Incidence of viral hemorrhagic disease in wild rabbit populations in Spain

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Summary. – The current distribution and rate of spread of rabbit hemorrhagic disease (RHD) has been studied in Spain using interviews with hunters and conservationists and field surveys. The disease is currently present throughout Spain. A low rate of expansion (2-15 km per month), yet long distances between simultaneous outbreaks, suggests human-related transmission of RHD. The disease appears annually, mainly during spring (52.5 %) and winter (33.8 %). Most of the interviewees (63 %) indicated that the first outbreak of the disease caused greater mortalities than successive outbreaks. To investigate this, we estimated the mortality rate from RHD ot one locality, six years after the initial outbreak. The mortality rate was approximately 30 %, and lower than that estimated during the first outbreak of RHD (55-75 %). Few populations of rabbits (7.5 %) have returned to levels they held prior to the onset of the disease. Recovery was greater among dense populations. Most sites with low population of rabbits prior to the arrival of RHD have not recovered.

Résumé. – La distribution actuelle et les taux de propagation de la maladie hémorragique virale (RHD) ont été étudiés en Espagne par des entrevues directes et un examen sur le terrain, après l'arrivée de la RHD en 1988. La maladie est maintenant présente partout en Espagne. La façon dont la RHD s'est répandue en Espagne, avec un faible taux d'expansion entre localités (2-15 km par mois) et une longue distance entre épidémies simultanées, semble indiquer un mode de transmission lié à l'activité humaine. La maladie apparaît annuellement, principalement au printemps (52,5 %) et en hiver (33,8 %). La plupart des personnes interrogées (63 %) ont indiqué que la première épidémie de la maladie a causé une plus forte mortalité que les épidémies suivantes. Afin de vérifier cela, nous avons estimé le taux de mortalité dû à la RHD dans une localité, six ans après la première épidémie. Le taux de mortalité était d'environ 30 % et inférieur à celui estimé lors de la première épidémie de RHD (55-75 %). Peu de populations de lapins (7,5%) sont retournées aux niveaux antérieurs à l'arrivée de la maladie. Le rétablissement a été le plus important là où la densité avant la maladie était la plus forte. La plupart des populations avec une faible abondance de lapins avant la RHD se sont rétablies complètement.

INTRODUCTION

Rabbit hemorrhagic disease (RHD) is an infectious disease caused by a virus. It was first described in the People's Republic of China in 1984 (Liu *et al.* 1984), and

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was detected in 1988 in Europe where it was possibly introduced with imported domestic rabbits. Currently the disease is present in Europe, from the Federal Czech and Slovak Federal Republics to the south of Spain, including the Balearic Islands (Argüello *et al.* 1988, Beltrán 1991, Rodak *et al.* 1991, Villafuerte and Moreno 1991). Recently, RHD has been reported in the British Isles (Fuller *et al.* 1993) and during the ellaboration of this paper it has been detected in Australia, where it is causing larges mortalities within wild rabbit populations.

Shortly after the first RHD outbreaks were described, various types of vaccines were made from inactivated virus. These provided a high degree of immunity and were very effective in controlling outbreaks that occurred in domestic rabbits breeding facilities (Pages Mante 1990, Rodak *et al.* 1991).

Because RHD was first described, many efforts have been made to understand it, with research aimed mainly at characterization of the causal agent as well as clinical and pathological aspects of the disease (Pu *et al.* 1985, Capucci *et al.* 1991, Du 1991, Ohlinger and Thiel 1991). However, with the exception of Villafuerte *et al.* (1994), there have been no published studies of the disease or its incidence and evolution in natural wild rabbit populations. This lack of information is especially critical if we consider that in Spain the wild rabbit (*Oryctolagus cuniculus*) is the staple prey of a wide variety of predators (Delibes and Hiraldo 1981), including some carnivores that are severely endangered (e.g. the Iberian lynx, *Lynx pardinus*). In addition, rabbits are the most important small game species in Spain.

Our study had two objectives : a) to describe the mortality caused by RHD in a wild population, during an outbreak in the northeast of Spain six years after its first appearance ; and b) in a wider context, to describe its distribution and occurrence among wild rabbit populations in Spain and trends in its epidemiology since it first appeared.

STUDY AREA AND METHODS

Radiotracking

From November 1992, we monitored mortality patterns of rabbits in a locality near Zaragoza (NE Spain) with telemetry. Adult and young rabbits were captured by ferreting. Rabbits were radiomarked with tags that had an activity posture sensor (BIOTRACK, Wareham, UK). During Jannuary 1994, 20 radiotagged adult rabbits (12 females and 8 males) were located once every three days. Rabbits found dead were autopsied and the cause of deaths was determined. When possible, a direct hemagglutination test (HA) was used to detect antigens in liver tissues. The tests, carried out in micromethod, were performed by Laboratorios HIPRA (Gerona, Spain).

Mortality rates were estimated for the time interval between the first and last radiotagged rabbit found dead from RHD (Heisey and Fuller 1985).

Surveys and interviews

The entire Iberian Peninsula was sampled using : 1:100.000 maps (Cartografía Militar), with > 300 localities selected for surveying, and the following desing : 1) a 4 km field transect to collect data on relative density of rabbits, and a number of ecological and land use variables (results not presented here) ; and, 2) one interview with either a local hunter or naturalist to determine the relative density of rabbits before RHD, the effects of the first outbreak and subsequent population trends.

All the field surveys were completed in a short period (to avoid possible seasonal variations), with 17 collaborators. They were selected according to their previous experience in field work with rabbits, and their knowledge of the survey area. All interviews and field data were collected between June and July 1993, when rabbit densities were presumably at their greatest (Villafuerte 1994).

The questionnaire were conformed by more than 30 questions. To avoid ambiguity in the questionnaire, the possible answers were ordinal (yes/no) or interval ones (i.e. actual abundance of rabbits : very low, few, some, or many). Among the most important questions in this study, were those regarding the presence of RHD and its arrival date to the area.

To determine the presence/absence of RHD through the interviews, massive mortalities of rabbits must have been detected, and the presence of blood in their noses or mouths also clarified. If only carcasses (no matter the abundance) or a few rabbits were detected dead, RHD was not regarded as present at the area.

A total of 311 interviewees were used in this study. The data obtained were analysed using distribution-free statistics (Siegel 1956), and log-linear models for the test of associations between variables (Fienberg 1970, Heisey 1985).

RESULTS

Mortality estimated by radiotracking

During the radiotracking study in Zaragoza, the first RHD victims were found on 12 December 1993, 7 km west of the study area. In the last week of December, more dead rabbits were found by hunters, and the description of some of them suggested that RHD was the cause of death. The dates of the first and last radiotagged rabbits found dead due to RHD were 8 January and 5 February, respectively. During this period, six radiotagged rabbits (four males and two females) were found dead. In three of these cases it was possible to diagnose RHD as the cause of death, their HA liver tissue titres being 2^{18}, 2^{16} and 2^{20} respectively. Two other rabbits were predated or scavenged by red foxes (*Vulpes vulpes*). The remaining rabbit died in a burrow, it was not possible to ascertain the cause of death. Since this predation pressure is too high when compared from our previous experiences in studying wild rabbits in absence of RHD (monthly rates of mortality usually lower to 2 %), to be conservatives and in order to hipothetize a lower mortality due RHD along time, we assumed that all six of the 20 rabbits died of RHD.

The mortality rate during this period (29 days from the first to the last radiotagged rabbit found dead from RHD) was 29.3 % (6.8 %-46.6 %, 95 % confidence limits). This mortality rate was higher ($Z = 2.2$; $p = 0.014$) than the rate calculated in the 29 days prior to the RHD outbreak (4.9 %), and was also higher than that corresponding to the period following the outbreak ($Z = 2.9$; $p = 0.002$), when no deads occured.

Surveys results

Most of the interviewees (79.4 % ; n = 311) noticed the initial arrival of RHD. Presently, there are only a few localities where it has not been reported. Moreover, after the survey, we received news that RHD had reached localities that reported negative in the survey.

The dates of the onset of the disease were very different throughout the country, appearing simultaneously but patchily in areas far apart (Fig. 1). The cumulative proportion of localities affected by the disease depending of the timing of first outbreak were : 1988 : 5.2 %, 1989 : 19.9 %, 1990 : 50.1 %, 1991 : 72.4 %, 1992 : 77.6 % and 1993 : 78.5 %. This indicates a rapid spread since 1990.

After the first outbreak, 12.1 % of interviewees never detected the disease again, 62.8 % have detected it again but with lower mortality, and the rest (25.1 %) detected further massive mortalities. The majority of interviewees (81.3 %) who detected new deaths after the first outbreak, believe that the disease appeated once a year. It seems that it mainly occurs in spring (52.5 %) or winter (33.8 %).

Population recovery and trends

Less than half of the interviewwes (41.4 %) said that rabbit numbers had not increased at all after the first outbreak ; 29.5 % said that there had been a moderate increase ; and 7.5 % that the population had returned to levels existing prior to the onset of the disease.

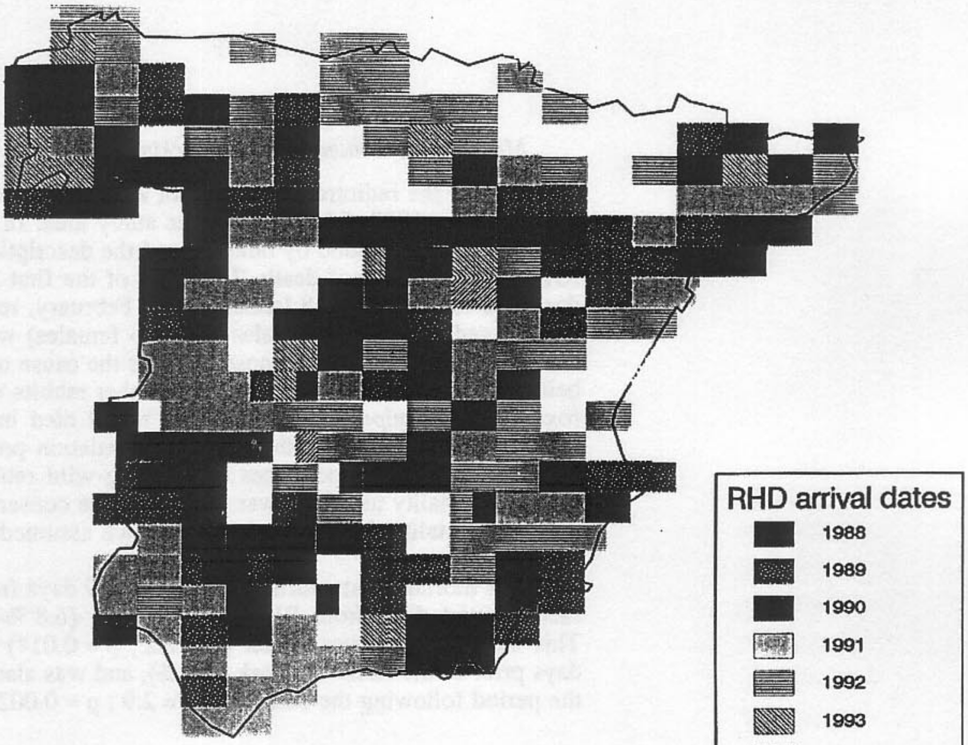

Fig. 1. – Distribution and dates of the first spread of the RHD, as reported by the interviewees.

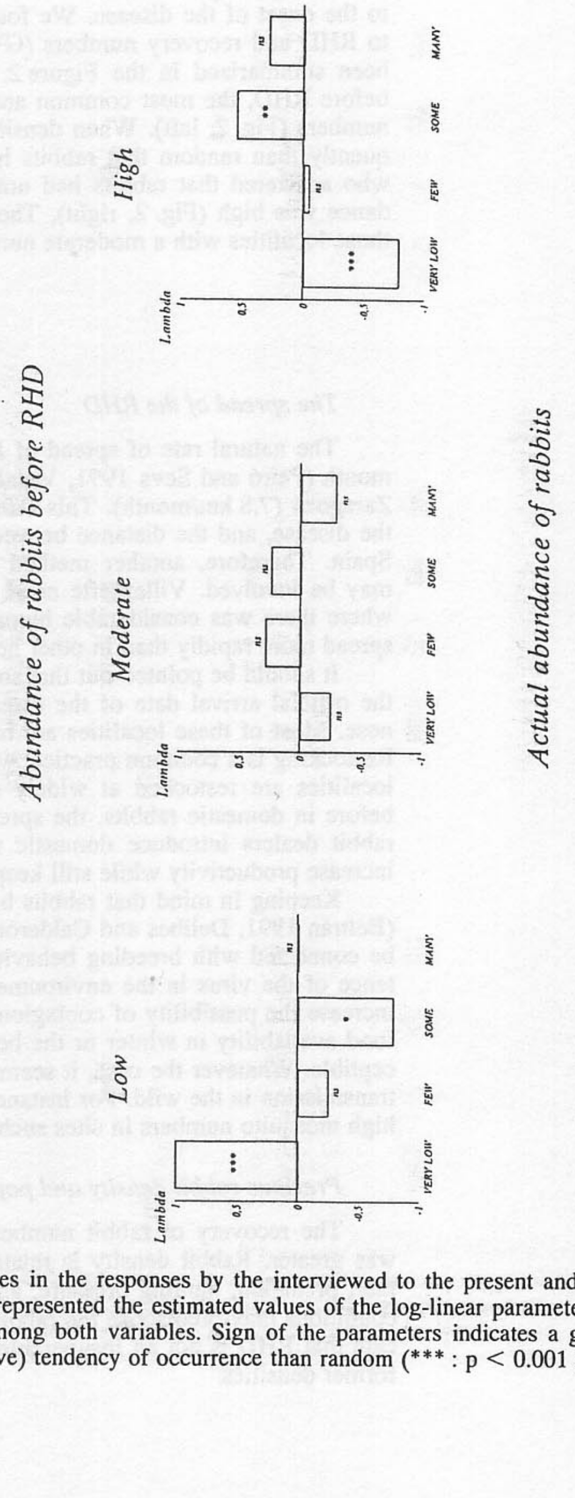

Fig. 2. – Tendencies in the responses by the interviewed to the present and past abundance of rabbits. It is represented the estimated values of the log-linear parameters (lambda) for the interaction among both variables. Sign of the parameters indicates a greater (positive) or lower (negative) tendency of occurrence than random (*** : $p < 0.001$; ** : $p < 0.01$; * : $p < 0.05$).

Using a log-linear model we analyzed rabbit recovery in relation to densities prior to the onset of the disease. We found a significant association between numbers prior to RHD and recovery numbers ($G^2 = 46.54$; d.f. = 6 ; $p < 0.0001$). The results have been summarized in the Figure 2. In the localities where rabbit numbers were low before RHD, the most common answer was that rabbits have not recovered to former numbers (Fig. 2, left). When densities were high, the interviewees answered more frequently than random that rabbits had recovered moderately, and there were very few who answered that rabbits had not recovered to former numbers when rabbits abundance was high (Fig. 2, right). There are no clear tendencies in the answers given on those localities with a moderate number of rabbit prior to RHD (Fig. 2, center).

DISCUSSION

The spread of the RHD

The natural rate of spread of RHD has been estimated to be around 2-15 km per month (Peiró and Seva 1991, Villafuerte *et al.* 1994), very similar to these obtained at Zaragoza (7.8 km/month). This rate is too slow to explain the dates of appearance of the disease, and the distance between outbreaks as the disease initialed spread through Spain. Therefore, another method of propagation, possibly connected with humans, may be involved. Villafuerte *et al.* (1994) showed that in the Doñana National Park, where there was considerable human activity, the disease colonized much earlier and spread more rapidly than in other nearby areas with minimal human activity.

It should be pointed out that some of the interviewees recalled that in 1987, before the official arrival date of the disease in, dead rabbits were found with blood in the nose. Most of these localities are in areas where rabbits were captured for restocking. Restocking is a common practice, with > 200.000 rabbits translocated each year. Many localities are restocked at widely scattered points across. Once RHD had appeared before in domestic rabbits, the spread to wild rabbits would have been rapid as some rabbit dealers introduce domestic rabbits (mostly females) into their populations to increase productivity while still keeping the « wild rabbit look ».

Keeping in mind that rabbits breed from early winter to the beginning of summer (Beltrán 1991, Delibes and Calderón 1979, Villafuerte 1994), the spread of RHD could be connected with breeding behavior. Another possibility could be the greater persistence of the virus in the environment during the winter and early spring. This would increase the possibility of contagion between the individuals as well as the decrease in food availability in winter or the beginning of spring that could make them more susceptible. Whatever the case, it seems that vectors do not play a decisive role in disease transmission in the wild. For instance, RHD outbreaks do not occur in association with high mosquito numbers in sites such as Doñana.

Previous rabbit density and population recovery

The recovery of rabbit numbers was greater where rabbit density prior to RHD was greater. Rabbit density is related to many factors such as food availability, weather, predation, hunting pressure, and soil quality. Therefore, after a catastrophe, these conditions may predispose the rabbits to a slower or quicker recovery. Our results indicate that RHD is not an insuperable disease because several localities returned to their former densities.

Current mortality due to RHD

In spite of the fact that our study at Zaragoza was carried out six years after the onset of the disease in the area, the estimated time period between the first and last findings of dead radiotagged rabbits (29 days) is very similar to those reported for domestics (42 days, Rossell *et al.* 1989) or for wild rabbit during the first outbreak (32 days, Villafuerte *et al.* 1994).

Since RHD was reported in the study area, wild rabbit numbers have decreased considerably and do not show clear sign of recovery. Although mortality from RHD during first outbreak was not estimated, the estimated mortality rate in this paper (probably over estimated), after six years and several known RHD outbreaks, suggests that mortality due RHD may be decreasing when compared with those reported during the first outbreak in other Spanish wild rabbit populations : 55 % at Doñana (SW Spain, Villafuerte *et al.* 1994) around 75 % at Navarra (N Spain, Ceballos, unpublished data) and at Alicante (SE Spain, Peiró and Seva 1991). This finding is supported by the interviewees since 63 % of them considered that the disease struck much harder during the first outbreak.

There are three possible non-exclusive explanations of these lesser mortality : a) a lower density of wild rabbits that makes transmission of the disease difficult ; b) a possible increasing of genetic or acquired resistance by rabbits to RHD, and c) a decrease in the pathogenicity caused from RHD. In this way, RHD resembles myxomatosis in the way that myxomatosis decreased also its effect on rabbit numbers (Trout *et al.* 1992), because a genetic resistance to the disease (Fenner and Ross 1994), and a lower virulence of the virus strains (Fenner and Chapple 1965).

Future of rabbit populations

Rabbit numbers will determine the future of other species (Villafuerte *et al.* in press). After myxomatosis many hunters and game keepers considered predators to be directly responsible for the lack of rabbits, and reinforced the traditional vermin control. Nevertheless, when prey populations are at a very low level, predators can delay recovery for a long time throughout the called predator pit (Newsome *et al.* 1989, Trout and Tittensor 1989).

As in the case of myxomatosis, RHD seems to have become an endemic disease for rabbits in the wild. While myxomatosis appears every year in summer or autumn, killing or affecting young rabbits, RHD appears every year and kills a high proportion of adults, being the susceptibility to RHD influenced by age (Xu 1991, Villafuerte *et al.* 1994). Myxomatosis is directly or indirectly (by facilitating the predation of infected animals) responsible for around 35 % of juvenile deaths (unpublished data). If RHD affects adults in the same way, its impact on rabbits dynamic must be greater than those produced by myxomatosis, considering the time of the year both diseases usually occurs (see the assumptions of Smith and Trout 1994). Moreover, while RHD reduces directly yearly rabbits' productivity by killing the reproductive animals just before or during the breeding season, myxomatosis reduces the number of reproductive rabbits that will be present during the next breeding season.

This study shows that populations with a higher density prior to the disease were able to recover better than those with lower densities, which, at least over the last five years, have not been able to recover at all.

Despite the small number of radiotagged animals used in this study, and the problems involved in obtaining informations from interviewees, present results about RHD are of a great value because epidemiological data are very scarce up to now. Therefore, more complete studies, with a more acute methodology, must be carried out to understand the evolution of the disease and the response by rabbits (incidence, possible immunity by rabbits, etc.).

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BIBLIOGRAPHY

ARGÜELLO, J.L., A. LLANOS PELLITERO and L.I. PEREZ ORDOYO GARCIA, 1988. – Enfermedad hemorrhagica del conejo en España. *Medicina Veterinaria*, 5 : 645-650.

BELTRÁN, J.F., 1991. – Temporal abundance pattern of the wild rabbit in Doñana, SW Spain. *Mammalia*, 55 : 591-599.

CAPUCCI, J.F., M.T. SCICLUNA and A. LAVAZZA, 1991. – Diagnosis of the viral hemorrhagic disease of rabbits and the European brown hare syndrome. *Revue Scientifique et Technique O.I.E.*, 10 : 347-370.

DELIBES, M. and J. CALDERÓN, 1979. – Datos sobre la reproducción del conejo *Oryctolagus cuniculus* (L.), en Doñana, S.O. de España, durante un año seco. *Doñana Acta Vertebrata*, 6 : 91-99.

DELIBES, M. and F. HIRALDO, 1981. – The rabbit as prey in the Iberian mediterranean ecosystem. *In* : Myers, K. and C.D. MacInnes (eds.). *Proceedings of the World Lagomorph Conference*, University of Guelph, Ontario. Pp. 614-622.

DU, N.X., 1991. – Molecular biology of the viral hemorrhagic disease of rabbits. *Revue Scientifique et Technique O.I.E.*, 10 : 325-336.

FENNER, F. and P.J. CHAPPLE, 1965. – Evolutionary change in myxoma virus in Britain. *J. Hygiene*, 63 : 175-185.

FENNER, F. and J. ROSS, 1994. – Myxomatosis, in : *The European rabbit. The history and biology of a successful colonizer* (eds H.V. Thompson & C.M. King), pp. 205-239. Oxford University Press.

FIENBERG, E.S., 1970. – The analysis of multidimensional contingency tables. *Ecology*, 51 : 419-433.

FULLER, H.E., D. CHASEY, M.H. LUCAS and J.C. GIBBENS, 1993. – Rabbit hemorrhagic disease in the United Kingdom. *Veterinary Record*, 25 : 611-613.

HEISEY, D.M., 1985. – Analyzing selection experiments with log-linear models. *Ecology*, 66 : 1774-1748.

HEISEY, D.M. and T.K. FULLER, 1985. – Evaluation of survival and cause-specific mortality rates using telemetry data. *J. Wildl. Manage.*, 49 : 668-674.

LIU, S.J., H.P. XUE, B.Q. PU and N.H. QUIAN, 1984. – A new viral disease in rabbits (in Chinese). *Animal Husbandry and veterinarian Medicine*, 16 : 253-255.

NEWSOME, A.E., I. PARER and P.C. CATLING, 1989. – Prolonged prey supression by carnivores in predator removal experiments. *Oecologia*, 78 : 468-467.

OHLINGER, V.F. and H.J. THIEL, 1991. – Identification of the viral hemorrhagic disease virus of rabbits as a calicivirus. *Revue Scientifique et Technique O.I.E.*, 10 : 371-392.

PAGES MANTE, A., 1990. – Control de la enfermedad hemorrágico virica del conejo (RHDV) mediante vacunación. *Medicina Veterinaria*, 7 : 93-96.

PEIRÓ, V. and E. SEVA, 1990. – Le lapin de garenne dans la province d'Alicante (sud-est de l'Espagne). *Bull. Mens. Off. Nat. Chase*, 182 : 189-196.

PU, B.Q., N.H. QUIAN and S.J. CUI, 1985. – Micro HA and HI test for the detection of antibody titers to so-called « hemorrhagic pneumonia » in rabbits. *Chinese Journal of Veterinary Medicine*, 11 : 16-17.

RODAK, L., B. SMID and L. VALICEK, 1991. – Application of control measures against viral hemorrhagic disease of rabbits in the Czech and Slovak Federal Republics. *Revue Scientifique et Technique O.I.E.*, 10 : 513-524.

ROSSELL, J.M., J.L. BADIOLA, J. PUJOLS, A. PÉREZ DE ROZAS, J.J. BADIOLA, J.A. GARCÍA DE JALÓN and M.A. VARGAS, 1989. – Enfermedad vírica hemorrágica del conejo. Epizootiológica y clínica. *Medicina Veterinaria*, 6 : 275-284.

SIEGEL, S., 1956. – *Nonparametric Statistic for the behavioural Sciences*. McGraw Hill Book Co. Inc. NY.

SMITH, G.C. and R.C. TROUT, 1994. – Using Leslie matrices to determine wild rabbit population growth and the potential for control. *J. Appl. Ecol.*, 31 : 223-230.

TROUT, R.C. and A.M. TITTENSOR, 1989. – Can predators regulate wild rabbit *Oryctolagus cuniculus* population density in England and Wales ? *Mammal Rev.*, 19 : 153-173.

TROUT, R.C., A.M. TITTENSOR and A.P. FOX, 1992. – The effect on a British wild rabbit population (*Oryctolagus cuniculus*) of manipulating myxomatosis. *J. Appl. Ecol.*, 29 : 679-686.

VILLAFUERTE, R. and S. MORENO, 1991. – Rabbit hemorrhagic disease (RHD) in Doñana National Park (DNP). *XXth Congress of the International Union of Game Biologist. Godollo, Hungary, August 21-26, 1991*, pp. 783.

VILLAFUERTE, R., 1994. – *Riesgo de predación y estrategias defensivas del conejo, Oryctolagus cuniculus, en el Parque Nacional de Doñana*. Ph. D. Diss. University of Córdoba.

VILLAFUERTE, R., C. CALVETE, C. GORTÁZAR and S. MORENO, 1994. – First epizootic of rabbit hemorrhagic disease in free-living populations of *Oryctolagus cuniculus* at Doñana National Park, S.W. Spain. *Journal of Wildlife Diseases*, 30 : 176-179.

VILLAFUERTE, R., D.F. LUCO, C. GORTÁZAR and J.C. BLANCO, In press. – Effect on red fox litter size and diet after rabbit hemorrhagic disease in North Eastern Spain. *Journal of Zoology*.

XU, W.Y., 1991. – Viral hemorrhagic disease of rabbits in the People's Republic of China : Epidemiology and virus characterisation. *Revue Scientifique et Technique O.I.E.*, 10 : 393-408.