Epidemiology of viral haemorrhagic disease and myxomatosis in a free-living population of wild rabbits

C. Calvete, R. Estrada, R. Villafuerte, J. J. Osácar, J. Lucientes

From January 1993 to June 1996, the epidemiology of myxomatosis and viral haemorrhagic disease (VHD) was studied in a free-living population of wild rabbits (*Oryctolagus cuniculus*) in Spain by means of serological surveys and radiotracking. Myxomatosis was endemic and associated with the breeding period. Its serological pattern was characterised by a 100 per cent prevalence of antibodies in adult rabbits and a rapid increase in antibodies in young rabbits in their first year. No mortality from myxomatosis was detected in adults, and mortality in young rabbits could not be estimated because of interference by predators and scavengers and the deaths of many radiotagged rabbits inside their burrows. VHD was also an endemic disease associated with the breeding period. Adults had a higher prevalence of antibodies against VHD than young rabbits, reaching values of 80 to 90 per cent. During the study, there was an increase in rabbit numbers as a result of a decrease in mortality from predation which was associated with an increase in mortality due to VHD and in the prevalence of antibodies to VHD. Mortality from VHD was lower in rabbits with VHD antibodies than in seronegative rabbits, but some mortality from the disease was also detected in seropositive rabbits. The annual mean mortality rate due to VHD in adult rabbits was estimated to be 21·8 per cent.

Veterinary Record (2002) **150,** 776-782

THE wild rabbit (*Oryctolagus cuniculus*) is one of the most important vertebrate species in Spanish Mediterranean ecosystems. Its size and relative ease of capture make it the potential prey of many predator species (Delibes and Hiraldo 1981), and the biodiversity of these ecosystems is usually associated with large numbers of rabbits. In addition, wild rabbits are the primary species of small game in sport hunting, and constitute the largest percentage of the game bag.

The appearance of myxomatosis in the 1950s led to a substantial reduction in the population density of rabbits and significant changes in their distribution in Spain (Muñoz 1960). Nevertheless, rabbit populations gradually increased until the arrival of viral haemorrhagic disease (VHD) at the end of the 1980s (Argüello and others 1988), which caused another considerable decrease in numbers and the extinction of many local wild rabbit populations (Villafuerte and others 1995). Since then, considerable efforts have been made to increase rabbit populations by restocking (Calvete and others 1997), by vaccination against VHD and myxomatosis, and by the control of predator populations. The results of these initiatives, however, have been inconclusive. Despite the importance of VHD in wild rabbit ecology and of its impact on other species which are affected either directly or indirectly by the decrease in the rabbit population, most studies of the disease have centred on its pathology and epidemiology in domestic rabbits, and data on wild populations are scarce. The aim of this study was to investigate some epidemiological parameters of VHD,

C. Calvete, PhD,
R. Estrada, PhD,
R. Villafuerte, PhD,
Instituto de Investigación de Recursos Cinégeticos, (IREC-CSIC-UCLM)
Ronda de Toledo s/n,
13005 Ciudad Real, Spain
J. J. Osácar, PhD,
J. Lucientes, PhD,
Departamento de Patología Animal,
Facultad de Veterinaria de Zaragoza, Zaragoza, Spain

and to estimate its effects on the survival rates of wild rabbit populations.

MATERIALS AND METHODS

Study area

The wild rabbit population chosen for study was located in a 250 hectare area representative of the Mediterranean ecosystem of the middle valley of the Ebro River, 15 km from Zaragoza in north-east Spain. The landscape consisted of low hillocks interspersed with small fields of wheat and barley. The natural vegetation was sparse steppe scrub with species such as *Genista scorpius*, *Rosmarinus officinalis* and *Thymus* species which were restricted to the hillocks where rabbit burrows are abundant. The climate was semi-arid Mediterranean, with an average of 300 mm of rain per year, and mean monthly temperatures ranging from 6·3°C in January to 25·9°C in August. Myxomatosis had been present in the rabbits in the area since the 1950s, whereas VHD was first detected in 1989.

Data collection

From March 1992 to September 1996, rabbit pellets were counted monthly in 50, 1 m^2 plots in the study area (Taylor and Williams 1956). The mean number of pellets deposited per day over 100 m^2 was used as a monthly index of rabbit numbers, and the annual accumulated mean of this index was used to survey the numbers throughout the study period.

To characterise population breeding, reproductive indices were estimated by regularly live trapping rabbits between January 1993 and June 1996. In 1993 and 1994, rabbits were captured by ferreting and baited cage traps. As these methods did not prove very effective, from the beginning of 1995 to the end of the study, only burrow fences were used. This method entailed surrounding the perimeter of each burrow with 1 m high wire mesh so that the rabbits, which could only cross through several 20 cm diameter holes in the lower part of the mesh, were trapped in cage traps placed outside each hole.

The captured rabbits were sexed, weighed and identified with a numbered ear tag (Presadom number 3; Chevillot); males with testicles in the scrotum and pregnant or nursing females were recorded. The reproductive indices of the population were calculated by using data from males weighing 880 g or more (the smallest male with testicles in the scrotum) and females weighing 960 g or more (the smallest pregnant female). The apophyseal line of the tibia was used as an indicator of age (Watson and Tyndale 1953). Two age classes were differentiated: adult rabbits without a palpable apophyseal gap and young rabbits with this gap.

Each captured rabbit was placed inside a cloth bag and a 1·5 ml blood sample was obtained from an incision in the auricular marginal vein. The blood samples were allowed to clot at room temperature, centrifuged and the serum obtained was frozen at –20°C and sent the Hipra SA Laboratory, Gerona, Spain, for the determination of antibody concentrations against VHD and myxomatosis by means of ELISAs (Pagés and others 1991). The antibody concentration was expressed in terms of a relative index of immunity (RI) ranging from 1 to 10, sera with an RI of 2 or more being positive. Blood samples were not taken from rabbits weighing less than 300 g (aged approximately under eight weeks) to avoid interference by maternal antibodies. A total of 344 sera were obtained during the study 156 from adults and 188 from young rabbits. Logistic regression was used to identify the factors associated with variations in the prevalence of the antibodies.

Rabbits found dead were examined postmortem, mainly to search for lesions due to VHD. When possible, a direct haemagglutination (HA) test was used to detect VHD antigens in liver tissues. The tests were carried out by the Hipra SA Laboratory, using a micromethod.

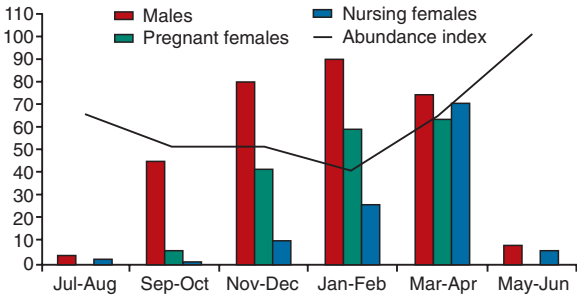

FIG 1: Mean percentages of male rabbits with testicles in the scrotum and pregnant and nursing females throughout the study, and the mean abundance index of the rabbit population, expressed as the number of pellets deposited per day per 100 m^2, estimated for periods of two months during the year

Radiotracking

From January 1993 until June 1996, the pattern of rabbit mortality was monitored by telemetry. Data on radiotagged adults and young were obtained throughout 1993, 1994 and 1995, but only data on adults were available in 1996. The rabbits were marked with transmitters equipped with an activity sensor (Biotrack). Rabbits weighing 600 g or more were tagged with 20 g radiocollars, but lighter rabbits were tagged with 5 g radiotags attached to their ears. The rabbits were located once every three days, to check whether they were alive or dead and, if dead, to determine the cause of death. When rabbits died inside a burrow, disease was assumed to be the cause. Mortality rates and their 95 per cent confidence intervals (CIs) were calculated for annual and two-month intervals by using the program Micromort, and compared by means of the one-way z-test (Heisey and Fuller 1985). The data obtained from young rabbits during the first week after they were tagged were excluded from the rate calculations to avoid any possible interference from an increase in mortality as a result of their increased vulnerability to predation while they adapted to the radiotags (Boag 1972, Gilmer and others 1974).

In all, data from 69 adults and 80 young were obtained during the study. Data for the young rabbits were added to those for the adults from January of the year after their birth. The adults were classified into two groups: 35 seronegative, with no VHD antibodies when tagged, and 34 seropositive, which had VHD antibodies when they were tagged.

RESULTS

Population dynamics

The reproductive indices showed that the population bred annually (Figs 1, 2). Males with testicles in the scrotum were captured throughout the year, with the largest percentage captured between September and April. Pregnant females were captured between October and April, especially between January and April, and the highest percentage of nursing females was captured in March and April.

The monthly variation in the population index (Fig 2) showed an annual cyclical pattern related to breeding. The index increased in February and March of each year and reached a maximum at the end of the breeding season in about May or June. This abundance pattern showed a maximum correlation with rainfall seven months earlier ($P<0·01$), such that the peaks in population numbers in the spring were correlated with the autumn rainfall in the previous year.

The autumn of 1995 was particularly dry, and the rainfall typical of autumn occurred in winter, causing a delay in the breeding season until the beginning of 1996. In 1996, the first pregnant females were captured in March, and the abundance index did not increase until April to May, later than in previous years.

The annual accumulated mean of the population index increased slightly during the study, the highest value occurring in 1995 before it decreased in the first half of 1996 (Fig 2).

Myxomatosis

During many visits to the study area, pathognomonic lesions due to myxomatosis were observed only during the breeding months, and only on young animals (Fig 2). The most common lesions were swellings (fibromas) on the nose, eyelids, external ear and anogenital region accompanied by mucopurulent blepharoconjunctivitis.

Throughout the study, 100 per cent of the adult rabbits had antibodies to myxomatosis and there was no variation either within or between years (Table 1). Young rabbits, however, had a lower prevalence ($P<0{\cdot}001$) that increased towards the end of every year ($P<0{\cdot}01$); no difference was found between the sexes, but the prevalence varied from year to year, having the lowest estimated value in 1996 ($P<0{\cdot}001$). However, this variation was caused by the design of the survey. All the rabbits sampled in 1996 were captured before the period of maximum incidence of myxomatosis, so that rabbits with myxomatosis were not detected until June of that year, which was later than in previous years. As the survey finished in July, a low antibody prevalence was estimated because of the high proportion of young not yet infected with the myxomavirus.

Viral haemorrhagic disease

From January 1993 to June 1996, 70 dead rabbits with gross lesions compatible with VHD were found (Fig 2). The most common lesions were in the trachea, lungs and liver, with hyperaemic dark reddening of the tracheal mucosa accompanied by abundant frothy fluid, lung congestion with multifocal haemorrhages, and a soft liver which was darkened or pale yellow with a marked lobular pattern. These lesions were frequently accompanied by poor blood coagulation and petechial haemorrhages in almost all the organs. Of the 70 rabbits, 46 (65·7 per cent) were found during the main breeding period (November to April, when the highest percentage of pregnant females was found), and the others during the rest of the year ($P<0{\cdot}01$). Twenty-five of the 45 young rabbit carcases, and 21 of the 25 adult carcases were found during the breeding season.

Thirty of the 70 carcases were in relatively good condition and liver samples were tested for VHD antigen by the HA test. Of the 19 samples obtained before May 1995, 12 were positive, but the 11 samples obtained later were negative ($P<0{\cdot}001$).

In general, the prevalence of antibodies to VHD (Table 1) increased during the study ($P<0{\cdot}001$), although the lowest prevalence for young rabbits occurred in 1994. Adult rabbits had a higher prevalence than young rabbits ($P<0{\cdot}001$), reaching values of around 80 to 90 per cent in 1995/96. No statistically significant differences were observed in the prevalence of VHD antibodies either within years or between the sexes.

Radiotracking

Three factors – flooding, predation and disease – were identified as causes of mortality, the last two being much more important than flooding (Table 2). Overall, the mortality among young rabbits was higher than among adults ($P<0{\cdot}01$), although there was a general decrease in annual mortality during the study owing to a considerable reduction in mortality from predation in 1994 and 1995. This reduction was partially compensated by an increase in mortality from disease, which was positively correlated with the increase in population (Fig 3).

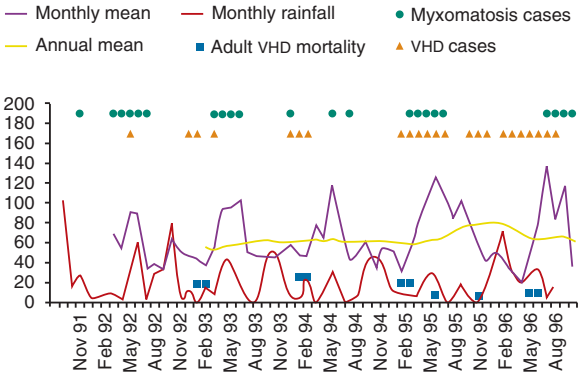

FIG 2: Monthly means and the annual mean rabbit abundance index, expressed as the number of pellets deposited per day per 100 m², the average monthly rainfall (mm) during the study, and the months during which rabbits were observed with myxomatosis or were observed with or died (per cent) of viral haemorrhagic disease (VHD)

For both age classes, there were no significant differences between males and females in the total or cause-specific mortality rates. In adult rabbits, there was no difference between the sexes in comparisons of the overall mortality rates with respect to the presence or absence of VHD antibodies.

Flooding Mortality due to flooding was observed only once. In May 1996, heavy rain flooded the burrows near the valley bottoms, and three of the 32 radiotagged adults died inside the flooded burrows. This represents a mortality rate of 9·4 per cent (95 per cent CI 0 to 19·5 per cent).

Predation For both adult and young rabbits, predation was the most frequent cause of death in the population. When possible, predation was assigned either to raptors (evidence of feathers, characteristic tufts of torn out hair and remains of long bones), or to carnivores (incisor marks on collars, scat, rabbit caecum, and sometimes burying of corpse remains). Red foxes (*Vulpes vulpes*) were the most frequently identified carnivore. During the study, 34 radiotagged adult rabbits were predated, foxes being identified as the species responsible for 21 of them and raptors for five. In the remaining eight cases, the species of predator could not be identified, although raptors were ruled out because of a lack of the obvious signs they leave in the remains. Fifty-eight young rabbits were predated, 18 by foxes, 14 by raptors and one by a feral dog and, in 25 cases, the predator could not be identified.

TABLE 1: Numbers of adult and young rabbits, the prevalence of antibodies to myxomatosis and viral haemorrhagic disease (VHD) and their mean (se) relative index of immunity (RI) to these diseases in successive years

	1993		1994		1995		1996	
	Adults	Young	Adults	Young	Adults	Young	Adults	Young
Number	28	25	46	22	33	98	32	43
Myxomatosis								
Seropositive	28	16	46	16	33	94	32	4
Prevalence (%)	100	64	100	73	100	96	100	9·3
RI	7·8 (0·4)	8·9 (0·6)	7·8 (0·4)	8·2 (0·6)	7·5 (0·4)	8·2 (0·2)	7·2 (0·5)	2·5 (0·5)
VHD								
Seropositive	13	11	29	3	28	55	31	24
Prevalence (%)	46	44	63	14	85	56	97	56
RI	5·1 (1·0)	4·7 (0·9)	4·3 (0·5)	3 (0)	6·7 (0·5)	5·8 (0·4)	7·7 (0·4)	7·2 (0·6)

TABLE 2: Total and cause-specific mean annual rates of mortality (per cent) and their 95 per cent confidence intervals of adult and young rabbits with and without antibodies to viral haemorrhagic disease

	Adults	Young	Adults		P
			Seropositive	Seronegative	
Total mortality	60·2 (45·7-70·8)	93·0 (79·0-97·7)	56·6 (28·0-67·3)	63·6 (43·3-76·6)	NS
Predation	36·8 (24·6-49·0)	69·7 (50·2-89·3)	43·1 (24·2-62·0)	29·3 (14·1-44·6)	NS
Diseases	21·8 (11·7-32·0)	23·2 (4·5-42·0)	13·5 (1·0-26·1)	31·1 (15·2-47·0)	<0·05
Flooding	1·5 (0-4·5)	–	–	3·1 (0-9·1)	NS

NS Not significant

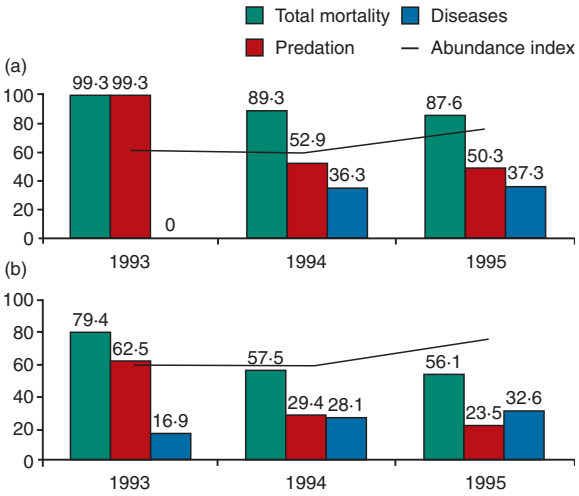

FIG 3: Total and cause-specific rates of mortality (per cent) among (a) young and (b) adult rabbits, and the annual mean abundance index of the rabbit population, expressed as the number of pellets deposited per day per 100 m^2

Disease Disease was considered to be the cause of death of 14 radiotagged adults. Five died inside a burrow, but only two of the carcases could be recovered because the size and complexity of the burrows hindered excavation. Death from disease was assumed in the remaining three cases. Postmortem examinations of the 11 recovered carcases revealed obvious lesions compatible with VHD in all of them. The presence of VHD antigen was confirmed by the HA test in three of the four carcases found in good condition.

The pattern of mortality from disease among adult rabbits was cyclical (Fig 2). In 1993, 1994 and 1995 the main periods of mortality were observed in January and February, just before the increase in the abundance index. The mortality rates from disease estimated for the adults in each of these two-month periods were 19·1 per cent (0 to 46·7), 25·5 per cent (6·3 to 44·7) and 20 per cent (0·4 to 35·8), respectively. In 1996, however, the main period of mortality was April to May, which correlated with the delay in reproduction. The estimated rate for this period was 9·6 per cent (0 to 22·1), lower than in the previous years. Other secondary peaks of mortality, detected in May and October 1995, were related to maximum population numbers.

None of the nine adults that died from VHD between January 1993 and February 1995 had antibodies against VHD when they were captured and radiotagged, whereas the five that died between May 1995 and May 1996 had VHD antibodies (RI in the range 2 to 10) when they were captured four to 10 months before their deaths. Seropositive adults suffered a lower annual mortality rate from disease than seronegative adults ($P<0·05$), although there was no significant difference between the total mortality rates of the two classes (Table 2).

Eleven of the 18 young rabbits classified as having died from disease did so inside burrows. Seven of the carcases could not be recovered, so the cause of their deaths was unknown. Another four were recovered in an advanced state of decay but with no signs of predation; again the cause of death remained unknown. Scavengers sometimes prevented the recovery of entire carcases of radiotagged rabbits; partially eaten carcases or carcases without viscera were often found. As a result, only three cases of lesions compatible with VHD were recorded in young rabbits. Mortality from disease in this age class occurred mainly during the breeding period, that is, March 1993, May 1994, and February, March and September 1995.

DISCUSSION

The reproduction of wild rabbits is associated with the growth of vegetation which, in turn, is related to rainfall and temperature (Poole 1960, Wood 1980). The breeding season of the study population started with the first substantial autumn rains, after which the proportion of pregnant females increased over the course of the breeding season. This dependence on rainfall delayed the start of reproduction in winter 1995/96 owing to the shortage of rain in autumn 1995. This pattern was similar to that observed in other populations of rabbits in southern Spain, in which the highest proportion of pregnant females occured in February to April, and the lowest in November to January (Soriguer 1981).

Predation and disease were the two main causes of mortality. The rates and yearly patterns of mortality were characteristic for rabbits, with lower mortality rates in adults than in young, and predation being the main cause in both age classes (Tyndale-Biscoe and Williams 1955, Parer 1977, Wood 1980, Wheeler and King 1985, Gibb 1993, Kunkele and Von Holst 1996). Nevertheless, the true impact of disease was probably underestimated because sick rabbits are more prone to predation, and rabbits that die from disease may be consumed as carrion and wrongly classified. This effect, especially in the young rabbits, is suggested by the results shown in Fig 3.

Clinical cases of myxomatosis were observed only in young rabbits in winter and spring. The serological survey showed that all the adult rabbits had myxoma antibodies with a high RI value throughout the year, signifying that most adults were probably resistant. The estimated prevalence of antibodies in the young rabbits was lower, although it increased during the year as they were gradually infected by myxomavirus. In general, myxomatosis was endemic, with outbreaks or increases in incidence associated with the recruitment of susceptible young rabbits, which were virtually all infected in the first year of life. This association was made evident in 1996, when the delay in breeding occurred simultaneously with the delay in the annual outbreak of myxomatosis.

These annual outbreaks of myxomatosis were probably initiated by transmission via fleas of the species *Spilopsyllus cuniculi* (Mead-Briggs and Vaughan 1975), whose numbers are associated with rabbit reproduction. This flea was the most abundant flea species in the area between March and May (Osácar 1996). The subsequent maintenance of the disease could depend on other flea species, such as *Xenopsylla cunicularis* (Launay 1982), which are very abundant in the area in spring and summer (Osácar 1996).

This pattern of myxomatosis is similar to that described for some French populations, which were found to be characterised by 100 per cent antibody prevalence in adult rabbits, and by the start of the annual outbreak when prevalence drops below 50 to 20 per cent in the whole population

as a result of the recruitment of young rabbits during the breeding season. After becoming infected, the young quickly reach antibody prevalence levels similar to those of the adults (Arthur and Louzis 1988, Rogers and others 1994, Marchandeau and Boucraut 1999).

All the radiotagged adult rabbits had high values of RI against myxomatosis, and no mortality from this disease was detected among adults. However, myxomatosis was probably an important cause of mortality among young rabbits because, despite the high proportion that died inside their burrows and the fact that carcases were quickly eaten as carrion, the buried heads of two predated radiotagged young rabbits were found with evident lesions of myxomatosis. This suggests that mortality due to myxomatosis was to a large extent hidden by predation. In contrast to the relative ease with which rabbits that died from VHD were found, the high prevalence of subacute or chronic forms of myxomatosis probably hindered the discovery of rabbits that died from myxomatosis; rabbits with myxomatosis were more likely to be predated before they died from the disease.

Although mortality from VHD among adult rabbits was included within mortality from disease, all the radiotagged adults found dead from disease had lesions compatible with VHD. The three adults that died inside the burrow and whose carcases could not be recovered, also probably died of VHD, because they died at the same time as other rabbits that died from this disease and all the radiotagged adults were resistant to myxomatosis. It may therefore be supposed that, among adult rabbits, mortality from disease was due exclusively to the VHD virus. However, the estimated mortality from disease in young rabbits probably had a more diverse aetiology. Past studies have shown that in addition to myxomatosis and VHD, other diseases of parasitic origin can be the cause of death of young rabbits (Tyndale-Biscoe and Williams 1955, Stodart 1968). Nevertheless, the annual mortality from disease among young rabbits increased progressively from 1993 to 1995, and coincided with the increase in the prevalence of VHD antibodies in both age classes and with the increase in mortality from VHD in adults. This correlation suggests that the increase in mortality from disease among young rabbits could also have been caused by an increase in mortality from VHD.

Throughout the study, a considerable increase in the prevalence of VHD antibodies occurred in parallel with an increase in population density. This trend coincided with the absence of positive results for the HA test in the samples analysed after May 1995, when the higher prevalence values and population numbers were estimated. Wild rabbit populations with a high prevalence of antibodies to VHD have been described by Dugast (1995), Marchandeau and others (1998) and Marchandeau and Boucraut (1999), who suggested that such high prevalence values could be caused by some strain of non-pathogenic rabbit calicivirus (RCV) (Capucci and others 1996b). This leaves the possibility that the observed increase in the resistance of the rabbits to infections by VHD virus could have been due to the appearance or generalisation of a new strain of less lethal non-haemagglutinating virus (Capucci and others 1996a, Kesy and others 1996). This possibility would account for the observed increase in antibody prevalence and population density, owing to the reduction in mortality from VHD in infected rabbits.

However, another hypothesis is that the increase in numbers from 1993 to 1995 could have been due to a decrease in predation. Although no special effort was made to monitor the fox population, in early 1994 there was an outbreak of sarcoptic mange among foxes, and sightings of foxes in the area decreased considerably in 1994 and 1995. This parasitic disease can cause high mortality in wild canid populations (Pence and Windberg 1994) and, since foxes were the most frequent predator of radiotagged rabbits, sarcoptic mange in foxes would have been expected to decrease rabbit mortality from predation and facilitate the increase in population density. This increase could have raised the reproduction rate of the VHD virus, leading to an increase in mortality due to VHD that partially compensated for the decrease in predation. Hence, despite the high mortality the virus causes in infected rabbits, the considerable increase in antibody prevalence could have been due to the increased incidence of VHD in young rabbits during the breeding period as a result of the increase in population density and, therefore, in the contact rate. These circumstances may have reduced the average age of infection and increased the proportion of rabbits infected at ages at which they were resistant to the disease, increasing the proportion of seropositives in the population (Anderson and May 1982, 1983). The observed epidemiological changes could, therefore, have been due to variations in population dynamics rather than to changes in the resistance of the rabbits to infection or to variations in the pathogenicity of the virus.

On the other hand, some of the liver samples may have given negative results in the HA test because the degradation of viral particles caused by sample decomposition disabled the haemagglutination reaction (Capucci and others 1991). However, subacute or chronic forms of VHD are associated with the appearance of degraded non-haemagglutinating virus particles, so the absence of haemagglutination in all of the samples analysed after May 1995 might have been due to the generalisation of these disease forms resulting from the high prevalence of antibodies to VHD in the population during that period (Capucci and others 1996a, Granzow and others 1996). However, this also leaves open the possibility of the appearance or generalisation of a new pathogenic non-haemagglutinating variant of the VHD virus (Capucci and others 1998, Schirrmeier and others 1999).

The presence of VHD antibodies in adult rabbits was associated with a higher survival rate due to a reduction in mortality from the disease, although death from VHD was still detected in seropositive rabbits. Rabbits with VHD antibodies that died from VHD may have suffered from persistent infection, although the observation of cyclical mortality from the disease, even in seropositive rabbits, suggests that these rabbits were newly infected during outbreaks. It remains unknown, however, whether the antibodies in seropositive rabbits originated from previous VHD infections or whether they were due to some non-pathogenic strain of RCV.

There was an annual cycle in the occurrence of VHD outbreaks, which were detected predominantly in winter and spring. This regular cycle indicates that VHD was an endemic disease, with endemic peaks or increases in incidence associated with rabbit reproduction. This relationship was evident in 1996, when the delay in breeding was associated with an equivalent delay in the beginning of the endemic peak of the disease. This epidemiological pattern suggests that the virus remains in the same area from one year to another, although the primary reservoirs of the virus from an epidemiological point of view are still unknown. The peaks of maximum mortality from VHD in adult rabbits were detected at the beginning of the second half of the breeding season, when the highest proportion of pregnant females was found, and when most young rabbits were still limited to the burrows. In these conditions, the rate of reproduction of the virus would have been less than that expected if only the numbers of rabbits were considered; the simultaneous appearance of numerous sources of virus could be suggested as the cause of the high mortality detected among adults at the start of the annual outbreaks. It is possible that rabbits with a persistent infection function as sources of the virus (Loliger and Eskens 1991), or that flies act as reservoirs and mechanical vectors (Asgari and others 1998). However, the small numbers of persistently infected rabbits in wild populations in Spain (Simón

and others 1998), and the limited winter activity of flies in the area, suggest a different reservoir for the virus. The persistence of the virus inside the burrows, associated with the large number of rabbit deaths from VHD inside them (Cooke 1996) may play an important role in VHD epidemiology because during the breeding season adult rabbits considerably increase their use of the burrows, especially for digging (Gibb 1990). This behaviour would facilitate the simultaneous infection of a high proportion of the adults, causing the annual winter outbreak, which would continue while there were sufficient susceptible young rabbits in the population (Cooke and others 2000).

The question of whether the burrows are the main reservoir of the VHD virus is an important point for future research, because the numbers of burrows might determine how the disease manifests itself and its impact on rabbit populations. If burrows acted as reservoirs of VHD, it would be expected that, in areas with many burrows, VHD would be endemic and associated with the breeding season when young and, therefore, resistant rabbits form a substantial proportion of the population. In areas where burrows are scarce or absent, however, VHD would be expected to be irregular and epidemic, and associated with successive reintroductions of the virus by carriers. In comparison with the great impact of VHD on wild rabbits, little is known about rabbit ecology, and more research is needed both on the epidemiology of VHD in wild rabbit populations and on possible measures to help them to recover.

ACKNOWLEDGEMENTS

Funding was provided by the Dirección General de Medio Natural (Gobierno de Aragón) and a postdoctoral fellowship from CSIC-Junta de Andalucía (IFD97-2299) to C. C. The authors are grateful to E. Espejo for the use of the facilities on his property. This study would have been impossible to carry out without the work of E. Escudero and J. Guiral.

References

ANDERSON, R. M. & MAY, R. M. (1982) Directly transmitted infectious diseases: control by vaccination. *Science* **215,** 1053-1060

ANDERSON, R. M. & MAY, R. M. (1983) Vaccination against rubella and measles: quantitative investigations of different policies. *Journal of Hygiene* **90,** 259-325

ARGÜELLO, J. L., LLANOS, A. & PEREZ, L. I. (1988) Enfermedad vírica hemorrágica del conejo en España. *Medicina Veterinaria* **5,** 645-650

ARTHUR, C. P. & LOUZIS, C. (1988) Myxomatose du lapin en France: une revue. *Revue Scientifique et Technique – Office International des Epizooties* **7,** 939-957

ASGARI, S., HARDY, J. R. E., SINCLAIR, R. G. & COOKE, B. D. (1998) Field evidence for mechanical transmission of rabbit haemorrhagic disease virus (RHDV) by flies (Diptera: Calliphoridae) among wild rabbits in Australia. *Virus Research* **54,** 123-132

BOAG, D. A. (1972) Effect of radio packages on behaviour of captive red grouse. *Journal of Wildlife Management* **36,** 511-518

CALVETE, C., VILLAFUERTE, R., LUCIENTES, J. & OSÁCAR, J. (1997) Effectiveness of traditional wild rabbit restocking in Spain. *Journal of Zoology, London* **241,** 271-277

CAPUCCI, L., CHASEY, D., LAVAZZA, A. & WESCOTT, D. (1996a) Preliminary characterisation of a non-haemagglutinating strain of rabbit haemorrhagic disease virus from the United Kingdom. *Journal of Veterinary Medicine* **43,** 245-250

CAPUCCI, L., FALLACARA, F., GRAZIOLI, S., LAVAZZA, A., PACCIARINI, M. & BROCCHI, E. (1998) A further step in the evolution of rabbit haemorrhagic disease virus: the appearance of the first consistent antigenic variant. *Virus Research* **58,** 115-126

CAPUCCI, L., FUSI, P., LAVAZZA, A., LODOVICA, M. & ROSSI, C. (1996b) Detection and preliminary characterisation of a new rabbit calicivirus related to rabbit haemorrhagic disease virus but nonpathogenic. *Journal of Virology* **70,** 8614-8623

CAPUCCI, L., SCICLUNA, M. T. & LAVAZZA, A. (1991) Diagnosis of viral haemorrhagic disease of rabbits and the European brown hare syndrome. *Revue Scientifique et Technique – Office International des Epizooties* **10,** 347-364

COOKE, B. D. (1996) Field epidemiology of rabbit calicivirus disease in Australia. European Society for Veterinary Virology Symposium on caliciviruses. University of Reading, Reading, UK, September 15 to 17, 1996

COOKE, B. D., ROBINSON, A. J., MERCHANT, J. C., NARDIN, A. & CAPUCCI, L. (2000) Use of ELISAs in field studies of rabbit haemorrhagic disease (RHD) in Australia. *Epidemiology and Infection* **124,** 563-576

DELIBES, M. & HIRALDO, F. (1981) The rabbit as prey in the Mediterranean ecosystem. Proceedings of the World Lagomorphs Conference. Eds K. Myers, C. D. MacInnes. University of Guelph, Ontario, Canada, 1979. pp 614-622

DUGAST, F. (1995) Etude de la prevalence serologique de la maladie haemorragique virale dans une population de lapins de garenne. PhD thesis, Faculté de Médecine de Nantes, France

GIBB, J. A. (1990) The European rabbit *Oryctolagus cuniculus*. In Rabbits, Hares and Pikas. Eds J. A. Chapman, J. E. C. Flux. Gland, UICN. pp 116-120

GIBB, J. A. (1993) Sociality, time and space in a sparse population of rabbits *(Oryctolagus cuniculus)*. *Journal of Zoology, London* **229,** 581-607

GILMER, D. S., BALL, I. J., COWARDIN, L. M. & RIECHMANN, J. H. (1974) Effects of radio packages on wild ducks. *Journal of Wildlife Management* **38,** 243-252

GRANZOW, H., WEILAND, F., STREBELOW, G. G., LIU, C. M. & SCHIRRMEIER, H. (1996) Rabbit haemorrhagic disease virus (RHDV): ultrastructure and biochemical studies of typical and core-like particles present in liver homogenates. *Virus Research* **41,** 163-172

HEISEY, D. M. & FULLER, T. K. (1985) Evaluation of survival and cause-specific mortality rates using telemetry data. *Journal of Wildlife Management* **49,** 668-674

KESY, A., FITZNER, A., NIEDBALSKI, W., PAPROCKA, G. & WALKOWIAK, B. (1996) A new variant of the viral haemorrhagic disease of rabbit virus. *Revue Scientifique et Technique – Office International des Epizooties* **15,** 1029-1035

KUNKELE, J. & VON HOLST, D. (1996) Natal dispersal in the European wild rabbit. *Animal Behavour* **51,** 1047-1059

LAUNAY, H. (1982) Données préliminaries sur l'écologuie de *Xenopsylla cunicularis* Smit, 1957 (Siphonaptera: Pulicidae) parasite du lapin de garenne. *Annales de Parasitologie* **57,** 145-163

LOLIGER, H. C. & ESKENS, U. (1991) Incidence, epizootiology and control of viral haemorrhagic disease of rabbits and the European brown hare syndrome in Germany. *Revue Scientifique et Technique – Office Internationale des Epizooties* **10,** 423-430

MARCHANDEAU, S. & BOUCRAUT, C. (1999) Epidemiology of myxomatosis and calicivirosis related to RVHD in a free-living population of European rabbits *(Oryctolagus cuniculus)*. *Gibier Faune Sauvage* **16,** 65-80

MARCHANDEAU, S., RICCI, J. C. & CHANTAL, J. (1998) Taux de prevalence serologique du virus de la maladie virale haemorragique (VHD) du lapin de garenne *(Oryctolagus cuniculus)* et de ses formes apparentees au sein de differentes populations sauvages en France. *Mammalia* **62,** 95-103

MEAD-BRIGGS, A. R. & VAUGHAN, J. A. (1975) The differential transmissibility of myxoma virus strais of differing virulence grades by the rabbit flea *Spilopsyllus cuniculi* (Dale). *Journal of Hygiene* **75,** 237-247

MUÑOZ, G. (1960) Anverso y reverso de la mixomatosis. Madrid, Dirección General de Montes

OSÁCAR, J. J. (1996) Ecología de las pulgas (Siphonaptera) del conejo silvestre *(Oryctolagus cuniculus)* en el Valle Medio del Ebro. PhD thesis, University of Zaragoza, Spain

PAGÉS, A., ARTIGAS, C. & ESPUÑA, E. (1991) Serological profile (by ELISA) of the active and passive immunity on rearing does vaccinated with an oil inactivated vaccine against RHD. Proceedings of the International Symposium on RHD. Beijing, China, August, 1991

PARER, I. (1977) The population ecology of the wild rabbit, *Oryctolagus cuniculus* (L), in a Mediterranean-type climate in New South Wales. *Australian Wildlife Research* **4,** 171-205

PENCE, D. B. & WINDBERG, L. A. (1994) Impact of a sarcoptic mange epizootic on a coyote population. *Journal of Wildlife Management* **58,** 624-633

POOLE, W. E. (1960) Breeding of the wild rabbit, *Oryctolagus cuniculus,* in relation to the environment. *CSIRO Wildlife Research* **5,** 21-43

ROGERS, P. M., ARTHUR, C. P. & SORIGUER, R. C. (1994) The rabbit in continental Europe. In The European Rabbit. The History and Biology of a Successful Coloniser. Eds H. V. Thompson, C. M. King. London, Oxford Science Publications. pp 22-63

SCHIRRMEIER, H., REIMANN, I., KOELLNER, B. & GRANZOW, H. (1999) Pathogenic, antigenic and molecular properties of rabbit haemorrhagic disease virus (RHDV) isolated from vaccinated rabbits: detection and characterisation of antigenic variants. *Archives of Virology* **144,** 719-735

SIMÓN, M. C., ORTEGA, C., MAYNAR, P., MUZQUIZ, J. L., DE BLAS, I., GIRONES, O., ALONSO, J. L. & SANCHEZ, J. (1998) Studies in wild rabbit *(Oryctolagus cuniculus)* populations in Navarra (Spain). I. Epidemiology of rabbit viral haemorrhagic disease. *Gibier Faune Sauvage* **15,** 47-64

SORIGUER, R. C. (1981) Biología y dinámica de una población de conejos *(Oryctolagus cuniculus,* L*)* en Andalucía Occidental. *Doñana Acta Vertebrate* **8,** 1-379

STODART, E. (1968) Coccidiosis in wild rabbits, *Oryctolagus cuniculus* (L) at four sites in different climatic regions in Eastern Australia. I. Relationship with the age of the rabbit. *Australian Journal of Zoology* **16,** 69-85

TAYLOR, R. H. & WILLIAMS, R. M. (1956) The use of pellet counts for estimating the density of populations of the wild rabbit, *Oryctolagus cuniculus* (L) *New Zealand Journal of Science and Technology* **38,** 236-256

TYNDALE-BISCOE, C. H. & WILLIAMS, R. M. (1955) A study of natural mortality in a wild population of the rabbit, *Oryctolagus cuniculus. New Zealand Journal of Science and Technology* **36,** 561-580

VILLAFUERTE, R., CALVETE, C., BLANCO, J. C. & LUCIENTES, J. (1995) Incidence of viral haemorrhagic disease in wild rabbit populations in Spain. *Mammalia* **59,** 651-659

WATSON, J. S. & TYNDALE, C. H. (1953) The apophyseal line as an age indicatory for the wild rabbit. *New Zealand Journal of Science and Technology* **34,** 427-435

WHEELER, S. H. & KING, D. R. (1985) The European rabbit in southwestern Australia. III. Survival. *Australian Wildlife Research* **12,** 213-225

WOOD, D. H. (1980) The demography of a rabbit population in an arid region of New South Wales, Australia. *Journal of Animal Ecology* **49,** 55-79