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Studies in the epidemiology of infectious myxomatosis of rabbits*

VII. The virulence of strains of myxoma virus recovered from Australian wild rabbits between 1951 and 1959

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A detailed analysis has been published of the virulence of ninety-two strains of myxoma virus obtained from the field in South America, California, Europe and Australia (Fenner & Marshall, 1957). This included forty-nine strains recovered from wild rabbits and thirteen from wild-caught mosquitoes in Australia between February 1951 and March 1955. Since then a further 610 strains from naturally occurring cases of myxomatosis in Australian wild rabbits have been tested; the material being sent from all States of the Commonwealth (except the Northern Territory), between March 1955 and July 1959. The overall results of the virulence tests on material obtained up to the end of 1957 have been briefly summarized elsewhere (Fenner, 1959*a*). This paper records the results of all tests made on the virulence of field strains of myxoma virus recovered from Australian wild rabbits (or mosquitoes) between February 1951 and July 1959.

MATERIALS AND METHODS

Rabbits

In the early stages of this investigation wild rabbits were sometimes used for the virulence tests (Fenner & Marshall, 1957). Since early in 1956 only adult laboratory rabbits have been used. These were bred in the University Animal Breeding Establishment from stock rabbits which had never been exposed to myxomatosis. Inoculated rabbits were housed in individual cages in animal rooms which were heated in winter. They were fed on a diet of pellets and water, supplemented daily with green feed.

Collection of virus samples

Since 1955 comprehensive collections of infected tissue have been facilitated by the distribution of small screw-capped bottles filled with 50% glycerol to individuals concerned with rabbit control. The bottles were accompanied by printed forms which gave instructions for the collection and mailing of infected tissue, and indicated data required on the history of myxomatosis in the area, and on the case from which the sample was obtained.

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On arrival at the laboratory in Canberra the pieces of infected tissue were washed and stored in the frozen state until they could be ground up, titrated on eggs, and tested in rabbits. Aliquots of the prepared samples were stored at $-70°$ C., so that they could be retested if necessary.

Inoculation of rabbits

Groups of three, four, or five rabbits were inoculated intradermally in one flank with about $20ID_{50}$ of the virus strain being tested. They were observed at regular intervals so that the course of the disease could be followed, and records were kept of their time of death or recovery.

Assessment of virulence

In the earlier report (Fenner & Marshall, 1957) we described a classification of viruses into five grades of virulence based on the mean survival times observed in groups of five laboratory rabbits. The same general method of handling the results has been used in this paper. The criteria used for grading are shown in Table 1.

Table 1. *The virulence of strains of myxoma virus recovered from Australian wild rabbits between* 1951 *and* 1959, *grouped according to the year of recovery*

Grade of severity* ...	I	II	III	IV	V	
Degree of virulence ...	Very high	Very high	Moderate	Low	Very low	
Mean survival time (days)† ...	$\leqslant 13$	$> 13 \leqslant 16$	$> 16 \leqslant 28$	$> 28 \leqslant 50$	—	
Case mortality rate (approximate)† ...	99·5 %	99 %	90 %	60 %	0–30 %	No. of strains tested
1950–51‡	100	0	0	0	0	1
1951–52	33	50	17	0	0	6
1952–53	4	13	74	9	0	23
1953–54	16	25	50	9	0	12
1954–55	16	16	42	26	0	19
1955–56	0	3	55	25	17	155
1956–57	0	6	55	24	15	165
1957–58	3	7	54	22	14	112
1958–59	0·5	16	53	16	14	179

* Allocation to grades follows Fenner & Marshall (1957).

† For adult laboratory rabbits never exposed to myxomatosis.

‡ Since in Australia epizootics almost always occur in the summer months, the reference year is 1 July to 30 June.

Regularity of the response of inoculated rabbits

Environmental factors, especially temperature, have a pronounced effect on the response of rabbits to infection with myxoma virus (Marshall, 1959). Animal rooms were always adequately heated in the winter, and even in midsummer the ambient temperature rarely rose above 90° F. Control groups of rabbits inoculated with a preparation of known virulence were usually tested at the same time as each batch of field strains. On one occasion when tests during the summer gave a high proportion of specimens producing mild infections the strains were retested, with similar results, during the cooler months of the year. We believe that conditions were sufficiently constant throughout the period of testing to make year-by-year comparison of the results valid.

RESULTS

The results of tests on all strains of virus recovered from the field in Australia are shown in Table 1. The results for the period 1950–55 have already been reported in detail (Fenner & Marshall, 1957), and results of testing up to 1957 have been briefly referred to elsewhere (Fenner, 1959*a*).

DISCUSSION

The five grades of virulence have no genetical meaning, they merely represent a convenient way of handling the results of a rather crude test of virulence carried out with 672 preparations of virus over a period of 9 years.

The numbers of samples available during the first 5 years (1950–55) are obviously inadequate to allow detailed conclusions to be drawn about changes in the virulence of myxoma virus during these years. Nevertheless, field observations throughout Australia during the first two seasons (1950–51 and 1951–52) attest to the very high mortality rates associated with myxomatosis then (Ratcliffe, Myers, Fennessy & Calaby, 1952; Myers, Marshall & Fenner, 1954). However, there was epidemiological and serological evidence of low case mortality rates in 1951–52 in a few localities in which extensive kills had occurred in the previous summer (Fenner, Marshall & Woodroofe, 1953), and from our experience since then it is probable that in some cases at least these were due to the occurrence of strains of considerably reduced virulence.

The first strain of grade III virulence was recovered from a rabbit captured in northern New South Wales in May 1952 (Marshall, Dyce, Poole & Fenner, 1955). During the next summer similar strains were recovered from several parts of Australia, and two strains of grade IV virulence were recovered from the Australian Capital Territory (Mykytowycz, 1953).

Greatly attenuated viruses (grade V) were recovered from several widely separated parts of Australia in 1955–56. They may have occurred earlier, but sampling was so restricted that the chance of finding them was very small.

During the last 4 years of the survey a large number of strains have been collected and tested. As can be seen from Table 1, the proportions allotted to the five groups have remained remarkably constant. In contrast to the first years, when practically all cases of myxomatosis were due to highly virulent strains of virus, the present situation is not unlike that of any established enzootic infection i.e. many subvarieties of the causative virus co-exist. Investigations at Lake Urana, reported in detail elsewhere (Fenner, 1959*b*), show that this situation is as true of small localities as of Australia as a whole.

This state of balance may be upset by changes in the genetic resistance of the rabbit population (Marshall & Fenner, 1958). Although at present it appears that slightly attenuated strains (grade III) are still the best adapted for transmission by mosquitoes (Fenner, Day & Woodroofe, 1956), the increasing genetic resistance of the rabbit might well favour viruses of higher virulence (Fenner, 1959*b*), and the slight increase in the percentage of strains of grades I and II in 1958–59 (Table 1) may foreshadow this. However, it appears that further major change

will be slow, and large scale surveys of virus virulence are now planned at 5-year intervals, instead of annually.

It remains to consider the effect of inoculation campaigns with highly virulent virus, which have been a feature of rabbit control operations, especially in Victoria and New South Wales. Analysis of 100 cases from the 1957–58 and 1958–59 collections showed that there was no relation between the interval since the last inoculation of highly virulent virus in a district and the virulence of the virus recovered. Highly virulent strains (grades I and II) were recovered no more commonly within 3 months of the last inoculation than they were 12 months or more after the last inoculation campaign, and attenuated strains were similarly distributed. This does not exclude the possibility that the introduced virulent virus may sometimes initiate a highly lethal epizootic, and eventually be replaced by a more attenuated strain. This is, indeed, what happened in a deliberate field experiment at Lake Urana (Fenner, Poole, Marshall & Dyce, 1957), in which the French strain of virus was introduced on a large scale into the small, localized area of rabbit infestation.

SUMMARY

During the 8½ years, February 1951 to July 1959, 672 strains of myxoma virus were recovered from the field in Australia, all but thirteen, which were derived from mosquito pools, being extracted from the tissues of infected wild rabbits. These were tested for virulence by the intradermal inoculation of small groups of rabbits with a small dose of virus. Based primarily on survival times, strains have been allocated to five grades of virulence, ranging from very high to very low. In spite of inadequate sampling during the first 5 years there has clearly been a trend from a predominance of highly virulent strains in the initial epizootics to a mixture of strains of virulence varying from very high (rarely) to very low. For the last 6 years the proportion of strains allotted to the different virulence grades has remained almost constant, just over half the strains tested each year being classified as moderately virulent.

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