STUDIES IN THE EPIDEMIOLOGY OF INFECTIOUS MYXOMATOSIS OF RABBITS

I. RECOVERY OF AUSTRALIAN WILD RABBITS (ORYCTOLOGUS CUNICULUS) FROM MYXOMATOSIS UNDER FIELD CONDITIONS

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(With 3 Figures in the Text)

Infectious myxomatosis has the reputation of almost invariably killing European rabbits (Oryctolagus cuniculus) which are infected with the virus. Thus Hyde (1939) recorded that there were no survivors among over 2000 rabbits infected with the virus in his laboratory over a period of 10 years. Hobbs (1928) reported one case of recovery out of 175 animals; and Fisk & Kessel (1931), working with the Californian strain of virus, two cases out of 150. More recently Ginder & Friedewald (1952) reported 100 % mortality amongst 200 normal laboratory rabbits inoculated with relatively small doses of myxoma virus.

These figures refer to laboratory rabbits inoculated with the virus. Of greater importance in the present context are the figures obtained when the disease was maintained in rabbit colonies under conditions of natural spread. Working with English wild rabbits, Martin (1936) recorded ten recoveries out of 312 rabbits infected by contact, or by the implantation of small amounts of one strain of virus which he regarded as less virulent than another, which killed 245 out of 248 rabbits similarly infected. Bull & Mules (1944) provide the best available baseline for predicting case mortality in the field. They noted two recoveries amongst 929 Australian wild rabbits exposed to contact infection in an enclosure about 400 square yards in area. Owing to the occurrence of some non-specific deaths within a week of exposure, and a few deaths not obviously due to myxomatosis, it was not possible to determine the exact case-mortality rate, but it was about 99-7 %.

While both the South American and the Californian strains of myxoma virus exhibit this very high lethality for European rabbits, two less virulent mutant forms of the virus have been obtained from the South American strain during laboratory manipulations. Hurst's (1937) ‘neuromyxoma’ killed ten out of thirty rabbits infected by intradermal inoculation, and Berry's (1937) ‘80A mutant’ killed only 15 % of rabbits infected with it.

When myxomatosis became epizootic in wild rabbits in south-eastern Australia during the summers of 1950-1 and 1951-2 (Ratcliffe, Myers, Fennessy & Calaby, 1952), it was obviously desirable that some estimate should be made of the mortality rates in exposed populations in the field. With one exception, which is discussed in detail later, this has not proved possible except in a very approximate way.
owing to the difficulty of determining the initial pre-epizootic population in any area. However, rough assessments of the percentage kill in various localities have been made by members of the Wildlife Section of the Commonwealth Scientific and Industrial Research Organization, and by the field officers of the departments concerned with rabbit control in the different States of the Commonwealth, outbreaks being graded as follows:

- Grade I kill: 90% or greater reduction in the rabbit population (occasional rabbits seen where there were hundreds previously).
- Grade II kill: intermediate (killed many but left many).
- Grade III kill: occasional cases only seen; no noticeable effect on population size.

No information was available as to the composition of the populations remaining after the epizootics, in terms of animals which had recovered from myxomatosis and those which had escaped infection.

In March 1951, shortly after the first outbreaks, several rabbits with severe scarring around the eyes and elsewhere on the face and body were forwarded to our laboratory. Their serum was found to contain antibodies to myxoma virus. Three of them were kept without challenge inoculation of myxoma virus so that the rate of disappearance of specific antibodies could be determined. On another occasion, wild rabbits showing much the same picture were found, by both challenge inoculation and serological tests, to be completely susceptible to myxomatosis, so that it became apparent that recoveries could not be diagnosed with certainty merely from the appearance of the animal. Serological tests over a period of 18 months on the first group of rabbits indicated that such tests should form a satisfactory basis for determining recovery from myxomatosis. In this paper an account is given of the application of these tests to sera collected from 824 rabbits killed in seventeen localities in which there were different histories of myxomatosis outbreaks.

MATERIALS AND METHODS

Myxoma virus. The virus used in laboratory experiments was the same South American strain as that used to initiate the field outbreaks. Its history was described in a previous paper (Fenner, Day & Woodroofe, 1952).

Complement-fxation test. The myxoma and normal control antigens used in the complement-fixation tests were prepared from the chorio-allantoic membranes of the developing chick embryos (Lush, 1939). The myxoma antigen was standardized by chessboard titration against a known immune serum to obtain the optimal dilution (usually 1:8) before use. The complement-fixation technique described by Donnelley (1951), utilizing small volumes of each reagent, was used. Serum was diluted 1/20 in saline and inactivated by heating at 62°C for 15 min. Two drops (each 0.04 ml. approximately) of the serum were diluted serially in twofold steps in tubes containing one drop of calcium-magnesium saline and one drop of complement, containing 3 HD₅₀. After the addition of one drop of optimally diluted antigen to each tube, fixation was allowed to take place overnight at 4°C. One drop of 3% sensitized sheep cells was added to each tube, and the tubes were incubated, with shaking, for 30 min. at 37°C. The dilution of serum showing 50%
haemolysis was taken as the end-point. Appropriate controls, including known potent immune serum and antigen prepared for normal chorio-allantoic membranes, were always used.

Neutralization test for myxoma virus. Neutralization tests were carried out by the pock-counting method on the chorioallantoic membrane of the developing chick embryo (Lush, 1937). Undiluted serum was heated at 56°C for 30 min. before mixture with the virus dilution. The virus-serum mixture was allowed to stand at room temperature (15–20°C) for half an hour before inoculation of the eggs. The degree of neutralization has been expressed as the percentage reduction in the pock count, compared with that obtained with normal serum. Results were usually clear-cut, reduction being either 0–50% (negative) or 95–99% (positive).

EXPERIMENTAL RESULTS

Persistence of antibody in rabbits which had recovered from myxomatosis

Only one record was discovered which indicated the persistence of circulating antibody to myxoma virus in recovered animals, namely, the report by Shaffer (1941) that antibody was detected by the complement-fixation test in an animal which had recovered from an attack of myxomatosis 5 years earlier. It was not clear whether this animal had been subjected to challenge inoculations during the intervening period.

Upon receipt of a number of recovered wild rabbits from the field in March 1951, three of these were set aside for periodic tests of complement-fixing and neutralizing antibodies. Tests were carried out by the standard procedure immediately after blood was drawn, and again about a year after receipt of the animals, the sera having been stored meanwhile in the frozen state. The results on both occasions agreed closely and are shown in Fig. 1.

It is apparent that after a relatively rapid decline from an initial high level, the titre of complement-fixing antibody fell very slowly, and in all three animals remained moderately high (1/80 and 1/160) for at least 19 months after their recovery from myxomatosis.

Nineteen months after recovery the two surviving rabbits were challenged by the intradermal inoculation of about 10 ID₅₀ of myxoma virus. One of them (R555) responded with a small nodule (6 mm. diameter on the sixth day) at the inoculation site, but the titre of complement-fixing antibody remained constant at 1/160. There was no local reaction in the other rabbit with this small dose, and no change in the antibody titre. Ten days after this inoculation a further inoculation was made, of about 10⁶ ID₅₀. A hard flat nodule, about 25 mm. in diameter, developed at the site of inoculation, and the antibody titre rose from 1/80 to 1/640. In neither rabbit were there any signs other than the nodules at the inoculation sites.

The neutralization index showed little change throughout the period of study. It was thought that the latter result might have been due to the lack of sensitivity of the technique used, so the sera from the March 1951 and May 1952 bleeds were tested. Egg neutralization tests were carried out with undiluted serum, and with serum diluted 1/10, 1/30 and 1/100, six eggs being used with each serum-virus
mixture. The results are shown in Table 1. In all cases the 1/100 dilution of the serum caused an appreciable reduction (over 60%) in the pock count, so another titration was made with the last bleed of R 556, in which the serum was tested at tenfold dilutions from undiluted to 1/10,000. R 556 serum diluted 1/1000 also reduced the count by more than 60%, but no difference could be detected between the membranes inoculated with the 1/10,000 serum-1/100 virus mixture and control membranes inoculated with 1/100 virus in normal serum. Following the observa-

Fig. 1. The persistence of complement-fixing (C.-F.) and neutralizing (N) antibodies to myxoma virus in wild rabbits which had recovered from natural infection contracted about one month before the first titrations. 19 months after infection two survivors (R 555 and R 556) were challenged with 10 ID₅₀ of myxoma virus, and 10 days later R 556 with 10⁵ ID₅₀ (see Table 2). The serological response is shown.

-, +, ++ indicate local reactions to challenge inoculations.
Table 1. Results of egg-neutralization tests with sera taken 1 and 15 months after recovery from myxomatosis, in the absence of reinfection

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Serum dilution</th>
<th>Virus dilution</th>
<th>Pre-inoculation treatment</th>
<th>Bled March 1951</th>
<th>Bled May 1952</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pock counts</td>
<td>Mean</td>
</tr>
<tr>
<td>R555</td>
<td>1/1</td>
<td>10^{-2}</td>
<td>30 min. room temperature</td>
<td>7, 8, 12, 10, 12, 5</td>
<td>9-0</td>
</tr>
<tr>
<td></td>
<td>1/10</td>
<td>10^{-3}</td>
<td></td>
<td>60, 58, 48, 30, 38, 25</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>1/30</td>
<td>10^{-4}</td>
<td></td>
<td>88, 40, 52, 52</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>1/100</td>
<td>10^{-5}</td>
<td></td>
<td>75, 74, 46, 104, 52</td>
<td>70</td>
</tr>
<tr>
<td>R556</td>
<td>1/1</td>
<td>10^{-1}</td>
<td>Do.</td>
<td>4, 3, 4, 8, 4</td>
<td>4-5</td>
</tr>
<tr>
<td></td>
<td>1/10</td>
<td>10^{-2}</td>
<td></td>
<td>27, 42, 31, 15, 21</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>1/30</td>
<td>10^{-3}</td>
<td></td>
<td>100, 64, 20, 65, 39</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>1/100</td>
<td>10^{-4}</td>
<td></td>
<td>80, 46, 58, 33, 53, 53</td>
<td>31</td>
</tr>
<tr>
<td>R557</td>
<td>1/1</td>
<td>10^{-1}</td>
<td>Do.</td>
<td>2, 12, 7, 10, 10</td>
<td>8-2</td>
</tr>
<tr>
<td></td>
<td>1/10</td>
<td>10^{-2}</td>
<td></td>
<td>16, 38, 15, 67, 18</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>1/30</td>
<td>10^{-3}</td>
<td></td>
<td>31, 33, 60, 41, 38, 41</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>1/100</td>
<td>10^{-4}</td>
<td></td>
<td>85, 69, 82, 17, 47, 73</td>
<td>62</td>
</tr>
<tr>
<td>Control</td>
<td>1/1</td>
<td>10^{-3}</td>
<td>Do.</td>
<td>3, 0, 4, 7, 3, 14</td>
<td>5-2</td>
</tr>
<tr>
<td>R556 (retest)</td>
<td>1/1</td>
<td>10^{-1}</td>
<td>Do.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1/10</td>
<td>10^{-1}</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1/100</td>
<td>10^{-1}</td>
<td></td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td>1/10,000</td>
<td>10^{-1}</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>1/1</td>
<td>10^{-4}</td>
<td>Do.</td>
<td>5, 5, 4, 10, 6, 16</td>
<td>7-6</td>
</tr>
<tr>
<td>R556</td>
<td>1/1</td>
<td>10^{-1}</td>
<td>Do.</td>
<td>25, 24, 15, 65, 32, 24</td>
<td>5-6</td>
</tr>
<tr>
<td></td>
<td>1/100</td>
<td>10^{-1}</td>
<td></td>
<td>31</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>1/1</td>
<td>10^{-2}</td>
<td>Do.</td>
<td>10, 35, 69, 18, 28, 29</td>
<td>33</td>
</tr>
<tr>
<td>R556</td>
<td>1/1</td>
<td>10^{-2}</td>
<td>Do.</td>
<td>93, 19, 29, 62, 100</td>
<td>63</td>
</tr>
<tr>
<td>Control</td>
<td>1/1</td>
<td>10^{-7}</td>
<td>Do.</td>
<td>2, 9, 8, 5, 7, 6</td>
<td>9-2</td>
</tr>
<tr>
<td>R556</td>
<td>1/1</td>
<td>10^{-4}</td>
<td>60 min.</td>
<td>14, 12, 2, 1, 6, 10</td>
<td>7-5</td>
</tr>
<tr>
<td>Control</td>
<td>1/1</td>
<td>10^{-4}</td>
<td>37°C</td>
<td>8, 28, 37, 24</td>
<td>24</td>
</tr>
<tr>
<td>R556</td>
<td>1/1</td>
<td>10^{-7}</td>
<td></td>
<td>24, 74, 23, 65, 57, 41</td>
<td>47</td>
</tr>
<tr>
<td>Control</td>
<td>1/1</td>
<td>10^{-7}</td>
<td></td>
<td>3, 5, 14, 11, 2, 14</td>
<td>8-1</td>
</tr>
</tbody>
</table>
obtained with either undiluted or dilute serum. Whatever technique was used, there were no differences between the first and last bleeds of any of the rabbits of anything approaching the same order of magnitude as those detected by the complement-fixation test.

From these results it is apparent that both the complement-fixation test and the neutralization test should serve to diagnose past infection with myxoma virus for a long period after recovery. In myxomatosis, as in many other viral diseases, the titre of the neutralizing antibody changes little for a long time after recovery, whereas the titre of complement-fixing antibody usually falls fairly rapidly and then reaches a steady rather low level.

The response of wild rabbits which had recovered from myxomatosis acquired naturally or from fibroma infection (752 and 758), at the indicated periods (interval in months) before challenge inoculation with myxoma virus.

- to ++++ are arbitrary measures of the extent of the local reaction at the site of intradermal inoculation of the virus. tr. = trace, g = generalized.

The serological response in wild rabbits which had recovered from myxomatosis and were subsequently inoculated with the virus

The clinical and serological response of two rabbits which had been challenged with myxoma virus after being kept in the laboratory for 18 months following their recovery from myxomatosis were described in the preceding section. In order to obtain more complete data upon this, five wild rabbits which had recovered from naturally acquired myxomatosis were bled and their sera stored. They were then inoculated with various amounts of myxoma virus. The responses at the inoculation sites were observed for 14 days or longer, and another sample of serum was then collected. All sera from each rabbit were tested simultaneously by the complement-fixation test, with the results shown in Table 2 and Fig. 2. The responses of another rabbit which had recovered from myxomatosis and was later challenged with several large doses of myxoma virus, and of two rabbits which had previously been vaccinated with fibroma virus (Fenner, Woodroofoe & Marshall, 1953) are also included.
Table 2. The response of rabbits previously infected with myxoma or fibroma virus to subsequent inoculations of myxoma virus

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>History to infection when caught</th>
<th>Estimated interval Infection to challenge</th>
<th>Date</th>
<th>Dose (ID&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>Complement-fixation test</th>
</tr>
</thead>
<tbody>
<tr>
<td>696</td>
<td>Scabs on back when caught</td>
<td>3 months</td>
<td>12 v. 52</td>
<td>7*</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27 v. 52</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>712</td>
<td>Scabs and loss of hair round eyes when caught</td>
<td>3 months</td>
<td>12 v. 52</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27 v. 52</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>649</td>
<td>Captured September 1951, Failed to become infected on inoculation then and on five subsequent inoculations</td>
<td>6 months</td>
<td>12 v. 52</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1. v. 52</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>672</td>
<td>Active disease when caught</td>
<td>7 months</td>
<td>19 v. 52</td>
<td>14*</td>
<td>12, 10, 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26 v. 52</td>
<td>480</td>
<td></td>
</tr>
<tr>
<td>605</td>
<td>Scarring round the eyes</td>
<td>7 months</td>
<td>19 x. 51</td>
<td>+++†</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 x. 51</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>713</td>
<td>Scarring and loss of hair around eyes when caught</td>
<td>14 months</td>
<td>19 v. 52</td>
<td>20, 11, 6</td>
<td>19, 5, 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26 v. 52</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>555</td>
<td>Extensive scarring, and loss of hair around eyes when caught</td>
<td>19 months</td>
<td>23 x. 52</td>
<td>7</td>
<td>14, 5, 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 x. 51</td>
<td>640</td>
<td></td>
</tr>
<tr>
<td>556</td>
<td>Extensive scarring, and loss of hair around eyes when caught</td>
<td>19 months</td>
<td>23 x. 52</td>
<td>7</td>
<td>14, 5, 2</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td></td>
<td>30 x. 51</td>
<td>640</td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>Laboratory rabbit fibroma virus 9. iv. 52</td>
<td>2 weeks</td>
<td>19 v. 52</td>
<td>12, 10, 7</td>
<td>16, 4, 2</td>
</tr>
<tr>
<td>758</td>
<td>Laboratory rabbit fibroma virus 14. i. 52</td>
<td>3 months</td>
<td>20, 11, 6</td>
<td>12, 10, 7</td>
<td>16, 4, 2</td>
</tr>
</tbody>
</table>

* Figures represent diameter in millimetres, when lesion was at maximum size. 7 etc. = small nodules; 21, 20, 14 etc. = cocarde with erythema 21 mm., pallor 20 mm., necrotic centre 14 mm. 0 = no response detected.
† ++ to +++ = reactions of varying severity, graded arbitrarily.
Some of the rabbits were completely resistant to reinfection, and others reacted by the production of a localized lesion which was always clearly demarcated from the adjacent normal skin. Such a reaction sometimes developed after the introduction of quite small doses of virus. Lesions remote from the inoculation site never developed in rabbits which had recovered from myxomatosis. In the absence of a local response the titre of complement-fixing antibody did not change, whereas if a local lesion developed there was an increase in the titre. The response of individual rabbits could not be predicted from the antibody level before the test, but the evidence suggested that the likelihood of a local response increased with the duration of the interval between the attack of myxomatosis and the subsequent challenge inoculation. The height of subsequent increase in the antibody titre appeared to be proportional to the severity of the lesions produced by the myxoma virus. This was most evident in rabbits previously vaccinated with fibroma virus. The results are rather similar to those obtained upon reinoculation with ectromelia virus of mice which had recovered from mousepox (Fenner, 1949a).

Neutralization tests on the chorionic membrane, using undiluted serum from the rabbits which had recovered from myxomatosis, showed no change, even in R605, in which there was an appreciable rise in the titre of complement-fixing antibody. Sera from the rabbits vaccinated with fibroma virus failed to neutralize myxoma virus before the challenge inoculation, but produced a reduction of 98–99% in the pock count after challenge.

From the point of view of field epidemiology the important feature of these experiments, apart from the fact that they demonstrate the high degree of resistance to generalized myxomatosis which follows recovery from the disease, is that doses comparable to those which a mosquito might introduce could, in some rabbits which had recovered from myxomatosis, produce a localized lesion and a rise in the titre of complement-fixing antibody. The likelihood of such a response appears to increase with the interval between first infection and subsequent exposure. This fact complicates to some extent deductions of the time of infection of rabbits based on antibody titres, for a moderately high titre of complement-fixing antibody might indicate recent infection (see Fig. 1), or might be due to reinfection of a rabbit which had been infected the previous summer.

The composition, in terms of immunity to myxomatosis, of the rabbit population surviving after outbreaks of varying severity

824 samples of rabbit serum were collected from seventeen different localities between February and July 1952, and tested for antibody either by the complement-fixation test, by the neutralization test, or by both. Sera were collected from animals which had been trapped, shot or poisoned. While the best kills were obtained by poisoning, serum from such animals, which could not be collected until several hours after death, was almost always badly haemolysed, and could not be tested by the complement-fixation test. Neutralization tests with these sera were satisfactory.

Sera came from three geographical regions (Fig. 3).
Fig. 3. Map showing the localities from which samples of serum were collected for testing.
Description of localities from which sera were collected

A. Bacchus Marsh District. Central Victoria (localities 1–3)

This region consists of a deeply dissected basalt plateau overlying sandstones (Hills, 1940). Rabbit control in the steep gorges has always been difficult and largely ineffective, and adjoining agricultural land is constantly reinfested from this source.

Myxoma virus was introduced to the area in March 1951 by eye-to-eye inoculation from rabbits obtained in the Kerang district. Although there was no appreciable kill, the disease survived over the winter in some of the gorges. A vigorous campaign to introduce the virus effectively was initiated by the Victorian Department of Lands and Survey in September 1951. Infected rabbits were available at the depot in Bacchus Marsh for eye-to-eye inoculation by landholders from September until late December 1951, and occasionally since that time. Spectacular outbreaks commenced in December 1951, and although there was considerable variation in effectiveness, grade I kills were reported in many localities. Outbreaks were still being reported in May 1952. The effective epizootics were generally confined to the plateau and gorge country, and the rabbit population on the plains to the south and east remained relatively free from the disease.

B. The Riverine Plain of south-eastern Australia (localities 4–13)

The Riverine Plain is an extensive alluvial plain bounded by the Great Dividing Range to the south and east, the McPherson Range to the north-east, and the Mallee to the west (Butler, 1950). The soils range from low ridges of light gravels and sands to broad plains of heavy clay. The stream system, based on the Murray River with its tributaries and anabranches, has been largely controlled and considerably modified by several irrigation projects.

The natural rainfall varies from 20 in. around the arc of foothills to the south and east to about 10 in. in the north-west. There is a sequence of plant associations that approximate the parallel arcs of diminishing rainfall; sclerophyll forest at the foothills followed by savannah woodland, open grassland, and finally a shrub steppe of saltbush to the north-west of the 14 in. isohyetal. This sequence is interlaced with the River Red Gum-Black Box association along the watercourses, and Murray or Cypress Pines on the sandy ridges. The forms of land utilization are also remarkably varied, so that an insect-borne epizootic would have to comply with a very wide range of environmental conditions before uniform results could be obtained over the whole area.

The Australian myxomatosis epizootic originated at the end of a series of experiments at Balldale on the eastern side of the plain in December 1950 (Ratcliffe et al. 1952). Natural spread of the disease was augmented with mass inoculations of rabbits by officers of the Commonwealth Scientific and Industrial Research Organization, Pastures Protection Boards (New South Wales) and Department of Lands and Survey (Victoria). These were begun early in 1951, ceased during the winter, and were then carried out intensively between September and December.
Epidemiology of infectious myxomatosis

C. The Moree district on the North Central Plain of New South Wales; and Texas, in the Downs Division of Queensland (localities 14–17)

The North Central Plain in the vicinity of Moree is composed of heavy, deeply cracking, intractable grey clays interspersed with low narrow ridges of soil of lighter colour and coarser texture. These ‘red ridges’, as they are locally known, are extensively colonized by rabbits which graze out on to the clay plains. The annual average rainfall of 22 in. is almost equally distributed between summer and winter.

The spread of myxomatosis assumed a different pattern in these districts. There were few of the short spectacular epizootics that were encountered in the southern regions. The usual observation was a slow progression of the disease over many months, which finally killed a large proportion of the rabbits present. This type of epizootic made accurate assessment of the kill difficult.

Locality 17 (Texas) is on the fertile flats of Dumaresq River and is surrounded by rough hill country.

The basic data relating to localities, past history of myxomatosis, and the serological findings are set out in Table 3, and the exact geographical location of the areas in which collections were made is shown in Fig. 3.

Before entering into a discussion of the results by areas, certain general questions may be considered.

Correlation between results of complement-fixation and neutralization tests

Some of the early groups of sera were tested by both techniques, and in later samples any specimens which gave low or doubtful results by complement-fixation were tested by neutralization also. In all, 135 of these sera have been tested by both techniques with the results shown in Table 4.

The results were unequivocal with the definitely negative and definitely positive complement-fixation tests. Of the complement-fixation tests recorded as doubtful, twenty were so classified because of non-specific fixation with the control antigen. Seven of these gave positive and thirteen negative results with the neutralization tests. The other twenty-three gave partial fixation only at the lowest dilution. Nineteen of these were classified as positive by the neutralization test and four negative. These last four sera are the only ones, out of the 115 not complicated by non-specific fixation, in which there was any conflict between the results of the two tests. Being simpler and cheaper, complement-fixation was preferred with all sera which proved suitable. Sera of animals killed by poisoning were tested by the neutralization test only.

The sex ratio of survivors

An accurate assessment of the sex was made in 593 rabbits, of which 282 were male and 311 female. The ratio was 49:51 in the uninfected survivors and 46:54 in the immune survivors. When assessed by localities the sex ratio was about the same throughout. Thus sex appears to have no influence upon the likelihood of recovery from myxomatosis.
Locality no. | Approximate area | Incidence of myxomatosis in 1951-2 season |
--- | --- | --- |
1 | 200 acres | Explosive outbreak at the end of Dec. 1951 which continued through Jan. 1952. Smouldering since then |
2 | 80 acres | Do. |
3 | $\frac{1}{4}$ mile of poison trail | Disease reported to be active over a part of the area during Feb. 1952 |
4 | 400 acres | Disease active in medium-sized population during summer of 1951-2 |
5 | 18,000 acres | Explosive outbreak in Dec. 1951 |
6 | $\frac{1}{4}$ mile of creek frontage in 400 acres paddock | Disease very active when first observed in Nov. 1951. One diseased rabbit seen in small surviving population whilst collecting serum samples |
7 | 2 miles of timbered roadway | Disease overwintered at low level, and flared in Nov. 1951. No diseased rabbits seen in small surviving population |
8 | $\frac{1}{4}$ mile of river frontage | Disease very active during Nov. and Dec. 1951. No diseased rabbits seen in fairly small surviving population while collecting serum samples |
9 | 2 chains of small channel bank | Disease active from Dec. 1951 to Mar. 1952, but no marked effect on population |
10 | 2 chains of small channel bank | Disease overwintered and became increasingly active during summer. Smouldering in Mar. 1952 |

**Table 3**

| Locality no. | Approximate area | Incidence of myxomatosis in 1951-2 season |
--- | --- | --- |
1 | 200 acres | Explosive outbreak at the end of Dec. 1951 which continued through Jan. 1952. Smouldering since then |
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4 | 400 acres | Disease active in medium-sized population during summer of 1951-2 |
5 | 18,000 acres | Explosive outbreak in Dec. 1951 |
6 | $\frac{1}{4}$ mile of creek frontage in 400 acres paddock | Disease very active when first observed in Nov. 1951. One diseased rabbit seen in small surviving population whilst collecting serum samples |
7 | 2 miles of timbered roadway | Disease overwintered at low level, and flared in Nov. 1951. No diseased rabbits seen in small surviving population |
8 | $\frac{1}{4}$ mile of river frontage | Disease very active during Nov. and Dec. 1951. No diseased rabbits seen in fairly small surviving population while collecting serum samples |
9 | 2 chains of small channel bank | Disease active from Dec. 1951 to Mar. 1952, but no marked effect on population |
10 | 2 chains of small channel bank | Disease overwintered and became increasingly active during summer. Smouldering in Mar. 1952 |

| Date of serum collection | Effectiveness of myxomatosis 1950-1 | 1951-2 |
--- | --- | --- |
19-21. iii. 52 | Absent | Grade I in large population |
18-22. iii. 52 | Absent | Grade I in large population |
21. iii. 52 | Absent | Grade III with small area of grade II in medium-sized population |
6-10. iii. 52 | Grade III | Grade II |
21. iii. 52 | Absent | Grade I in large population |
19-20. ii. 52 | No record | Grade I in medium-sized population |
18-22. ii. 52 | Absent | Grade I in large population |
19-21. iii. 52 | Absent | Grade I in large population |
18-22. ii. 52 | Absent | Grade I in large population |
21. iii. 52 | Absent | Grade III with small area of grade II in medium-sized population |
22. ii. 52 | Disease present. Not classified | Grade I in large population |
22. ii. 52 | Disease present. Not classified | Grade I in large population |
3. iv. 52 | Grade I in very large population | Grade III in large population |
28. iii. 52 | Grade II in large population | Grade II in medium-sized population |

| No. of serum samples | No. showing antibody to myxoma |
--- | --- |
42 | 40 |
15 | 11 |
46 | 3 |
67 | 31 |
109 | 26 |
20 | 17 |
18 | 13 |
30 | 23 |
42 | 16 |
53 | 25 |
11 8000 acres enclosed by three poison trails

Disease overwintered and was present at a low level throughout the summer. No diseased rabbits seen whilst collecting serum samples

1-3, iv. 52  Grade I in large population  Grade III in medium-sized population

12 1 mile of poison trail and two warrens in channel bank

Disease overwintered and became increasingly active during the summer. No diseased rabbits seen in the small surviving population whilst collecting serum samples

27, iii. 52  Disease present. Not classified  Grade I in large population

13 2000 acres

Disease overwintered at low level and flared spectacularly in Dec. 1951. No diseased rabbits seen in the very small surviving population whilst collecting serum samples

29-31, iii. 52  Grade III in very large population  Grade I in very large population

14 1500 acres

Disease first observed in mid Jan. 1952, and has been quietly active since then. Six diseased rabbits seen whilst collecting serum samples

1-2, v. 52  Disease absent  Grade II in large population

15 3 miles of creek frontage

Disease has been smouldering in the small population surviving from flare-up in Feb.-Mar. 1951. No diseased rabbits seen whilst collecting serum samples

3-4, v. 52  Grade I in large population  Grade III in small population

16 100 acres

Disease first reported in Aug. 1951. Slow progression resulting in effective kill by Dec. 1951. No diseased rabbits seen whilst collecting serum samples

5, v. 52  Disease not reported  Grade II in large population

17 600 acres

Except for a mild flare-up in April 1952, the disease has only smouldered in the very large population that has built up since an effective kill during the summer of 1950-1. Eleven diseased rabbits seen whilst collecting serum samples

6-8, v. 52  Grade II in very large population  Grade III in very large population

Totals

824 370
Table 4. Correlation between results of complement-fixation and neutralization tests

<table>
<thead>
<tr>
<th>Complement-fixation test</th>
<th>Neutralization test</th>
<th>Negative*</th>
<th>Doubtful*</th>
<th>Positive*</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (0-80% reduction)</td>
<td>31</td>
<td>17</td>
<td>0</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Positive (&gt;90% reduction)</td>
<td>0</td>
<td>26</td>
<td>61</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>31</td>
<td>43</td>
<td>61</td>
<td>135</td>
<td></td>
</tr>
</tbody>
</table>

* Negative = no fixation at serum dilution of 1/20.
Doubtful = partial fixation at serum dilution of 1/20, or definite fixation at this dilution with both the myxoma antigen and a control antigen prepared from normal chorio-allantoic membrane.
Positive = fixation at serum dilution of 1/40 or higher, at least two tubes greater with myxoma antigen than with normal control.

The presence of external lesions attributable to myxomatosis

Some of the rabbits secured were suffering from active myxomatosis. They have not been included in this series, but the opportunity was taken to recover virus from the organs of such animals in several localities (nos. 1, 2, 9, 14 and 17). Other rabbits appeared to have recovered recently from myxomatosis, the diagnosis being based upon the presence of distinctive scabs on the head and body of an otherwise healthy rabbit. Among other animals again there were less distinctive signs suggestive of older infection such as fur missing from large areas on the head, scarred eyelids, and scarring in the ano-genital region. The majority, however, showed no signs whatever which could be attributed to past infection with myxoma virus. In Table 5 the relations between signs of presumed previous infection and serological results are set out.

It is apparent that, while diagnosis of past infection from external signs was usually correct, only 20% of the rabbits which had actually recovered from myxomatosis acquired a few months earlier showed characteristic scars or scabs.

Table 5. Relations between signs of past infection with myxomatosis and the results of serological tests

<table>
<thead>
<tr>
<th>Results of complement fixation or neutralization</th>
<th>Signs of presumed past infections with myxoma virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>261</td>
</tr>
<tr>
<td>Negative</td>
<td>330</td>
</tr>
</tbody>
</table>

The effect of prior infection with myxomatosis on fertility

The opinion has been expressed that because of the invariable involvement of the external genitalia in myxomatosis, the fertility of surviving males might be diminished, at least temporarily. No evidence bearing upon this belief was obtained in any of these surveys. However, definite evidence was obtained from localities 16, 17, 18, in area C, that prior infection with myxomatosis had no effect
Epidemiology of infectious myxomatosis

upon the fertility of the females. The relevant data are shown in Table 6. Not enough data were available to determine whether there had been any reduction in the average numbers of foetuses per pregnant female.

Table 6. Incidence of pregnant or lactating does amongst female rabbits examined in localities

<table>
<thead>
<tr>
<th>Prior infection with myxomatosis</th>
<th>Pregnant or lactating</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>18 10</td>
</tr>
<tr>
<td>-</td>
<td>39 28</td>
</tr>
</tbody>
</table>

$\chi^2 = 0.3038$, therefore no significant difference.

DISCUSSION

The actual numbers of sera tested for the presence of antibody to myxoma virus, and the results of these tests, are shown in Table 3. In this discussion an attempt will be made to relate these results to the observed kill in the localities studied, and the case-mortality rate of the disease. The difficulty of obtaining anything like an accurate assessment of the kill due to an epizootic was mentioned in the introduction. I am indebted to Mr K. Myers, of the Wildlife Survey Section of the Commonwealth Scientific and Industrial Research Organization (personal communication), for data upon the only locality for which accurate counts were made of the pre- and post-epizootic rabbit populations. Regular counts were made on standard walks at two areas on Lake Urana (locality 5, Fig. 3). In two samples, each of 50–60 sera, from areas adjacent to those in which the counts were made (and experiencing, according to Myers, an epizootic of comparable severity) there were 20 and 30% containing antibody to myxoma virus. Using an average figure of 24%, and Myers’s (1952, unpublished) data, it was possible to determine case-mortality rates for these two areas as shown in Table 7. The rates agree remarkably with the figure of about 99.7% recorded by Bull & Mules (1944) for naturally transferred disease amongst Australian wild rabbits in a large colony experiment.

Table 7. The percentage kill and the case-mortality rate in two areas at Lake Urana (locality 5)

<table>
<thead>
<tr>
<th>Area</th>
<th>Rabbit population</th>
<th>Case-mortality rate (assume 25% of survivors immune)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Before epizootic</td>
<td>99</td>
</tr>
<tr>
<td>II</td>
<td>After epizootic</td>
<td>97.6</td>
</tr>
</tbody>
</table>

*Case-mortality rate = \(\frac{\text{total number of survivors} \times \text{percentage of immune survivors}}{\text{total number of exposed}}\).
Section of the Commonwealth Scientific and Industrial Research Organization who knew the localities under consideration. From these a figure for the pre-epizootic population per 100 survivors was determined, and from this figure and the immune rate the case-mortality rate was calculated.

### Table 8

<table>
<thead>
<tr>
<th>Locality</th>
<th>Epizootic history 1950-1</th>
<th>Epizootic history 1951-2</th>
<th>Recoveries Percentage amongst survivors (%)</th>
<th>Recoveries Percentage kill (field observation)</th>
<th>Calculated pre-epizootic population per 100 survivors</th>
<th>Calculated case-mortality rate* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2</td>
<td>0 I</td>
<td>1</td>
<td>88</td>
<td>99</td>
<td>10,000</td>
<td>99.1</td>
</tr>
<tr>
<td>5</td>
<td>0 I</td>
<td>1</td>
<td>24</td>
<td>99</td>
<td>10,000</td>
<td>99.8</td>
</tr>
<tr>
<td>6</td>
<td>? I</td>
<td>1</td>
<td>85</td>
<td>&gt;95</td>
<td>&gt;2,000</td>
<td>&gt;96</td>
</tr>
<tr>
<td>7</td>
<td>+? I</td>
<td>1</td>
<td>72</td>
<td>&gt;95</td>
<td>&gt;2,000</td>
<td>&gt;96</td>
</tr>
<tr>
<td>8</td>
<td>+? I</td>
<td>1</td>
<td>77</td>
<td>&gt;95</td>
<td>&gt;2,000</td>
<td>&gt;96</td>
</tr>
<tr>
<td>12</td>
<td>+? I</td>
<td>1</td>
<td>87</td>
<td>&gt;95</td>
<td>&gt;2,000</td>
<td>&gt;96</td>
</tr>
<tr>
<td>13</td>
<td>III I</td>
<td>1</td>
<td>78</td>
<td>99</td>
<td>10,000</td>
<td>99.2</td>
</tr>
<tr>
<td>14</td>
<td>0 II</td>
<td>1</td>
<td>21</td>
<td>85</td>
<td>666</td>
<td>96</td>
</tr>
<tr>
<td>16</td>
<td>0 II</td>
<td>1</td>
<td>37</td>
<td>70</td>
<td>333</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>0 II-III</td>
<td>1</td>
<td>7</td>
<td>20</td>
<td>125</td>
<td>92</td>
</tr>
<tr>
<td>4</td>
<td>III II</td>
<td>1</td>
<td>46</td>
<td>75</td>
<td>400</td>
<td>86</td>
</tr>
<tr>
<td>10</td>
<td>II II</td>
<td>1</td>
<td>47</td>
<td>80</td>
<td>500</td>
<td>90</td>
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<tr>
<td>9</td>
<td>I III</td>
<td>1</td>
<td>38</td>
<td>20</td>
<td>125</td>
<td>40</td>
</tr>
<tr>
<td>11</td>
<td>I III</td>
<td>1</td>
<td>52</td>
<td>30</td>
<td>143</td>
<td>43</td>
</tr>
<tr>
<td>15</td>
<td>I III</td>
<td>1</td>
<td>25</td>
<td>20</td>
<td>125</td>
<td>50</td>
</tr>
<tr>
<td>17</td>
<td>I-III</td>
<td>1</td>
<td>26</td>
<td>30</td>
<td>143</td>
<td>62</td>
</tr>
</tbody>
</table>

*Case mortality rate* = \(100 - \frac{\text{total number of survivors} \times \text{percentage of immune survivors}}{\text{total number of exposed}}\)

There is little doubt that, in general, assessments of the percentage kill are too low, and it is probable that in all areas in which a grade I kill was recorded the percentage kill was probably nearer 99 than 90 %, and in all these areas the case-mortality rate was probably of the same order as found at Lake Urana (locality 5), i.e. over 99 %.

There remain, however, several localities, in which either the case-mortality rate was much lower than usual, the field assessment of the percentage kill was much too low, or the sampling was not representative of the population under study. Some of the discrepancies between the calculated and the observed percentage kills shown in Table 8 are much too great to be explained by sampling errors or inaccurate estimates of the percentage kill, and it seems impossible to avoid the conclusion that in some cases the case-mortality rate was in fact much less than 99 %. The consequences of any significant decrease in the case-mortality rate are of the utmost importance in determining the future behaviour of myxomatosis as a method of rabbit control, and the situation in certain of these localities therefore merits more detailed discussion.

Locality 3 adjoined localities 1 and 2, in which there had been very high kills in large rabbit populations. The physiography differed from that of these two areas, however, and myxomatosis had not caused more than a grade II kill in locality 3. Only three out of forty-six sera collected contained antibody. The area had not
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been closely observed and assessment of the kill was only very approximate. In addition, sampling errors assume more importance when the proportion of positive sera is low. As myxomatosis had not occurred in the area during the previous season, and the area closely adjoined others in which there had been extremely high mortality rates during the period in which occasional cases only were observed in locality 3, it seems reasonable in this case to ascribe both the relatively ineffective performance of the disease in the field, and the small number of recovered animals, to failure of effective spread by insect vectors rather than to any change in the host-parasite relationship.

In all the localities in which the proportion of immune animals was much higher than expected on the basis of the kill in the 1951–2 epizootic (localities 9, 11, 15, 17) it is noteworthy that there had been a very effective kill in the previous (1950–1) summer. This immediately raises several problems for which we do not yet know the answers. The first is the possible survival, through the period of the second epizootics, of rabbits which had recovered from myxomatosis in the first. If the immune rates after the grade I kills in 1950–1 were of the same order (70–90%) as found in 1952 in localities 1, 2, 6, 7, 8, 12 and 13, the majority of rabbits left after the first outbreak would be immune. There is no information on the life-span of Australian wild rabbits under natural conditions, still less under the conditions which might exist after an epizootic had reduced a large population to a small remnant. It is apparent, however, that a few immune survivors of the first outbreak would greatly affect the proportion of immunes left after the second outbreak, and thus make calculation of the case-mortality rate based on the assumption of uniform susceptibility of the population practically meaningless.

The difficulties of interpretation are increased by the fact that it is in just such areas as these in which there had been a violent outbreak in the summer of 1950–1, overwintering through 1951 (Ratcliffe et al. 1952), and another small outbreak in the summer 1951–2, that various other factors could operate which would alter the host-parasite balance.

The first we might consider is active immunization of suckling rabbits due to infection while they were partially protected by antibody received from their immune mothers. This is almost certainly the mechanism by which mousepox survives in mouse colonies (Fenner, 1949b). In the very few tests made of transmission of protective antibody from mother to young in myxomatosis the results were negative (Martin, 1936), and all the inoculated offspring died. We have recently obtained evidence of the protective effect of passive immunization (by the injection of potent antiserum) in suckling rabbits. Under certain rather limited conditions of dosage and timing of the inoculation of myxoma virus some animals sustained non-lethal immunizing infections. In other experiments the young born by rabbits which had recovered from myxomatosis were shown to possess specific antibodies in their circulation, but so far none has survived challenge inoculation.

Secondly, if the majority of survivors of the first epizootic had recovered from myxomatosis rather than escaped infection, it is possible that they transmitted to their progeny an appreciably greater genetic resistance to the disease than occurred in the original population. In a single experiment Martin (1936) failed to find any
evidence of this, but further investigation is obviously necessary. It would seem unlikely, however, that the genetic constitution of the exposed herd could be altered as greatly by the selective action of a single epizootic as the altered case-mortality rates would suggest.

Thirdly, a variant of the original virus which has a lower virulence may have become dominant. Virus was recovered from sick rabbits in some of the areas in which this lower case-mortality rate occurred, and it may be possible to determine whether there has been any fall in its virulence, although the technical difficulties of measuring a drop from 99.5% case-mortality rate to say 95%—a drop which would be of importance in the field—are considerable.

Finally, certain environmental factors could reduce the case-mortality rate in fully susceptible rabbits. Amongst these are the effect of temperature (Parker & Thompson, 1942), and of 'interference' by certain neurotropic viruses (Ginder & Friedewald, 1952). In none of the areas of study did climatic conditions approach those which would be necessary to effect the change in body temperature of the rabbits described by Parker & Thompson. An Australian neurotropic virus, that of Murray Valley Encephalitis (French, 1952), has been found (French & Fenner, 1952, unpublished) to exert the same effect on rabbit fibroma (and possibly therefore on myxoma also) as did Semliki Forest Virus, but its absence from southern Australia during the 1951—2 summer (Anderson & Eagle, 1953) excludes the possibility that it might have caused a lowered mortality from myxomatosis.

Thus it is not possible to account for the discrepancies between observed kills and the immune rate amongst survivors in some localities. We believe that in some cases survival through the second epizootic of a relatively high proportion of the immune survivors of the previous epizootic may be the explanation for the figures obtained, but it is practically certain that this is not the whole explanation in every locality. In particular, we would mention locality 9, in which the field observations were quite definite—there was a ‘phenomenal result’ in autumn 1951, but in autumn 1952 the disease played a ‘negligible part’ (Hoatson, 1952, personal communication). Nevertheless, early in March 1952 twelve rabbits were sent to our laboratory from this area, and all proved to have recovered from myxomatosis acquired in the previous month or so. These twelve were selected from thirty animals showing signs of recent infection which were captured during one day, along two miles of an irrigation channel. Such a large number of recently recovered animals is not compatible with a low-grade kill and a case-mortality rate of more than 99%.

In myxomatosis, as in other virus infections, reinoculation of rabbits some months after recovery may cause a local lesion to develop at the inoculation site, and induce an increase in the titre of the complement-fixing antibody, or the animal may be completely resistant and the antibody titre may remain unchanged. For this reason, and because of the comparatively small numbers of positive sera in any one group, analysis of the titres of complement-fixing antibody have not been of much use in elucidating the significance of our results. However, there was suggestive evidence, in sera from locality 17, that the frequency distribution of titres formed a bimodal curve with one mode at a low and one at a high level.
Much larger samples of serum have now been collected from that area, and the results of their titration will be reported in detail in a later publication.

From the foregoing discussion the impression may have emerged that it was usual for a grade I kill in the first epizootic to be followed by a much less effective kill during the next outbreak. We have been particularly concerned with such occurrences, partly because of their importance for a long-term assessment of the effectiveness of myxomatosis, and partly because under these conditions there remained a reasonable population of rabbits from which to obtain samples of serum. It is important to emphasize that in many areas in which there was a grade I outbreak in 1950–1 the rabbits were virtually eliminated, and there was no build-up of population which could support an epizootic during the next summer. In many other areas there was, in 1951–2, an effective kill in the small population which had built up, by reproduction, since the devastating outbreaks of 1950–1. We have been concerned with the exceptional rather than the usual occurrence.

SUMMARY

1. Australian wild rabbits which had recovered from myxomatosis acquired in the field contained in their serum antibodies which could be detected by complement-fixation or neutralization tests for a long period (more than 18 months) after their recovery. The titre of complement-fixing antibody fell fairly rapidly during the first few months, and remained at a steady level thereafter. No change could be detected in the titre of neutralizing antibodies throughout the observation period.

2. Inoculation of such rabbits with myxoma virus was sometimes followed by the development of a local lesion at the inoculation site, and in these rabbits the titre of complement-fixing antibody rose, but there was no alteration in the neutralizing power of the serum. In other animals no lesion developed and there was no change in the antibody titre.

3. Serum was collected from a total of 824 wild rabbits from seventeen localities in eastern Australia with varying histories of myxomatosis since December 1950. Examination of 135 of these sera by both neutralization and complement-fixation tests showed that the results obtained by the two methods were in close agreement.

4. In many areas in which the disease was absent in the summer of 1950–1 and produced a violent epizootic in 1951–2, the majority (70–90%) of the sera collected from rabbits 2–5 months after the height of the epizootic contained antibody to myxoma virus, i.e. the majority of the survivors had recovered from the disease.

5. Counts of the rabbit population before and after a violent epizootic in the summer of 1951–2, and the proportion of immune animals amongst the survivors in these areas showed that the case-mortality rate was between 99.4 and 99.8%.

6. Consideration of the results obtained in the serological surveys showed that the case-mortality rate was probably of this order (about 99.5%) in all areas in which there had been no disease or a negligible outbreak in 1950–1, whether they had a grade I or grade II kill in 1951–2.

7. In certain other areas, in which a grade I outbreak in 1950–1 was followed by a grade II or poorer kill in 1951–2, the observed immune rate was considerably
higher than would be expected if the case-mortality rate (assuming that the whole rabbit population was susceptible) was 99.5%. The possible causes of this are discussed. Survival of immune survivors from the first epizootic through the second may be a factor of some importance, but it is probably not the only factor involved.

8. The areas just mentioned were exceptional. In most places there was either no build-up of population after the 1950–1 epizootic, or a second effective epizootic destroyed the majority of rabbits in the small population which had developed by reproduction of the survivors of the first outbreak.

We are indebted to many individuals for aid in the collection of sera and the assessment of the results of myxomatosis in the field. Especially would we thank Messrs A. Dyce, B. V. Fennessy, K. Myers, W. Poole and G. Wright, of the Wildlife Survey Section of the Commonwealth Scientific and Industrial Research Organization, and Messrs B. D. Robinson and G. Douglas, Research Officers of the Vermin and Noxious Weeds Branch of the Department of Lands and Survey, Victoria.

We are grateful to Sir Macfarlane Burnet, Director of the Walter and Eliza Hall Institute, for making laboratory space and facilities available to us pending the completion of the University laboratories in Canberra.

REFERENCES


(MS. received for publication 19. xi. 52)