STUDIES IN THE EPIDEMIOLOGY OF INFECTIOUS MYXOMATOSIS OF RABBITS

IV. OBSERVATIONS OF DISEASE BEHAVIOUR IN TWO LOCALITIES NEAR THE NORTHERN LIMIT OF RABBIT INFESTATION IN AUSTRALIA, MAY 1952 TO APRIL 1953

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(With 2 Figures in the Text)

Within three months of its recognition, some miles from an experimental site in December 1950, myxomatosis had spread so widely that cases had been recognized in south-eastern Australia over an area of over 600,000 square miles (Ratcliffe, Myers, Fennessy & Calaby, 1952; Fenner & Day, 1953). Detailed investigations on the disease performance would be impossible over so wide an area, and it was decided to concentrate intensive observation at two localities. The results of observations made at one of these localities (Lake Urana) have been described in the previous paper of this series (Myers, Marshall & Fenner, 1954).

The present paper records the data collected at the other area, near the New South Wales-Queensland border, between May 1952 and April 1953. A locality map showing the observation sites, and the results of a serum survey there in May 1952, were published in the first paper of this series (Fenner, Marshall & Woodroofe, 1953). Attention was concentrated on two sites, Texas and Dunroy Stations.

MATERIALS

Myxoma virus

Virus introduced artificially into the rabbit population was supplied as a freeze-dried powder, prepared from the South American strain of the virus (strain B of Martin, 1936) by one of us (F. F.) and dried by the Commonwealth Serum Laboratories, Melbourne. Virus of similar origin was maintained in the laboratory at $-70°$ C, and designated as the standard laboratory strain of the virus.

Experimental rabbits

Laboratory rabbits bred in the Animal Breeding Establishment of the University, and elsewhere, were used for the isolation and subsequent testing of strains of virus obtained in the field. Because of a shortage of these rabbits, and also because it was thought that laboratory and wild rabbits might react rather differently to infection with myxoma virus, the initial testing of field strains was carried out in wild rabbits captured in the field and screened for immunity by

serological tests. As wild rabbits were rather variable in their response, and the incidence of non-specific deaths was high, the strains were finally tested by challenging groups of laboratory bred rabbits.

METHODS

Field

Population counts

In contrast to the situation at Lake Urana (Myers *et al.* 1954) the nature of the vegetation and topography at Texas and Dunroy rendered impossible sight counts of the rabbit population. Disturbance of the population by continuous trapping also interfered with population counts. No satisfactory estimates of population size could be made except for a comparison of the times taken to shoot rabbits for serum samples during successive visits to the area. The assessment of the rabbit population shown in Figs. 1 and 2 was derived from this information, as well as general observations on rabbit density and the reports of the local station managers.

Age structure of rabbit populations

Rabbits were aged empirically by an approximate relationship between sexual maturity and paunched weight. Rabbits in the May 1952 sampling were not weighed, and only 'live' weights were noted in August 1952. Both live and paunched weights were taken in all subsequent samplings, a spring balance being used at the time of blood collection. The relationship between live and paunched weight was found, and the data of the August 1952 sampling corrected accordingly. All weighed rabbits were placed into two groups, those with a paunched weight of more than 1·15 kg. being classed as adults, and those below this weight as sub-adults. Rabbits which weighed 1·15 kg. were divided equally between the two groups. It is thought that this weight approximates to an age of 4 months. Eleven out of 443 pregnant or lactating does weighed less than this, six weighed 1·15 kg. and ten weighed 1·20 kg.

Sampling of rabbits

One hundred and sixteen rabbits were obtained by daylight shooting and 1372 by spotlight shooting at night.

Normally the shooting party consisted of three: driver, marksman and light operator. The vehicle used was a Land Rover, and a housed sealed-beam headlamp unit equipped with a wandering lead powered by the vehicle supply was used as a spotlight. A repeating 0·22-gauge rifle was used in all except the final shoot when, because of the lower density of rabbits, a 12-gauge shotgun was substituted. The latter reduced stalking and shooting time to a minimum.

Despite a suspected bias during the breeding season, which is discussed later in the text, shooting was superior to other practicable methods for obtaining a large series of rabbits for blood samples.

Collection of blood samples

Carcasses were opened at the thorax and blood taken from the heart by Pasteur pipette. This was usually done within an hour of shooting. The samples were centrifuged within the next 36 hr. and the serum separated, merthiolated (to a final concentration of 1/10,000 merthiolate) and stored in corked Wasserman tubes in Dewar flasks containing ice.

Collection of virus samples

Testes, lungs, liver or skin tumours were harvested from rabbits shot in the active stage of the disease. The material was stored in 5 ml. bottles in Dewar flasks of ice until arrival at the laboratory. Here it was ground and inoculated into a laboratory rabbit. If myxomatosis developed virus-containing material, usually from the inoculation site, was removed about 7 days after the inoculation. This material was ground in saline, dispensed in several ampoules and stored at $-70°$ C. The titre of the suspension was determined before the virulence tests, and checked when these tests were performed by titration of the contents of one ampoule on the chorioallantois.

Laboratory

Serological studies

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).

Assessment of the virulence of field strains of the virus

An account was given previously (Myers *et al.* 1954) of the methods adopted for comparing the virulence of strains of myxoma virus recovered in the field with that of the standard laboratory strain of the virus. Briefly, groups of adult rabbits were inoculated intradermally in a single site with small known doses (about 10 ID_{50}) of each of the field strains under study, and with a similar dose of the standard laboratory strain of myxoma virus. Each field strain had been passed once in a laboratory rabbit before testing. All rabbits were observed daily and records maintained of the rate of progress and severity of the different symptoms of myxomatosis. The most valuable objective criterion for the comparison of strains was the survival time. In the analysis of survival times a transformation of the data should be made which normalizes the distribution curves and makes comparable the variances of different series. The metameter generally applicable is $y=(u-u_0)^i$, as suggested by Kapteyn (1903). In the present instance we have never observed the death of an adult rabbit from myxomatosis, after the intradermal inoculation of a small dose of virus, in less than 8 days. This period was therefore taken as u_0. The survival time thus became $(u–8)$ days and a log transformation was carried out on the data, i.e. the value of $i=0$. Rabbits which survived were assumed to have a survival time of 60 days. Calculations of means and

variances, and comparisons of the different groups, were made with these transformed figures, but in Table 3 and Figs. 1 and 2 the parameters have been expressed in terms of the original data.

RESULTS

The inability to make population counts made it impossible to determine at Texas and Dunroy case mortality rates of the type which Myers *et al.* (1954) had calculated at Lake Urana. In addition, the disease persisted throughout the year, and severe summer epizootics with virtual absence of the disease during the winter months, such as characterized the epidemiology in the Murray Valley, did not occur. The results here recorded therefore consist in the main of the serological findings and the determination of the virulence of strains of the virus recovered from the field, correlated with some relevant biological observations.

Detailed entomological observations were made at Texas and Dunroy, as well as at other places in the Moree district, but no particular insects or groups of insects were positively incriminated as the vectors of myxomatosis. These observations will be reported elsewhere (Dyce, in preparation).

Texas

The study area at Texas consisted of about 2500 acres of river flats belonging to the Texas Station. These were used for the breeding and grazing of beef cattle and were heavily stocked throughout the year. During the sampling period, 300 acres were under cultivation to lucerne and cereals and the remainder was unimproved pasture.

Extensive floods during 1950 were followed by a dense growth of woody annuals which reached a height of 6 ft. over much of the area. When the first sampling was made, in May 1952, most of this growth was dry but still standing. Good autumn rains had germinated the winter annuals which subsequently dominated the area, and by August 1952, barley grass and medics formed a dense cover which matured and dried by October 1952. Dry conditions prevailed until February 1953, when heavy rains induced prolific, lush growth which was still persisting in April 1953. Some regeneration of the tall summer-growing annuals occurred but not to the same extent as following the 1950 floods. In addition to Dumaresq River itself, there were many semi-permanent flood-water lagoons scattered over the area.

Epizootic history of myxomatosis

Scarcity of labour during the recent war and in the subsequent years allowed the rabbit population to multiply almost unchecked except by seasonal conditions, and by the summer of 1950–1 the population had reached plague proportions. The first epizootic of myxomatosis occurred in March 1951, and it is estimated that more than 90 % of the population succumbed during this month (Elliott, personal communication), but no diseased rabbits were reported during the following 11 months despite repeated artificial introduction of the virus. During this time the population was replenished by breeding and probably by immigration from disease-free areas in the surrounding hills until very high numbers were again

attained by January 1952. A minor epizootic lasting about a fortnight occurred in March 1952. From this time on the disease was always present in the population and although very few infected rabbits could be seen at any one time, numbers had dwindled to an insignificant level by April 1953. It will be shown that serological tests revealed fluctuations in the intensity of the epizootic not readily apparent by visual observation.

Interference with the rabbit population by measures other than myxomatosis

Between 2000 and 4000 rabbits were poisoned and trapped on the sampling area during April and May 1952. During the following 3 months many of the burrows and warrens were ploughed out, but some were missed and there was considerable re-opening of those ploughed. From September 1952 to April 1953, a pack of dogs was worked on the property, but the amount and nature of the rabbit harbour

Table 1. *Sex ratios, age structure, and immune status of the rabbit population at Texas, southern Queensland, from May* 1952 *to April* 1953

Month	Adults			Sub-adults	Percentage of sub-adults in sample	Antibody to myxoma virus					
						Adults			Subadults		
	Bucks	Does	Total			+	−	% immune	+	−	% immune
1952											
May	46	57	103	4	4	27	76	26	0	4	0
August	190	248	438	64	13	277	161	63	13	51	20
October	29	48	77	26	25	47	30	61	2	24	8
November	12	26	38	16	30	18	20	47	0	16	0
December	21	25	46	26	36	32	14	70	9	17	35
1953											
January	23	21	44	14	24	33	11	75	9	5	64
April	96	91	187	0	0	130	57	70	0	0	—
Total	417	516	933	150	—	564	369	—	33	117	—

hampered their effectiveness. The kill was never more than twenty or thirty rabbits a week from the area under observation.

Feral cats, foxes, and predatory and scavenging birds were all abundant and hawks in particular were observed to effect rapid removal of dead rabbits.

Biological observations on the rabbit

Between May 1952 and April 1953, seven samples yielded 1083 rabbits. Of these 933 were classified as adults and 150 as subadults. The overall sex ratio of the adults was 81 bucks to 100 does. The data are shown in Table 1. The discrepancy from a 50–50 sex ratio was greatest in the collections made between May and November. Two explanations are suggested. First, the extensive trapping and poisoning carried out in April 1952, may have removed more bucks than does, for trappers in Australia usually trap many more bucks than does. Secondly, it is possible that pregnant does are less apt to run when picked up by a spotlight at night. The high percentage of pregnant animals during August, October and November is apparent in Table 2. The more even sex ratio obtained in the April

1953 sample might be due, amongst other factors, to the greater efficiency of the shotgun against moving animals.

The immune status of the pregnant and lactating does is set out in Table 2, animals being grouped into those which showed serological evidence of recovery from myxomatosis and those which had not been infected. The data confirm the smaller series published earlier (Fenner *et al.* 1953) and indicate that myxomatosis is without effect on the fertility and fecundity of female rabbits. The mean number (± standard error) of embryos borne by recovered does was $5{\cdot}8 \pm 0{\cdot}12$ and by normal does $5{\cdot}4 \pm 0{\cdot}15$.

Serological studies

The results of serological tests on the seven samples taken from Texas during the year are set out in Table 1 and Fig. 1. After breakdown into adult and sub-

Table 2. *Data on reproduction at Texas*

	Recovered does				Susceptible does				
Month	Lactating	Pregnant	Mean no. of embryos	Percentage pregnant + lactating	Lactating	Pregnant	Mean no. of embryos	Percentage pregnant + lactating	Overall % pregnant
1952									
May	8	2	4·0	67	20	7	3·6	64	16
August	46	70	5·8	81	36	43	5·5	76	46
October	4	14	6·6	69	5	10	5·6	75	50
November	4	1	5·0	62	2	9	6·6	61	38
December	6	2	6·5	50	4	1	4·0	50	12
1953									
January	1	1	5·0	12	1	0	—	16	5
April	14	10	5·3	36	3	5	4·8	31	16
Totals	83	100	5·8	—	71	75	5·4	—	—

adult categories, some of the groups were unfortunately very small, and the 'percentage immunity' is therefore subject to considerable error. Nevertheless, the curve showing the geometric mean complement-fixation titres of immune animals confirms the trends apparent in the percentage immunity figures. In general, high titres indicate recent recovery from infection (Fenner *et al.* 1953) so that the peaks of the curves, which correspond with peaks in the percentage immunity figures (particularly those of subadult animals) indicate recent increases of disease activity.

The virulence of virus strains recovered from the field

Six strains of myxoma virus were recovered from material obtained from sick rabbits shot during the sampling period. The dates of collection of these strains, and the results of tests designed to compare their virulence with that of the standard laboratory strain of the virus are shown in Table 3 and Fig. 1.

Virus was first obtained from rabbits at Texas about 14 months after the first epizootic. These strains were slightly attenuated, when compared with the standard

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laboratory strain of virus. Not only were the mean survival times slightly longer, but the survival times of several individual rabbits were significantly longer than those of any rabbits infected with the standard laboratory virus during this or previous experiments. Strains isolated subsequently were all definitely attenuated when compared with the standard strain, and there was an impression that there was a gradual increase in the degree of attenuation observed (Fig. 1).

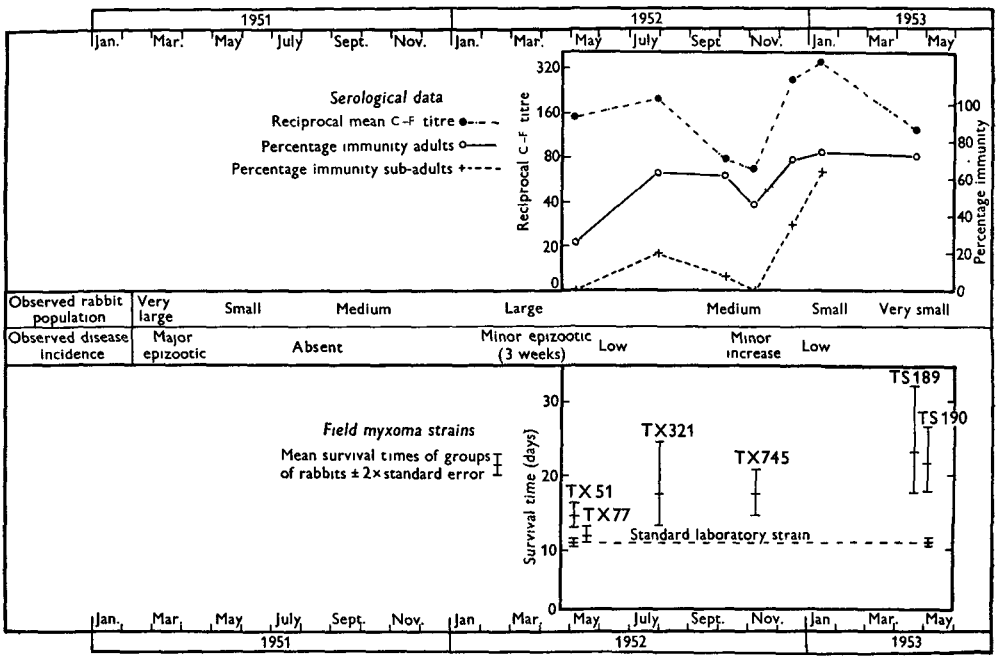

Fig. 1. The relationship between the incidence of myxomatosis, the size and immune status of the rabbit population, and the virulence of field strains of myxoma virus at Texas.

Table 3. *Survival times of rabbits challenged with myxoma virus strains isolated at Texas, southern Queensland, and at Dunroy, northern New South Wales*

Date isolated	Strain	Survival times (days)		Mean ± standard error*	Average survival time and 95% fiducial range
		Laboratory rabbits	Wild rabbits		
May 1952	TX51	12, 13, 16, 17, 21	12, 13, 14, 16	0·80 ± 0·06	10·9, **14·4**, 22·1
May 1952	TX77	11, 12, 13, 13, 13, 17	10, 10, 11, 13	0·59 ± 0·06	9·6, **11·9**, 17·2
August 1952	TX321	12, 18, 25, 31, S†	11, 13, 13, 16	0·97 ± 0·14	9·7, **17·3**, 61·2
November 1952	TX745	16, 17, 19, 19	11, 19, 20, 25	0·97 ± 0·08	11·6, **17·3**, 32·4
April 1953	TS189	19, 23, 28, 54	14, 15, 25, 35	1·18 ± 0·10	12·2, **23·1**, 62·8
April 1953	TS190	15, 16, 17, 24, 27	23, 26, 34	1·13 ± 0·07	13·5, **21·5**, 40·8
May 1952	DY10	13, 15, 18, 18, 18, 19, 41, S†	20, 21, 31	1·12 ± 0·09	11·8, **21·3**, 54·5
December 1952	DY329	12, 12, 13, 14, 14, 18, 20, 21, 22, 28	23	0·93 ± 0·07	11·0, **16·6**, 32·4
	Standard	10, 10, 10, 10, 10, 11, 11, 12, 13	12, 12, 12, 12	0·47 ± 0·04	9·6, **11·0**, 13·7

* Expressed as log (survival time in days − 8).

† S = rabbit recovered from infection. Survival time evaluated at 60 days.

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Freeze-dried standard virus has been inoculated into groups of rabbits which were subsequently released on many occasions since early in 1951, but this had no apparent effect on the emergence and survival of the less lethal strains.

Comment

No dramatic outbreaks of myxomatosis were observed or reported during the year that serum samples were collected. Minor fluctuations of disease activity were apparent and the serological data indicate that these were more intense than was suggested by field observation alone. Eleven diseased rabbits were seen during the week's intensive observation in May 1952, in a very heavy rabbit population. This was the highest incidence recorded during the year of observation. It was very low compared with the striking seasonal outbreaks in southern Australia, where at the height of the epizootics hundreds of diseased rabbits could be seen within a few hours walking (Myers *et al.* 1954). Population immunity and mean antibody titre reached peaks in August 1952 (Table 1 and Fig. 1), so that the observed disease incidence persisted and probably increased during June and July. Impetus was possibly given to the disease during this period by the autumn breeding activity and consequent supply of fresh susceptible population. This new population provides the most sensitive index to disease activity, and it can be seen from Fig. 1 and Table 1 that during the winter months the immunity in this group practically disappeared as the relics of the immune subadults were absorbed into the adult group.

The lowered activity of the disease and the diluting effect of maturing susceptible subadults caused a steady decline of immunity in the adult population until November. Following heavy rains in October more diseased rabbits than usual were reported, and the curves for population immunity and antibody titres rose sharply to reach peaks in January. With the highly immune population and the diminution of breeding during the summer the progress of the disease slowed down so that the mean antibody titre had fallen by April 1953, whilst the percentage immunity remained approximately constant. It is significant that in the very small population surviving at this time the only sick rabbits encountered were two seen and shot on the fourth night of hunting. It is this incidence of about 1 % that understandably passes unnoticed in the field.

The striking features of the course of the disease during the year are the reduction in the number of rabbits from near-plague proportions in May 1952, to the lowest numbers in living memory in April 1953; and the fact that the occurrence of the disease was almost imperceptible during that time.

Traditional methods of control such as trapping and poisoning were continued during the study period, but such activity was certainly no greater than in previous post-war years when rabbits got completely out of control.

The season was highly favourable to the maintenance of the rabbit population, and although there are no previous records with which to compare the reproductive potential of the rabbits in this area, the percentage of pregnant and lactating does and the embryo counts during the breeding season leave no doubt as to their fecundity. The incidence of disease as assessed by observation could not account

for the remarkable reduction of the size of the rabbit population. However, in the field the disease incidence is estimated by the number of sick or dead rabbits seen in a particular area at a particular time. This method excludes most of the diseased kittens, which frequently die within a week of infection and before obvious symptoms have developed (Fenner & Marshall, 1954). In this area many sick animals would probably be removed by predators before death. The serological findings indicate clearly that fluctuations in disease activity not apparent to field observers did occur, and also that the subadult population was involved in these fluctuations.

Passive immunity due to the transfer of maternal antibodies would partially protect the subadult population (Fenner & Marshall, 1954), but the case mortality rate in this group would still be sufficiently high to effect a large measure of control over the new generation. The percentage of immune adults at Texas during the main breeding period indicates that about 60% of kittens would be born with maternal antibodies to myxoma virus (Table 1). On the basis of laboratory experiments about 75% of these passively immunized kittens would die if infected with strains of myxoma virus with the level of virulence found at Texas at that time, as would all the infected normal kittens (Fenner & Marshall, 1954). Recently recovered rabbits have high complement-fixing antibody titres and frequently bear recognizable scars of external symptoms, and can be fairly readily distinguished from the passively immune kittens in which detectable maternal antibodies are short lived and of relatively low titre. By these criteria at least fourteen of the forty subadults listed in December 1952 and January 1953 (Table 1) had actually recovered from myxomatosis, and this suggests that there was a high infection rate amongst kittens. After allowing for protection by passive immunity there would still be an overall case mortality rate of the order of 80%, so that the effect of the disease on the subadult section of the population must have been an important factor contributing to the decline in the number of rabbits observed in the area.

Dunroy

The Dunroy property is situated 6 miles east of Moree, and the entire area of 2800 acres was used for the study. The area was chosen as a marked environmental contrast to Texas. Until 1951 it had been used exclusively for sheep grazing, but since then 600 acres have been sown to wheat each year. There is no permanent natural water on the property.

Pasture growth in 1951 had been limited by low rainfall, and by February 1952, when soaking rains fell, the pasture had been so heavily overgrazed that recovery was slow. With effective winter rainfall winter-growing annuals provided a dense cover 6–15 in. high over most of the property by August 1952. These were drying out at the beginning of October, were completely dry by the end of November, and all except the most woody plants had collapsed into litter by mid-January. However, during this period the favourable spring and summer rains allowed abundant growth of perennial summer grasses which were mature and drying in April 1953,

Epizootic history of myxomatosis

Rabbits had been virtually uncontrolled until the introduction of wheat growing in 1951, but despite the action initiated at that time, considerable numbers were present on the property in May 1952.

Myxomatosis penetrated into the Moree district in March 1951 during the general advance along the watercourses of eastern Australia at that time (Ratcliffe *et al.* 1952; Brereton, 1953) and effected rapid and significant reductions in the rabbit populations on many properties. Diseased rabbits were seen within 2 miles of Dunroy at this time, but not on the property itself. There were artificial introductions of myxoma virus at Dunroy during November 1951, but whether the epizootic that occurred during January 1952 was due to these, or to the migration of infected rabbits or vectors from surrounding areas, is not known. This initial epizootic was unlike the short and spectacular outbreaks reported at Texas and in the southern areas, but over a period of more than 20 weeks it killed more than 90% of the original population, a performance that was typical of a large area of rabbit-infested land east and south of Moree. Infected rabbits were seen throughout the year, and the disease was active at a low level of intensity when the last sample was taken in April 1953. As at Texas there were fluctuations in the intensity of the disease incidence that became apparent only by analysis of serological surveys.

Biological observations on the rabbit

Seven samples taken between May 1952 and April 1953 yielded 405 rabbits. Of these 320 were adults and 85 subadults (Table 4). The sex ratio of the adults was 66 bucks to 100 does, the most abnormal ratios occurring between May and November, as at Texas.

The data on reproduction were analysed in the same manner as for Texas (Table 5). The numbers of animals in the different categories were often very small, but the results were essentially the same as at Texas, i.e. the reproduction rate was high during August–October 1952 and was rising again in April 1953. The mean number ($\pm$ standard error) of embryos borne by recovered does was $6{\cdot}2 \pm 0{\cdot}25$ and by normal does $5{\cdot}3 \pm 0{\cdot}14$.

Serological studies

The results of the serological tests on the seven groups of sera collected during the year are presented in Table 4 and Fig. 2. The percentages of immune rabbits and the levels of serum antibody are again mutually confirmatory and allow conclusions to be drawn on the course of the disease which would be unjustified if based on one set of figures only.

The virulence of virus strains recovered from the field

Two strains of myxoma virus were recovered from sick rabbits shot at Dunroy. The results of tests of their virulence are shown in Table 3. Both were attenuated when compared with the standard laboratory virus, but they did not differ significantly from each other.

Comment

The first serological analysis of the rabbit population at Dunroy was made about 4 months after the start of the initial epizootic. It revealed a relatively low level of immunity in the population, but a high mean antibody titre (Fig. 2). The enzootic nature of the outbreak is reflected in the gradual increase of the immunity percentages through the winter, whilst the accumulating older immune rabbits caused a decline in the mean antibody titres over the same period. The sharp

Table 4. *Sex ratios, age structure, and immune status of the rabbit population at Dunroy, northern New South Wales, from May* 1952 *to April* 1953

	Adults				Percentage of subadults in sample	Antibody to myxoma virus					
						Adults			Subadults		
Month	Bucks	Does	Total	Subadults		+	−	% immune	+	−	% immune
1952											
May	17	27	44	4	8	10	34	23	0	4	0
August	42	96	138	16	10	39	99	28	4	12	25
October	14	23	37	8	18	14	23	38	0	8	0
November	8	11	19	17	47	3	16	16	0	17	0
December	7	7	14	19	58	7	7	50	3	16	16
1953											
January	11	8	19	18	49	8	11	42	8	10	44
April	28	21	49	3	6	35	14	71	3	0	100
Total	127	193	320	85	—	116	204	—	18	67	—

Table 5. *Data on reproduction at Dunroy*

	Recovered does				Susceptible does				
Month	Lactating	Pregnant	Mean no. of embryos	Percentage pregnant + lactating	Lactating	Pregnant	Mean no. of embryos	Percentage pregnant + lactating	Overall % pregnant
1952									
August	4	15	5·9	79	11	56	5·4	94	74
October	3	5	6·8	100	2	13	5·2	100	78
November	1	1	7·0	66	1	5	5·4	75	55
December	0	1	8·0	17	0	0	—	0	14
1953									
January	1	0	—	25	0	0	—	0	0
April	2	5	5·6	50	1	2	4·5	43	38
Total	11	27	6·2	—	15	76	5·3	—	—

decline in immunity percentages shown in the small November sample is probably due to the addition of susceptible animals of the maturing new generation as well as a diminution of disease activity. Diseased rabbits were seen in October and November and there were corresponding increases in the immunity percentages for both subadults and adults in December, whilst the mean antibody titre also rose. No diseased rabbits were seen during the visit made for sampling in April 1953 although they were reported to be present in the area. The lower mean antibody

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titre suggests that disease activity had slackened earlier in the year, and that the 70 % immunity level represents the pre-breeding status of the population.

The population at April 1953 was estimated to be about half that at May 1952, so that the disease did better than control the natural increase by breeding. However, there were still a considerable number of rabbits remaining and the attenuated virus strains present from the first outbreak of the disease probably contributed to the high percentage of immune animals.

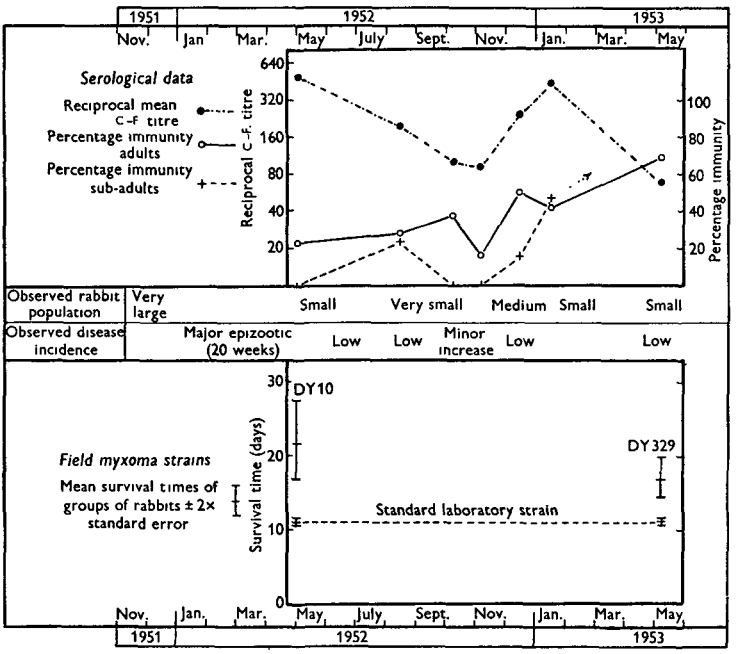

Fig. 2. The relationship between the incidence of myxomatosis, the size and immune status of the rabbit population, and the virulence of field strains of myxoma virus at Dunroy.

DISCUSSION

The study sites at Texas and Dunroy were chosen for serological and biological observations of rabbit populations exposed to myxomatosis because they offered contrasting climatic and environmental conditions near the northern limit of rabbit infestation in Australia. Only minor differences in disease behaviour were distinguished between them. The reduction in the rabbit population at Texas was considerably greater than at Dunroy during the year under observation. In all probability, this can be accounted for by the different sizes of the susceptible populations at the beginning of the observation year. The number of susceptible rabbits at Dunroy in May 1952 was small, after the recent protracted epizootic (Fig. 2), compared with a large susceptible population at Texas (Fig. 1). It is possible, also, that somewhat attenuated strains of the virus became dominant earlier at Dunroy than at Texas.

In some southern parts of Australia in which the epidemiology of myxomatosis has been studied there are violent fluctuations in rabbit population, vector activity, and the incidence of myxomatosis. The disease persists at a low level

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through the winter, and it is the short summer epizootics that cause dramatic reductions in the size of rabbit populations (Myers *et al.* 1954). In southern Australia breeding activity and the maturing of most of the new generation of rabbits usually proceed unhindered by epizootics. At Texas and Dunroy the periods of rabbit breeding and vector activity were less sharply defined and myxomatosis exerted a marked influence on the young animals. This was demonstrated principally by serological analysis. Passive immunization by transfer of maternal antibodies from the recovered does would afford some protection to the young exposed to the attenuated virus strains operating at both areas, but judging from laboratory experience (Fenner & Marshall, 1954) the percentage mortality in this group would probably be no less than in susceptible adults exposed to the same strain.

Attenuation of myxoma virus in the field has now been reported on two occasions (Mykytowycz, 1953; Myers *et al.* 1954). The results recorded here and those to be reported elsewhere (Fenner & Marshall, in preparation) indicate that during 1952 and 1953 attenuated virus strains were recovered from widely separated localities, in northern New South Wales and southern Queensland, in the Australian Capital Territory, on the southern tablelands of New South Wales, on the Murray River in New South Wales and South Australia, and in Gippsland in southern Victoria. Further, it is of considerable significance that in spite of the repeated release of fully virulent standard virus in inoculation campaigns in all these areas, during and preceding the time of the investigations, the great majority of the strains of virus recovered from the field during 1952 and 1953 showed some degree of attenuation. Current experiments on quantitative aspects of mosquito transmission (Day, Fenner and Woodroofe, in preparation) suggest that the explanation for the replacement of the original virulent strain by these attenuated variants lies in the longer survival of animals bearing highly infectious skin lesions of myxomatosis. A progressive attenuation is suggested in the virus strains isolated at Texas between May 1952 and April 1953. The survival times of rabbits challenged with the most recent strains isolated from Texas were about the same as those of rabbits challenged with strains isolated at Lake Urana and Corowa in December 1952 (Myers *et al.* 1954). While these strains are attenuated when compared with the standard laboratory strain of virus, they are still highly virulent. There were only two survivors out of seventy-four laboratory and wild rabbits infected with small doses of the strains of virus recovered from Texas and Dunroy under the sheltered conditions of laboratory maintenance, so that myxomatosis due to these attenuated strains is still a highly lethal infection. This is borne out by the continued reduction of rabbit populations at Texas, Dunroy and elsewhere, in spite of their great reproductive capacity..

Despite extensive involvement of the external genitalia during the generalization of myxomatosis, fertility of the doe is not permanently impaired by the disease. This observation is based only on embryo counts, for there was no opportunity to assess numbers reared per litter or numbers of litters per season. The slightly greater numbers of embryos found in pregnant does which had recovered from myxomatosis, compared with normal does, may be due to the greater average age

of the recovered animals, for the litter size increases with repeated pregnancies (Brambell, 1944). Observations of recovered bucks in the laboratory indicate that their fertility is often unaffected, although there might be occasions when damage to the external genitalia will prevent coitus after recovery. There are indications (Sobey, personal communication) that some bucks are sterile immediately after recovery but subsequently become fertile.

SUMMARY

Field observations supplemented by laboratory tests were carried out on the occurrence of myxomatosis at two sites with contrasting environments near the northern limit of rabbit infestation in Australia, during the period May 1952 to April 1953.

In spite of the very low incidence of cases of myxomatosis observed in wild rabbits during that period great reductions in the rabbit population occurred.

Analysis of the results of serum surveys indicates that myxomatosis was present and caused minor unobserved epizootics during this period, The overlap in this area of rabbit breeding and active transmission of myxomatosis probably led to a high death rate in the sub-adult rabbit population.

In spite of continued reintroduction of fully virulent standard virus into the areas, all strains of virus recovered were somewhat attenuated. There was a suggestion that at Texas the degree of attenuation increased during the study period.

Sex ratios and data on reproduction are presented for the 1488 rabbits shot at the two areas. Recovery from myxomatosis appeared to have no adverse effect on the fecundity of the female rabbit.

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