Changes in the virulence of myxoma virus strains in Britain

By J. ROSS AND M. F. SANDERS

M.A.F.F., Worplesdon Laboratory, Tangley Place, Worplesdon, Guildford, Surrey

(Accepted 30 July 1986)

SUMMARY

National surveys of the virulence of field strains of myxoma virus were carried out in 1975 (128 virus strains) and in 1981 (123 strains), using the virulence testing method employed in a similar survey in 1962. Results showed that the virulence of field strains had increased between 1962 and 1975, and again between 1975 and 1981. The increases in virulence are thought to be a result of the development of resistance to myxomatosis in wild rabbit populations. The effects of the changes in virulence and resistance are discussed.

INTRODUCTION

Myxomatosis first appeared in Great Britain in late 1953 and, in the major epizootic that followed, it is estimated to have reduced the rabbit population by 99% (Lloyd, 1970). Since then, despite the continuous presence of the disease, rabbit numbers have increased gradually although they are nationally still much lower than the pre-myxomatosis levels. The disease continues to be an important factor regulating rabbit numbers (Ross & Tittensor, 1981).

The natural evolution of myxomatosis has been the subject of considerable research in both Australia and Great Britain. In particular, changes in the virulence of field strains of myxoma virus and the development of inheritable resistance to the disease have been monitored. In Great Britain inheritable resistance was first detected in rabbits from one site in England in the early 1970s (Ross & Sanders, 1977) and subsequently from several other sites, including one in Scotland (Ross & Sanders, 1984). It is reasonable to assume that a degree of resistance is common throughout Britain.

Changes in the virulence of field strains of virus occurred quickly in Britain, as in Australia, with attenuated strains being detected within two years of the appearance of the disease (Hudson & Mansi, 1955). In 1962 a national survey of field strains was conducted and it was shown that fully virulent strains, similar to that which caused the first outbreaks, had almost disappeared. Virus strains of moderate virulence, killing 70–95% of susceptible rabbits were found most commonly (Fenner & Chapple, 1965).

It was shown that the predominance of such strains was due to more effective transmission by mosquitoes in Australia (Fenner, Day & Woodroffe, 1956) and by the rabbit flea in Britain (Mead-Briggs & Vaughan, 1975). Fenner & Ratcliffe (1965) postulated that, in resistant rabbits, strains of higher virulence would be more effectively transmitted and they predicted that the development of resistance

in wild rabbits would lead to an increase in the virulence of field strains of virus. There is some evidence that such a change has occurred in Australia where virus strains collected in 1970–1974 from an area of Victoria with very resistant rabbits were of higher virulence than strains from other areas in Victoria where the rabbits were less resistant (Edmonds *et al.* 1975).

To monitor changes in the virulence of field strains in Britain, national surveys (similar to the 1962 survey) were carried out in 1975 and in 1981, and the results of these surveys are reported here.

METHODS

Collection of samples

During October to December 1975 and October to December 1981 two diseased rabbits were obtained from locations at least 10 miles apart within each 'old-style' county in Great Britain. The rabbits, showing obvious symptoms of myxomatosis, were killed and immediately placed in insect-proof linen bags and sent to the laboratory. Eyelid lesions were taken from each rabbit and stored dry at -70°C until processed.

Preparation of virus samples

Virus was extracted from the eyelid lesions into 5 ml of phosphate buffered saline (pH 7.2) by Colworth Stomacher (Sharpe & Jackson, 1972). The suspension was centrifuged at 1000 g for 10 min to remove cell debris. Each virus sample was passaged in a New Zealand White rabbit using 0.1 ml of a 10^{-1} dilution of suspension. The rabbits were killed 14 days after infection and eyelid lesions taken.

Virus was then extracted from the eyelid lesions of each of these rabbits as before, and the resulting suspension was titrated by injecting serial 10-fold dilutions on to the shaved flank of another white rabbit. Six intradermal injections of 0.1 ml of each dilution enabled the virus titre of the original suspension to be estimated using the method of Reed & Muench (1938).

Virulence test

The virulence of 128 virus samples collected in 1975 and 123 samples collected in 1981 was tested using the method of Fenner & Marshall (1957). For each virus sample, six (or occasionally five) New Zealand White rabbits were injected intradermally with 0.1 ml of a diluted suspension containing 10–50 rabbit infectious doses (RID_{50}) and kept at a temperature of $17 \pm 4^{\circ}\text{C}$. Development of symptoms and day of death were noted for each rabbit. The mean survival time (MST) was calculated for each virus strain and, from this, each strain was assigned to one of six virulence grades as described by Fenner & Marshall (1957).

Statistical analysis

The distributions of field strains in different virulence grades in 1962, 1975 and 1981 were compared by fitting a log-linear model using Poisson error distribution by means of generalized linear interactive modelling (GLIM) (Baker & Nelder, 1978).

Table 1. *The virulence of field strains of myxoma virus in Britain in 1962, 1975 and 1981*

(The number of strains falling into each virulence grade is expressed as a percentage of the total sample.)

	No. of strains tested	Virulence grade					
		I	II	IIIa	IIIb	IV	V
MST (days)...		≤ 13	14-16	17-22	23-28	29-50	—
% mortality...		> 99	95-99	90-95	70-90	50-70	< 50
Year							
1962*	222	4.1	17.6	38.8	24.8	14.0	0.9
1975	128	1.6	25.8	48.4	18.0	5.5	0.8
1981	123	0	35.8	54.5	8.1	1.6	0

* From Fenner & Chapple (1965).

RESULTS

Calculation of mean survival time

The distribution of times to death (survival times) of all rabbits that died after infection with all strains collected in the 1975 and 1981 surveys was skewed, indicating that transformation of the data is required before mean survival times can be calculated. The methods of Jeffers (1959) and of Kirk (1968) both indicated that a reciprocal transform would be most appropriate, but, in almost every case, this gave an identical virulence grade to that obtained by the modified logarithmic transform used by Fenner & Marshall (1957). For the sake of continuity therefore, the logarithmic transform, $\log[(ST-8)]$ (where ST = survival time in days) was used in this paper.

When one or more of the six rabbits injected with each strain survived infection, Fenner & Marshall (1957) used the method of Sampford (1954) to calculate MST. However, in analysing the results of the 1975 and 1981 surveys, Sampford's method was found to be inappropriate (giving negative third moments) in some cases. Eighteen sets of data from the 1975 survey and two from the 1981 survey were re-analysed using the more appropriate method of Cohen (1957) and in only three cases did this result in a change in virulence grade. It was not possible to reanalyse the results from the 1962 survey and therefore, to make direct comparison possible, the results of the 1975 and 1981 surveys, as presented in Table 1, were analysed using the Sampford method in all cases in which rabbits recovered.

Virulence of field strains of myxoma virus

The percentage of field strains in each virulence grade are given in Table 1 with the results of the 1962 survey (Fenner & Chapple, 1965) shown for comparison.

The distribution within virulence grades changed significantly between 1962 and 1975 ($\chi^2 = 13.91$, D.F. = 5, $P < 0.02$) with most of the difference being due to Grade II strains becoming more common (17.6-25.8%) and Grades IIIb and IV less common (24.8-18.0% and 14.0-5.5%) in 1975. There was also a significant

change in virulence between 1975 and 1981 ($\chi^2 = 14.03$, D.F. = 5, $P < 0.02$) with 90% of field strains being of Grade II or IIIa virulence in 1981.

DISCUSSION

It has been suggested that random collection of field strains for virulence testing is likely to be biased against fully virulent strains because such strains kill rabbits within a few days of the appearance of symptoms, and hence there is less chance of such strains being included in the survey sample. However, in an outbreak caused by a fully virulent strain, spread of the disease would be less effective than with a moderately attenuated strain (Mead-Briggs & Vaughan, 1975) and, although individual infected rabbits would die more quickly, the length of the outbreaks would probably be similar.

In an outbreak involving a fully virulent strain and a moderately attenuated strain, the former would infect relatively few rabbits (Parer, 1983) and would probably disappear (Shepherd & Edmonds, 1977). Therefore it may remain undetected but, since it contributed little to the mortality of rabbits, any resulting bias in the survey would be of little practical importance. The results of the surveys should thus perhaps be considered to reflect the relative importance of field strains of different virulence, rather than the actual percentage occurrence of these strains.

In addition, any comparison between the virulence surveys should take account of the development of innate resistance in the rabbit population in Britain. In 1975 and in 1981, after the development of resistance, rabbits infected with virulent strains (of Grades I and II particularly) would have survived longer than non-resistant rabbits infected with the same strains, and such strains, being more effectively transmitted, should then have been more easily detected. This may partly explain the increases in occurrence of strains of Grade II virulence in 1975 and again in 1981, but also underlines the significance of the continuing rarity of fully virulent strains (see below).

The results presented in Table 1 indicate that the changes in virulence predicted by Fenner & Ratcliffe (1965) have indeed taken place in Britain. The increase in virulence of field strains between 1962 and 1975 correspond well with the detection of innate resistance to myxomatosis in about 1970 (Ross & Sanders, 1977). There have since been further increases in resistance (Ross & Sanders, 1984) resulting in an increase in virulence between 1975 and 1981, because selection favours more virulent strains that are transmitted more effectively in rabbits with a degree of resistance.

However, despite the increases in occurrence of field strains of Grade II virulence, fully virulent (Grade I) strains have remained rare. Thus there appears to be a limit, at least temporarily, to the increase in virulence of field strains, until selection increases the prevalence of fully virulent strains or mutation produces even more virulent strains. The development of resistance may also stop when the limit of selection of pre-existing genotypes is reached (Fenner, 1983), though mutation may later allow a further increase in resistance.

Nevertheless resistance has already developed to the level where rabbits in at least one area are showing significant resistance to a fully virulent strain. As a consequence the influence of myxomatosis on rabbit populations is likely to

continue to decline, and the gradual increase in rabbit numbers evident since the mid-1960s in Britain is likely to accelerate with serious implications for agriculture and forestry in particular.

We are grateful to the field staff of MAFF and Department of Agriculture and Fisheries for Scotland who collected infected rabbits, and to Dr L. Huson and Dr I. R. Inglis for statistical advice.

REFERENCES

- BAKER, R. J. & NELDER, J. A. (1978). *The GLIM system; Generalised Linear Interactive Modelling*. Oxford: Numerical Algorithms Group Ltd.
- COHEN, A. C. (1957). On the solution of estimating equations for truncated and censored samples from normal populations. *Biometrika* **44**, 225–236.
- EDMONDS, J. W., NOLAN, I. F., SHEPHERD, R. C. H. & GOCS, A. (1975). Myxomatosis: the virulence of field strains of myxoma virus in a population of wild rabbits with high resistance to myxomatosis. *Journal of Hygiene* **74**, 417–418.
- FENNER, F. (1983). Biological control as exemplified by smallpox eradication and myxomatosis. *Proceedings of the Royal Society, Series B* **218**, 259–285.
- FENNER, F. & CHAPPLE, P. J. (1965). Evolutionary changes in myxoma virus in Britain. *Journal of Hygiene* **63**, 175–185.
- FENNER, F., DAY, M. F. & WOODROOFE, G. M. (1956). Epidemiological consequences of the mechanical transmission of myxomatosis by mosquitoes. *Journal of Hygiene* **54**, 284–303.
- FENNER, F. & MARSHALL, I. D. (1957). A comparison of the virulence for European rabbits (*Oryctolagus cuniculus*) of strains of myxoma virus recovered in the field in Australia, Europe and America. *Journal of Hygiene* **55**, 149–191.
- FENNER, F. & RATCLIFFE, F. N. (1965). *Myxomatosis*. Cambridge: University Press.
- HUDSON, J. R. & MANSI, N. (1955). Attenuated strains of myxomatosis virus in England. *Veterinary Record* **67**, 746–747.
- JEFFERS, J. N. R. (1959). *Experimental Design and Analysis in Forestry Research*, pp. 74–75. Stockholm: Almqvist & Wiksell.
- KIRK, R. E. (1968). *Experimental design: procedures for the behavioural sciences*. Belmont, California: Brooks/Cole.
- LLOYD, H. G. (1970). Post-myxomatosis rabbit populations in England and Wales. *EPPO Publications Series A*, No. 58, 197–215.
- MEAD-BRIGGS, A. R. & VAUGHAN, J. A. (1975). The differential transmissibility of myxoma virus strains of differing virulence grades by the rabbit flea *Spilopsyllus cuniculi* (Dale). *Journal of Hygiene* **75**, 237–247.
- PARER, I. (1983). The introduction of two strains of myxomatosis into field populations of rabbits. *7th Australian Vertebrate Pest Control*, Working papers, 79–85.
- REED, L. J. & MUENCH, H. (1938). A simple method of estimating 50% endpoints. *American Journal of Hygiene* **27**, 493–497.
- ROSS, J. & SANDERS, M. F. (1977). Innate resistance to myxomatosis in wild rabbits in England. *Journal of Hygiene* **79**, 411–415.
- ROSS, J. & SANDERS, M. F. (1984). The development of genetic resistance to myxomatosis in wild rabbits in Britain. *Journal of Hygiene* **92**, 255–261.
- ROSS, J. & TITTENSOR, A. M. (1981). Myxomatosis in selected rabbit populations in southern England and Wales 1971–1977. *Proceedings of the World Lagomorph Conference*, Guelph 1979, 830–833.
- SAMPFORD, M. R. (1954). The estimation of response time distribution. III. Truncation and survival. *Biometrics* **10**, 531–561.
- SHARPE, A. N. & JACKSON, A. K. (1972). Stomaching: a new concept in bacteriological sample preparation. *Applied Microbiology* **24**, 175–178.
- SHEPHERD, R. C. H. & EDMONDS, J. W. (1977). Myxomatosis: the transmission of a highly virulent strain of myxoma virus by the European rabbit flea (*Spilopsyllus cuniculi*) in the Mallee region of Victoria. *Journal of Hygiene* **79**, 405–409.