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## Susceptibility of wild rabbits (*Oryctolagus cuniculus*) in the United Kingdom to rabbit haemorrhagic disease (RHD)

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**Summary** — Adult wild rabbits from the southern UK, previously unexposed to rabbit haemorrhagic disease (RHD), were experimentally challenged with a UK strain of the virus in laboratory conditions. Initial serum antibodies were measured by an haemagglutination inhibition test and all seropositive rabbits, with reciprocal titres > 10, were protected against fatal infection. These results are consistent with the behaviour of laboratory and commercially bred rabbits in similar circumstances, and are relevant to consideration of the overall impact of RHD in wild populations.

**rabbit haemorrhagic disease / calicivirus / wild rabbit**

**Résumé** — **Sensibilité du lapin de garenne (*Oryctolagus cuniculus*) du Royaume-Uni à la maladie hémorragique du lapin.** Des lapins de garenne adultes du sud du Royaume-Uni, qui n'avaient jusqu'alors pas été exposés à la maladie hémorragique virale du lapin (RHD), ont été infectés expérimentalement par une souche du virus provenant du Royaume-Uni, dans des conditions de laboratoire. Les anticorps sériques initiaux ont été mesurés par un test d'inhibition hémagglutination, et tous les lapins séropositifs, dont les titres réciproques étaient >10, étaient protégés contre une infection mortelle. Ces résultats correspondent au comportement de lapins de laboratoire et de lapins élevés commercialement dans des circonstances similaires, et s'appliquent à la considération de l'impact global de la RHD dans les populations sauvages.

**maladie hémorragique virale du lapin / calicivirus / lapin de garenne**

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## INTRODUCTION

Since rabbit haemorrhagic disease (RHD) was first described in 1984 as a highly infectious fatal condition, it has become widespread throughout much of Asia and Europe. The disease has recently been reviewed by Ohlinger et al (1993) and Chasey (1997). Despite the considerable impact of RHD on domestic and commercial rabbits, and extensive investigation of the causative calicivirus, there is comparatively little published data on the epidemiology of the disease among European wild rabbits (Maess et al, 1990; Villafuerte et al, 1994; Simon et al, 1995). This is, however, an important area from which to obtain information as wild rabbit populations are expected to act as continuing major reservoirs of infection for pet, show and commercial rabbits, and implications for conservation, with respect to both fauna and flora, could be considerable.

Morbidity and mortality rates among susceptible adult domestic rabbits challenged with RHD can be as high as 90-100% but in some populations many individuals appear to be unaffected. It is clear that where there has been no previous exposure to the clinical disease, RHD-specific serum antibody may already occur in a proportion of European rabbits (Ohlinger et al, 1989; Rodak et al, 1990; Capucci et al, 1991; Smid et al, 1991; Trout et al, 1997), and this was also described in the People's Republic of China (Huang, 1991). Retrospective examination of stored sera has indicated that RHD antibody has been present many years before the emergence of recognisable disease (Rodak et al, 1991; Chasey et al, 1995). Importantly, experimental studies on farmed (Rodak et al, 1991) and laboratory (Chasey et al, 1995) rabbits have shown that seropositive animals are effectively protected against fatal infection when challenged with RHD virus, and this finding may explain the variation in survival rates sometimes observed in the field.

A recent seroepidemiological survey of wild rabbits in the UK found that more than 60% of the population overall in late winter was seropositive to the disease (Trout et al, 1997). Sera in this study were collected from areas where RHD had not been reported, since its appearance in 1992 (Fuller et al, 1993). The data suggested that by no means all adult wild rabbits across the UK would be susceptible to the disease. There is, however, little evidence to indicate whether seropositive, or indeed seronegative, wild rabbits respond to virus challenge in the same manner as laboratory bred or farmed animals (Simon et al, 1995). Information in this area would be useful in predicting likely environmental effects, and could help in planning the strategic overall response to the continuing spread of RHD. The purpose of the present study was to investigate the susceptibility of seropositive wild rabbits to experimental challenge with a UK strain of RHD virus.

## MATERIALS AND METHODS

A total of 27 adult wild rabbits were obtained from the counties of Surrey and Hampshire in the southern UK. Rabbits were either newly trapped (Surrey) or taken from the Central Science Laboratory's wild colony at the Agricultural Development and Advisory Services Bridgets Farm (Hampshire). There was no known record of RHD at any of the trap sites and the disease had not occurred at Bridgets Farm. Rabbits weighed between 1.25 and 1.53 kg, and were estimated to be at least 8 months of age.

Four laboratory-bred RHD-seronegative adult New Zealand (NZ) White rabbits, 2.5-2.9 kg in weight and over 8 weeks of age, were obtained as virus controls.

Blood samples from all the rabbits were taken initially and serum antibody levels to RHD virus were measured by a haemagglutination inhibition test (HIT) based on a previously described haemagglutination assay (Chasey et al, 1995). Sera were heat inactivated at 56 °C for 30 min, kaolin extracted for 1 h and adsorbed with human type 'O' erythrocytes. Haemadsorption times were either 1 h at ambient temperature or

overnight at +4 °C, and the final starting dilution of the sera was 1/10. Sera were examined using eight haemadsorption units of antigen, and titrated in doubling dilutions in microplates. Titres were defined as the highest dilution giving complete inhibition of haemagglutination.

Twenty-two seropositive rabbits, with titres > 1/10, and five seronegatives, with titres < 1/10, were selected and held in open pens for 5-10 days, in order to accustom them as far as possible to captivity, before transfer to individual cages in a high disease security laboratory. They were fed on pelleted food and carrots throughout the experimental period, and none of the rabbits died before inoculation.

Challenge virus was prepared from a laboratory New Zealand White rabbit that had died after experimental infection with the UK Ascot strain of RHD virus. Liver tissue was homogenised in a 1:10 dilution in phosphate buffer, and clarified and filtered (0.22 µm) to produce an infectious virus suspension. RHD virus cannot be titrated *in vitro*, but titres in liver tissue homogenates prepared in this manner are usually sufficiently high to produce fatal infection when administered to susceptible rabbits. Wild rabbits in this experiment were challenged in two consecutive groups (14 and 13 rabbits respectively), 30 days apart, because of animal accommodation restrictions. The four seronegative New Zealand White laboratory rabbits were included with the first group to act as a virus control, and the inoculating virus suspension, prepared as a single aliquoted batch, was held at -20 °C for the duration of the experiment to ensure no significant change in titre. All rabbits in both groups were inoculated intramuscularly with 1 mL of the virus suspension.

Blood samples were taken immediately before inoculation and also from surviving rabbits 11 days later.

Rabbits were examined at regular intervals up to 11 days post-inoculation, sufficient to establish survival after virus challenge, and all pre-inoculation sera and sera from surviving animals at 11 days were examined by the HIT. Liver tissue from rabbits that died, or were humanely killed, during the course of the observation period was examined for RHD virus by haemagglutination tests (HA) and negative contrast electron microscopy (EM) (Chasey et al, 1995).

Surviving rabbits at the end of the experiment were humanely killed.

## RESULTS

The results for the 27 wild rabbits, and the four controls, are summarised in table 1, which also lists corresponding antibody titres before and 11 days after virus inoculation.

Three of the four NZ White control rabbits died between 32 and 42 h post-inoculation. Calicivirus particles were demonstrated by EM in liver tissue from all of the three, and HA tests were positive. The fourth rabbit showed transient mild signs of depression and survived.

The 22 initially seropositive wild rabbits remained seropositive immediately prior to inoculation and the five seronegative rabbits remained seronegative. All the seropositive rabbits and two of the seronegatives ate normally, appeared healthy, showing no clinical signs throughout the 11 day observation period, and survived. Two of the remaining three seronegative rabbits showed no preliminary signs but died approximately 43 and 65 h respectively after inoculation. The third seronegative rabbit became recumbent with paddling movements of the limbs, and was humanely killed in extremis 32 h after inoculation.

At post mortem, gross liver and tracheal changes were variable in all three rabbits, with some evidence of haemorrhage and engorgement, but there were no other remarkable features. RHD virus was demonstrated, by both HA and EM, in liver tissue from each of the three rabbits.

Antibody titres in surviving wild rabbits 11 days after inoculation did not show large rises (threefold median increase), and two animals appeared to show no response. By comparison, the single laboratory bred New Zealand White to survive, initially seronegative, produced a considerably higher response with a final titre > 2 560.

**Table I.** Results of challenge experiment on UK wild rabbits. HIT antibody titres are expressed as reciprocal values.

<i>Rabbit</i>	<i>Initial titre</i>	<i>Final titre</i>	<i>Survival</i>
Control			
1	< 10 <sup>a</sup>	–	–
2	< 10	–	–
3	< 10	–	–
4	< 10	> 2560	+
Wild			
5705	20	80	+
5709	40	80	+
5710	20	160	+
7382	40	160	+
7430	40	320	+
7540	20	40	+
7543	20	80	+
7544	20	80	+
7559	10	40	+
7565	20	160	+
7566	40	80	+
7571	40	160	+
7576	40	80	+
7580	160	160	+
7586	80	320	+
7588	10	320	+
7590	10	10	+
7598	20	160	+
7600	20	80	+
7604	10	160	+
7635	640	1280	+
7638	80	320	+
5702	< 10	–	–
5704	< 10	–	–
5707	< 10	–	– <sup>b</sup>
7599	< 10	80	+
7602	< 10	80	+

<sup>a</sup> < 10 = negative; <sup>b</sup> killed humanely in extremis.

## DISCUSSION

All 22 seropositive wild rabbits in this investigation survived challenge with virulent RHD virus after receiving identical inocula, each sufficient to induce fatal infection in susceptible control animals of approximately two to three times the weight.

Throughout the study the wild rabbits remained quiescent with a subdued demeanour, and it is not clear whether the artificial conditions of captivity may have had any significant influence on their response to infection. It is not known whether surviving rabbits remained infected after inoculation.

Initial antibody levels in the surviving rabbits were not high, only two having reciprocal titres greater than 80, but titres as low as 10 were still protective against fatal infection with the acute form of RHD. Although the number of available animals was limited, these results are consistent with our previous data on commercial laboratory bred rabbits. Initial serum titres of 10, as measured in our HI test, are considered positive as they have, in our laboratory, always prevented fatal infection in rabbits challenged with RHD virus (Chasey et al, 1995). In some vaccine studies post-immunisation titres greater than 20 have been found to protect (Huang, 1991; Simon et al, 1993). Other researchers consider serum antibody titres up to a value of 80 to represent non-specific reactions, but there have been no comparisons between laboratories, and no standardisation of test protocols. Rodak et al (1991) also found that natural antibodies in commercially farmed rabbits in the Czech Republic conferred immunity to fatal infection with RHD virus. In their study three categories, strongly positive, weakly positive and negative, were defined based on an enzyme linked immunosorbent assay. Survival rates of experimentally challenged rabbits in each group were proportional to the antibody titres, and there was 97% mortality among those that were seronegative.

Two of the five seronegative wild rabbits in the present study survived. Although the group size was small this figure is consistent with survival rates among seronegative laboratory rabbits of approximately 10% (Chasey et al, 1995), and is comparable with the 1/4 survival of the NZ White controls.

At the end of the 11-day period all but two of the initially seropositive rabbits showed an expected, but moderate, increase in serum antibody titre to the virus. The response in the single, initially seronegative, surviving NZ White was considerably greater but the significance of this observation is not known.

The main conclusion from this study is that the response of UK wild rabbits in terms of mortality rates on exposure to the acute fatal form of RHD virus is broadly similar to that of laboratory rabbits. In particular, wild rabbits that are initially RHD-seropositive, but coming from colonies with no RHD-history, are immune. Coupled with the observation in Britain that more than 60% of adult wild rabbits in winter contain 'naturally occurring' antibody (Trout et al, 1997) this could, in conjunction with environmental factors, explain the relatively slow spread of disease throughout wild populations which have not, overall, shown obvious reductions in rabbit numbers. Clusters of high mortality leading to local population effects have, however, occurred (Chasey and Trout, unpublished observations). Similar levels of immunity have been described for wild rabbits in Spain, although it is not clear to what extent these reflected previous exposure to clinical disease (Simón et al, 1995). Rabbit losses are thus unlikely to reach the levels (99%) seen originally from myxomatosis in the 1950s; in recent years myxomatosis field mortality has been closer to 20-30% (Trout et al, 1992). Protective immunity to RHD is likely to increase further through survivors after passage of virulent virus, and circulation of virus among seropositive rabbits may boost existing immunity. There has been no detectable attenuation of RHD virus isolates in the 12 years of its history but future mutation, coupled with changes in host genetic resistance, may also lessen future effects of the disease. It is instructive to compare qualitatively the relatively low mortality and slow spread of RHD in the UK with the rapid dissemination in Australia after accidental release of the virus in late 1995. Australian wild rabbits are free of naturally-occurring RHD-specific antibody, and after only a few weeks mortality from the disease had been severe and widespread amongst susceptible adults (Chasey, 1997).

The origin of pre-existing RHD-specific antibody in European rabbits is not clear (Ohlinger et al, 1989; Rodak et al, 1990), but it is probable that undetected non-pathogenic RHD-like viruses are implicated (Capucci et al, 1991; Smid et al, 1991; Chasey et al, 1995), and a recent study provides more substantial evidence (Capucci et al, 1997). The significance of these putative viruses in terms of their protective role against fatal disease indicates the need to investigate further and identify other caliciviruses that may circulate within rabbit populations. This information is also necessary in the wider context of calicivirology, the relationships between existing members of the group and changes in host specificity for viruses that may infect a wide range of wildlife species.

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