**Myxomatosis: the transmission of a highly virulent strain
of myxoma virus by the European rabbit flea
Spilopsyllus cuniculi (Dale) in the Mallee region of Victoria**

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SUMMARY

The European rabbit flea *Spilopsyllus cuniculi* (Dale) was introduced into Australia to act as a vector of myxoma virus. It was first released in the semi-arid Mallee region of Victoria in 1970 where epizootics caused by field strains of myxoma virus occur each summer. Introductions of the readily identified Lausanne strain were made annually following the release of the flea. The introductions were successful and the strain persisted for up to 16 weeks despite competition from field strains.

The Lausanne strain is more readily spread by fleas than the Glenfield strain which has been widely used in rabbit control. The ability of the Lausanne strain to persist and its effective transmission compared with the Glenfield strain may be due in part to the more florid symptoms of the disease.

INTRODUCTION

When myxoma virus was first introduced into Australia in 1950 little was known about the transmission of the virus from rabbit to rabbit although Bull & Mules (1944) had stressed the necessity for the presence of insect vectors. The role of certain species of mosquito vectors became apparent when the first epizootics occurred and it was established that myxomatosis would develop in association with local and seasonal vector activity (Fenner & Ratcliffe, 1965). Early workers in the United Kingdom believed that there were very few if any mosquito vectors in that country, although Service (1971) found that several species of *Aedes*, *Culiseta*, *Culex* and *Anopheles* were more common than originally thought. When myxomatosis spread through the rabbit population of the United Kingdom it was found that the European rabbit flea, *Spilopsyllus cuniculi* (Dale) was the important vector (Andrewes, Thompson & Mansi, 1959). The rabbit flea was introduced into Australia in 1966 to act as a permanent vector of myxomatosis within the rabbit population (Sobey & Menzies, 1969). When the flea was introduced little was known in Australia about its ability and requirements as a vector. It was thought that the flea would possibly tend to select myxoma virus strains of higher viru-

lence than mosquito vectors selected, and that it might be possible to maintain the virulence of field strains at a higher level in regions where the flea could be established (Fenner & Ratcliffe, 1965). It was also thought that the flea might transmit myxomatosis throughout the year causing 'trickle' outbreaks rather than epizootics. Consequently the rabbit flea was released at several sites in the Mallee region of Victoria in 1970 and became well established in about three years (Shepherd & Edmonds, 1976).

A readily identified and highly virulent strain of myxoma virus (Lausanne) was introduced into the rabbit population at intervals from 1970 on and the population was counted monthly and sampled at regular intervals for strains of myxoma virus. The Glenfield strain was used for comparison on some occasions.

This paper reports on the techniques used to introduce the Lausanne strain and the effectiveness of the rabbit flea in maintaining the strain in the rabbit population in competition with field strains.

MATERIALS AND METHODS

Site

The rabbit flea was released at seven sites at 'Pine Plains' in the Mallee region (Shepherd & Edmonds, 1976).

Rabbit counting transects were established covering three of the release sites.

Myxoma virus was introduced either as spot introductions into specific warren complexes or along the regularly marked transect.

Myxoma virus

The Lausanne strain was used as either a freeze-dried powder prepared by the Commonwealth Serum Laboratories, Melbourne, or as virus in scarified tissue from a multiply-infected rabbit. The Glenfield strain used was C.S.L. freeze-dried powder.

Introduction of virus

In most cases the virus was introduced into the rabbit population on rabbit fleas. One vial of C.S.L. virus or 1 g of scarified tissue was mixed with sufficient Hanks' General Medium plus horse serum to make a viscous paste and then mixed with 1000 fleas. One thousand fleas were liberated into 10-15 active warrens per 400 m of the transect during September-December before the normal summer myxomatosis epizootics occurred.

Virus was also introduced by catching rabbits, inoculating them with Lausanne strain and releasing them where they had been caught. The rabbits for inoculation were caught by trailing with alpha-chloralose along a 1.6 km trail of which half was in a flea-free area. Each rabbit was tagged, a blood sample taken, inoculated with Lausanne strain and released. The blood samples were returned to Keith Turnbull Research Institute and tested for antibodies to the soluble antigens of myxoma virus.

Collection and testing virus from the field

Virus present in the rabbit population was collected by shooting infected rabbits, cutting an eyelid from each rabbit, placing it in a bottle and returning it to the laboratory in a portable refrigerator. Each sample was tested by passaging through one laboratory rabbit and then into five laboratory rabbits. The symptoms were observed and the survival time was used to grade the strains from Grade I (highly virulent) to Grade V (severely attenuated) (Fenner & Ratcliffe, 1965).

RESULTS AND DISCUSSION

The first attempts to put the Lausanne strain of myxoma virus into a susceptible rabbit population were made in November 1970 using both inoculation of wild rabbits and distribution of fleas carrying the Lausanne strain provided by Dr W. R. Sobey, C.S.I.R.O., Sydney. Twenty-seven rabbits from the flea-free trail and 30 rabbits from the trail where fleas were present, including 21 carrying fleas, were inoculated. At the same time fleas carrying the Lausanne strain were released into warrens in two areas, Blow Hole, where fleas were already present and Mt Jenkins where there were no fleas.

The data on virus samples collected after these introductions are shown in Table 1. In the inoculation area the Lausanne strain was recovered after two or three cycles. At Blow Hole, in the Lausanne flea area, where fleas carrying virus were released, it was recovered after sufficient time for four or possibly five cycles but we cannot be sure that the virus did not persist on the mouth parts of the fleas we released and cause new infections after several weeks.

Similar releases were made in subsequent years. Virus strains were collected as observed and no attempt was made to select for the Lausanne strain in particular. Each year the Lausanne strain was released before field strains were observed but the releases were followed by the annual summer outbreak of field strains.

The grades of the strains collected from 1971 to 1974 are shown in Table 2. No Grade I strain other than those with typical Lausanne symptoms were found. The occurrence of attenuated field strains was similar whether they were collected close to the flea release sites or not and whether the rabbits carried fleas or not. However, most Lausanne samples were collected close to the flea release sites and on rabbits carrying fleas.

Although the Lausanne strain was successfully established in the rabbit population it did not spread widely in competition with the field strains. However, it has persisted in the population for up to 16 weeks after one release of fleas carrying the strain despite the occurrence of an epizootic of field strains six weeks after the fleas were released.

It has been generally accepted that the Glenfield strain which was widely used for field releases by rabbit control authorities does not persist in the field in competition with field strains in the presence of mosquito vectors. The longest recorded period between field release and recovery was 10 weeks (J. Edmonds & I. Nolan, unpublished data) and the strain usually disappeared within three or four weeks.

Table 1. *Samples of the Lausanne strain of myxoma virus collected from the field following introduction of the virus, 21-25 November 1970*

Area	Rabbit number	Date collected	Mean survival time of lab. rabbits tested (days)	<i>S. cuniculi</i> present on the rabbit
Inoculation area	P 3	15. xii. 70	11.5	Yes
	P 11	16. xii. 70	11.5	Yes
	P 97	8. i. 71	10.0	Yes
Lausanne flea area	P 153	24. i. 71	13.0	Yes
	P 2	15. xii. 70	12.5	No
	P 183	24. i. 71	12.0	No

Table 2. *Myxoma virus strains collected on Pine Plains from 1971 to 1974*

Grade of virus strain	Collected within 0.8 km of flea release point		Collected further than 0.8 km from flea release point	
	Rabbits with <i>S. cuniculi</i>	Rabbits without <i>S. cuniculi</i>	Rabbits with <i>S. cuniculi</i>	Rabbits without <i>S. cuniculi</i>
Lausanne	14	3	1	0
Grade I	0	0	0	0
Grade II	5	9	1	3
Grade III	17	29	8	16
Grade IV	2	5	0	2
Grade V	0	0	0	0

We have made four releases of the Glenfield strain simultaneously with the Lausanne strain but have failed to recover it. The maintenance of the Lausanne strain for up to 16 weeks, and its persistence through several cycles in competition with field strains, suggest that, in the presence of field strains, there is less strong selection against the Lausanne strain than against the Glenfield strain. This may be due to the more florid symptoms of the disease produced by the Lausanne strain than by the Glenfield strain and hence to a greater availability of virus particles to a biting insect.

Experiments carried out on the transmission by *S. cuniculi* of various grades of myxoma virus (Mead-Briggs & Vaughan, 1975) showed that it was more efficient at transmitting strains in the middle range of virulence, i.e. in the range of virulence that has evolved in the field in Australia. The ability of the Lausanne strain to persist for several cycles in the field in competition with field strains may be due in part to differences in flea behaviour on severely and moderately diseased rabbits. We have observed that some severely diseased rabbits carry fewer fleas than moderately diseased rabbits, possibly because the fleas leave the severely diseased rabbits in response to high body temperature. There may be, therefore, a greater movement from rabbit to rabbit of fleas carrying the Lausanne strain than of fleas carrying field strains.

In laboratory trials no wild rabbit has recovered from infection with the Lausanne strain. If no Lausanne infected rabbits recover in the field then the fleas from almost all these rabbits become available to be picked up by other rabbits, whereas there may be little movement of fleas from those field strain infected rabbits which survive.

It is difficult to determine the numbers of rabbits infected with the Lausanne strain or field strains when both are circulating. The shorter period during which rabbits infected with the Lausanne strain show symptoms means that collection is biased against the Lausanne strain. Fenner, Poole, Marshall & Dyce (1957) estimated that, in a system where the Lausanne strain and field strains were circulating together, 70 % of cases were caused by the Lausanne strain although only 39 % of the samples collected were the Lausanne strain. If this sort of relationship holds for our releases then the Lausanne strain may have caused about 50 % of the cases.

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