STUDIES IN THE EPIDEMIOLOGY OF INFECTIOUS MYXOMATOSIS OF RABBITS

III. OBSERVATIONS ON TWO SUCCEEDING EPIZOOTICS IN AUSTRALIAN WILD RABBITS ON THE RIVERINE PLAIN OF SOUTH-EASTERN AUSTRALIA 1951–1953

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(With Plates 12 and 13, and 4 Figures in the Text)

The circumstances under which myxomatosis escaped from certain trial sites in the Murray Valley in south-eastern Australia have already been described (Ratcliffe, Myers, Fennessy & Calaby, 1952). Observations in the eastern Riverine Plain immediately before and after the epizootic spread of the disease in the summer of 1950–1 were reported in the previous paper of this series (Myers, 1954).

In this paper the summer epizootics of 1951–2 and 1952–3 in the eastern Riverine Plain are described, together with the results of serological surveys at selected areas, and the assessment of the virulence of strains of myxoma virus recovered from mosquitoes caught in the region.

The Riverine Plain of south-eastern Australia (Butler, 1950) was described briefly in an earlier paper (Fenner, Marshall & Woodroofe, 1953). Superficial observations were made over a large area in the south-eastern portion of the Plain, referred to in subsequent discussion as the 'regional' study. The extent of this area, and the localities where monthly observations of the incidence of rabbits, insect vectors, and myxomatosis were made, are shown in Text-fig 1. Climatically, the region falls within a winter rainfall-summer drought zone. It is bounded along its eastern and southern margins by the foothills of the Great Dividing Range at an average elevation of 1500 ft. and with an average annual rainfall of 30 in. To the north and west the foothills give way to the Western Plains which have an average altitude of about 500 ft. The average annual rainfall decreases steadily with distance from the hills until it reaches 16 in. in the north-west corner of the region studied. The region is dissected by one large river, the Murray, which is usually in flood in late winter and spring (July to November).

Within this region more detailed studies were made at four sites.

(1) *Lake Urana.* (Pl. 12, figs. 1, 2)

This is a lake of internal drainage, about 18,000 acres in extent. Over the years, wind action has built up a great rim of sand around the lake and the area now takes the form of a saucer-shaped depression elongated north-south, with the lake itself in the centre.

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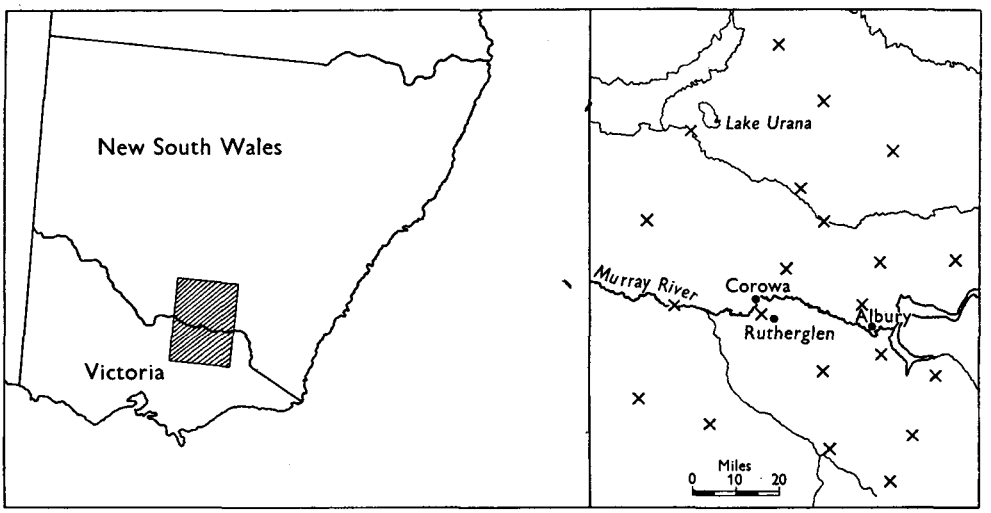
The sandy rim has been colonized by the native pine, *Callitris glauca* R. Br., and by the rabbit, which finds the sand easy to burrow and obtains food and water from the lake floor. Dead trees (red gum—*Eucalyptus camaldulensis* Denh.) cover the lake floor, killed by previous heavy floods, but the same species is re-establishing itself at the base of the sandy rim.

In a region where the average annual rainfall is 17 in., with eleven dry months (less than 2 in. of rain per month) each year, the lake has been an oasis for large rabbit populations, which have been present in pest proportions for the last 25 years, almost irrespective of conditions in the surrounding country.



Text-fig. 1. Maps showing the area over which the observations were made. The places indicated by a cross in the detail map are those regularly visited each month during the regional survey. Localties at which more detailed observations were made are indicated by name.

(2) *Albury* (Pl. 13, fig. 1)

The site thus designated is a valley in the foothill country of the Great Dividing Range, cleared of timber and deeply dissected by eroding gullies. These are spring fed, and hold water later into the summer than is the case in the open plains country. This has an important effect on the activity of the insect vectors. Rabbits live in warrens along the gullies, both in the banks and on the flat valley floor.

(3) *Rutherglen*

This site is very close to that described in a previous paper as the location of early field experiments with myxomatosis (Myers, 1954). It consists of open rolling pastures with small stands of box (*Eucalyptus melliodora* A. Cunn.) along the roads. The rabbits are distributed sparsely in warrens in the open paddocks and along the edges of a few small creeks.

(4) *Corowa* (Pl. 13, Fig. 2)

The Corowa site is in typical river-frontage country of the River Murray. The rabbits occur in a high bank overlooking the flood plain of the valley, which is wooded with red gum (*E. camaldulensis*) and broken by meanders and the weedy lagoons and billabongs of the Murray River.

MATERIALS

Myxoma virus

Virus introduced artificially into the rabbit population was prepared from the South American strain of the virus (strain B of Martin, 1936) by one of us (F.F.), and freeze-dried by the Commonwealth Serum Laboratories, Melbourne. The method of maintenance of the seed virus and preparation of material for freeze drying has been described elsewhere (Fenner & Woodroofe, 1954).

Similar material stored at $-70°$ C. constituted the standard laboratory strain of myxoma virus used in the virulence tests.

Experimental rabbits

Laboratory rabbits bred in the Animal Breeding Establishment of the University were used for the isolation of virus from mosquitoes and for testing the virulence of field strains. Animals 3–4 months old, weighing about 1·5 kg., were used. Owing to the difficulty of obtaining enough laboratory rabbits for the virulence tests, wild rabbits caught near Canberra were also used. Only adult animals, free from serological evidence of myxomatosis, were employed. They were not very satisfactory experimental animals, because of the frequency with which deaths due to causes other than myxomatosis occurred.

METHODS

Field

The field workers were faced with several difficult technical problems, due to the absence of any previous ecological study of wild rabbits in Australia and early observations suggesting that many of Southern's (1940) findings could not be transferred from Britain to the very different conditions obtaining in Australia. Assessment of the size and age structure of populations of wild rabbits on the study sites was urgently required so that reliable information on the results of epizootic myxomatosis could be accumulated. Crude methods of estimation of the population size were therefore used and attempts were made to assess the age structure of the population. These were improved with increased experience.

Estimation of the size of rabbit populations

The difficulties involved in the performance of population counts of rabbits were discussed in the previous paper of the series (Myers, 1954). Subsequent observations (Ratcliffe, personal communication, 1953) have underlined these difficulties. Nevertheless, we believe that the population counts at Lake Urana, made by sight counts on standard transects, under uniform weather conditions, were sufficiently accurate for our purposes, i.e. to provide basic information on the case-mortality rates during two successive epizootics of myxomatosis.

Age structure of rabbit populations

Southern (1940) calculated that the life span of the rabbit in nature in Britain was probably quite short, the majority which reach adult life living only about a year. In the study of the epidemiology of myxomatosis, the major requirement

was the separation of rabbits into those which had lived through the epizootic of the previous summer and those which had been born since the end of that epizootic. Southern's weight–age formula did not give this information, as it applied only to the growth of kittens.

A series of observations was therefore made on the rabbits which yielded the monthly serum samples, about fifty rabbits each month from June, 1952 to March 1953, at each of three sites. The detailed results of these observations will be presented elsewhere (Myers, in preparation). Suffice it to say here that observation of the paunched weight (after removal of gut and uterine horns of pregnant does), the condition of the pelage, and the size and condition of the reproductive organs were sufficient to provide a reasonably accurate division of the observed rabbits into those which were old enough to have lived through the November–December 1951 epizootic (which ceased by the end of December, 1951) and those born since that outbreak.

Sampling of rabbits

Samples of rabbits were taken for serum tests, and for determination of the age structure of the population. They were not taken from the areas in which population counts were made but from nearby areas which had a similar epizootic history. Various methods of collection were used. During winter the ground was soft and samples were obtained by digging out warrens. Animals which attempted to escape were caught by a dog pack. During summer digging-out was impossible, and gin-traps were used. They were used in such a way as to minimize the selection of any particular group in the population—e.g. buck-hills were not trapped—but it is impossible to say whether the animals caught were in fact a random sample of the population.

Collection of blood samples

The captured rabbits were killed by manually breaking the neck, and were immediately bled from the great vessels, which were severed with a scalpel. Blood was collected in small tubes which were appropriately numbered, corked, placed on ice in a thermos flask, and sent by air to the laboratories in Canberra.

Sampling of mosquitoes

No ecological surveys have been made on the mosquitoes of the countryside in southern Australia, and there was no precedent upon which to base sampling methods. As in the case of rabbit counts and sampling, methods were devised and improved upon with increasing experience, and a knowledge of the epidemiological importance of the various species was gradually built up.

There are at least sixteen species of mosquitoes in the region and it was impossible to obtain random samples of insects so variable in their activity patterns and habitat and host preferences. Collection of active adults was carried out continuously throughout the year, whenever the opportunity offered and without regard to mosquito habitat or the major reason for the field excursions. The total number of adult mosquitoes collected was not large, but more systematic larval collections indicated that, on the whole, they reflected fairly accurately the general succession of the different species throughout the year.

Catching at any one site was limited to 15 min. Owing to wide differences in the feeding habits of the different species, some were collected from their resting places. While not 'active adults' in the usual sense of the term, the high proportion of recently engorged females amongst them made it reasonable to include these catches with those taken on the wing.

These methods of mosquito survey were able to give only a rough idea of the relative proportions of different groups of mosquitoes throughout the year. Nevertheless, it will be apparent that they indicated a close correlation between the activity of certain species and the outbreaks of myxomatosis, and a complete absence of such correlation in the case of other common species.

Collection of mosquitoes for the recovery of myxoma virus

During the epizootics, adult mosquitoes were collected for the recovery of myxoma virus. They were lightly anaesthetized with chloroform, grouped in batches of about fifty in 6×1 in. tubes, packed in an ice cold thermos flask, and sent by air to Canberra. *Anopheles annulipes* was invariably collected from its resting place in rabbit warrens during daylight hours. Once on the wing, in the evening, this species could not be collected in large numbers. *Culex annulirostris* and all the *Aedes* sp. were caught from livestock and human bait during their periods of feeding activity. *Culex pipiens australicus* (Dobrotworsky & Drummond, 1953) accompanied *Anopheles annulipes* as warren-resting adults. This was the only place in which they were taken. These methods of collection favoured the recovery of virus from *A. annulipes* and *Culex pipiens australicus*, which were collected from rabbit burrows, but the adult mosquitoes present in the area during the epizootic could be collected in appreciable numbers by no methods other than those described for each species.

Laboratory

Serological studies

Sera were stored in the frozen state. The methods employed for testing the serum samples for antibodies to myxoma virus were fully described in the first paper of this series (Fenner *et al.* 1953).

Neutralization tests were performed only on those specimens of serum which gave doubtful results in the complement-fixation test (low titres or some non-specific fixation of complement).

Comparison of titres of complement-fixing antibody tested on different occasions

The titre of complement-fixing antibody has been used as a basis for comparison between batches of serum collected at different times of the year. Since these batches were titrated with different preparations of reagents a test was set up involving the simultaneous titration of fifty-three samples of serum collected and previously titrated at different times. The results were compared with the titres obtained at the time of the first titration of each of these sera. The titres with thirty-two out of the fifty-three sera were the same in both tests, to within one twofold dilution. Five out of the eight samples showing the greatest variation

between the two titrations (i.e. between two and three twofold dilutions) were from one batch of serum. The whole of this batch was retitrated and the new titres taken as correct. In addition, a sample of serum of known high titre was included in each day's tests. If the titre of this control serum differed from its usual figure by more than one twofold dilution the whole batch was retitrated.

Recovery of virus from mosquitoes

Mosquitoes received in ice-cold thermos flasks were sluggish when first removed, but the majority became active when the temperature rose. Within a few hours of receipt they were quickly cooled and then ground with alundum in a cold mortar and pestle in batches of about fifty insects, taken up in 2 ml. of saline containing 10% normal horse serum, 2000 units of penicillin per ml. and 20,000 units of streptomycin per ml. After centrifugation a portion of the supernatant material was ampouled and stored in a dry ice cabinet. The rest was inoculated intradermally in the rabbit, several pools being tested in one animal. If only one sample gave a positive result, virus-containing tissue was taken from that rabbit. If more than one sample tested on a single rabbit yielded virus, the stored ampoules were opened and the contents of each inoculated into different rabbits, from which virus-containing material, usually the local lesion at the inoculation site, was obtained 6–7 days after the inoculation. This material was ground in saline, dispensed in several ampoules, and stored on dry ice. Next day the titre of the suspension was determined by titration of one of these ampoules on the chorioallantois.

Assessment of the virulence of field strains of virus

Attempts to compare the virulence of field strains of myxoma virus with that of the standard laboratory strain were based on the reaction of susceptible adult rabbits to the intradermal inoculation of small doses (10–20 ID_{50}) of virus. Field strains were passed once through rabbits in the laboratory as described in the previous paragraph, and the titre of the suspension of infected subcutaneous tissue used as a virus source determined before each test.

We have now had considerable experience with the development of myxomatosis in laboratory rabbits inoculated intradermally in one site with small (10–20 ID_{50}) doses of standard fully virulent myxoma virus (e.g. Fenner & Woodroofe, 1953). Each field strain was tested by intradermal inoculation in groups of four or five rabbits, each rabbit receiving 0·1 ml. of a suspension containing 10–20 ID_{50} of virus. During daily observation the size of the local lesion at the inoculation site was measured and its character noted, the time of appearance and the severity of signs of generalization were observed (blepharitis, swelling in the anogenital region, and secondary skin lesion), and the survival time determined.

Some of the strains were first tested in susceptible wild rabbits, but finally all field strains referred to in this paper were tested in a homogeneous batch of laboratory rabbits of about the same age and from the same source.

RESULTS

Lake Urana

The most detailed observations were made at Lake Urana, and they will be described first.

Epizootic history

Apart from an occasional case of myxomatosis noticed in March and April of 1951, the first great epizootic of 1950–1 did not affect the rabbits at Lake Urana, so that the population on which regular observations were commenced in two sample areas in August 1951 consisted entirely of susceptible rabbits.

Population counts of the rabbits were carried out on two sections of the sandy rim of the lake. Sample area I, on the southern edge of the lake, consisted of about 400 yd. of the rim, sloping towards the lake bed with its bordering stand of young red gum. Sparsely scattered native pine covered the area but did not interfere greatly with the performance of population counts (see Pl. 12, figs. 1, 2).

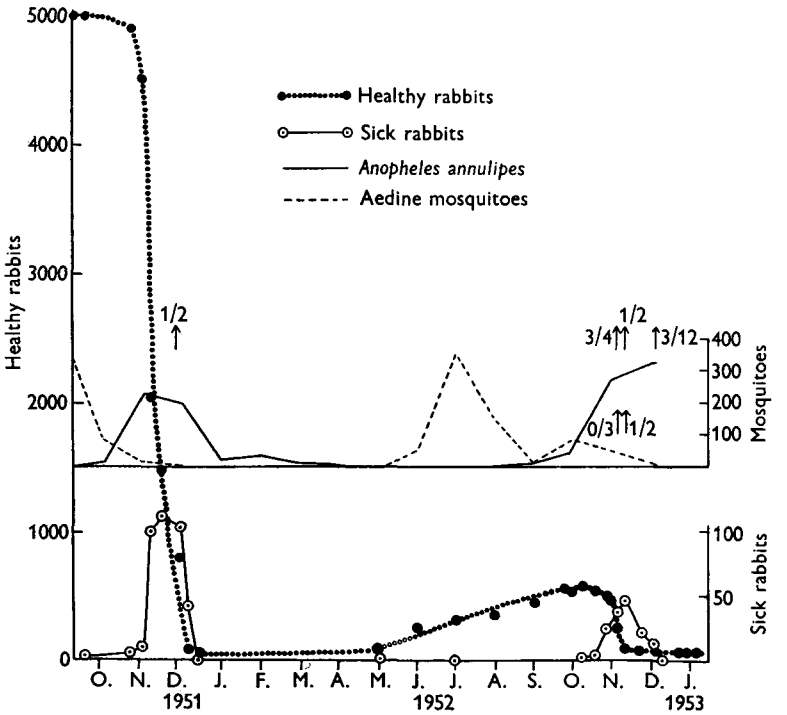
Sample area II, on the south-western edge of the lake, was similar in every respect except for an even lighter covering of pine. In this section of the rim, 300 yd. long, there were no obstructions to the counting operations.

During September and October 1951, hundreds of rabbits in the sand-hills around the rim of the lake were inoculated subcutaneously with reconstituted freeze-dried virus.

The first natural cases of myxomatosis occurred in October, and the epizootic rose to a peak in November, during which month there was a catastrophic fall in the rabbit population, so that there was a count of only about 50 rabbits in sample area I in which there had previously been counts of about 5000 (Text-fig. 2); and there was a drop from 500 to 12 in sample area II.

The disease probably persisted at a very low level of transmission throughout the winter of 1952, for single diseased rabbits were seen during population counts in sample area I in May and July, and at least one diseased rabbit was seen at Lake Urana every month from October 1951, to December 1952, except for June, August and September (Table 2). The landowner destroyed the remaining few rabbits in sample area II, so that no further observations could be made on that site. The population in sample area I was supplemented in June 1952, by a number of adult rabbits which immigrated to the area due to flooding of the lake floor, and this increased the population count by about 150. Young rabbits emerging from the warrens were first seen in July, and their numbers rose steeply in September and October. The population reached about 550 in October, when fifty rabbits were inoculated with virus and released. Observations at this time showed that the epizootic had commenced due to the natural spread of virus which had survived the winter before the inoculated virus could have been transmitted to normal rabbits. The epizootic reached a peak in November and had reduced the population to about 60 by December. It remained at this level for the rest of the summer. At each of the counts made in January about two-thirds of the rabbits counted showed obvious signs of recent recovery from the disease.

Unfortunately, on the night of 15 December, when the epizootic was at its height, a group of rabbit trappers deliberately trapped an area which included the experimental site, and removed 600 rabbits. Examination of a sample of 74 of these showed that they appeared to be a random sample of the population, and included rabbits of all ages and at all stages of the disease. Counts the day before and 2 days after the trapping showed that there was a drop of 246 in the count. About 70 could be accounted for as expected deaths from myxomatosis. If the calculated 176 rabbits removed by the trappers included old immune rabbits and potential recoveries at the same incidence as they occurred in the general population, the final population figure (62) should be increased by 35% (176/500) to 84, so that comparisons can be made between pre-epizootic and post-epizootic population levels.



Text-fig. 2. Population counts of rabbits and adult mosquitoes at sample area I, Lake Urana. The arrows indicate the occasions on which virus was isolated from batches of mosquitoes (*A. annulipes* in upper line, aedine mosquitoes in lower) and the proportion of isolations to batches tested.

Age-structure of the rabbit population

Batches of 60 to 100 rabbits were collected from parts of Lake Urana some distance from sample area I at approximately monthly intervals between June 1952 and March 1953, samples being taken by digging out warrens in the winter and by trapping in the drier weather. All these areas had experienced major epizootics comparable to that just described.

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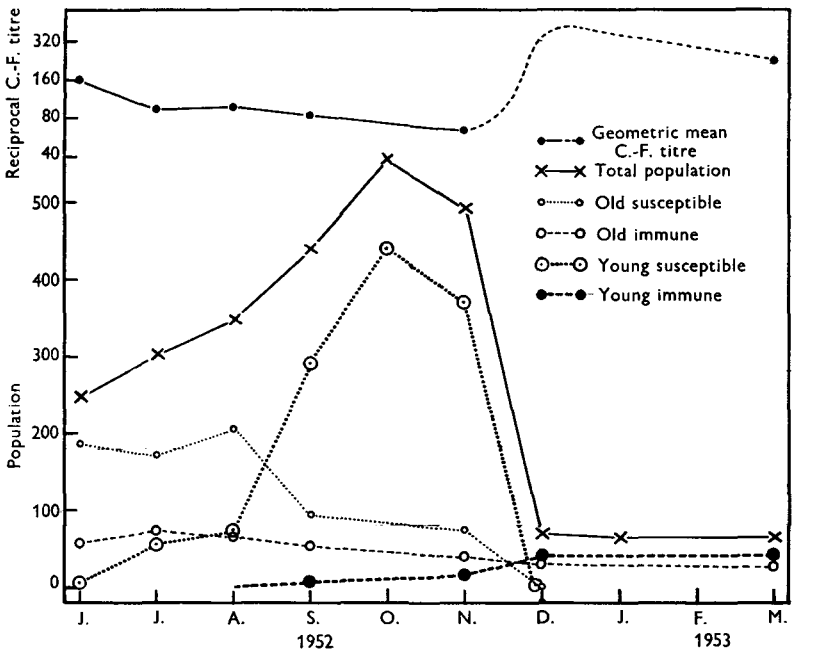
The criteria described elsewhere (Myers, in preparation), were applied to the estimation of the age of rabbits in the samples, and serum was taken from the animals and tested for antibodies to myxoma virus. In Text-fig. 3 are set out the population counts in sample area I dissected into four groups: (*a*) rabbits which had lived through the 1951–2 epizootic but had not been infected ('old susceptible'); (*b*) recoveries infected during the 1951–2 epizootic ('old immune'); (*c*) rabbits born since the end of that epizootic, which had not been subsequently infected with myxomatosis ('young susceptible'); and (*d*) rabbits born since the 1951–2 epizootic which had recovered from myxomatosis acquired since then ('young immune').



Text-fig. 3. The age structure and immune status of rabbits in the population at sample area I, Lake Urana, between June 1952 and March 1953. The geometric mean antibody titre for complement-fixing antibody is also shown.

The assumptions have been made that the data gathered from the monthly samples were directly applicable to sample area I and that the findings in late December (after the epizootic) would have been essentially the same as in March. Sample area I was quite similar in terrain and epizootic history to the surrounding areas from which rabbit samples were taken. The epizootic had died out in December, and there was no breeding of rabbits in the summer so that the relict rabbit population remained static in numbers throughout the period, except for the immigration of adult rabbits from surrounding areas, due to flooding of the lake in June. As no serum samples were collected just after the end of the outbreak in December, when the population count was the same as in March, the age structure observed in March has been plotted in Text-fig. 3 for both December and March.

The population rose due to the emergence of kittens from the burrows in August, September and October, and this rise was followed by an abrupt fall of this group to zero by December, and its replacement by the very much smaller group of young recovered animals. There was a steady fall in the numbers of old susceptible and old immune rabbits between June and November, when the old susceptible group disappeared due to the epizootic but contributed a proportion of animals to maintain the level of the old immune group.

Serological observations

The complement-fixation test had been carefully standardized and comparable titres were obtained on retest of the same sera. The geometric mean titres in the monthly samples are shown in Text-fig. 3. The trend shown, a steady fall from 1/160 in June 1952, to 1/60 in November 1952, followed by a rise to about 1/200 after the November–December 1952 outbreak, confirms other evidence that there was negligible disease activity between the major epizootics. In the March 1953 sample there was little difference in titres of antibody between the older rabbits which had survived two epizootics and younger rabbits which had lived through only one. With the intensity of infection that characterized the 1952 outbreak it is highly likely that every rabbit, immune and otherwise, was bitten by at least one infected mosquito. Earlier experiments (Fenner *et al.* 1953) showed that in a proportion of cases old immune adults responded to such infection by a rise in antibody titre. It is not possible, therefore, to distinguish serologically, in the old rabbits of the March 1953 sample, those which had first been infected in the November–December 1951 epizootic and those which had just recovered from myxomatosis. The estimated fall in old immune rabbits from 50 in November 1952, to 34 in March 1953, shown in Table 1, must be ascribed to natural (non-myxomatosis) mortality in this age group, and not to fatal myxomatosis in serologically positive animals.

Antibody was present in sera of two young rabbits of the August sample, at low concentration. These animals were kittens about 5 weeks old, and the antibody was almost certainly passively transferred from the immune mothers (Fenner & Marshall, 1954). A single young immune animal of the September sample was at least 4 months old, and showed no signs of having recently recovered from the disease. Nevertheless, this animal and the two 4 to 5-month-old animals of the November sample with antibody were almost certainly recoveries from infections contracted in the winter or early spring, for none showed signs of recent infection, and passively acquired antibody would certainly not persist as long as four months.

All the young animals of the March sample (i.e. those which had experienced only the November–December 1952 epizootic) were immune.

The case-mortality rate.

The data obtained at Lake Urana in 1951–2 were used previously for estimating the case-mortality rate of myxomatosis at the time of the first contact of a previously unexposed population to the fully virulent virus (Fenner *et al.* 1953). We may now, with the data on population counts and the immune state of the rabbits

before and after the epizootic, determine the case-mortality rates in the same locality at the time of the second epizootic, when there had been a drastic selection imposed by the first contact with the disease, and when the survival of immune rabbits through a second epizootic complicated the situation. The data are set out in Table 1.

Table 1. *The case-mortality rates due to myxomatosis at sample area I at Lake Urana, in two succeeding epizootics*

	Rabbit population							Case-mortality rate (%)	
	Before epizootic			After epizootic					
	Total	Susceptible	Immune	Total	Susceptible	Old Immune	Recent recoveries	Overall	Excluding old immune rabbits
Nov.–Dec. 1951	5000	5000	0	50	38	0	12	99·8	99·8
Nov.–Dec. 1952	550	500	50	84	0	34	50	85	90

* Old immune = rabbits immune before the commencement of the 1952–3 epizootic.

The accumulation of immune rabbits has an appreciable effect on the case-mortality rate, but even the corrected rate was much lower in 1952 than in the previous epizootic. The extent of the change is better appreciated when it is expressed as a change in the recovery rate from 2 per thousand to 100 per thousand. On evidence available at the moment it is not possible to evaluate the possible contribution of increased host resistance (due to the drastic selection imposed on a proportion of the relict population by the first epizootic), but the observations reported in a later section indicate that a change in the virulence of the virus is at least partly responsible for the reduction in the case-mortality rate.

The correlation between insect vector activity and the epizootics of myxomatosis

Regular catches of adult mosquitoes gave information on the abundance of various species of mosquitoes throughout the year. Detailed observations on mosquito ecology will be published elsewhere (Myers, in preparation). The monthly catches at Lake Urana over the period under consideration are set out in Table 2, together with the number of sick rabbits counted, as an indication of the prevalence of myxomatosis. The monthly incidence of *Anopheles annulipes*, and of the three *Aedes* species is also shown graphically in Text-fig. 2. It is apparent that *Anopheles annulipes* density is closely correlated with the epizootics and that the peaks of activity of the *Aedes* species show no relation to the disease incidence.

In December 1951 two batches each of 60 *Anopheles annulipes* caught at Lake Urana were ground up and inoculated each into three rabbits. All rabbits inoculated with one batch developed typical local and general lesions of myxomatosis and died 8–11 days after the inoculation. None of the animals inoculated with the other batch was affected.

Table 2. *Total numbers of adult mosquitoes of different species caught each month at Lake Urana, and the total numbers of sick rabbits counted each month*

Month	*Culex annuli-rostris*	*C. pipiens australicus*	*Anopheles annulipes*	*Aedes alboannu-latus*	*A. theobaldi*	*A. sagax*	Total sick rabbits counted
1951							
Aug.	0	0	0	137	179	204	0
Sept.	0	0	6	51	191	83	0
Oct.	0	2	9	13	64	11	14
Nov.	8	28	230	1	10	3	421
Dec.	12	32	197	1	2	1	434
1952							
Jan.	8	5	21	0	0	0	7
Feb.	3	3	32	0	0	0	3
Mar.	4	7	11	0	0	0	3
Apr.	0	7	10	2	1	0	1
May	0	0	0	2	0	0	2
June	0	0	0	35	7	5	0
July	0	0	0	120	83	140	3
Aug.	0	0	0	32	41	76	0
Sept.	0	0	12	2	2	12	0
Oct.	0	11	47	0	18	68	7
Nov.	45	35	270	0	30	25	220
Dec.	100	29	325	0	2	7	32

Table 3. *Recovery of myxoma virus from batches of mosquitoes collected at Lake Urana (about fifty mosquitoes per batch)*

Date of collection	Species of mosquito	Result	Totals
Dec. 1951	*Anopheles annulipes*	1/2*	1/2
20 Nov. 1952	*A. annulipes*	1/2	1/5
	Aedes sagax	0/1	
	A. theobaldi	0/2	
27 Nov. 1952	*Anopheles annulipes*	3/4	5/7
	Aedes sagax	0/1	
	A. theobaldi	1/1	
	Culex annulirostris	1/1	
25 Dec. 1952	*Anopheles annulipes*	3/12	3/19
	Culex annulirostris	0/6	
	C. pipiens australicus	0/1	
Totals	*Anopheles annulipes*	8/20	10/33
	Aedes sagax	0/2	
	A. theobaldi	1/3	
	Culex annulirostris	1/7	
	C. pipiens australicus	0/1	

* Numerator = number of mosquito pools from which virus was recovered; denominator = number of mosquito pools tested.

During November and December 1952, mosquitoes caught at Lake Urana were sent to the laboratories in Canberra, and attempts were made to isolate myxoma virus from them. The number of successful recoveries is shown in Table 3.

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The importance of *A. annulipes* as the major vector in the Lake Urana epizootics is indicated by the correlation between their abundance and the incidence of myxomatosis; by the observation, derived from testing their blood meals, that they fed predominantly on rabbits; and by the frequent isolations of virus from batches of these mosquitoes. It was also observed that they rested during the day in rabbit burrows. *C. annulirostris*, which was thought on epidemiological grounds to be the most important mosquito vector in the 1950–1 outbreak (Ratcliffe *et al.* 1952) did not appear to be of great importance here. Epizootics of myxomatosis had broken out before the night-biting *Culex annulirostris* was active, and the epizootic peaks were reached while its density was still low. However, it was taken feeding on rabbits and during both years must have played a minor part in the transmission of myxomatosis. *C. pipiens australicus* was never observed to feed on rabbits, livestock, or man, although the blood recovered from engorged adults taken from rabbit warrens was shown to be that of the rabbit. All the available evidence indicated the unimportance of this species as a vector. The isolation of virus from one batch of *Aedes theobaldi* at the height of the epizootic is not surprising, for it indicates only that some individuals of that species had fed on an infected rabbit. The conclusion that these aedine species are not important vectors of myxomatosis is based on the lack of any evidence of disease activity associated with their widespread prevalence in winter and spring, and the observation, from testing their blood meals, that they rarely bit rabbits.

The maintenance of a steady count of about 60 rabbits after the end of November in the presence of large numbers of *Anopheles annulipes*, some of which harboured virus as late as 25 December, is explicable only on the assumption that all these survivors were immune. This was borne out by subsequent serum sampling of neighbouring populations.

The virulence of virus strains isolated at Lake Urana

The virulent standard laboratory strain of myxoma virus was introduced artificially at Lake Urana in September–October 1951, and re-introduced in October 1952. It was noted that natural cases of the disease were abundant at the time of the second introduction of virus, and it is unlikely that virus introduced at this late stage contributed substantially to the epizootic. The virus strains recovered from mosquitoes were therefore probably representative of the strains which had survived at a low level of activity through the winter and spring of 1952, and caused the epizootic in November–December. The virulence of eight of the virus strains recovered from Lake Urana was tested, with the results recorded in Table 4.

None of them killed the inoculated animals as rapidly and regularly as did the standard laboratory strain of the virus. If the irregular results due to non-specific deaths are excluded (as they have been in Table 4) the survival times of wild rabbits agree reasonably well with those of the laboratory rabbits. As might be expected, the slight attenuation of the virus permitted the expression of differences in the innate resistance of the hosts which were obscured by the the high virulence of the standard laboratory strain of virus, and there was a good

Table 4. *The virulence of strains of myxoma virus recovered from mosquitoes caught at Lake Urana in November–December* 1952, *and at Corowa in December* 1952

Virus strain				Survival time (days) of rabbits inoculated with about $10ID_{50}$ of virus	
Place	Date	Mosquito Pool	Key no.	Laboratory rabbits	Wild rabbits
Lake Urana	20 Nov.	*Anopheles annulipes*	K.M.1	15, 16, 17, 20, 21, 23	16, 25
	25 Nov.	*A. annulipes*	K.M.2	18, 20, 20, 21, 21	25
	25 Nov.	*A. annulipes*	K.M.4	13, 17, 17, 20, 25	20
	25 Nov.	*Culex annulirostris*	K.M.5	13, 21, 26, 28, 28, S	14, 24, 27
	25 Nov.	*Aedes theobaldi*	K.M.7	10, 14, 14, 18, 21, 23	14, 17, S
	25 Nov.	*Anopheles annulipes*	K.M.10	12, 13, 15, 19, 19, 27, 34, 45	15, 16, 19
	25 Dec.	*A. annulipes*	K.M.36	15, 19, 20, 22, 22, 28	14, 18
	25 Dec.	*A. annulipes*	K.M.37	13, 13, 15, 16, 18, 20	12, 14
Corowa	16 Dec.	*A. annulipes*	K.M.12	11, 13, 14, 14, 30, S	18, 25
	16 Dec.	*A. annulipes*	K.M.13	18, 18, 22, 23, 37	19, 23, 23, 24
	16 Dec.	*A. annulipes*	K.M.17	10, 10, 12, 13, 18, 19	15, 24
	22 Dec.	*A. annulipes*	K.M.20	15, 19, 20, 29, S	—
Standard laboratory strain				8, 10, 10, 10, 10, 11, 12, 13, 15	11, 12, 12, 12, 12 12, 12, 12

S = survivor.

deal of scatter in the survival times. Only slight differences could be detected between the eight strains of virus recovered at Lake Urana, and all caused a less rapidly progressive disease than the standard laboratory strain, which had been used to initiate the epizootics at Lake Urana in 1951. The failure to recover virus with this degree of virulence in 1952 is further evidence of the unimportance of the second introduction of standard virus in October 1952.

Epizootic history *Albury*

Occasional sick rabbits were observed at this site during the first epizootic in the summer 1950–1, but there was no outbreak at that time.

In October 1951 the simuliid fly *Austrosimulium furiosum* became active throughout the region, but at the Albury site no epizootic developed, although more sick rabbits than usual were seen. In January–February 1952 *Anopheles annulipes* became active in this area and a major epizootic of myxomatosis developed. Regular counting of the rabbit population was not carried out, but sporadic counts indicated that the reduction of population was of the order of 2000 to 40.

During the following winter the population rose by breeding to about 300, and in October 1952 there was again some increase in the numbers of diseased rabbits seen contemporaneously with the obvious activity of *Austrosimulium furiosum*.

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In January 1953 *Anopheles annulipes* became prevalent and an epizootic developed. The population reduction was much less than in the previous year, and about 100 rabbits remained. No regular counts were performed, due to lack of protection of the site from casual shooters and trappers.

Two differences were apparent between the behaviour of myxomatosis at Lake Urana and at Albury, namely the slight increase in the prevalence of the disease in October, associated probably with the activity of *Austrosimulium*, which was captured in large numbers while feeding on sick rabbits, and the later occurrence of the main epizootic associated with the later appearance of large numbers of *Anopheles annulipes*.

Age structure and immune status of the population

As detailed population counts were not available, the history of the rabbit population at the Albury site cannot be reconstructed in detail.

Table 5 shows the changing age structure and immune status of the population. As at Lake Urana, the winter breeding season was reflected by a great increase in the percentage of susceptible young, which constituted 91 % of the population just before the epizootic. Almost all the surviving rabbits tested in March 1953 had recovered from myxomatosis.

Table 5. *Age structure and immune status of rabbits at Albury site, August* 1952 *to March* 1953

(Figures indicate total numbers of rabbits.)

Sample	Old rabbits*		Young rabbits*		Totals	Geometric mean titre of C.F. antibody of immune rabbits
	Immune	Susceptible	Immune	Susceptible		
August 1952	9	37	0	6	52	1/120
September 1952	4	25	0	36	65	—
October 1952	2	10	2	62	76	—
January 1953		5†		48†	53	—
March 1953	13	0	31	3	47	1/240

* Old rabbits = survivors of 1951–2 epizootic. Young rabbits = rabbits born since the 1951–2 epizootic.

† Sera not tested.

Apart from one high titre (1/960) specimen, the titres of the August 1952 sample were much the same at Albury as at Lake Urana, and there was no serological evidence of winter epizootics of myxomatosis. After the summer outbreak in December 1952 the mean antibody titre was much higher, due to the large proportion of recently recovered rabbits.

Case-mortality rates

In the absence of population figures it is not possible to determine the case-mortality rates at Albury, but it was clear from field observations that whereas the kill in the first epizootic (1951–2) was comparable to the very high kill at Lake Urana (> 99 %) there was a distinct reduction in the crude mortality rate in 1952–3

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(approximately 70%). The contribution to this of immune survivors of the first epizootic was small, and as virtually all the survivors of the second epizootic had been infected and recovered it is necessary to seek for factors other than the accumulation of immunes to explain the changed host-parasite relationship. No virus strains were obtained from this site for the comparison of their virulence with that of the standard laboratory strain.

Rutherglen

Epizootic history

This site was included among those regularly examined as offering an example of the behaviour of myxomatosis in rabbit populations of low density in cleared paddocks. Counts of the rabbits were not possible under these circumstances, and changes in the location of sampling areas were necessitated by the activity of some land-owners in eliminating rabbits from their properties.

In the summer months of 1951–2 myxomatosis was widespread, but it was not possible to estimate the mortality rate. In September 1952 samples were taken from two areas: (*a*) a paddock in which the disease had been active during the winter, (*b*) another population in which no such winter outbreak had occurred. The situation of the previous summer was repeated in the summer of 1952–3. Diseased rabbits were present in small numbers right through the summer.

Age structure and immune status of the population

The age structure and immune status of each sample taken is set out in Table 6.

Table 6. *Age structure and immune status of rabbits at Rutherglen site August* 1952 *to March* 1953

(Figures indicate total numbers of rabbits.)

	Old rabbits*		Young rabbits*		
Sample	Immune	Susceptible	Immune	Susceptible	Totals
August 1952	3	13	0	7	23
September 1952 (*a*) †	12	3	3	2	20
September 1952 (*b*)	2	9	0	20	31
October 1952	1	3	1	22	27
March 1953	25	1	25	6	57

* Old rabbits = survivors of 1951–2 epizootic. Young rabbits = rabbits born since the 1951–2 epizootic.

† (*a*) From a paddock where the disease had been active during the winter. (*b*) From a population in which no winter outbreak had occurred.

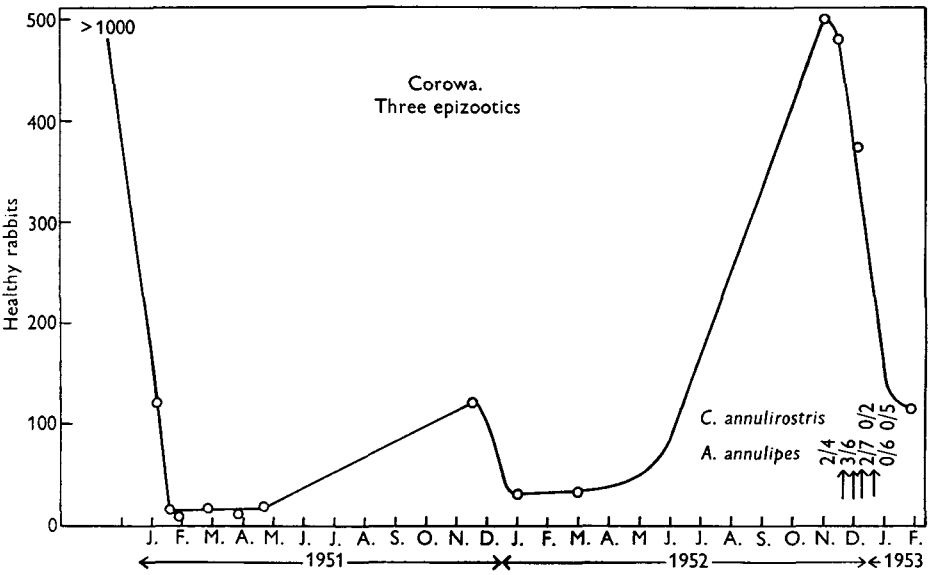
The winter epizootic is well reflected in the immune sample September (*a*) in which the age structure was reversed, compared with a control group September (*b*), and the majority of rabbits, both old and young, were immune. After the long-drawn-out disease incidence of summer 1952–3 most of the survivors showed serological evidence of previous infection. Thus even under conditions in which mosquito vectors might be thought to be at a disadvantage, almost universal infection occurred.

Corowa

Epizootic history

Virus has never been introduced artificially into this area by rabbit inoculation.

When observations commenced here (Myers, 1954) the first great epizootic (1950–1) was waning. Counts of healthy and diseased rabbits were made and are plotted in Text-fig. 4. Judging from the number and size of the warrens the pre-epizootic population must have been very large, certainly more than 1000. The



Text-fig. 4. Population counts of rabbits at Corowa. The arrow indicates the occasions on which virus was isolated from batches of *Anopheles annulipes* and *Culex annulirostris*, and the proportion of isolations to batches tested.

count of the relict population showed an increase due to breeding to 120 in November 1951. A second epizootic occurred in the greatly reduced population, and the count in March 1952 was 30. Winter-spring breeding of this relict population, augmented by an undetermined number of immigrant rabbits, brought the population up to 500 by November, when the third epizootic commenced. Several counts were made, and the pre-epizootic population of 500 was reduced to 114 by February. Owing to interference (trapping and shooting) with the rabbit populations at this site the counts give only a rough impression of rabbit numbers, but this is the only area in which three epizootics have been observed.

Rabbit populations in this area have not been sampled, so that no comment is possible on age-structure or immune status.

Case-mortality rate

Owing to our ignorance of the immune status of the population, case-mortality rates after the third epizootic cannot be determined. There is circumstantial evidence, however, that all rabbits surviving in mid-February were immune, because *A. annulipes* was present in appreciable numbers, and was feeding on

rabbits until the end of February. Virus was recovered from seven out of seventeen pools of *A. annulipes* caught during December.

The survivors of the second epizootic at Lake Urana, Rutherglen and Albury were practically all immune, and it is not unreasonable to suppose that the same situation obtained after the second (1951–2) epizootic at Corowa. All 'old' rabbits present at the beginning of the third epizootic would therefore be immune, and this group would be expected to survive intact, except for incidental deaths from other causes, until after the epizootic. The contributions of this 'old immune' group to the 114 survivors might therefore amount to about 20. The case-mortality rate in the susceptible population would then be about 80 %, somewhat lower than that found after the second epizootic at Lake Urana. The inaccuracy of the figures due to the infrequent counts is such that it is hard to attach much significance to this possible difference in the case-mortality rate.

The virulence of virus strains recovered from mosquitoes

Virus was recovered from pools of *A. annulipes* on seven occasions, the mosquitoes being collected during December 1952. Six pools of *A. annulipes* caught early in January failed to yield virus. The detailed results are shown in Table 7.

Table 7. *Recovery of myxoma virus from batches of mosquitoes collected at Corowa (about fifty mosquitoes per batch)*

Date of collection	Species of mosquito	Result	Totals
6 Dec. 1952	*Anopheles annulipes*	2/4	2/4
16 Dec. 1952	*A. annulipes*	3/6	3/6
22 Dec. 1952	*A. annulipes*	2/7	2/9
	Culex annulirostris	0/2	
2 Jan. 1953	*Anopheles annulipes*	0/6	0/9
	Culex annulirostris	0/3	
Totals	*Anopheles annulipes*	7/23	7/28
	Culex annulirostris	0/5	

Symbols as in Table 3.

The virulence of four of these seven strains was determined, with the results shown in Table 4. As in the case of the eight strains recovered from Lake Urana, all are slightly less virulent than the standard laboratory strain of myxoma virus. They appear to have the same degree of virulence as the Lake Urana samples, although the latter were recovered only 14 months (two epizootics) after the standard laboratory strain of virus was liberated in the neighbourhod, whereas at Corowa the virus had never been artificially introduced, but there had been three epizootics since its first appearance there 24 months before the samples were collected.

The regional situation

The more detailed accounts just given of the epizootics of myxomatosis at Lake Urana, Albury, Rutherglen and Corowa reflect fairly accurately the behaviour of the disease over the whole of the region shown in Text.-fig. 1. General observations

were made over the greater part of this region. Mosquito catches were carried out, and the incidence of epizootics noted, during regular larval survey trips. In Table 8 are set out the results of mosquito catches over the whole of the region (excluding Lake Urana, the results of which are shown separately in Table 2), and the numbers of major local outbreaks occurring in the region each month. The latter figure was based upon the number of such outbreaks observed during each monthly larval survey trip, and is included as a rough index of the disease activity at different times of the year.

Table 8. *The monthly incidence of adult mosquitoes of different species and of major epizootics of myxomatosis in the regional survey*

Month	*Culex annulirostris*	*C. pipiens australicus*	*Anopheles annulipes*	*Aedes alboannulatus*	*A. theobaldi*	*A. sagax*	No. of major outbreaks recorded
1951							
Apr.	3	—	—	178	86	3	5
May	0	—	—	57	23	0	3
June	0	0	0	29	10	0	3
July	0	0	0	46	31	11	0
Aug.	0	0	0	250	272	334	1
Sept.	0	0	7	62	282	131	1
Oct.	0	2	12	7	135	10	8
Nov.	0	57	383	0	25	1	34
Dec.	57	83	276	0	1	0	37
1952							
Jan.	174	78	87	0	0	0	21
Feb.	192	62	100	0	0	0	18
Mar.	120	74	63	0	0	0	5
Apr.	4	32	142	0	0	0	10
May	0	3	20	9	0	0	4

Whereas small differences in the topography, cover, water supply, etc., affected the local epizootic behaviour of myxomatosis, there was an underlying unity in the disease performance over the region as a whole. After the wet winter of 1951 and 1952, the waterlogged countryside produced large populations of mosquitoes in spring and early summer. The usual summer droughts followed, and the country dried up, restricting mosquito activity to a few permanent swamps, the river flats, and the drying creeks in the foothill country. Coinciding with the activity of the summer mosquitoes, myxomatosis flared up over the whole region, then contracted to the swamps and permanent waters as the countryside dried out, and died away with the disappearance of mosquitoes as winter approached.

The relative importance of *A. annulipes* and *Culex annulirostris* as vectors was demonstrated during this regional survey. *C. annulirostris* bred in the permanent swamps and flood water lagoons of the river-flats, and near these restricted habitats it was the most important vector during the hot summer months. *Anopheles annulipes*, a product of the extensive surface waters present on the plains after the heavy winter rains, occurred throughout the region. Wherever virus was present it caused major epizootics of myxomatosis. *A. annulipes* adults were

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active about 1 month earlier than *Culex annulirostris*, and initiated outbreaks even in the restricted habitat range of the latter species before it had appeared in appreciable numbers. During the advanced stages of outbreaks occurring near permanent waters both mosquitoes must have acted as vectors. Away from the rivers and swamps, however, *Anopheles annulipes* acted alone.

Within the region it was possible to define a seasonal 'front' which advanced from the lower northern and western areas to the foothills in the south and east, bringing to life the vector populations as it passed and initiating a wave of disease which advanced with it. This was particularly evident in the summer of 1951–2, when the disease broke out for the first time in dense populations of susceptible rabbits, commencing in October in the far north-west and ending in mid-summer (January–February) in the foothills where a new phase of vector emergence commenced with the drying-up of the spring-fed streams.

Dense populations of *A. annulipes* usually occurred in the same areas as dense rabbit populations, both finding shelter and food in the same type of habitat, such as stands of timber bordering swamps.

DISCUSSION

The major point of interest arising in the observations just described is the changed case-mortality rate occurring after the initial very destructive epizootics. Calculation of the crude case-mortality rate has been based on population counts. These are subject to large errors, which were discussed in some detail previously (Myers, 1954). It was pointed out then, however, that the errors in the post-epizootic counts, which are the more important from the point of view of case-mortality rates, were not large enough to invalidate the conclusion reached about a change in the case-mortality rate. In the same way, any errors due to failure of the rabbit population to return to normal social conditions following the trapping episode at Lake Urana would diminish rather than exaggerate the observed change in the case-mortality rate. The observations that there were many more chronic cases in the later epizootics, and that all field strains of the virus were slightly attenuated, support our confidence in the case-mortality rates estimated by the population counts.

The regional study showed that over the whole of that part of south-eastern Australia, myxomatosis had by March 1953 reduced the rabbit population to an estimated 10–20 % of the 1950 figure. Nevertheless, when more detailed examinations of populations were made, as at Lake Urana, Corowa and Albury, it was apparent that the case-mortality rate of the disease during the 1952–3 epizootic was distinctly lower than it was in previous outbreaks. Possible explanations of this changed case-mortality rate have been discussed elsewhere (Fenner, 1953). The only factor for which positive evidence has been obtained is the changed virulence of the virus. Immunological mechanisms, either the accumulation of old immunes or the immunization of young rabbits while they were partly protected by maternal antibody, have been excluded as major factors at Lake Urana, and there is no evidence that environmental factors (excessive heat or concurrent infection with Murray Valley Encephalitis virus) had any effect. An assessment of the innate

resistance of the susceptible rabbit populations is being carried out at the present time and will be reported in a later paper.

The cost and availability of experimental rabbits necessarily limited the number which could be used for comparison of virulence of field strains, but within these limitations there appeared to be only minor differences between the strains isolated from Lake Urana and Corowa. This conclusion was borne out by daily observations of the course of the disease in the inoculated rabbits. The field strains tested were certainly not as virulent as the standard laboratory strain of virus and were much more virulent than neuromyxoma, and somewhat more virulent than the Uriarra strain described by Mykytowycz (1953). Some data are available on the mortality rates of rabbits inoculated with these field strains: there were three recoveries out of 70 laboratory rabbits and one out of 25 wild rabbits.

In addition, strain KM 13 (from Corowa) had been used in experiments designed to assess the innate susceptibility of rabbits from areas with different epizootic histories of myxomatosis. So far there have been seven recoveries out of 45 susceptible young adult wild rabbits tested. As the mean survival times and the number of recoveries appear to be independent of the origin of these rabbits, this figure may be taken as a measure of the average survival times of fatal cases (26 ± 10 days) and case-mortality rate (86%) among susceptible wild rabbits infected with this field strain and maintained under laboratory conditions which of course permitted the survival of some rabbits which would certainly have died under natural conditions. This may be compared with a mortality rate of 100% and average survival time of 11 ± 2 days observed when another group of 25 susceptible young adult wild rabbits from the Albury site were challenged with the standard laboratory strain of the virus.

It is of interest that field strains of virus showing a similar or greater degree of attenuation were also recovered during the summer of 1952–3 from several other parts of Australia (northern New South Wales—Marshall, Dyce, Poole & Fenner, 1955; Canberra—Mykytowycz, 1953), in fact from all areas in which suitable virus samples were obtained (Fenner & Marshall, in preparation). Tests with one such slightly attenuated strain showed that it attained titres in the rabbit skin comparable to those attained by the highly virulent standard laboratory strain of virus, and was equally well transmitted by mosquitoes (Day & Mykytowycz, unpublished observations). The longer survival of infected rabbits as sources of virus available to the insect vectors explains the replacement of the highly virulent laboratory strain by these somewhat attenuated variants. It is possible, indeed, that these strains represent a more stable form of the virus than the standard laboratory strain which might then be considered a hypervirulent laboratory variant.

The similar degree of attenuation of the four Corowa strains (from an area in which virus had never been artificially introduced, but where there had been major epizootics in 1950–1, 1951–2, and 1952–3) and the eight strains from Lake Urana (where there was no outbreak in 1950–1 and standard virus was artificially introduced in October 1951, and again, in the presence of naturally occurring cases, in October 1952), is suggestive evidence of the stability of a variant with this degree of attenuation under field conditions in Australia.

The entomological data indicate the outstanding importance of *A. annulipes*, and to a lesser extent *Culex annulirostris*, as vectors of myxomatosis in this area. Since transmission of myxomatosis is mechanical in nature (Fenner, Day & Woodroofe, 1952) this can only be the result of the feeding habits of these mosquitoes. In restricted areas close to permanent water *C. annulirostris* was seen to feed at night on the rabbits which frequent the same areas, and is undoubtedly a vector of some importance close to water and during the hot summer months. The results of blood meal tests which showed that practically all engorged *Anopheles annulipes* caught in rabbit burrows had fed on rabbits, and the observation that the preferred resting place of *A. annulipes* was in rabbit burrows, explain its efficiency as a vector. This is further borne out by the failure of myxomatosis to occur at Lake Urana during November–December 1953. The previous winter was very dry and there was a widespread failure of breeding of *A. annulipes* during the early summer. In February 1954, however, the disease broke out again in areas adjacent to permanent water, and *Culex annulirostris* appeared to be much the most important vector species.

It is of interest that during the second epizootic at Lake Urana (November–December 1952), the incidence of *Anopheles annulipes* actually reached a maximum after the epizootic had declined. All engorged specimens of this mosquito taken from rabbit burrows on 21 December and 7 January had fed on rabbits, and virus was isolated from three out of twelve batches of *A. annulipes* collected on 25 December, after the epizootic was over. The cessation of the outbreak was obviously not due to a decline in vector activity, and serological tests showed that the whole of the remaining rabbit population consisted of animals which had recovered from the disease. At Corowa no serological surveys were undertaken but the vector picture was similar. Eighty-four out of 90 engorged *A. annulipes* caught in rabbit burrows on 19 February had recently fed on rabbits, although the epizootic was over at this time.

The observations at Lake Urana provided the first indication that *A. annulipes* might not merely be using rabbit burrows as convenient and suitable daytime resting places, but might actually feed underground on the rabbits lying up in the warrens during the day. This possibility was suspected when it was observed that the epizootic rose to a peak in November 1952, despite a long succession of cold and windy nights which reduced outdoor mosquito activity to a minimum. Later, in another locality, the large numbers of anophelines caught as they issued from the burrow mouths at dusk were frequently found to include individuals with such fresh blood in the gut that it seemed safe to conclude that they had fed less than 12 hr. previously. Other observations are available which provide indirect evidence that this mosquito may have acquired the habit of taking its blood meals underground; and the possibility is of such interest and epidemiological importance that it is being investigated in greater detail.

At Albury epidemiological evidence suggested that in 1951–2 and 1952–3 *Austrosimulium furiosum* was responsible for a somewhat early increase in the disease incidence.

SUMMARY

Field observations on myxomatosis in wild rabbit populations of the Riverine Plain of south-eastern Australia, extending between August 1951 and March 1953, were reported. General observations on vector abundance and disease incidence were made over a large area. Detailed analysis of the population counts, age structure and immune status of the rabbit population, and the occurrence of insect vectors, were made at one site, Lake Urana.

The case-mortality rate in the first epizootic was in the neighbourhood of 99·8%, whereas in the second epizootic it was about 90%, and many more chronically sick rabbits were observed in the later outbreaks. The virulence of eight strains of virus recovered from mosquitoes at Lake Urana in December 1952 and four strains recovered at Corowa at the same time was found to be somewhat reduced when they were compared with the original standard laboratory strain of virus. It is thought that this attenuation is the major reason for the observed change in the case-mortality rates.

The mosquitoes, *Anopheles annulipes* and *Culex annulirostris* were by far the most important insect vectors of myxomatosis in this region.

Thanks are due to Messrs F. N. Ratcliffe, B. V. Fennessy, and H. J. Frith of the Wildlife Survey Section of C.S.I.R.O. for helpful discussion and assistance during the field work and in the preparation of the paper. The technical assistance in the field of Messrs C. S. Hale and K. L. S. Harley is gratefully acknowledged, as is the generous help afforded by the landholders of Lake Urana, especially Mr J. Muldoon, and by Mr W. Quirk, rabbit inspector.

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ERRATUM

In the first paper of this series, Vol. **51**, no. 2, p. 225, the word 'wild' in the twelfth line should have been omitted; the sentence should read: 'Working with English rabbits, Martin (1936) recorded ten recoveries out of 312 rabbits infected by contact....'

EXPLANATION OF PLATES 12 AND 13

Plate 12

Fig. 1. Lake Urana. Sample area I, part of the sandy rim of the lake, where population counts were made. (Photo K. Harley, December 1951.)
Fig. 2. Lake Urana. View from sample area II looking north across the lake. (Photo K. M. December 1952.)

Plate 13

Fig. 1. Albury. Rabbit warrens are situated in the eroded gullies and along the valley floor. Mosquito breeding (*Anopheles annulipes*) occurs in the spring-fed pools which constitute the only surface water in the area after the summer has set in. (Photo K. M.)
Fig. 2. Corowa. The rabbit burrows were situated in the flood bank on the left, the rabbits fed on the river flats, and mosquito breeding (*Culex annulirostris* and *Anopheles annulipes*) occurred in the numerous lagoons. (Photo K. M.)

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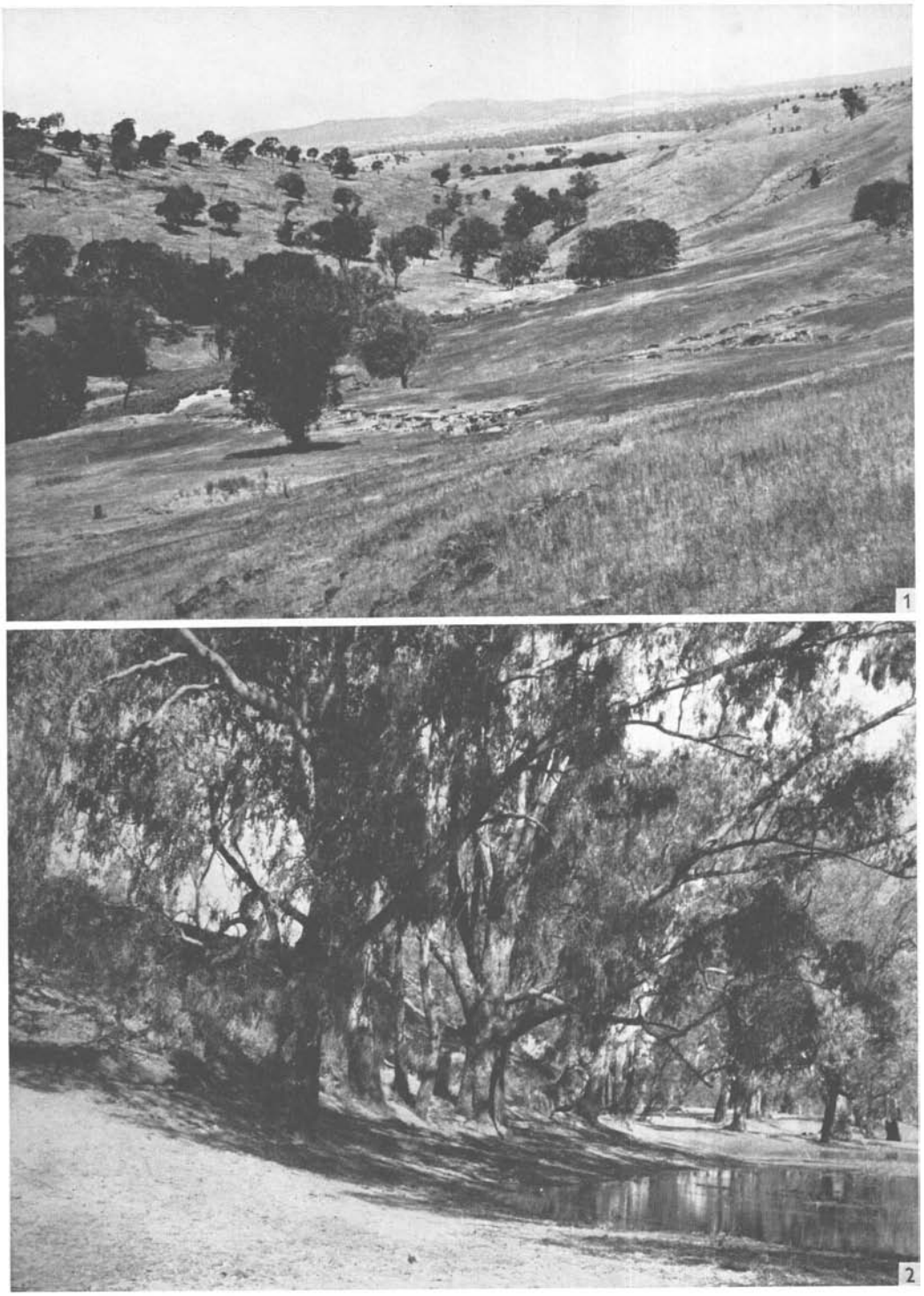

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