The development of genetic resistance to myxomatosis in wild rabbits in Britain

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SUMMARY

The presence of genetic resistance to myxomatosis in a sample of wild rabbits from one area in England was reported in 1977. Rabbits from three other areas in Great Britain have been tested subsequently, and all cases showed similar resistance to a moderately virulent strain of myxoma virus. Rabbits from one area also showed a significant degree of resistance to a fully virulent strain of virus.

It is concluded that genetic resistance to myxomatosis is widespread in wild rabbit populations in Britain. The implications of the results are discussed in relation to the co-evolution of the disease and its host.

INTRODUCTION

Myxoma virus occurs naturally as a mild infection of certain *Sylvilagus* species of rabbits in South America and in California, and might have remained undiscovered had it not spread to stocks of laboratory rabbits in which it caused the lethal disease which we know as myxomatosis (Sanarelli, 1898; Kessel, Prouty & Meyer, 1931). When introduced into wild rabbit (*Oryctolagus cuniculus*) populations in Australia, France and Britain, the disease initially killed almost every rabbit which became infected. However, it soon became clear that changes were occuring indicative of an accommodation in the virus–host relationship: (*a*) the appearance of less virulent types of virus and their establishment as the most common form (Marshall & Fenner, 1960; Joubert, Leftheriotis & Mouchet, 1972; Fenner & Chapple, 1965) and (*b*) the development in the rabbit of a considerable degree of inherited resistance to myxomatosis within a few years in Australia (Marshall & Fenner, 1958), and apparently much more slowly in Great Britain (Ross & Sanders, 1977). Possible reasons for the delayed appearance of resistance in Britain are discussed in this paper.

In the first two years following the appearance of myxomatosis in Britain, the rabbit population was reduced by an estimated 99 % (Lloyd, 1970). Since then, the effects of the disease have been less drastic but they have remained an important factor influencing rabbit numbers. The demonstration of genetic resistance to a moderately attenuated virus (Brecon) strain in rabbits from one area in Norfolk (Ross & Sanders, 1977) was a significant development and it was considered important to determine whether genetic resistance to myxomatosis was becoming

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widespread in Britain, and whether the degree of resistance was changing. We report here the results of investigation of rabbits from three other widely separated areas in Britain.

Ross & Sanders (1977) reported that small numbers of rabbits from the Norfolk study area were inoculated with a fully virulent strain of virus in 1974 and 1975. All these rabbits died, indicating that there was no significant resistance in that population to fully virulent virus, though it was noted that mean survival times were greater than for domestic rabbits used as controls. We report on the results of inoculating rabbits from one other site with virulent viruses.

In earlier investigations of resistance to myxomatosis in Great Britain (Vaughan & Vaughan, 1968: Ross & Sanders, 1977) it was found that, although mortality rates were not affected, mean survival times of wild rabbits were significantly longer than those of domestic rabbits used as controls. It was suggested that such lengthening of survival times could be an early indication of developing resistance. This possibility is investigated by analysis of all the results of tests for resistance to the moderately attenuated (Brecon) strain of virus.

MATERIALS AND METHODS

Rabbit collection areas

Rabbits were obtained from three areas where relatively high population densities of rabbits exist despite the continued presence of myxomatosis, with major epizootics at least once a year.

One site was centred on a small (7 ha) Forestry Commission plantation near Micheldever, Hampshire; another site was situated on chalk downland at Porton Down near Salisbury, Wiltshire, and the third site consisted of an upland farm, mainly rough grazing, in Glen Esk, Angus, Scotland.

Virus strains

The Brecon strain (Grade III a virulence as defined by Fenner & Marshall, 1957) (Chapple & Bowen, 1963) was from the same batch as that used in 1969 and 1970 (Ross & Sanders, 1977).

The Cornwall strain (Grade I virulence) (Fenner & Marshall, 1957) had been passed twice in eggs, twice in rabbits, once in rabbit kidney cell culture and one further passage in a rabbit.

The Wiltshire strain (Grade II virulence) was isolated from a rabbit, caught on the site in 1979, which developed symptoms of myxomatosis after capture. The virus was passed once in a domestic rabbit before being used.

Virus strains were passed by intradermal injection on the shaved flanks of domestic (New Zealand White) rabbits, which were killed 14 days later. Eyelid lesions were taken and virus extracted using a Colworth stomacher (Sharpe & Jackson, 1972). Virus preparations were titrated by intradermal injection of series of dilutions on the shaved flanks of domestic rabbits and the number of rabbit infectious doses (RID_{50}) were calculated by the Reed & Muench (1938) method. For testing resistance, rabbits were injected intradermally on the shaved flanks with 0·1 ml of virus suspension diluted to contain 10–100 RID_{50}/0·1 ml.

Rabbits

Young rabbits (generally 4–6 weeks old) were caught alive in the three areas, using ferrets and purse nets or cage traps. The rabbits were dusted with pyrethrum powder to remove fleas, transported to the laboratory and held in cages for approximately 3 months, to ensure that any myxoma virus antibodies passively acquired from immune does would have been lost. They were then bled from the marginal ear vein and sera were tested by the Sobey method of agar gel diffusion (Sobey, Conolly & Adams, 1970) and by a serum neutralization test (Vaughan & Vaughan, 1968). Any rabbits that possessed detectable levels of myxoma virus antibodies were rejected. The remaining rabbits were injected with the Brecon strain of virus, as were groups of New Zealand White rabbits used as controls. Groups of rabbits from Wiltshire were also infected with virus strains of virulence Grades II and I. The onset of symptoms, times of death and numbers of survivors were recorded and mean survival times calculated using a logarithmic transformation [log (ST-8)] (Fenner & Marshall, 1957).

RESULTS

The results of inoculation with the Brecon strain of virus of non-immune wild rabbits from the three areas are summarized in Table 1*a* along with the results of simultaneous infection of fully susceptible domestic rabbits. In each case the mortality rate of the wild rabbits was very significantly lower ($P < 0.001$) than the mortality rates obtained when non-resistant wild rabbits were tested previously (84–94 %) (Ross & Sanders, 1977). Because of the small sample size no special significance can be given to the survival of all 16 rabbits from the Hampshire site.

In order to determine whether resistance to more virulent strains of virus had developed subsequently, rabbits from the Wiltshire site were infected with a Grade II strain (Wiltshire) in 1979 and with a Grade I strain (Cornwall) in 1980. The results are summarized in Table 1*b*. Mortality rates (45 % and 52 %) were very much lower than in Norfolk rabbits in 1974 and 1975 (100 %) (Ross & Sanders, 1977).

In order to investigate the possibility that the lengthening of survival times (as noted by Vaughan & Vaughan, 1968 and Ross & Sanders, 1977) could be an early indication of developing resistance and to see what effect the increase in resistance may have on transmissibility of virus strains, the results of all tests for resistance to the Brecon strain of virus were analysed. Mead-Briggs & Vaughan (1975) showed that the survival time of an infected rabbit influenced the ability of rabbit fleas to transmit virus from that rabbit: rabbits which died between 17 and 44 days (and particularly between 17 and 28 days) after infection had the highest proportion of infective fleas. Table 2 gives the percentages of rabbits dying between 17 and 44 days, and between 17 and 28 days after infection with the Brecon strain (Grade IIIa virulence). The earlier results are from the work of Vaughan & Vaughan (1968) and also include unpublished work by Vaughan & Vaughan (rabbits from Skokholm, 1967). The results are illustrated in Fig. 1. With both sets of data, the percentage of rabbits dying within the stated period decreases as resistance increases (i.e. mortality rate decreases) but the rates of decrease are different. The relationship between the percentage dying in 17–44 days and the mortality rate

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Table 1. *Tests for genetic resistance to myxomatosis in wild rabbit populations in Britain*

Location	Year	Strain and virulence grade	No of. rabbits tested	No. of rabbits which died	Mortality rate (%)	Mean survival time (days) of rabbits which died
			(a)			
Wiltshire	1978	Brecon III a	71	32	45	28·6
Domestic controls		Brecon III a	18	18	100	18·1
Hants	1978	Brecon III a	16	0	0	—
Domestic controls		Brecon III a	9	9	100	17·3
Angus	1979	Brecon III a	45	20	44	31·5
Domestic controls		Brecon III a	12	12	100	17·9
			(b)			
Wiltshire	1979	Wilts II	53	24	45	29·4
Domestic controls		Wilts II	18	18	100	16·3
Wiltshire	1980	Cornwall I	44	23	52	22·1
Domestic controls		Cornwall I	9	9	100	11·3

Table 2. *The mortality rates and percentages of rabbits dying between 17 and 28 days (and 17 and 44 days) after infection with a myxoma virus strain of Grade III a virulence*

Location	Year	Mortality rate (%)	% dying in	
			17–28 days	17–44 days
Norfolk[1]	1966	90	51	90
Norfolk[1]	1967	94	89	94
Skokholm[2]	1967	97	85	95
Norfolk	1968	86	56	83
Norfolk	1969	84	51	74
Norfolk	1970	59	37	59
Norfolk	1976	21	5	14
Wilts	1978	45	20	38
Angus	1979	44	11	42

[1] Data from Vaughan & Vaughan (1968).
[2] Data from Vaughan (unpublished).

is linear ($y = -6·83 + 1·05x$, $r^2 = 0·99$) and due solely to the decrease in mortality rate, i.e. the ratio of the number of rabbits dying in 17–44 days to the total number of rabbits which die remains constant. However, the percentage dying in 17–28 days decreases more quickly than the mortality rate ($\log y = 0·49 + 0·015x$, $r^2 = 0·93$).

DISCUSSION

The results presented here, along with the results given by Ross & Sanders (1977), using rabbits from an area in Norfolk, demonstrate that a considerable degree of resistance exists in four widely separated rabbit populations to a
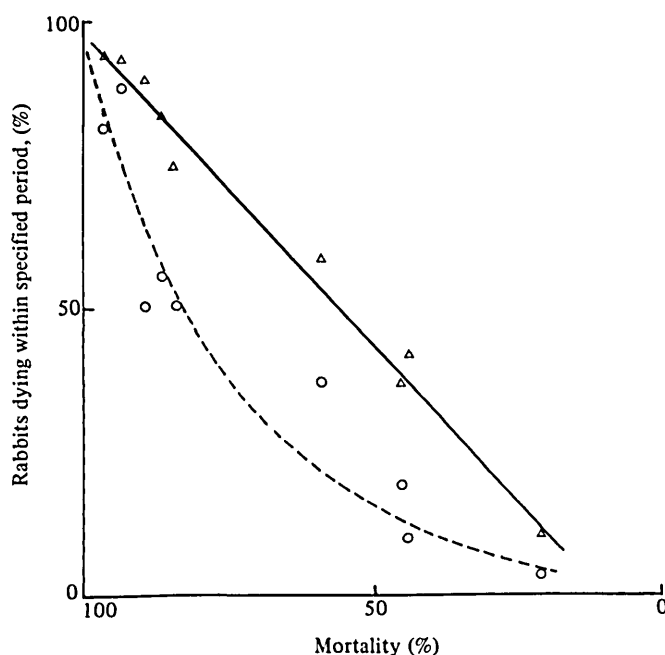
Fig. 1. Relationships between mortality rate of rabbits infected with a virus strain of Grade IIIa virulence and the percentages of rabbits dying in 17–44 days (△——△) and in 17–28 days (○----○).

moderately virulent strain of myxoma virus. There is no reason to suppose that the four rabbit populations are exceptional, and it is reasonable to conclude that such resistance to myxomatosis is now widespread in wild rabbits in Britain. In addition, it has been shown that by 1980, rabbits from the Wiltshire site had developed considerable resistance even to a fully virulent strain of virus.

In Australia, genetic resistance to myxomatosis developed much earlier. Marshall & Douglas (1961) reported that by 1958 (only eight years after the introduction of the disease) the mortality rate in rabbits from one area, when inoculated with a Grade IIIa strain, had decreased from the expected 90 % to 26 %: in Britain, such a degree of resistance was not definitely demonstrated until over 20 years after the appearance of the disease. Subsequent work, referred to by Fenner & Myers (1977) has shown that by 1961–6 rabbits from one area in Victoria were resistant to a fully virulent (Grade I) strain (mortality decreased from 100 to 68 %). The level of resistance had not increased in rabbits from the same area by 1971–5, and this is in agreement with the results of Sobey (1969 and pers. comm.), who showed that selection for resistance in domestic rabbits initially increased with each successive generation, but appeared to reach a plateau after about six generations. Although resistance to a Grade I strain has now been demonstrated in rabbits from one area in Great Britain (Table 1b), it is not possible at this stage to say whether such a plateau has been reached in wild rabbits in Britain.

There are several possible explanations for the delay in detecting genetic resistance in wild rabbits in Britain compared with Australia. Most of the earlier investigations of genetic resistance in Britain were restricted to rabbits from one area in Norfolk (though Vaughan & Vaughan (1968) did test rabbits from an area

in Kent in 1965 and 1966) and resistance could have developed more quickly elsewhere but remained undetected. Alternatively, conditions in Australia may have allowed resistance to develop more quickly than was possible in England; more attenuated strains of virus quickly became more common in Australia than in Britain (Marshall & Fenner, 1960; cf. Fenner & Chapple, 1965); until recently mosquitoes were the main vectors of myxomatosis in Australia and major epizootics occurred mainly in the summer months, when the high temperatures experienced in many parts of Australia were likely to lead to lower mortality rates (Marshall, 1959).

The speculation by Vaughan & Vaughan (1968) that the longer survival times of rabbits might indicate that resistance was beginning to develop is supported by analysis of all tests for resistance to the Brecon strain of virus: from Fig. 1 it can be seen that the percentage of rabbits dying between 17 and 28 days decreased before any decrease in mortality rate was detected. Also, from the work of Mead-Briggs & Vaughan (1975), the decrease in the proportion of rabbits dying between 17 and 28 days should mean that transmissibility decreases. Fenner & Ratcliffe (1965) predicted that, since selection of virus strains depends on transmissibility, increased resistance in wild rabbits should select for more virulent strains of virus. An analysis by Anderson & May (1982) supports this view, and recent data from Australia (Edmonds *et al.* 1975) and Britain (Ross, 1982) indicate that the virulence of field strains of virus is changing as predicted.

This trend towards higher virulence in field strains of virus may mean that, in the short team at least, myxomatosis will continue to be an important cause of mortality in wild rabbit populations. The longer-term developments depend on whether resistance to the disease continues to increase and on the ability of mutation to produce ever more virulent strains of virus. If both processes can occur, the present 'dynamic equilibrium' (increasing resistance counterbalanced by increasing virulence) could continue. If resistance can continue to develop but not increasingly virulent strains of virus (*which can be transmitted effectively*) then the climax association would be similar to that of *Sylvilagus* species in South America and California, a mild infection causing very few deaths in very young rabbits.

The latter possibility would have grave consequences for British agriculture. At present myxomatosis continues to be directly responsible for the death of large numbers of rabbits (Ross & Tittensor, 1981), yet it is generally accepted that rabbit numbers are increasing despite the present level of rabbit control. Rabbits are already causing damage to cereals estimated to be costing tens of millions of pounds annually; there is no estimate for rabbit damage to grassland. If myxomatosis were to become less effective, an increase in rabbit numbers (and in rabbit damage) could be prevented only by a major change in the methods and the organization of rabbit control.

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