

## Studies in the epidemiology of infectious myxomatosis of rabbits

### VIII. Further observations on changes in the innate resistance of Australian wild rabbits exposed to myxomatosis\*

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(Received 6 September 1960)

#### INTRODUCTION

Populations of wild rabbits (*Oryctolagus cuniculus*) in areas of Australia where there have been severe annual outbreaks of myxomatosis exhibit a significant increase in genetic resistance to that disease (Marshall & Fenner, 1958). Although the experiments which led to this conclusion allowed selection for resistance to take place under natural conditions, the successive generations of rabbits were challenged with myxomatosis under the artificial conditions of an animal house; and this led to some doubt as to whether the demonstrated increase in resistance would greatly affect the mortality rate of naturally occurring epizootics.

In addition to presenting further observations on progressive changes in the resistance of wild rabbits, this paper records the results of experiments designed to correlate the effects of resistance under laboratory and field conditions.

#### MATERIALS AND METHODS

##### *Myxoma virus*

Three strains of myxoma virus were used, the highly virulent standard laboratory strain prepared, ampouled and lyophilized for field distribution by the Commonwealth Serum Laboratories, the slightly attenuated Aust/Corowa/12-52/2, (KM 13) (Fenner & Marshall, 1957), and Aust/Corowa/12-52/2A which is a more attenuated variant of KM 13 (Marshall, 1959). Tumour extracts of the last strain were lyophilized and ampoules of the same batch used for both field and laboratory inoculations. The KM 13 strain was the same preparation as was used in previous studies of innate resistance of Australian wild rabbits (Marshall & Fenner, 1958).

##### *Serology*

The Ouchterlony technique of plate gel diffusions as modified by Mansi (1957) was used to test pre-inoculation sera for antibodies to myxoma virus.

\* Supported by grants from the Wool Research Trust Fund.

*Rabbits*

Laboratory rabbits bred in the University Animal Breeding Establishment were used when 4 months old. Their reaction to both virulent and attenuated strains of myxoma virus is similar to that of wild rabbits whose forbears have never been exposed to the disease (Fenner & Marshall, 1957).

Australian wild rabbits were captured by netting at night with the aid of spotlights (Marshall & Fenner, 1958) in two districts in the state of Victoria and at Lake Urana in New South Wales. After capture, the rabbits for the combined field and laboratory trials were released into large (approximately two acres) compounds which were double fenced and designed to prevent both the escape of rabbits from within, and the invasion of predators from without. One of these enclosures was constructed at each of the two areas in Victoria where rabbits were being collected. Drinking water was provided and the natural grazing was supplemented by cereal oats when necessary. Whenever rabbits were required for individual handling they were caught in the artificial warrens provided for their cover or were driven into small temporary netting enclosures through conical entrance runways.

Only young adult rabbits were used in the experiments.

*Lake Urana**Sources of rabbits*

The history of myxomatosis in this area up to 1956 has been summarized elsewhere (Marshall & Fenner, 1958). Further epizootics occurred in December 1956 and November 1957. The results of serological surveys carried out after these epizootics indicated that the infection rates were again extremely high, 84% of 154 serum samples having antibodies to myxoma in February 1957, and 93% of 108 samples in February 1958. The latter sample was from the parents of the group of 148 young rabbits collected during October 1958.

*Maryvale station*

The rabbit population in this area had experienced five annual summer epizootics when last sampled early in 1956 (Marshall & Fenner, 1958). A sixth epizootic occurred in 1956–57 during the summer prior to the collection of rabbits for the experiments described here. Serological surveys were carried out in April 1955 (79% immune), July 1956 (62%) and March 1957 (98%). There was a very severe outbreak in the summer of 1956–57 which accounted for the very high immunity rate in the last sample. Usually the infection rates were lower and the genetic selection less intense than at Lake Urana, where almost universal infection occurred each year.

Approximately 250 rabbits were collected in this area in November–December 1957, and 206 of these were used in the experiments. The remainder were immune, were judged by weight to be progeny of an earlier breeding season, or died during adjustment to captivity.

*Ouyen*

This area is in the Mallee district of north-western Victoria, which is a semi-arid region of light soils and sand dunes. In their native state the dunes are stabilized principally by scrubby eucalypts which give the district its name. The Mallee was opened for dry wheat farming after the First World War, but extensive clearing, erratic rainfall and rabbits in plague proportions led to serious wind erosion and sand drift. It is only in relatively recent years that judicious land management has bestowed some measure of stability on the region.

Myxomatosis was successfully introduced during the summer of 1951-52, and there have been annual epizootics since then (Douglas, in preparation). Serological surveys were conducted in the Ouyen area in June 1956 (84 % immune) and March 1957 (88 %).

Of the total of approximately 300 rabbits collected in this area in October 1957, 233 were used in the experiment.

## EXPERIMENTAL RESULTS

Two series of experiments were carried out. The first consisted of an extension of earlier observations of changes in the innate resistance of successive generations of wild rabbits exposed to naturally occurring outbreaks of myxomatosis (Marshall & Fenner, 1958), and the second an attempt to correlate the laboratory findings with the performance of the disease in the field.

(1) *Further observations on the innate resistance of wild rabbits to myxomatosis*

Fifty-one of the rabbits collected at Maryvale station in 1957 were used in this experiment and all those collected at Lake Urana in 1958. As in the earlier experiment all rabbits were reared in the laboratory to the age of at least 4 months and were shown by serological testing to be susceptible to myxomatosis before challenge with 10-20 ID<sub>50</sub> of myxoma strain Aust/Corowa/12-52/2 (KM13).

In Fig. 1 the current results have been added to the graph originally presented by Marshall & Fenner (1958). The numbers of epizootics (selection pressure) have again been adjusted according to the immune rate in the survivors of each successive outbreak.

Thirty-six (70 %) of the rabbits from Maryvale station died of myxomatosis (L, Fig. 1); the same percentage mortality as in a group of 76 rabbits (G, Fig. 1) tested the previous year (Marshall & Fenner, 1958).

Only 37 (26 %) of the 142 rabbits from Lake Urana (M, Fig. 1) died compared with 54 % in a group of 35 rabbits collected there in October 1956 (J, Fig. 1) and 45 % in a group of 31 collected in September 1955 (K, Fig. 1).

During the period of the current experiments a total of 50 laboratory-bred rabbits were inoculated with myxoma strain Aust/Corowa/12-52/2 (KM13). Forty-four (88 %) of these died, compared with 89 % of a total of 93 similar rabbits tested in previous years.

(2) *Combined field and laboratory experiments*

It was necessary to time the experiments so that rabbits being held in the field would be inoculated early enough to avoid expected natural summer epizootics in the districts, but late enough to ensure a reasonable abundance of young adult rabbits from the spring and autumn breeding. Rabbits were therefore collected at Ouyen in October, and at Maryvale in November, and the experiments were completed in both districts some weeks before natural epizootics occurred.

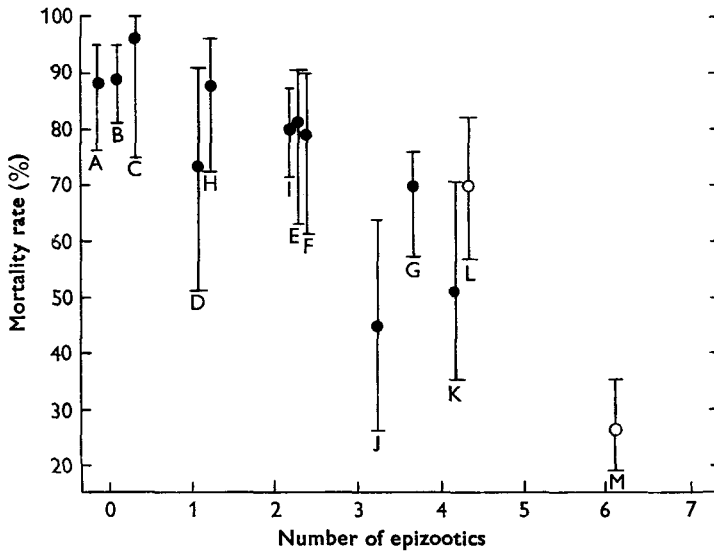


Fig. 1. The relation between mortality rates of Australian wild rabbits after challenge infection with small doses of Aust./Corowa/12-52/2 (KM 13) strain myxoma virus, and the selection pressure for increased genetic resistance exerted by exposure of the antecedents of the tested rabbits to severe epizootics of myxomatosis. Data from text (L and M) and from Marshall & Fenner, 1958 (A to K). Ordinates: mortality rates (%). Abscissae: number of annual epizootics which occurred in areas from which rabbits were obtained, corrected for immune rates in survivors of each epizootic. Results from Lake Urana are represented by H (1953), I (1954), J (1955), K (1956) and M (1958), and from Maryvale by G (1956) and L (1957).

During this series of experiments, and others carried out under similar conditions, a total of 70 laboratory rabbits were inoculated with the attenuated myxoma strain Aust./Corowa/12-52/2A. Forty-seven of these died, giving a mortality rate of 67% in rabbits unselected for resistance to myxomatosis (Table 1).

The rabbits at Ouyen were divided into three groups. Of the group of 89 which were taken to Canberra, one died shortly after arrival and four were found to be immune by the gel diffusion test. After a period of 2 weeks to allow adjustment to cage life, the remaining 85 rabbits were inoculated intradermally at a shaved area of the right flank with 20 ID<sub>50</sub> of myxoma strain Aust./Corowa/12-52/2A. Ampoules of the same batch of lyophilized virus suspension were used to inoculate a group of 106 non-immune rabbits in the field enclosure at Ouyen with a similar

dose. Eighteen (21 %) of the rabbits in the animal house died, compared with six (6 %) in the field enclosure (Table 1).

The third group of 43 rabbits was held in the field enclosure and inoculated with 20 ID<sub>50</sub> of the standard laboratory strain (C.S.L.) virus concurrently with the group inoculated with Aust/Corowa/12-52/2A. All developed severe symptoms and thirty-seven (86 %) died, although many survived for a considerably longer period than the usual 10-12 days. Temperatures in the animal house ranged from 68° to 73° F., whilst maximum afternoon shade temperatures in the field enclosure ranged between 60° and 100° F.

Table 1. *Mortality rates in groups of rabbits held in the animal house or in field enclosures and inoculated with a small (20 ID<sub>50</sub>) dose of myxoma virus*

Group	Conditions	Virus strain	No. of rabbits	No. of deaths	Mortality rate (%)
Domestic rabbits (control)	Animal house	Aust/Corowa/12-52/2A	70	47	67
Ouyen	Animal house	Aust/Corowa/12-52/2A	84	18	21
Ouyen	Field enclosure	Aust/Corowa/12-52/2A	106	6	6
Maryvale	Animal house	Aust/Corowa/12-52/2A	52	20	38
Maryvale	Field enclosure	Aust/Corowa/12-52/2A	103	7	7
Domestic rabbits (control)	Animal house	Standard Lab. Strain (C.S.L.)	—	—	99.8 *
Ouyen	Field enclosure	Standard Lab. Strain (C.S.L.)	43	37	86
Maryvale	Field enclosure	Standard Lab. Strain (C.S.L.)	20	18	90

\* Fenner & Marshall (1957).

Both strains of virus were also used in the field enclosure of Maryvale station. Here seven (7 %) of the 103 non-immune rabbits inoculated with the attenuated strain died. One of the 110 rabbits taken to Canberra was immune and six died shortly after arrival. Fifty-two rabbits were inoculated with Aust/Corowa/12-52/2A and twenty (38 %) of these died (Table 1). Twenty rabbits in the field enclosure were inoculated with the highly virulent standard laboratory strain. All showed very severe symptoms but several lived for 15-20 days and two recovered.

#### DISCUSSION

There had been two summer epizootics of myxomatosis at Lake Urana since the previous sampling of the population for resistance to the disease (Marshall & Fenner, 1958), and the immunity rates in survivors indicate that infection in both of these was almost universal. This continued high selection pressure resulted in a further increase in the innate resistance of the rabbit population to myxomatosis, with no suggestion of a levelling out of the effect of this resistance. At Maryvale, on the other hand, no further increase in resistance was detected in the year since the previous sample. It can be expected that the resistance of rabbits to myxomatosis throughout Australia will vary considerably according to the degree of exposure to the disease over the years.

The results of the combined field and laboratory trials leave no doubt that under closely simulated natural conditions genetic resistance to myxomatosis is a significant factor in reducing mortality. The fact that proportionately fewer rabbits died at both field enclosures than in the sheltered environment of the animal house was quite unexpected, but this might have been due to high ambient temperatures experienced during the course of the field experiments. Using the same strain of virus (Aust/Corowa/12-52/2A) it has been shown that fluctuating high temperatures (78° F. min.-99° F. max.) exert a pronounced sparing effect in laboratory rabbits (Marshall, 1959). The temperatures experienced in the field enclosures favoured a high recovery rate. The extent of the influence of temperature when compounded with genetic resistance cannot be estimated because appropriate controls (wild rabbits from an area not subject to myxomatosis) were not available. It is apparent, however, that temperature was not the only factor inducing recovery, for the overwhelming disease consequent on infection with a highly virulent strain of myxoma virus is not altered by moderate elevation of the ambient temperature (Marshall, 1959). Yet in the groups of rabbits at Ouyen and Maryvale infected with the standard laboratory strain the mortality rate was reduced from the expected 99.8% to 86% and 90%, and many of the fatal cases survived for longer than 13 days. This reduction is as strongly indicative of increased host resistance as the more striking reduction of mortality rates observed using attenuated virus strains.

#### SUMMARY

1. In one Australian study area where the selection pressure of annual epizootics of myxomatosis has been extremely high, the innate resistance of the rabbits was found to have increased to such an extent that only 26% died when inoculated with a strain of myxoma virus which kills 88% of genetically unselected rabbits.
2. The level of innate resistance in a rabbit population varies with the total experience of the disease.
3. In combined field and laboratory trials it was shown that the mortality rate in the field is influenced by innate resistance at least as much as under the sheltered conditions of an animal house. The effect was apparent with both an attenuated and a highly virulent strain of virus.
4. The moderately high ambient temperatures during the field experiments probably contributed to the sparing effect.

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