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Myxomatosis: the Effects of Annual Introductions of an Immunizing Strain and a Highly Virulent Strain of Myxoma Virus into Rabbit Populations at Urana, N.S.W.

I. Parer, Dorothy Conolly and W. R. Sobey

Division of Wildlife and Rangelands Research, CSIRO, P.O. Box 84, Lyneham, A.C.T. 2602.

Abstract

A very attenuated strain of myxomatosis was introduced annually into two rabbit populations from 1978 to 1980, to protect susceptible rabbits from the effects of the field strains of myxomatosis. Within 2 years one population had increased by a factor of eight and the other by a factor of 12. The results indicate that myxomatosis is still an important factor in suppressing those populations of rabbits. After 4 years of introducing a highly virulent strain of myxomatosis into two populations the number of adult rabbits had not declined.

Introduction

After the successful establishment of myxomatosis into Australia in 1950, there was a rapid attenuation in the virulence of viruses circulating in the field (field strains) and an increase in the innate resistance of rabbits to myxomatosis (Fenner and Ratcliffe 1965). As predicted by Rendel (1971), there is evidence that field strains prevalent in areas with highly resistant rabbits are more virulent than those from areas with less resistant rabbits (Edmonds *et al.* 1975; Sobey and Conolly, unpublished). If this is so then myxomatosis may be killing a similar proportion of challenged rabbits throughout its range in Australia.

The relatively precise measurements of mortality rates due to myxomatosis in enclosures or animal houses can give only an indication of what is happening in the field. Field estimates of the mortality due to myxomatosis are imprecise because of the confounding effects of other mortality factors (Parer 1977; Shepherd *et al.* 1978). Even accurate estimates of high mortality rates in the field do not demonstrate that a particular agent of mortality is necessarily a major factor in regulating a population of animals (Varley *et al.* 1973). One way of estimating the extent of the effect of a mortality factor is to measure the change in the population when that mortality factor is eliminated. A method of minimizing the effects of field strains of myxomatosis is to immunize the susceptible rabbits in a population, before the local field strains become active in the area, with a strain sufficiently attenuated to be regarded as an immunizing virus.

As soon as it was realised that the virulence of field strains was declining in Australia (Mykytowycz 1953), the introduction of highly virulent strains into the field became an attractive proposition. State authorities in New South Wales and Victoria have provided highly virulent strains to farmers for many years but although there have been several experimental introductions the usefulness of this practice has not been demonstrated (Fenner *et al.* 1957; Sobey and Conolly 1971; Shepherd and Edmonds 1977). Parer *et al.* (1981) demonstrated that fleas, *Spilopsyllus cuniculi*, coated with a virus suspension (inoculated fleas) were an effective means of introducing a virulent virus into a free-living population of rabbits.

Our objectives in this study were to test the usefulness of annual introductions of a highly virulent virus (Lausanne) as a method of rabbit control, and also to observe the consequences of minimizing the effects of field strains of myxomatosis by annual introductions of an immunizing

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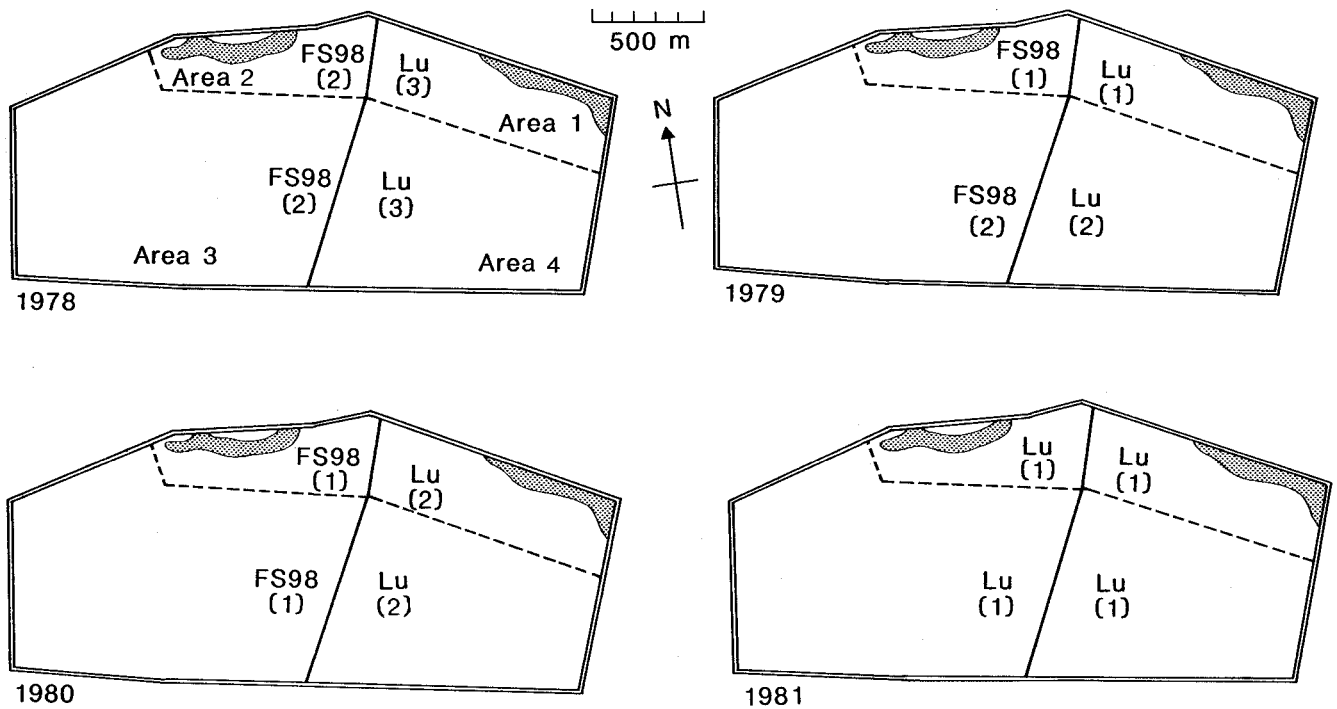


Fig. 1. Maps of the study site showing the double rabbit-proof fence (two unbroken lines), the single rabbit-proof fence (single unbroken line) and the sandhills (hatched). The strain of myxoma virus introduced into each area in each year is indicated (Lu, Lausanne; FS98, field strain 98); the number of occasions that the strain was introduced in a year is shown in parentheses.

virus. Both experiments required the populations to be infested with the European rabbit flea, *S. cuniculi*, which had been introduced onto the study site in 1968 and was well established by 1970 (Williams and Parer 1971).

Materials and Methods

A full description of the study area has been given by Parer (1977). The site is situated on the edge of Lake Urana, 11 km west of the township of Urana. Two rabbit-proof fences, 10 m apart, surrounded the 280-ha site, and since 1977 a single rabbit-proof fence has separated areas 1 and 4 from areas 2 and 3 (Fig. 1). The greatest concentrations of warrens were on the sandhills on areas 1 and 2. In August 1978 on areas 1 (37 ha), 2 (23 ha), 3 (138 ha) and 4 (82 ha) there were 68, 30, 24 and 30 warrens respectively. At the commencement of the study in March 1978 areas 1 and 2 had low- to medium-density populations (1–2 rabbits ha^{-1}) on predominantly sandy soils, and areas 3 and 4 had low-density populations (0·2 rabbits ha^{-1}) on a clay loam.

At the commencement of the study in March 1978 over 95% of the adult rabbits carried ear-tags, because the site had been used for previous studies (Daly 1979; Parer *et al.* 1981). Live-traps, baited with oats, were set on warrens for four nights every two weeks from August to January inclusive in the years 1978–81. At other times of the year traps were set four nights a month. Traps were inspected in the morning and in the afternoon. The traps were covered with the nylon fabric from wool packs to protect the rabbits from direct insolation and from wind. On areas 1, 2, 3 and 4 there were 90, 64, 82 and 41 traps respectively. The number of rabbits was determined by enumeration and thus it represents the minimum number known to have been alive at any given time. Rabbits were not weighed, because we wished to minimize the possibility of transporting infective fleas from one warren to another on clothing or on weighing bags. On their initial capture rabbits were tagged and their weight estimated. The age of a rabbit was calculated from this estimated weight on the basis of a growth rate of 10 g day^{-1} (Parer 1977; Daly 1979; Wheeler and King 1980). As the rabbit breeding season may commence in March, the number of rabbits known to have been alive on 1 March was used as an index of the potential breeding population. Counts were made of the number of active warren entrances in February, March or April from 1978 to 1982.

Viruses

The Lausanne strain (Lu) of myxoma virus (strain No. 7; Fenner and Marshall 1957) was chosen as the highly virulent virus because the symptoms it causes differ from those resulting from infection with any of the Australian field strains and are easily recognized. Experimental infections with Lu of rabbits bred from Urana stock produced a mortality rate of 100% in winter and 90% in summer in outdoor enclosures (Sobey, unpublished data). In 1978 and 1979 the Lausanne preparation was made in our laboratory from scarified skin material as described by Sobey and Conolly (1975). The commercial preparation of the Lausanne strain used in 1980 and 1981 was obtained from the Commonwealth Serum Laboratories (CSL) in Melbourne, Vic.

Field strain 98 (FS98), an attenuated field strain, was collected at Canowindra, N.S.W., in 1967. Very few rabbits of Urana stock were killed when infected with FS98 in Canberra (Sobey *et al.* 1983). A few rabbits showed severe symptoms in enclosures, and under field conditions may have been susceptible to predation by cats, *Felis catus*, or foxes, *Vulpes vulpes*.

Viral Introductions

European rabbit fleas, coated with a concentrated suspension of myxoma virus (Sobey and Conolly 1975), were used to introduce Lu and FS98 into the rabbit populations in 1978, 1979 and 1980 (Fig. 1). Because the CSL preparation used in 1980 had a much lower titre than our preparation, a more viscous suspension than usual was made in an attempt to increase the virus concentration in the suspension. The fleas were placed about 0·5 m down the entrance of a burrow where they should have been easily able to find a host (Mead-Briggs 1964). Details of the number of fleas used and the number and timing of the introductions are shown in Table 1.

Each year fleas, inoculated with Lu, were selected at random from the fleas to be released on the study site and were allowed to feed on marked positions on the back of a laboratory rabbit. The number of lesions 4 days later compared with the number of feeding sites gave a measure of the infectiousness of the inoculated fleas. In 1978 and in 1979, 20–25% of the fleas were infectious. In 1980 half of the fleas died before testing, possibly due to the viscosity of the suspension, and only one of 37 feeding sites produced a lesion. It is possible that we released no more than 10 fleas capable of producing an infection in 1980 compared with about 180 and 230 in 1978 and 1979 respectively.

In 1978, fleas were put out on a high proportion of the warrens on several occasions, to ensure a well distributed number of infections in time and space. In 1979, only one release was planned but, due to the

apparent failure of the first release on areas 4 and 3, another release was made on each of these areas. In 1980, we wanted to be certain of infecting rabbits on three large warrens on area 1 and five large warrens on area 4, so 100 inoculated fleas were introduced into each of these eight warrens. It was hoped that we could trace the spread of Lu from these warrens. As this first introduction of Lu in 1980 was almost a complete failure, nine rabbits on area 1 and three rabbits on area 4 were injected intradermally with Lu 4 weeks after the initial introduction.

By 1981, the rabbit populations on areas 2 and 3 were very high, and Lu was introduced into all four areas to compare its effect on high- and low-density populations. The CSL preparation of Lu was introduced by intradermal injection of young rabbits on all four areas.

Some of the rabbits trapped in January–March of each year were bled from the marginal ear vein, and the blood, collected on filter paper, was tested for antibody to the 'd' soluble antigen by the method of Sobey *et al.* (1966).

In September 1982, a year after the final introductions of Lu onto the study site, 198 young rabbits from areas 1 and 4, from areas 2 and 3, and from an adjacent property were transported from Urana to Canberra. By means of the techniques described by Sobey *et al.* (1983) the rabbits were challenged in outdoor enclosures with either FS 634 or FS 638 (CSIRO accession numbers for field strains of myxomatosis).

Table 1. Timing of the releases of inoculated fleas and the injections of rabbits

Under 'No. of fleas per warren': (*a*) indicates our preparation of Lausanne virus; (*b*) CSL Lausanne; (*c*) field strain 98. 'Injection' indicates that one rabbit on each warren was injected

Year	Introduction type	Week of year	No. of warrens treated				No. of fleas per warren	
			Area 1	Area 2	Area 3	Area 4	Areas 1 and 4	Areas 2 and 3
1978	Fleas	35	17	9	18	17	25(*a*)	25(*c*)
	Fleas	39	19	5	17	19	25(*a*)	25(*c*)
	Fleas	41	2	9	—	—	50(*a*)	—
1979	Fleas	38	12	7	13	9	50(*a*)	50(*c*)
	Fleas	42	—	—	—	19	—	50(*c*)
	Fleas	44	—	10	—	—	50(*a*)	—
1980	Fleas	39	3	5	13	8	100(*b*)	50(*c*)
	Injection	43	9	3	—	—	—(*b*)	—
1981	Injection	37	8	5	8	7	—(*b*)	—(*b*)

Identification of Viruses

As there is no immunological technique available to classify the various strains of myxoma virus, the following criteria were used to differentiate the strains on the basis of the observed symptoms.

Lausanne (Lu). Lausanne is characterized by large hemispherical swellings above the eyes and at the ear base (Fenner and Ratcliffe 1965). The eyes were generally closed in animals with advanced symptoms and at this stage the rabbit was most unlikely to be trapped again. The rabbits usually died before becoming emaciated.

Lausanne type (LuT). Tumours were generally smaller than in classical Lu, often of irregular shape, and their occurrence on ears and eyes variable. Sometimes only a single small discrete swelling was found at the base of an ear or on a toe. When rabbits were recovering the lesions could become encrusted and they could remain obvious for up to 12 weeks. Lu and LuT symptoms may be caused by the same virus strain whose expression has been modified by environmental conditions; on two different occasions when LuT cases were sampled and tested in Canberra they could not be differentiated from Lu. However, this does not rule out some LuT being mutants, recombinants or mixtures.

Local field strains (LFS). Early symptoms were a thickening of the eyelids and the perineum becoming swollen and red. As the disease advanced the eyes often exuded pus and were partly closed. The eyes eventually closed, at which stage the animal was emaciated.

Field Strain 98 (FS98). In most cases there were mildly swollen eyelids. A range of symptoms from tiny tumors on the eyelids to general involvement similar to the LFS has been observed in experimental infections in enclosures.

Unidentified Strains. Symptoms that could not be classified were designated as being caused by an unidentified virus. Although misclassification certainly occurred, the probability was lessened because there were usually only one or two myxoma strains in an area at any one time.

Results

Success of the First Introductions in Each Year

An introduction of myxomatosis onto a warren was regarded as a definite success if a primary infection was seen within 16 days of an inoculated flea introduction (1978–80) or within 30 days of a rabbit being injected (1981). Injected rabbits seen with symptoms were not regarded as primary infections. A longer period was allowed for primary infections after injection because insect vectors feeding on injected rabbits would not have become infectious until 9–14 days after the rabbit was injected. An introduction onto a warren was regarded as a probable success if most of the susceptible young rabbits on a warren disappeared from the trapping record around the time of the introduction of the virus.

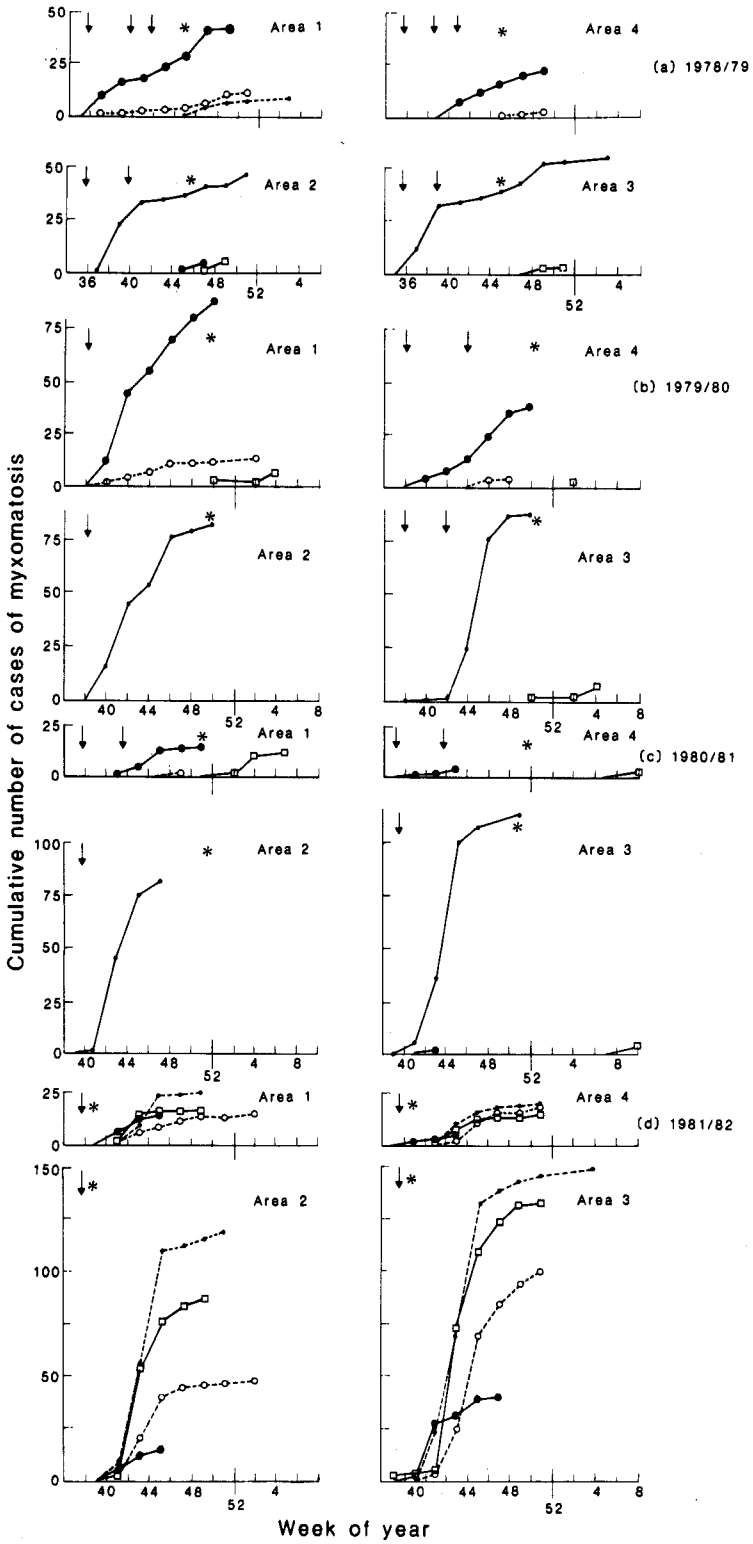
Table 2. Number of primary infections and the number of warrens onto which Lausanne or Field Strain 98 was successfully introduced in each year from 1978 to 1981

Year	Area	Virus	Treated warrens				Primary infections	
			Total	With no susceptible rabbits	Successful introduction	Probably successful	On treated warrens	On untreated warrens
1978	1	Lu	17	6	4	4	7	3
	4	Lu	9	4	0	6	0	0
	2	FS98	18	4	2	—	2	0
	3	FS98	17	0	5	—	13	0
1979	1	Lu	12	1	4	3	9	6
	4	Lu	7	0	1	4	2	2
	2	FS98	13	0	9	—	15	2
	3	FS98	9	0	1	—	1	0
1980	1	Lu	3	0	0	2	0	0
	4	Lu	5	0	1	0	1	0
	2	FS98	13	0	2	—	2	0
	3	FS98	8	0	4	—	6	0
1981	1	Lu	8	0	4	3	4	3
	4	Lu	5	0	1	1	1	1
	2	Lu	8	0	4	1	4	2
	3	Lu	7	0	4	3	27	2

(i) *Lausanne*

Lausanne was successfully introduced into 24% (4 of 17) of the treated warrens in area 1 in 1978 and into 33% (4 of 12) in 1979 (Table 2). Introductions were apparently successful on 47% (8 of 17) and 57% (7 of 12) of the treated warrens in area 1 in 1978 and 1979 respectively. The success rate was lower in 1978 because of the larger number of treated warrens with no susceptible rabbits at the time of treatment in 1978 (Table 2). In area 4, apparently successful introductions were observed on 67% (6 of 9) and 71% (5 of 7) of the treated warrens in 1978 and 1979 respectively. In 1981, 100% (7 of 7) of the introductions into area 3 and 40% (2 of 5) of the introductions into area 4 were apparently successful—area 1 (87·5%) and area 2 (62·5%) had intermediate values.

Of the 39 rabbits seen with primary Lu infections over the four years, 31 were 5 weeks of age or older at the time of infection. Many of the kittens in this age class disappeared at about the time of the Lu introductions and some of the deaths may have been due to unobserved primary infections. Very young rabbits, less than 5 weeks of age, were unlikely


Area 1
Area 4
(a) 1978/79
Area 2
Area 3
Area 1
Area 4
(b) 1979/80
Area 2
Area 3
Area 1
Area 4
(c) 1980/81
Area 2
Area 3
Area 1
Area 4
(d) 1981/82
Area 2
Area 3
Cumulative number of cases of myxomatosis
Week of year

to come into contact with inoculated fleas, because kittens use a restricted number of warren entrances.

Four of the 12 rabbits injected with Lu in 1980 were subsequently trapped; three showed symptoms. One of the 28 rabbits injected with Lu in 1981 was trapped again and it showed symptoms of Lu.

(ii) *Field Strain 98*

In 1978 seven of the 35 introductions onto warrens in areas 2 and 3 were successful. The success rate was higher in 1979 in area 2 (9 of 13) than in area 3 (1 of 9) but in 1980 the success rate was higher in area 3 (4 of 8) than in area 2 (2 of 13).

Introductions of both FS98 and Lu were more successful on warrens with three or more susceptible rabbits than on warrens with one or two susceptible rabbits. If the number of kittens on a warren is low relative to the number of adults, then inoculated fleas may lose their virus load by feeding on the, more attractive, immune adults.

Rate of Transmission of the Introduced Strains

(i) *1978–79*

After the introduction of Lu into area 1 in week 35 of 1978, there was a steady increase in the cumulative number of cases of Lu until week 45 when there was a sudden increase. There was a similar increase in FS98 cases at this time in areas 2 and 3 (Fig. 2). These increases coincided with the first observations of the mosquito *Anopheles annulipes*, which is a very effective vector of myxomatosis (Fenner and Ratcliffe 1965). Only rabbits seen in the active stages of the disease are included in Fig. 2. Rabbits first seen as recoveries were not used in Fig. 2, because scars on recovered rabbits may be obvious for up to 3 months and their inclusion would not give an accurate picture of the time when the various strains were spreading through the population.

In week 45 the first observations of symptoms of the unidentified strain or strains of virus were made. The unidentified cases could have been due to one of the introduced viruses, a mixture of viruses, or to the local field strain which was first identified in area 1 in week 3 of 1979 but was present in area 2 in week 47 of 1978. Forty-two rabbits were seen in area 1 with Lu in the active stages, and two of these recovered. Of the 14 rabbits seen with symptoms of active LuT virus, four recovered, and 11 rabbits were first seen as recoveries to LuT. In area 4 the first case of Lu was seen in week 41 and the last case in week 49.

In areas 2 and 3 the cumulative number of rabbits seen with FS98 increased rapidly from week 37 to week 41, and then the increase was less rapid until the last cases were seen in week 51 (1978) in area 2 and week 4 (1979) in area 3. Three cases of Lu were seen in area 2 in weeks 45–47. The virus may have been carried there by *A. annulipes*, which was first seen at that time.

(ii) *1979–80*

After the introduction of Lu into areas 1 and 4 and FS98 into area 2 in week 38, the cumulative number of cases of Lu in areas 1 and 4 and FS98 in area 2 increased until week 50. The introduction of FS98 into area 3 in week 38 was not very effective but that in week 42 was highly successful. Rabbits with the local field strain were first seen on the site in week 50; this coincided with the first observations of *A. annulipes*. Lausanne was seen by week 42 on only one-third of the warrens in area 1; mainly those at the eastern end. Five warrens were so successfully seeded that almost all rabbits born in the vicinity up to week 38 had disappeared. Warrens towards the west became progressively infected during weeks 42–48, and then in week

Fig. 2. Cumulative number of observed infections with the different strains of the virus. Virus strains: ●——● Lausanne; ○--○ Lausanne type; □——□ local field strain; ●——● field strain 98; ●--● unidentified strain. Arrows indicate introductions of Lu or FS98; * *A. annulipes* first seen on the study site.

51 symptoms of Lu were seen again on the eastern warrens coincident with the arrival of *A. annulipes*.

In week 38 the numbers of susceptible rabbits known to have been alive in areas 1, 2 and 3 were 186, 192 and 206 respectively, yet the number of rabbits seen with Lu in the active stages was 88 in area 1 and the numbers seen with FS98 in the active stages in areas 2 and 3 were 83 and 87 respectively. If rabbits first seen as recoveries are included, then the total numbers of cases of Lu and LuT were 89 and 25 respectively compared with 132 and 165 cases of FS98 in areas 2 and 3 respectively. This is surprising, as Lu kills quickly and, given a similar number of susceptible rabbits on the three areas, the number of observed cases of Lu should have been much fewer than those of FS98. To account for the large number of Lu cases it seems probable that the duration of Lu symptoms was extended as compared with that found in small enclosures in Canberra.

(iii) *1980–81*

The introductions of Lu by fleas in week 39 and by injection in week 43 were not very successful; only 15 cases of Lu were seen in area 1 and 4 cases in area 4. Mosquitoes (*A. annulipes*) were first noticed in week 51 (1980) and the first cases of the local field strain were seen in week 2 (1981). Only 17 cases of the LFS were seen in areas 1 and 4. However, many cases would have been missed because, after December, visits were made monthly instead of the fortnightly routine used during the breeding season. The last active cases of LFS were seen in week 10 of 1981. The ages of rabbits exposed to the LFS were largely between 4 and 6 months and rabbits in this age class are less trappable than younger rabbits.

After the introduction of FS98 into areas 2 and 3 in week 39 the attenuated strain spread rapidly through the populations, 89% (74 of 83) and 83% (94 of 113) of all new cases being seen in weeks 43–45. In week 43 one case of Lu was seen in area 3 on a warren where a fox had a litter of cubs. The adult foxes could have brought an infected rabbit with its infective fleas from areas 1 or 4. Another possibility is that the infective fleas were transported on the adult foxes.

(iv) *1981–82*

Lausanne was introduced into all four areas in week 37. The local field strain was first seen in area 3 in week 37 and *A. annulipes* in week 39. The local field strain and Lu were spreading through the populations at the same time, and they appeared to be recombining or infecting simultaneously because many rabbits were seen with unusual symptoms. Because of this a large number of cases were classified as being caused by an unidentified strain. In all areas the greatest numbers of new cases caused by Lu, LuT, the local field strain and the unidentified strain were in weeks 43–45. No new cases of Lu were seen in areas 1, 2 and 4 after week 45. In area 1 the number of cases of Lu, LuT and field strain were about the same. In areas 2 and 3 the unidentified strain and the local field strain predominated.

Patchy Survival Patterns within Areas

Of the young born in 1978 in area 1 that survived to March 1979, 83% were tagged on six warrens which produced 20% of the young. Four of these warrens were located on the eastern edge of the area. Survivors from the 1979 breeding season were also concentrated on the eastern edge of area 1; five warrens produced 18% of the young and 59% of the survivors. In area 4 three warrens produced 57% of the survivors and 15% of the young in 1978.

Survival rates on warrens varied considerably within areas and even between adjacent warrens. In many cases these variations may have represented chance events because of the small number of animals involved. Each of the five most western warrens (A, B, C, D and E) in area 3 produced many kittens in 1981, and the survival rates of young to March differed markedly between the warrens. The two warrens (B and E) with high survival rates had mainly LuT seen on them, whilst the three warrens with low survival rates either had many Lu cases seen on them or else LFS was the predominant strain (Table 3).

One of the many causes of patchy survival rates in 1981 was the injection of rabbits on some warrens (e.g. A and D above) with Lu. The survival rate on warrens where rabbits were injected with Lu was lower (7%) than on warrens where they were not injected (14%).

Table 3. Survival rates of kittens in five warrens in relation to strain of myxomatosis virus

	Warren A	Warren B	Warren C	Warren D	Warren E
Young tagged	208	71	99	127	36
Survival (%)	2·4	32·4	7·1	8·7	25·0
Lu cases	15	2	13	0	0
LuT cases	16	11	16	9	3
LFS cases	25	4	6	21	0
Unidentified	26	12	8	19	8

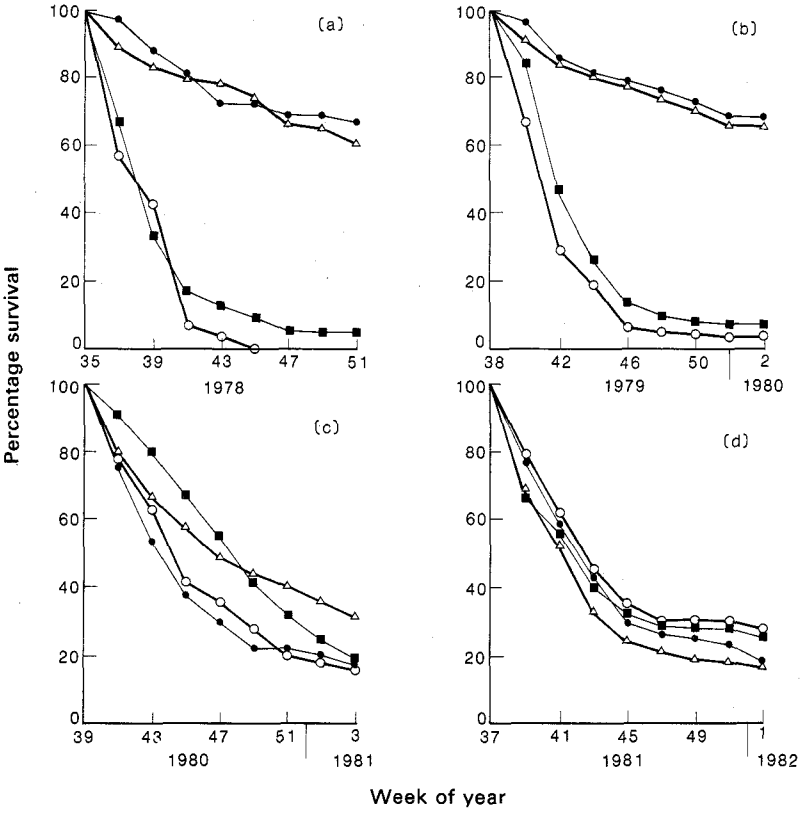


Fig. 3. Percentage survival of susceptible rabbits which were emerged and alive at the time of the introduction of Lausanne or field strain 98: (*a*) 1978; (*b*) 1979; (*c*) 1980; (*d*) 1981. ■——■ area 1; ●——● area 2; △——△ area 3; ○——○ area 4.

There was remarkably little variation in the percentage survival of susceptible rabbits between warrens in the FS98 treatment area in 1978 and 1979. Only one of the 14 warrens with more than 15 offspring in 1978 had a survival rate of less than 20%, and none of the 22 warrens in 1979. In the drought of 1980 some warrens were obviously more affected than others and survival rates were more variable.

Survival of Susceptible Rabbits

In 1978 and 1979 the survival rate of young rabbits which had emerged and were alive at the time of virus introductions was much higher in the areas treated with FS98 than in those treated with Lu, and the survival rates in the replicate areas was very similar (Figs 3*a*, 3*b*). In area 4 in 1978 all of the rabbits which were emerged and alive on week 35 had disappeared by week 45.

In 1980 the disappearance rates of young rabbits in areas 2 and 4 were very similar (Fig. 3*c*). The survival rate was initially better in area 1 but by week 3 (1981) the percentage survival in area 1 was the same as in areas 2 and 4. Only limited growth of pasture occurred in the spring of 1980 because of low winter and spring rainfall. The pasture hayed off during weeks 41 and 42—a month earlier than is usual for the area. Area 2 was overgrazed and lacked high-quality

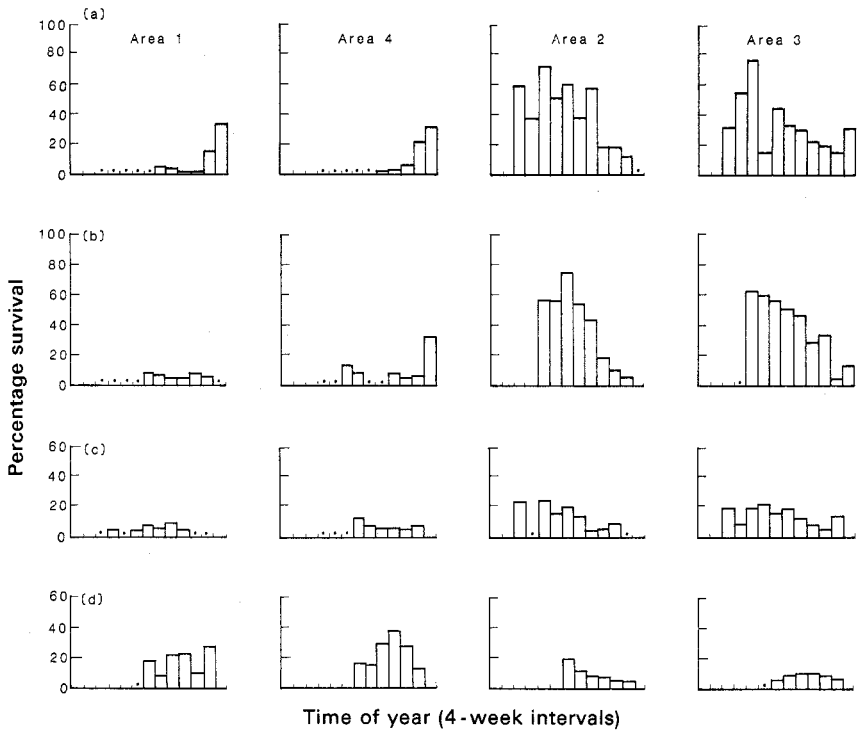


Fig. 4. Percentage of the young born at different times of the year in areas 1–4 surviving to March in the year following their birth. Young born: (*a*) 1978; (*b*) 1979; (*c*) 1980; (*d*) 1981. Dots indicate that no rabbits survived.

food; this probably accounted for the poor survival in that area. Population density was lower in area 3 and the survival rate was higher there than in area 2, but it was much lower than in 1978 or 1979. A heatwave in week 47 with temperatures up to 45°C killed eight rabbits in the traps and a further 10 were obviously stressed. In 1981 the survivorship curves were similar in all four areas, areas 1 and 4 having slightly higher survival rates (Fig. 3*d*).

Survival of young rabbits to March in the year following their birth is shown in Fig. 4. Normally late-born young have a low rate of survival but in 1978 in areas 1 and 4 they survived better than those born earlier in the season. Rabbits born in the last 10 weeks of 1978 were the only ones without antibodies to myxoma virus in areas 1 and 4 in January 1979.

In 1978 and 1979 rabbits born early in the breeding season in areas 2 and 3 survived better than those born later in the season. Survival rates were low in areas 1 and 4 regardless of the time of birth. In January 1980 most late-born young on the Lu-treated areas did not have antibodies

to myxomatosis but all rabbits bled in March had antibodies to myxomatosis. The late-born young that escaped infection with Lu were probably infected with field strain in February.

Survival rates of young in areas 2 and 3 were lower in 1980–81 than in the two previous years. Young born early in the season generally survived better than young born later. In the first week of 1981, 86% (25 of 29) of the young caught in areas 1 and 4 were without antibodies to myxomatosis—6 of the 25 without antibodies were bled in the following 4 weeks; four had symptoms of LFS and two had antibodies. The low survival rate of young born throughout 1980 was due to a combination of the effects of drought and infection with Lu or field strain.

From a total of 1871 rabbits tagged in areas 1 and 4 in 1978, 1979 and 1980, 83 (4·4%) survived to enter the adult population; of these, 27 were seen with Lu–LuT symptoms and six others probably recovered from Lu–LuT infections; six were seen with field-strain symptoms and 26 others probably recovered from field-strain infections; six were seen infected with an unidentified strain and 12 were neither seen with myxomatosis nor was it possible to guess the infecting strain. Thirteen of the 83 survivors were tagged very much later than their contemporaries and could have been on isolated warrens and exposed to Lu at an age (10–18 weeks) at which they would have had a survival advantage (Sobey *et al.* 1970).

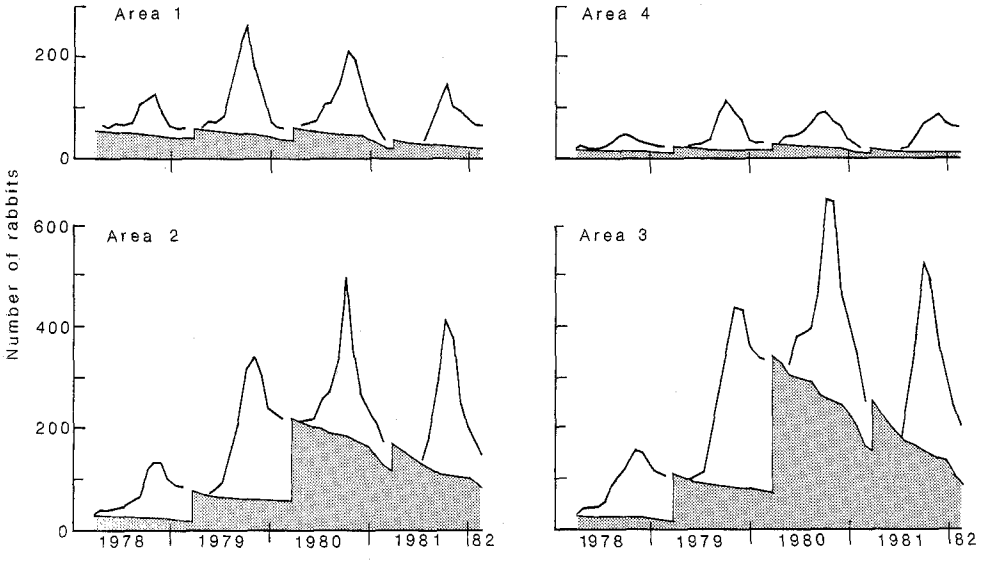


Fig. 5. Graphs of the number of adults (dark areas) and emerged young (open areas) known to have been alive in areas 1–4 from 1978 to 1982.

Of the 27 definite Lu–LuT survivors, 16 (59%) were infected in the warm to hot months of November–January, and 14 (52%) were infected at 10 weeks of age or more; only eight were infected both at less than 10 weeks of age and in the cooler weather at the age and temperature that the majority of the offspring were exposed to the Lu strain. Two-thirds of the Lu–LuT survivors were seen as recoveries when first trapped.

The suggestions from these data are that about half of the annual increment of yearling breeding rabbits could have recovered from a field strain, and that the majority of those that survived Lu–LuT infections did so because of their age at infection and/or the warmer ambient temperatures (Marshall 1959).

Annual Fluctuations in Numbers

In 1979 the main breeding season commenced 3 weeks earlier, and Lu was introduced 3 weeks later, than in 1978. This resulted in peak populations of 213 and 118 in areas 1 and 4 in 1979

compared with those of 78 and 47 in 1978, even though the number of adults was similar in both years (Fig. 5). In 1978 the greatest numbers of rabbits known to have been alive in areas 2 and 3 were 135 and 155 respectively. At the high point in the population curves in 1981, 491 and 658 rabbits were known to have been alive in areas 2 and 3. In 1978 and in 1979 the populations declined earlier in areas 1 and 4 than in areas 2 and 3.

Adult numbers in March in areas 1 and 4 showed only minor fluctuations from 1978 to 1980. Over the same period adult numbers in March increased by a factor of eight (222/28) in area 2 and by a factor of 12 (338/27) in area 3 (Fig. 6). After the dry year of 1980 adult numbers in 1981 were 70–74% of their 1980 levels in areas 2 and 3 and 55–61% in areas 1 and 4. After the introduction of Lu into all four areas in 1981 adult numbers increased in areas 1 and 4 and decreased in areas 2 and 3.

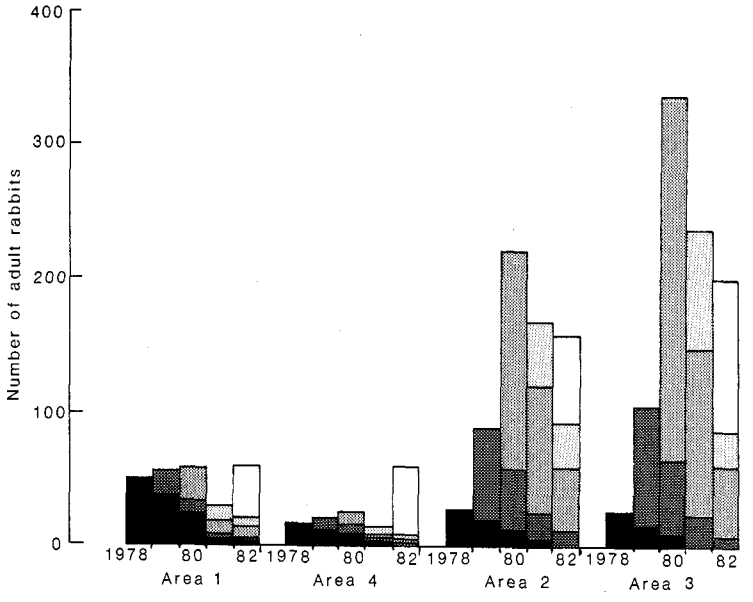


Fig. 6. Age structure of adult rabbits in areas 1–4 in March of the years 1978 to 1982. Year born: black, 1977 or earlier; heavy stippling, 1978; medium stippling, 1979; light stippling, 1980; open, 1981.

In the first two years of the study the annual adult survival rate was high (>58%) in all of the areas. In the dry year of 1980 the survival rate of adults was 48% on the areas treated with FS98, and 33% in the areas treated with Lu. Older adults survived poorly in this period. Of the 54 adults born in 1977 or earlier and known to have been alive in March 1980, only 10 (19%) survived to March 1981.

Over the four years 21 rabbits dispersed from area 1 to area 4, and 30 dispersed from area 4 to area 1. Of the 21 moving from area 1 to area 4, two rabbits survived to breed, and of the 30 moving from area 4 to area 1, twelve survived to breed. In area 1 there were 50 adults in 1978, and 57 in 1979 of which seven were immigrants. Thus the increment in the number of adults on area 1 from 1978 to 1979 was entirely due to immigration.

Changes in the Size and Distribution of Warrens

From 1978 to 1982 there was little alteration in the number, size and distribution of warrens in areas 1 and 4 (Fig. 7). The substantial increase in the rabbit population in areas 2 and 3 was not matched by a corresponding increase in the number of warrens, although many of the warrens

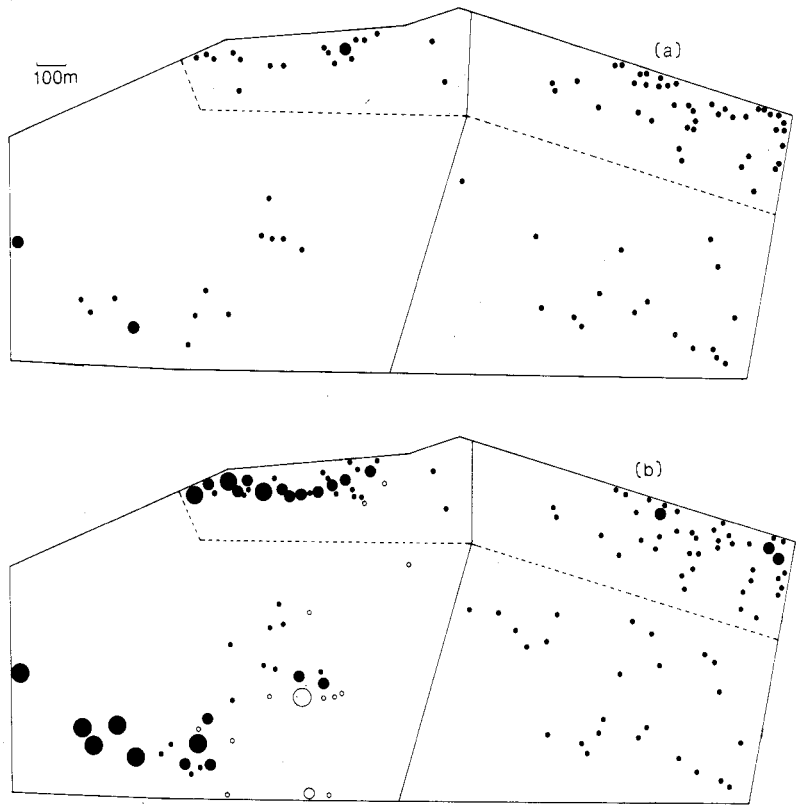


Fig. 7. Spatial distribution and size of warrens with at least one active entrance in: (*a*) April 1978; (*b*) February 1982. Number of active entrances: ● 1–5; ●● 6–10; ●●> 10. Open symbols represent warrens which were established after April 1978.

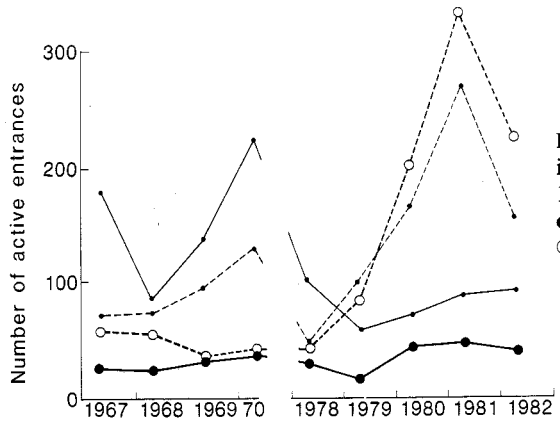


Fig. 8. Number of active entrances in February–April on areas 1–4 from 1967 to 1970 and from 1978 to 1982. ●——● area 1; ●- - -● area 2; ○- - -○ area 3; ●——● area 4.

were larger in 1982 than in 1978. Of the 1176 kittens tagged in 1981 in area 3, 615 were trapped on five warrens. These five warrens were on a soil type that was so restricted in distribution that in area 3 these five warrens occupied most of it. The soil was dark brown to black with calcareous nodules. One of these warrens, first noted in late 1978, had 105 kittens tagged on it in 1981.

A serious defect in this study is that there were no untreated areas to act as controls for the treated areas. We do have what might be termed an historical control, because the number of warren entrances used by rabbits (active entrances) on the study site were monitored from 1967 to 1970 (Parer 1977). The number of active entrances is an index of the number of rabbits (Parer 1982*a*). As the number of active entrances in area 1 from 1979 to 1981 was about half that in 1978, and about half the numbers for 1967–70 with 1968 excluded, it appears that annual introductions of Lu kept the population below its long-term potential (Fig. 8). The low number of active entrances in 1968 in area 1 was the result of a very severe drought, during which many rabbits moved from area 1 to the other areas (Parer 1982*b*). The pattern of change in the numbers of active entrances in area 4 in 1967–70 compared with 1978–82 suggests that the Lu introductions had little effect on the population.

From 1967 to 1978 there was little change in the number of active entrances in area 3, and then numbers increased to a level in 1981 which was about six times higher than those observed previously. In area 2 the number of active entrances increased by a factor of 4·8 from 1978 to 1981, and the 1981 value was about three times higher than the average for 1967–70.

Table 4. Percentage survival of rabbits from areas 1–4 and from Cocketgedong after challenge with two test viruses

FS634, field strain collected at Cocketgedong in 1977; FS638, collected at Yathong Nature Reserve in 1980. Values in the same column with the same superscript are not significantly different

Source of rabbits	FS634		FS638	
	Number tested	Survival (%)	Number tested	Survival (%)
Areas 1 and 4	39	95[a]	43	37[a]
Cocketgedong	28	86[a]	19	58[a]
Areas 2 and 3	27	59[b]	28	14[b]

Changes in Innate Resistance to Myxomatosis

Young rabbits in areas 1 and 4 survived better than those in areas 2 and 3 in 1981. An obvious explanation for this is the lower density of rabbits in areas 1 and 4. Another explanation is that during the course of the experiment there had been an increase in genetic resistance to myxomatosis in the rabbit populations in areas 1 and 4, or a decrease in genetic resistance in those in areas 2 and 3. To test this possibility 198 kittens were taken in September 1982 from the Urana study site and Cocketgedong, a neighbouring property, to enclosures in Canberra for a comparison of their survival rates to two Australian field strains, FS634 and FS638. The survival rates of rabbits from areas 1 and 4 were not significantly different from those of rabbits from the adjacent property, but the survival rates of rabbits from areas 2 and 3 were significantly different with both test viruses (Table 4). However, the bulk of the parents in areas 1 and 4 were survivors from the very mixed epizootic of 1981, but those from areas 2 and 3 were predominantly FS98 survivors (Fig. 6).

One possible explanation for the low survival rate of kittens from areas 2 and 3 to the test viruses could be that FS98 treatments of these areas reduced the innate resistance by removing selection pressure and allowing back-selection to occur. Alternatively it could have been due to a physiological difference in the young rabbits born into high-density populations with very little food available and that of poor quality. Less rain was recorded at Urana in 1982 than in any other year since recording commenced in 1871. When the young rabbits from areas 2 and 3 were

transferred to outdoor enclosures in Canberra they gained significantly more weight than those from areas 1 and 4 or those from Cocketgedong.

Discussion

After examining a number of strains of the myxoma virus, Sobey *et al.* (1983) suggested that FS98 was the most suitable virus for immunizing field populations of rabbits. In enclosures with no cats or foxes 100% of rabbits of Urana stock heavier than 1000 g survived challenge with FS98 in autumn, spring and summer. In our data there was a suggestion that mortalities of kittens in the nest might be higher than this, as there was a depression in the numbers of emergent kittens 2–3 weeks after FS98 was first introduced each year. Kittens born later could have been protected by the elevated levels of maternal antibodies in adult females re-infected with FS98.

The observed rates of population growth in the areas treated with FS98 may have been higher if a totally benign virus (e.g. Shope's fibroma) had been used. It is not permissible to use Shope's fibroma in the field in New South Wales, but if it had been used it is almost certain that it would not have spread from rabbit to rabbit (Sobey 1981). Indeed it is surprising that FS98 spread as well as it did, because Mead-Briggs and Vaughan (1975) showed that fleas were not very infective after feeding on rabbits infected with this strain.

As there were no experimental control areas it can only be presumed that the increase in the populations in the FS98 areas was due to the imposed treatment. Area 1 on the site has been monitored by trapping since 1967, and the adult population has remained relatively stable except for droughts and experimental manipulations (Parer 1977; Daly 1979; Williams and Dunsmore, unpublished). For 7 of the 11 years from 1967 to 1977 the adult population at the start of the breeding season was between 46 and 81 animals. In 1968 and 1973 the population was 25 and 24, respectively, after the droughts of 1967 and 1972. In 1971 and in 1972 the population was 112 and 110. Mammalian predators on the site were poisoned during the 1970 breeding season; this was the cause of the increased breeding population in 1971.

Control areas were established on a large adjacent property in 1978. Unfortunately the warrens on these areas were fumigated and ripped by the property owner. Control areas were not established on the site because it was possible that FS98 or Lu might be accidentally introduced into the control areas. Our observations of the surrounding rabbit populations, and the observations of the rabbit inspector for the Urana district, did not suggest that there was any change in these populations from 1978 to 1980.

The rapid increase in the number of rabbits in the areas treated with FS98 was unexpected, because Parer (1977) had concluded that myxomatosis was not controlling rabbit numbers at Urana. Further experiments using FS98 are desirable to try to understand the role of myxomatosis in other parts of Australia. It is a widespread belief that commensalism is the ideal evolutionary endpoint for both host and parasite (Dubos 1965); however, Ewald (1983) has shown that a severe disease can be an evolutionary endpoint if the disease is more or less host-specific and if insects are the major route of transmission. Myxomatosis in Australia meets both of these requirements, and it is possible that myxomatosis will continue to play an important role in the control of rabbit populations for many years.

Except for the low population in 1981 there were only minor changes in the adult population in area 1 during the course of the experiment. The average adult population from 1978 to 1982 was 52, compared with an average adult population in 1967–77 of 67. Annual introductions of Lu did not change population numbers significantly in the short term, and the average adult population was only 22% below the long-term average for the area. In area 4 numbers increased for the first two years, declined in the third year and increased sharply in the fourth year. The most successful introduction of Lu was in 1978, when infective fleas were released at the end of August, 10 weeks before mosquitoes were observed and well before the high summer temperatures. In spite of these favourable conditions some of the late-born kittens either escaped infection or received summer infections of LFS. In 1979 the majority of kittens were not infected before November, and the recovery rate was increased by both the high ambient temperatures

and by their being in an age group conferring a survival advantage (Sobey *et al.* 1970). In 1980 half of the fleas inoculated with Lu were dead when put down the burrows. In 1981 the very early appearance of mosquitoes and LFS coincided with our introduction of Lu, and many of the infections in that year could have been due to recombinant strains or to mixtures of strains.

The CSL preparation of Lu failed in the two years in which it was released, whether because of chance incidents or because of its inherent qualities is not known. Perhaps the release in 1980 would have had more success if a less viscous preparation had been made with which to inoculate the fleas, and if more warrens had been treated. The drought early in the breeding season in 1980 removed many of the kittens before they could become infected; this factor also could have affected the transmission of the CSL Lausanne.

Most of the LuT recoveries were first seen as recovered animals in late November or early December. The LuT virus in 1978, 1979 and 1980 may have been the same as Lu, the expression of the virus being different in the warmer weather, or else by late November the introduced virus had been passaged through sufficient rabbits for a more attenuated mutant to have become prevalent. In 1981 some of the LuT symptoms might have been caused by a virus that was a recombinant between the local field strain and Lu.

Bults and Brandon (1982) showed that a field strain collected in December 1979 at a site about 8 km from the study site was more similar to Lausanne in its antigenic determinants than to an Australian field strain. It was suggested that this similar strain might have been derived from the Lausanne release at Urana by Fenner *et al.* (1957) in 1954, or that it might have been derived from our release on the study site in 1977 (Parer *et al.* 1981) or 1978–79 (this paper).

The Use of Virulent Viruses in Rabbit Control

Before the introduction of the European rabbit flea into Australia, it was recommended that virulent viruses be released at a time of the year when mosquitoes were likely to be active (Douglas 1965). Our experiences at Urana suggest that this would not produce an effective epizootic of the virulent virus, because the virulent virus would have to compete with field strains. Even though the survival rate of tagged young in areas 1 and 4 in 1978 and in 1979 was 4–5%, the breeding population did not decline. Actual survival rates would have been lower because many young rabbits die before they are trapped (Parer 1977; Daly 1979). It appears that an introduction would have to reduce the survival rate of young rabbits to less than 4% to be successful. This would be most difficult to achieve.

The timing of our first introduction of Lu in each year was constrained by two factors: the expected time of the appearance of LFS and the number of kittens in the warrens. From 1966 to 1977 the earliest appearance of LFS was in week 40. Our introductions had to be made before week 40, but if an introduction were made much before then there would not have been sufficient kittens in the warrens to carry an effective epizootic. Therefore all our first introductions in each year were made between week 35 and week 39. Even with our background knowledge of the site and with our experience in infecting rabbits with myxomatosis we were not successful in all of our releases. As our experimental introductions of Lu on a site on which the epizootiology of myxomatosis was well known did not produce evidence of a reduction in the number of adult rabbits, we consider that releases by landholders are most unlikely to be a worthwhile method of rabbit control.

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