD, while only 30% of juveniles were seropositive. The prevalence of antibodies in each population was not related to the proportion of hunted adults (Spearman $R$=0.20, $n$=11, $p$=0.55); however, there was a significant positive correlation with population abundance (Spearman $R$=0.64, $n$=11, $p$=0.03). Therefore, rabbit abundance was associated with a higher sero-prevalence of RHDV antibodies (Fig. 1). Similarly, the rate of population change was also related to antibody prevalence, showing a positive, significant correlation (Spearman $R$=0.83, $n$=11, $p$<0.01). Most of the studied populations showed negative population trends between 1993 and 2002. Five of 11 showed a rate of population change between −0.9 and −0.6 (black in Fig. 1) and two populations showed lower negative trends (grey; Fig. 1). Only three increased (white, Fig. 1). All populations that have a low current abundance and decreased significantly between 1993 and 2002, showed prevalence of antibodies to RHD of less than 40% (Fig. 1).

Modeling results also indicated that antibody prevalence should increase with population density. Moreover, as shown in Fig. 2, this is not only confined to simulated populations at the beginning of the general hunting season (November), but would also apply to samples taken during the period of maximum abundance (July). Furthermore, the

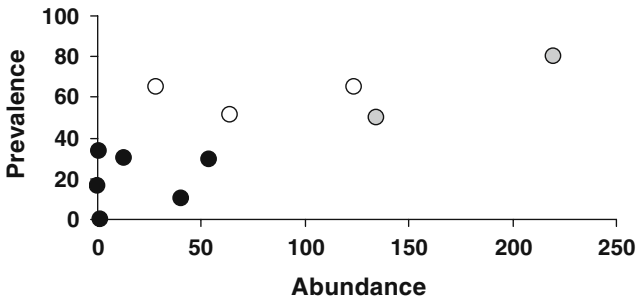


**Fig. 1** Prevalence (percentage of rabbits with antibodies to RHD) and population abundance (pellets/$m^2$) obtained for each of the 11 populations sampled. Population trend very negative, (*black*), slightly negative (*grey*), and positive (*white*)

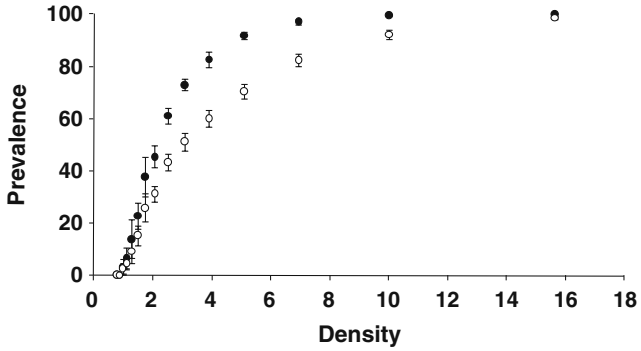


**Fig. 2** Prevalence (percentage of rabbits with antibodies to RHD) and population density (rabbits per hectare), according to the results of sampling simulations which will be carried out in July (*white*) or November (*black*)

relationship between population density and the prevalence is not linear but follows a logistic function for both dates (Fig. 2).

Antibody prevalence in both July and November, is predicted to approach 100% at densities exceeding ten rabbits/ha, but at a population density of less than two rabbits per hectare seroprevalence can be very variable, although not exceeding 50% even in November. It is interesting that the standard deviation is higher in populations of rabbit densities between two and five individuals per hectare with very low deviation for densities higher and lower this value (Fig. 2).

The prevalences obtained by simulating different sampling fortnights in the general hunting period are represented in Fig. 3. The proportion of animals with antibodies grows as the hunting period continues. However, differences between the prevalences obtained in the models are smaller at the extremes of the densities that have been considered in the study, becoming almost negligible differences at low and high densities.

Finally, to assess more clearly the differences in the prevalence peak (end of hunting season) and minimum (start), we have represented this value (range) in Fig. 4. It shows clearly that the greatest bias in sampling due to different dates would occur in populations of rabbits approximately 3/ha (12% range), dropping sharply above and below this density. In fact, the average rank obtained in all the simulations is less than 5%.

## Discussion

The introduction of new animal health monitoring protocols is a tremendous challenge for authorities, not only in an operational sense but also in terms of design. The choice of an appropriate methodology, spatial planning, and use of time as well as the coordination with staff working to obtain samples are perhaps the weakest points. It is not surprising, therefore, that up until now, there are few plans for health surveillance of wild species (Kuiken et al. 2005). In an operational sense, models combined with field tests such as those illustrated in this work may be very useful not only in planning the most appropriate strategy, but also for correctly interpreting results.

The epidemiology of RHD is known well enough to determine the expected mortality based on the prevalence, abundance of susceptible animals, and population abundance (e.g., Cooke 2002; Calvete 2006; Mutze et al. 2008). Thanks to this it should be possible to be forewarned and make the necessary management for the conservation of the species (Cooke 2002). However, there are certain gaps in the epidemiology of RHD that, if not resolved, could cause a major obstacle in the design of a plan for monitoring. For example, although it is assumed that the impact of RHD must be relatively low in areas where rabbits remain abundant (Blanco and Villafuerte 1993), theoretical studies

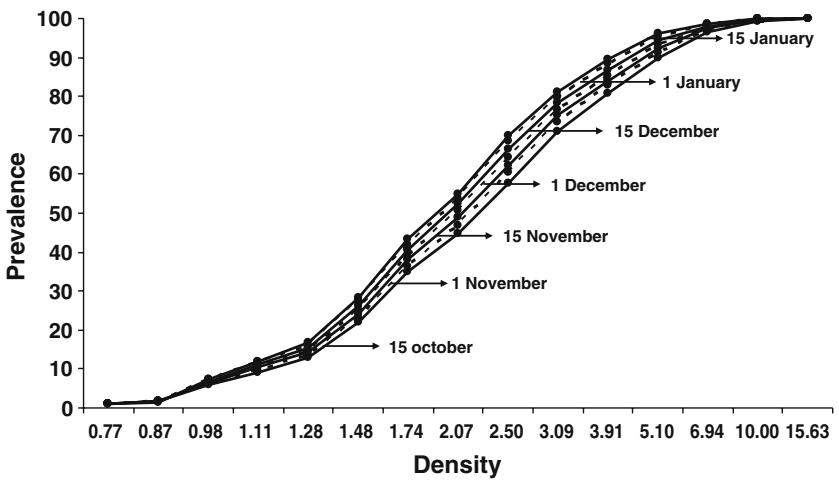


**Fig. 3** Prevalence (percentage of rabbits with antibodies to RHD) and population density (rabbits per hectare), according to the results of sampling simulations which will be carried out at different fortnights within the general game period in Spain

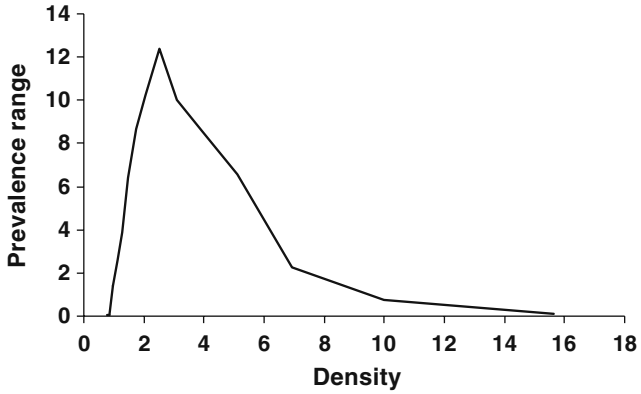


**Fig. 4** Prevalence range of rabbits with antibodies to RHD versus density (rabbits per hectare) in this work

based on mathematical models predict high prevalence antibodies to RHD in such populations (Calvete et al. 2002; Calvete 2006). This has not been empirically verified yet and even if it were done, it would be relatively difficult to measure the average annual prevalence (Calvete 2006). It is urgent to closely study RHD epidemiology to better understand and compare the results gathered from wild rabbit populations sampled on different locations, periods of the year, and in different age classes.

The average rabbit density in areas where hunting takes place is about five rabbits per hectare in central Spain (Villafuerte and Delibes-Mateos, 2008). Yet, despite working in the area of highest rabbit density in the Iberian Peninsula, only three of 11 sampled populations probably exceed these densities (Fig. 1), and in no case were any of the populations close to the maximum densities simulated. Because of this, we should have been working within the range where there is a more or less linear relationship between prevalence of antibodies and density (Fig. 2). On the other hand, more than half of surveyed populations had suffered a significant decline between 1993 (several years after the outbreak of RHD) and 2002. Delibes-Mateos et al. (2009b) observed that positive rabbit population trends have been recorded in favorable habitat. Our findings are in agreement with what predicted by theoretical models as populations with high prevalence in November have lower population declines, and moderate or high population abundance (Fig. 2).

The variation in the prevalence of RHD in the wild is truly remarkable, not only among populations, as shown in this study, but also within the same population depending on the sampling date. It has been observed that prevalence ranged from 17% to 100% in France and from 10% to 80 % in Australia depending on the sampling date (Marchandeau et al. 2000; Mutze et al. 2002, respectively).

Nevertheless, given the strong evidence and expectations of the correlation between the average prevalence of antibodies to RHD and abundance and population trend, it seems possible to establish a protocol for health monitoring of the disease, based on relatively simple estimates of both abundance and prevalence. On the one hand, the determination of rabbit abundance in this study was carried out using pellet counts during the time of maximum population, methodology relatively easy to perform by un-trained staff, and fairly accurate (Moreno and Villafuerte 1995; Palomares 2001). Unlike the sampling based on counting individuals and their distances to the line of travel, pellet counts enable good estimates even in areas with low abundance (e.g., Moreno and Villafuerte 1995). Additionally, tests for the presence of antibodies to RHD in serum are relatively simple, and there are now different commercial kits on the market. Obviously, sample size may compromise the ability to survey acutely the prevalence, but in our case, 40 animals would be more than enough to obtain acute results with a 15% of precision and a confidence interval of 95% (Magnani 1997).

During the general hunting season (October-January) the proportion of juveniles is usually very low, being considered "pre-breeding" phase of the rabbit (e.g., Angulo and Villafuerte 2003). This may be one of the reasons we have not found any relationship between antibody prevalence and age structure of populations, implying that it may not be necessary to determine the age of the rabbits sampled and this could greatly simplify methodology. Most samples needed for analysis in this study were obtained in a single day of hunting, although some populations with lower abundance required more visits. However, the simulation results of this study showed that it would be possible to collect samples on multiple visits because there are no significant differences between the prevalence of antibodies obtained in different dates for the hunting period (Fig. 3). Furthermore, the smallest differences occur in populations of very low density (<5%).

Notwithstanding the above, it is possible that in some areas the density of rabbits on some hunting reserves is so low that it does not allow sampling of a sufficient number

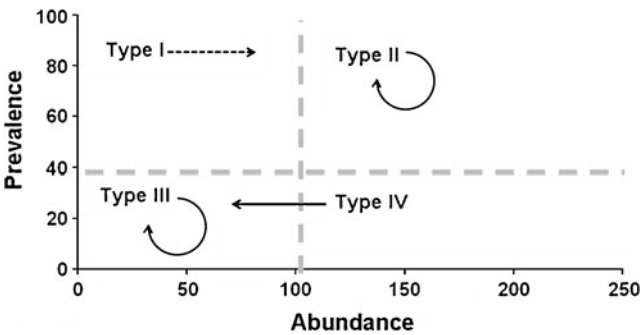


**Fig. 5** Interpretation of prevalence of antibodies and abundance that could be obtained from sampling performed during the monitoring plan of RHD. The *arrows* indicate the trend to vary from one population to another type

of rabbits for meaningful results. For example, in 12% of the 3,878 game reserves in Castilla-La Mancha (central Spain) where the rabbit is listed as a hunting species, the number of individuals hunted is currently less than 20 across the whole hunting season (A. Rios-Saldaña, personal communication). In these situations, it may be possible to sample when rabbits are normally more abundant (e.g., June or July), so reducing the sampling efforts, but also reducing the negative effect of hunting extraction, as previously recommended by Angulo and Villafuerte (2003). In these cases, the resulting prevalence would be lower than the one obtained during the winter hunting season (Fig. 2).

The protocol for animal health surveillance

In conclusion, it is possible to establish a protocol for monitoring the RHD based on the prevalence of antibodies to this disease and the abundance of rabbits. There are many techniques described for measuring rabbit abundance, such as line transects (e.g. Caley and Morley 2002), warren abundance and use (e.g., Parer and Wood 1986), pellet counts (e.g., Moreno and Villafuerte 1995), etc. Obviously each methodology has pros and cons (see Palomares 2001). In our case, we counted the pellets inside the 40 stations of 0.5 $m^2$ carried out during the maximum abundance period, because this technique is useful at both low and high rabbit densities, unlike most of the others, that require a minimum abundance (or a greater sampling effort) to avoid the "problem of many zero counts". Moreover, this low-cost methodology does not require highly trained personnel, may be conducted quickly, and is not seriously biased by weather factors (Fernández de Simón et al. 2008).

The estimates of the prevalence of antibodies can be obtained from blood (or liver) of hunted (or captured) individuals in the general hunting season. It would be desirable, in areas with high density, that the sampling were conducted in a single day at the start of the season. Obviously, in non-hunting areas, live capture (collection of blood) or hunting for scientific purposes should take place during this period. However, our models showed that in areas of low density samplings could take place throughout the whole hunting period, reducing the problem of getting an adequate sample. In those areas where it was not possible to obtain samples because of the low number of rabbits, it could be performed during the period of greatest abundance, but in such cases, it has to be taken account that the prevalence would be lower.

Depending on the estimates of abundance and prevalence obtained, it should be possible to divide each population according to the criteria set out in Fig. 5. Populations with high prevalence (type I and II), remain stable or show positive population trends. Type I populations probably increase more than those of type II, which are in equilibrium with the virus, and remain more stable in their abundances. Populations with low prevalence and abundance (type III), show very negative trends, and without any intervention, abundance is unlikely to increase. Some of these populations may also be in a predator pit (i.e., also effectively held low by predators). In this regard, as in the case of Doñana (Moreno et al. 2007), RHD appears to stop population growth because, although the population momentarily increases in abundance during favorable breeding seasons becoming a population of type IV (low number of animals with immunity to RHD and moderate or high abundance), most animals die suddenly because of the effect of RHD. Consequently, populations of type IV are unlikely to be detected in the Iberian Peninsula (none were found in this study) because their trends would be one of rapid decline.

Recently, Ferreira et al. (2009) showed that the myxomatosis immunization campaigns (vaccination) of wild rabbit populations might be considered as ineffective, mainly because the difficulties in capturing non immune rabbits before the arrival of the disease. Therefore, managers and conservationists should focus their most urgent efforts in those populations with low prevalence, base their strategies in tested management measures (see review of Delibes-Mateos et al 2009b), and assume that the increase in abundance will involve significant effort to overcome the suppressive impact of disease.

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