Evolutionary aspects of Myxomatosis in Australia*

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INTRODUCTION

Myxomatosis was first recognized in Montevideo, Uruguay, in 1896 by Sanarelli. During the next twenty years Brazilian workers, notably Dr. H. Beaurepaire Aragão and Dr. A. Moses, produced detailed accounts of the symptomatology and pathology of the disease in laboratory rabbits and Dr. S. Torres demonstrated that it was transmited by mosquitoes. In the course of his researches Dr. Aragão was so impressed with the very high lethality of the disease, and its specificity for the European rabbit (*Oryctolagus cuniculus*) that in 1918 he suggested to the Australian Government that it might be useful in combatting the rabbit plague in Australia. Prolonged correspondence followed, and after initial difficulties a specimen of the virus was successfully transported to Sydney, New South Wales, and in 1926 this was tested by officers of the Department of Agriculture of the State. The initial experiments led the Australian workers to believe that it would not spread successfully from one warren to another, and little further work was carried out in Australia for about ten years.

In 1933 Dame (then Doctor) Jean Macnamara persuaded the Australian Government to resume the study of myxomatosis as a method of rabbit destruction; and Sir Charles Martin, who had recently retired from the directorship of the Lister Institute in London, undertook intensive studies in Cambridge. His report (1936) was the basis of subsequent Australian work, and following its publication the Council for Scientific & Industrial Research of Australia (C.S.I.R.) brought the virus from Cambridge to Melbourne. Dr. L. B. Bull undertook studies relating to the specificity of the disease and its mode of spread; and made field trials in arid pastoral country in South Australian. The

* Recebido para publicação a 31 de Outubro de 1955.

war interfered with this work after 1942 but in 1949 field experiments were extended by the C.S.I.R. (now the Commonwealth Scientific & Industrial Research Organization — C.S.I.R.O.) in well- watered areas of the Murray Valley. Intensive efforts to establish the virus in the Australian rabbit population, carried out by the newly formed Wildlife Survey of C.S.I.R.O., under the leadership of Mr. F. N. RATCLIFFE, eventually succeeded, and in the summer of 1950-51 the disease spread with amazing speed over the greater part of the Murray-Darling basin of south eastern Australia. The first outbreak was confined to the environs of rivers and creeks, but later summer outbreaks carried the disease throughout the rabbit-infested areas of south eastern Australia.

THE EVOLUTION OF MYXOMATOSIS IN AUSTRALIA

In this paper I will attempt to synthesize information on the evolution of the virus, the rabbit, and the product of their interaction, the disease, in the five years that have elapsed since the introduction of this new, highly lethal virus into the vast population of fully susceptible host animals. Before doing that it is necessary to refer again to an investigation by Dr. ARAGÃO. The natural history of myxomatosis in South America had always been obscure, and for forty years the disease was recognized only by sporadic highly fatal outbreaks indomestic or laboratory rabbits. In 1942 Dr. ARAGÃO published papers which described two major discoveries, namely that myxomatosis survived in nature in South America as a benign tumour-producing disease of the tapeti (*Sylvilagus minensis* syn. *braziliensis*), and that it could be transferred from the wild rabbit to other tapetis, or to laboratory rabbits, by mosquitoes. He further showed that in all probability mosquito transmission was mechanical in nature.

Apart from its practical value in reducing the ravages of Australia's most serious pest, the rabbit, the occurrence of myxomatosis offered a unique opportunity to study the adjustments of host and parasite which might follow the introduction of a high lethal new disease into a large and widespread population of mammals. This opportunity was seized in a collaborative study carried out by three groups of. workers in C.S.I.R.O. (Wildife Survey Section, Division of Entomology, and Section of Animal Genetics), and the Department of Microbiology of the Australian National University; and the present interim report summarizes the changes which have been recognized so far. There is ample evidence that the final balance has not yet been attained, but important trends have now become apparent.

To study evolutionary changes in a virus and its animal host is necessary to use techniques which will allow the independent examination of these two components. Virus strains can be tested in rabbits

of reasonably uniform genetic makeup, of a uniform age, and in a uniform physiological condition. Laboratory rabbits fulfil these requirements. Changes in the genetic resistance of rabbits exposed to myxomatosis can be studied by planned breeding experiments, or by periodic testing of suscetible wild rabbits obtained from localities in which the parent population has been universally infected with myxomatosis, using a stable preparation of virus which has a uniform and constant virulence for unselected (laboratory) rabbits.

CHANGES IN THE VIRULENCE OF THE VIRUS

Soon after myxomatosis became widespread in Australia there was evidence that changes were occurring in the case-mortality rate. Since the evidence from the field suggested that the initial change was from over 99.5% to 90% the problem arose of comparing two strains of virus of such high virulence. As mosquito bite infection was by far the most important mode of natural infection in Australia, it was decided to mimic this by inoculating viruses of known and unknown virulence intradermally, in doses of 5-10 rabbit-infectious particles. It was not possible to compare the mortality rates associated with different virus strains, for the numbers of rabbits required to detect significant differences would be proibitive. The symptomatology and survival times of rabbits infected with small doses of different strains of virus, inoculated intradermally, were therefore compared and considerable and reproducible differences were found in both. The easiest to measure was the survival time, and experiments were therefore conducted with a few selected strains to see how accurately differences in the survival times reflected differences in the mortality rates. The results are summarized in Table I and provide a basis for using the survival time of rabbits infected in a uniform manner as a measure of the lethality, or virulence, of different strains of virus.

TABLE 1.

COMPARISON OF THE MORTALITY RATES AND AVERAGE SURVIVAL TIMES OF RABBITS INOCULATED INTRADERMALLY WITH SMALL DOSES (10 ID_{50}) OF THE VIRULENT STANDARD LABORATORY STRAIN AND THE SLIGHTLY ATTENUATED KM13 STRAIN OF MYXOMA VIRUS. SURVIVORS ALLOTED A SURVIVAL TIME OF 60 DAYS.

NUMBER OF RABBITS	STRAIN OF VIRUS	MORTALITY RATE	AVERAGE SURVIVAL TIME	
			ALL RABBITS	FATAL CASES ONLY
34	standard	100%	10.9 days	10.9 days
180	KM13	81%	28.0 "	24.0 "

By this means it was possible to examine the virulence of fifty eight strains of virus recovered from the field in Australia, as well as three strains from South America, two from California, and fifteen from

Europe. The results are shown in Tables 2 and 3. It is clear that attenuated strains appeared quite early all over Australia. For exemple

TABLE 2.

THE MEAN SURVIVAL TIMES OF GROUPS OF FIVE RABBITS INFECTED BY THE INTRADERMAL INOCULATION OF SMALL DOSES (10 ID_{50}) OF STRAINS OF MYXOMA VIRUS USED FOR DISTRIBUTION IN THE FIELD IN AUSTRALIA AND ISOLATED FROM NATURALLY INFECTED CASES IN THE FIELD DURING THE LAST FIVE YEARS.

ORIGIN OF VIRUS	MEAN SURVIVAL TIME		
	15 days	15—30 days	30 days
Virus for distribution	1		
C.S.L.	1		
Glenfield	1		
I.M.V.S.	1		
Virus recovered from field			
1950—1	1	0	0
1951—2	5	0	0
1952—3	1	18	1
1953—4	4	11	2
1954—5	3	12	0
TOTALS — field recoveries	14	41	3

TABLE 3.

THE MEAN SURVIVAL TIMES OF GROUPS OF FIVE RABBITS INFECTED BY THE INTRADERMAL INOCULATION OF SMALL DOSES (10 ID_{50}) OF STRAINS OF MYXOMA VIRUS RECOVERED FROM SOUTH AMERICA, CALIFORNIA AND EUROPE.

ORIGIN OF VIRUS	MEAN SURVIVAL TIME	
	15 days	20—30 days
Brazil	3	0
California	2	0
Europe		
1952 (inoculated)	1	0
1953	3	0
1954	7	0
1955	2	2

the first epizootic at Lake Urana in southern New South Wales occurred in October-November, 1951. It was caused by the fully virulent virus inoculated into rabbits there, and was associated with a case mortality rate greater than 99.5%. A second epizootic, wich occurred spontaneously a year later, was characterized by a case mortality rate of 90%, and 8 strains of virus recovered from mosquitoes or rabbits were all associated with survival times of about 20 days (compared with 11 days for the fully virulent strains). Myxomatosis first ocurred at Dunroy, in northern New South Wales, in March 1951. Virus recovered from there in May 1952 was definitely attenuated, the mean survival time being 21 days. This pattern, the emergence and establishment

of slightly attenuated strains, in spite of widespread inoculation campaigns in which the fully virulent virus was used, was almost universal in Australia.

In Europe, on the other hand, the virulence appeared to be much more stable, a fact which is accentuated by the absence of artificial reintroduction of a fully virulent strains of the virus. The first attenuated strain to be recovered, from the department of Loiret, was obtained in April 1955; two years after the first appearance of the virus in that area.

Another feature of interest has been the failure of more attenuated strains of still lower virulence to spread. Ocasional recoveries have been made of strains associated with mean survival times of 30-40 days, and mortality rates of about 70%. Although the first such strain was recovered in February, 1953 (Uriarra strain of Dr. MYKYTOWYCZ), it was subsequently replaced in that area by a less attenuated strain which resembled the KM13 type.

CHANGES IN THE GENETIC RESISTANCE OF RABBITS

The possibility that the genetic resistance of rabbits may be altered by the selective action of myxomatosis is being investigated from two aspects. Members of the Animal Genetics Section of C.S.I.R.O. are conducting extensive breeding experiments on lines of rabbits selected for high resistance to myxomatosis, using one of the slightly attenuated Australian field strains of virus. No results are available yet from this work, but it promises to give accurate information on the degree of hereditability of genetic resistance, and on the rapidity with which a high degree of resistance, and on the rapidity with which a high degree of resistance can be built up.

The other approach is based upon tests of wild rabbits from various parts of Australia where the epizootic history is accurately known. In the areas selected, the principal one being Lake Urana in southern New South Wales, epizootics occur each summer. After the epizootics the relict population has been sampled serologically to determine the proportion of recovered animals. In the areas used this has been almost 100%. During the following spring, when the progeny of these animals are a few weeks old an are leaving the burrows, they are captured and reared in the laboratory until they are about four months old. In this way a group of young rabbits can be assembled which are the offspring of parents which have recovered from myxomatosis, and which are themselves old enough to have lost any antibody acquired from the mother, and to give an adult reaction to infection with myxoma virus. These rabbits are then challenged with a slightly attenuated Australian field strain of the virus, a single preparation stored at —70°C being used throughout all the experiments. So far it has been possible to test no more than a "normal" wild rabbit population, reared in the laboratory from animals which had never been exposed to myxomatosis, and groups of rabbits from areas which have undergone one, two or three epizootics. Nevertheless, the evidence obtained is highly sug-

gestive. Whether based on mortality rates or survival times, it seems certain that the rabbit population left after three severe epizootics is genetically more resistant than the original population.

It is important to note that all challenge experiments have been made with an attenuated strain of virus, for this allows the expression of minor degrees of genetic resistance which would be obscured if the highly virulent standard laboratory strain of virus were used. The design of the experiments precluded the confusion of genetic resistance and acquired immunity of the immunological kind.

THE TRANSMISSION OF MYXOMATOSIS

It has been clearly established that in Australia the major epizootics of myxomatosis are due to the activity of mosquitoes, and detailed laboratory studies have fully confirmed ARAGÃO's contention that mosquito transmission is mechanical in nature, and that the important source of virus for the mosquitoes is the skin lesions.

From the point of view of natural selection of the virus, the dominant feature of the host-parasite relationship is the transmissibility of the virus by mosquitoes. It is rather difficult to reconstruct the process by which a mutant strain of virus, of lowered virulence, becomes established in the rabbit population. We know that there is an irregular infection of cells in the superficial layers of the skin, and that usually the clone of virus particles in one cell arises from a single virus particle which infects the cell. If we imagine that this infecting virus particles is the one in million particles which has undergone a mutation, occasionally a probing mosquito may become infected with the progeny of this mutant strain of virus, and with no other virus particles. It may then infect one or several susceptible rabbits with this mutant strain. If the mutation is, as is almost certainly the case, of diminished virulence, the infected rabbits will live longer than those infected with fully virulent strains. At the end of the transmission season the only infected rabbits left may be those bearing the mutant strain, which may then die out, or may persist through the winter, perhaps by contact infection. With the onset of the next mosquito transmission season this mutant strain may be widely spread.

In Australia the situation has been complicated by the extensive and very large scale inoculation campaigns which have been conducted each year. In these the fully virulent laboratory strain has been used, and attempts are usually made to spread the inoculations over the expected period of mosquito transmission. We are better informed on the results of competition between an attenuated and a virulent strain of virus than we are on the actual emergence of an attenuated mutant strain. We know that the skin titre, and the transmissibility of all the common Australian field strains, and of the European strains are the same. Of the virus strains tested only the grealy attenuated neuromyxoma variant, which is rarely fatal after the intradermal inoculation of small doses of virus, multiplies to such low titre in the skin as to render

mosquito transmission difficult. If the skin titres attained by the slightly attenuated strains are much the same as those found with the fully virulent strains other features of the disease must dominate the competition for transmission. The obvious one is the period of infectivity of an infected rabbit, and it is in this feature that the slightly attenuated strains are in an advantageous position. Rabbits infected with the standard laboratory strain become infectious for mosquitoes about five days after they are infected, and are usually dead five days later. Rabbits infected with a slightly attenuated strain become infectious about the same time, but may be highly infectious for mosquitoes for a much longer period — an average of about twenty five days. Further, during much of this period they are very sick, and will not disturb mosquitoes attempting to feed on them. With greater degrees of attenuation healing of skin lesions occurs even in rabbits which eventually die, and the period of infectivity then terminated by the fall in skin titre which accompanies healing of the lesion. The important features of several virus strains, from the viewpoints of virulence and transmissibility, are shown in Table 4.

TABLE 4.

THE PRINCIPAL CHARACTERISTICS OF SIX STRAINS OF MYXOMA VIRUS.

STRAIN OF	ORIGIN	Mean survival time (days)	First signs of generalization (day)	Eyes completely closed (day)	Field mortality rate	Period of infectivity (days)
Standard	Initiated Australian epizootics	11	6—7	9—11	99%	5
Neuromyxoma	Laboratory variant	a l survive	6—7	never	0	0
MK13	Australian field strain (attenuated)	23	6—8	14—18	90%	30
Uriarra	Australian field strain (attenuated)	38	6—7	11—12*	60%	12
Lausanne	Initiated European epizootics	13	6—7	9—11	99%	7
Loiret 55	European field strain (attenuated)	27	7—8	17*	90%	30

* eyes sometimes do not close completely.

FUTURE TRENDS IN THE HOST-PARASITE BALANCE IN MYXOMATOSIS IN AUSTRALIA

In 1955, five years after the introduction of myxomatosis into Australia, we can list three features of major importance in determining changes in the host-parasite balance. Firstly there is a strong selective advantage for a disease which combines extensive skin lesion bearing large amounts of virus with prolonged survival of the rabbit, preferably in a sick condition. This situation may be attained in two ways. In the first few years of myxomatosis in Australia the rabbit population was uniform by very highly susceptible, and the type of disease just described was produced by the emergence of attenuated strains of the virus. Now, however, there has been a trend towards increased genetic resistance in the rabbit population, at least in areas where annual epizootics occur. It must be emphasised here that there are very large areas of rabbit-infested land in Australia where only one or two outbreaks have occurred, so that the changes to be described may be deferred for several years in these areas. If one can legitimately extra-

polate from the available data obtained from areas subjected to severe epizootics each year, it can be predicted that the motality rate of rabbits in these areas, where they are infected with the slightly attenuated strains of virus at present dominant in Australia, will reach a level of 50% after a further 3-5 years. But the situation is not simple, for it is possible that in such resistant rabbits infection with the highly virulent standard laboratory strains would cause a disease resembling that due to the slightly attenuated virus in a fully susceptible population, and the selective advantage would then swing to the highly virulent strain. It is possible but not certain that resistance to the now dominant highly virulent strain would also become progressively greater, so that in time even the most highly virulent strain (for laboratory rabbits not subjected to selection) might cause a mortality of 50% of less.

LESSONS TO BE LEARNT FROM SOUTH AMERICA

In the previous section I have discussed evolutionary trends in Australia, as we see them five years after the introduction of the disease into the wild rabbit populations. It is not inconceivable, however, that some of the answers we seek may be immediately available from surveys made in South America. Perhaps the situation may best be summed up by posing a number of questions.

(1) What is the natural history of myxomatosis in *Sylvilagus*, and in particular, how does the disease over-winter? Does prolonged mild disease due to infection of young animals play the part here that KILAM suspects it does in fibroma of cottontail rabbits in North America?

(2) What is the geographical range of myxomatosis of *Syvilagus* in South America? Is the diesase coexistensive with the rabbit?

(3) What is the explanation of the occasional outbreaks of myxomatosis in European rabbits in places like Montevideo (1896 and 1947)?

(4) Has myxomatosis been the controlling influence which has prevented the colonization of parts of South America apparently favourable for *Oryctolagus*? What is the status, as far as genetic resistance to myxomatosis is concerned, of the wild *Oryctolagus* rabbits which occur at the southern border of *Sylvilagus* occupation, as in Northern Uruguay?

(5) What is the pathogenic behaviour in *Silvilagus* of strains of myxoma virus which are attenuated in their virulence for *Oryctolagus*?

Such questions present a challenge to scientists in South America. Myxomatosis was a disease of the tapetis of Brazil which occasionally ravaged colonies of domestic rabbits, as ARAGÃO showed thirteen years ago. Now it is a disease of four continents, South and North America (California), Autralia and Europe. And in the latter two continents it has undoubtedly caused the most destructive outbreaks of any infectious disease of mammals, and has killed more animals in a shorter time than any other disease known to man.