

An outbreak of myxomatosis caused by a moderately attenuated strain of myxoma virus

BY PAUL J. CHAPPLE* AND N. D. LEWIS

*Ministry of Agriculture, Fisheries and Food, Infestation Control Laboratory,
Worplesdon, Surrey*

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INTRODUCTION

Since the original epizootic of myxomatosis in this country (1953–55) carcasses of rabbits from outbreaks of the disease have been sent regularly to the Central Veterinary Laboratory, Weybridge, but no detailed investigations have been carried out on outbreaks of disease in natural populations in Great Britain since 1954 (Armour & Thompson, 1955). In July 1962 a rabbit was sent from the East Riding of Yorkshire and as a matter of routine this establishment was informed of the outbreak. The population of rabbits concerned was large and occupied an area of approximately 200 acres. The strain of virus causing the outbreak was attenuated and when this was discovered efforts were made to secure samples for as long as the outbreak lasted.

MATERIALS AND METHODS

Carcasses were obtained from July 1962 until January 1963 at fortnightly intervals. The majority were shot or taken by dogs.

Rabbit fleas

In a disease the spread of which is influenced to a large extent by its vectors, it was thought advisable to examine the numbers of the principal vector (the rabbit flea—*Spilopsyllus cuniculi*) on individual rabbits.

Counts of fleas were made on rabbit carcasses which had been put into flea-proof linen bags immediately after killing.

Virulence tests

With a large initial population it was hoped that the outbreak would be sufficiently prolonged for the detection of any change in the virulence of the virus strain concerned. Samples of rabbits were obtained at fortnightly intervals and some of these were used for carrying out virulence tests.

These were carried out after the manner used in Australia by Fenner & Marshall (1957) and later in Great Britain by Chapple & Bowen (1963):

(a) *Rabbits*. New Zealand Whites weighing 4–4½ lb. kept in individual cages in a room with a constant temperature (60° F.).

* Present address: The World Health Organization, International Reference Centre for Respiratory Virus Diseases, Common Cold Research Unit, Salisbury, Wiltshire.

(b) *Virus preparation.* Virus was extracted by grinding lesion material with sterile sand in a cold pestle and mortar. The homogenate was suspended in a diluent containing phosphate buffer, skim milk, penicillin and streptomycin (Westwood, Phipps & Boulter, 1957). Sand and debris were removed by light centrifugation (2000 r.p.m. for 10 min.). The supernatants were stored at -20° C. and titrated immediately before use.

(c) *Titration of virus preparations.* Virus suspensions were titrated on the chorio-allantoic membrane of 11-day-old chick embryos according to the technique of Westwood, Phipps & Boulter (1957).

(d) *Route of inoculation.* Groups of rabbits were inoculated intradermally into the shaved right flank with 0.1 ml. quantities of the virus suspensions. Five rabbits were used for each virus preparation. A record was kept of the clinical symptoms and survival times.

Gel diffusion precipitin reaction

We wanted to examine the usefulness of the gel diffusion technique in the study of myxomatosis. With this in view 124 rabbits were obtained during 3 consecutive days in September. Each rabbit was examined against positive antigen and anti-serum for the presence or absence of myxoma antigens and antibodies. From an economic point of view it was also important to know the recovery rate in the population.

The technique as described by Chapple, Bowen & Lewis (1963) was used. Pieces of lesion material, lung and blood clot were tested against known myxoma antigen and antiserum.

A watch was also kept on the behaviour of the disease and on any possible correlation between sex, disease incidence and recovery rate. A record was kept of the weight of each unpaunched carcass.

EXPERIMENTAL RESULTS

Field history and rapidity of spread

(Numbers in brackets refer to points on Fig. 1. The quotations are from the field officer's reports.)

January 1962—myxomatosis was seen near the railway line, but apparently died out. The field officer reported that twenty-four bodies were seen but that the disease was leaving 'far too many' apparently healthy rabbits.

March 1962—the disease was first officially observed at Jackdaw Plantation (1), but there was no evidence of spread. The farmer said he first saw it in this area about May.

2 July 1962—rabbit from point (2) examined by gel test in the laboratory with a positive result.

19 July 1962—three infected rabbits seen.

23 July 1962—four infected rabbits seen.

7 August 1962—'disease spreading' (but no bodies found).

13 August 1962—approximately twelve infected rabbits seen. Diseased rabbits seen in East Dale for the first time (3).

20 August 1962—'disease seems to have passed its peak as less infected rabbits and no bodies seen'.

21 August 1962—approximately twelve infected rabbits seen.

27 August 1962—approximately twelve infected rabbits seen.

On the first visit beyond the north-west end of Jackdaw Plantation (point 4) five infected rabbits were seen and disease may have been present there for some time.

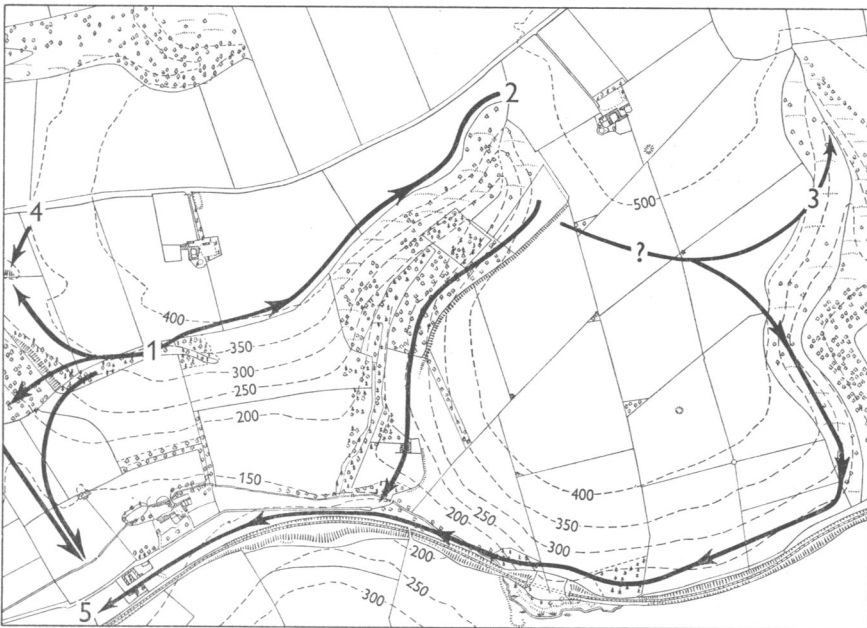


Fig. 1. Map showing St Austin's Dale, and direction of spread of outbreak. Scale, 3 in. = 1 mile. Reduced from the 6 in. = 1 mile ordnance survey map, by kind permission of H.M. Stationery Office.

3 September 1962—'disease position seems static'.

17–19 September 1962—sample of 124 rabbit carcasses obtained. The field officer considered that the peak infection was reached about 10 days before.

12 November 1962—disease thought to be approaching its peak in south-west corner (point 5).

10 December 1962—only one rabbit was seen and as a result the regular sampling of the population was terminated.

The field officer said he had not seen a 'typically' infected rabbit since the big sample. In fact the last was apparently obtained on 15 October. Others obtained were infected but seemed to be recovering.

On 6 March 1963 the keeper said that over the preceding 5 weeks he sold 234 rabbits in excellent condition. He also rejected some twelve or fifteen that showed symptoms of apparent recovery (one was sent to the laboratory on 6 March 1963). He also mentioned that three of the healthy ones contained 'a very hard ball-like object sort of gathered on the backbone which on being cut produced a white curd-like foul-smelling substance'. Presumably this was a chronic abscess produced

as a result of secondary infection of a regressing myxomatosis lesion. He also said at this time (6 March 1963) that the Jackdaw Plantation rabbits were all poor, undersized specimens though normally with no evidence of any disease.

Direction of spread

We have no knowledge of spread from the railway line and presumably the January outbreak died out there. According to the keeper it did not spread from Jackdaw Plantation. However, the farmer observed its spread along the edge of the dale to point (2), where a field officer first saw an infected rabbit.

From St Austin's Dale the disease was thought to have spread to East Dale (3). Throughout, all unwanted rabbits killed by dogs, shooting and by hand were buried in rabbit holes in the vicinity.

From East Dale, St Austin's Dale and Jackdaw Plantation the disease spread to the south-west corner of the estate (5)—on the site of the January outbreak. It seemed to be approaching a peak here on 12 November 1962.

Gel diffusion precipitin test

The studies on the precipitin reaction in agar gel have been reported in detail elsewhere (Chapple *et al.* 1963) and it is sufficient here to outline the results. Of the 124 rabbits examined there were twenty which appeared clinically healthy, showed no detectable antibody or antigen, and so were presumed to be susceptible to myxoma virus. A further group of fifteen apparently healthy rabbits could be divided into a group of ten which showed only antibody in the tissue examined and were therefore presumed recovered from infection; the remaining five showed the presence of antigen only and were presumed to be in the early stages of infection.

Of the rabbits which were clinically infected seventy-eight showed antigen, antigen and antibody or antibody in other tissues as well as blood. One had no antigen in its tissues and antibody was found only in the blood. One had neither antigen nor antibody in its tissues.

Table 1. *Summary of test results and clinical diagnosis*

(Based on Chapple *et al.* (1963), Tables 3 and 4.)

Clinical condition	Gel test diagnosis			Total
	Infected	Recovered	Susceptible	
Clean	5	10	20	35
Infected	78	1	1	80
Uncertain (?infected)	3	0	1	4
Uncertain (?clean)	1	0	2	3
Unknown	2	0	0	2

Another two groups with provisional clinical diagnoses (?infected, ?clean) were also examined, but the numbers were too small to draw any conclusion save that the gel test tended to confirm the provisional clinical diagnosis.

The remaining two rabbits' condition on receipt was such that a clinical diagnosis was not possible. The results are summarized in Table 1.

Table 2. Summary of virulence tests on the St Austin's Dale and Devon Strains of myxoma

Virus	Date of isolation	No. of rabbits inoculated	Amount of virus inoculated (pock-forming units)	Time to primary lesion (days)	Time to secondary lesion (days)	Time to severe generalization (days)	Mean survival time (days)
1	TPM/13/62	5	30	2 (2)	4.8 (4-6)	6 (6)	16.6 (12-21)
2	TPM/15/62	5	50	—	—	—	21.6 (14-28)
3	TPM/15/62	5	15	NK	4.0 (4)	6.2 (6-7)	16.2 (11-22)
4	TPM/18/62	4	10	NK	NK	NK	22 (21-23)
5	TPM/19/62	4*	10	NK	NK	NK	22 (20-24)
6	TPM/26/62	5	50	—	—	—	23.3 (19-27, 2S)†
7	TPM/34/62	5	50	—	—	—	23.4 (19-34)
8	TPM/44/62	5	50	—	—	—	25.4 (19-30)
9	TPM/149/62	5	1,600	NK	4 (4)	6 (6)	19 (18-21)
10	TPM/175/62	5	50	—	—	—	20.4 (17-23)
11	TPM/185/62	5	50	—	—	—	23.7 (17-33)
12	TPM/195/62	5	100,000	NK	4.75 (4-5)	6 (6-7)	13.9 (11-16)
13	TPM/195/62	5	50	—	—	—	22.3 (20-25, 1S)
			Devon				
	TPM/3/62	5	—	2.2 (1-5)	6 (7-8)	8.2 (8-10)	18.8 (16-27)

* One died of pneumonia. † S = rabbit recovered from infection. NK, not known.

Attenuation of virus

In order that any attenuation of virus could be detected virulence tests were carried out using material obtained at intervals during the course of the disease. The first sample for such testing was obtained on 23 July 1962 and this gave a mean survival time (M.S.T.) of 16.6 (range 14–21) days. Samples were then tested which had been taken at fortnightly intervals over a period of approximately 4 months.

The results have been summarized in Table 2, and they indicate that no further attenuation of virus occurred during the period in question. Table 2 also illustrates the finding of Fenner & Marshall (1957) that quite large variations in the dose of virus make little difference to the survival times of the rabbits inoculated.

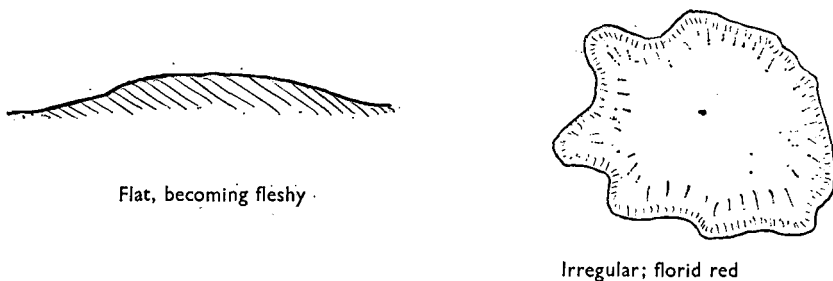


Fig. 2. Diagrammatic representation of the primary lesion caused by the St Austin's Dale myxoma virus strain.

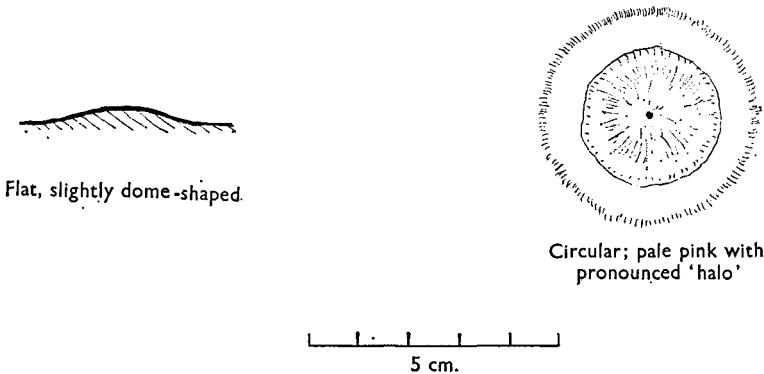


Fig. 3. Diagrammatic representation of the primary lesion caused by the Devon myxoma virus strain.

In all cases the primary lesion was large and irregular in outline with a florid red colour. In contour the primary lesion was flat at first, becoming rather raised and fleshy later. Because of the irregular shape it was not possible to obtain useful measurements of the lesions. A summary of the results of the virulence tests, including times for the onset of secondary lesions and for serious involvement of the head, is given in Table 2. Details of the Devon strain are also given in Table 2. Figs. 2 and 3 are diagrammatic representations of the differences in primary lesions of the St Austin's Dale and Devon strains of virus.

The clinical symptoms of the animals infected in the outbreak were indistinguishable from those observed during the epizootic of 1953–55.

Counts of fleas

Early in the course of the outbreak the field officers who were keeping watch on this rabbit population thought that the disease was not spreading as quickly as was usual in such a situation. It was suggested that the numbers of the principal vector (the rabbit flea) might not have been sufficiently large to initiate a rapid spread. To check this suggestion rabbit carcasses were obtained which had been put, immediately after killing, into flea-proof linen bags. Twenty-three rabbits were obtained in this way and 392 fleas were found, giving an average per rabbit of 17 (range 1–36). There were no rabbits completely devoid of fleas.

DISCUSSION

The area from which these rabbit samples were drawn is a part of the East Riding of Yorkshire known as St Austin's Dale, situated about 9 miles north-east of South Cave. The grid reference for the area is 92/93:33/35 and a map of the area (taken from 6 in. = 1 mile ordnance survey map by kind permission of H.M. Stationery Office) is shown in Fig. 1. This shows the large area of scrub in which the rabbit colony was based. The majority of the rabbits used the scrub for cover and the surrounding arable land as a source of food. The damage to the surrounding crops was considerable, some 5–10 acres of barley and about 5 acres of roots being rendered useless, besides damage to pasture. It is not possible to give an accurate estimate of the rabbit population at the beginning of the outbreak of myxomatosis. However, when the large sample of rabbits was obtained in September 1962 (approximately 6 months after the start of the outbreak) two independent observers counted 195 and 210 rabbits, respectively, on the combined west and north fringes of the dale. Even after the sample of 124 rabbits was obtained the numbers were not markedly diminished. Taking into consideration the time the disease had been present, the amount of damage, the effect of obtaining the large sample and the counts made, the initial population was estimated to be in the region of 1000 rabbits.

The mean survival times of all the samples, except one, are of the same order and it was thought that all showed the same strain of virus and that no further attenuation had occurred. The sample which gave a mean survival time of 13.9 days was retested using a smaller inoculum (50 instead of 10^5 p.f.u.) and the result indicates that this sample was not significantly different from the other samples. In the light of Fenner & Marshall's (1957) evidence that quite large variations in dosage do not make great differences to the survival times, an additional explanation must be sought. The experiment in question was carried out in the winter of 1962–63 when air temperatures were extremely low. Although there was heating in the animal house the temperature outside dropped sufficiently to cause the freezing of the water supply. Previous experience has shown that environmental temperature can have a profound effect on survival times (Mykytowycz, 1956; Fenner & Marshall, 1957; Chapple & Bowen, 1963). We feel that the mean survival time of 13.9 days could have been produced as a result of both the large dosage of virus and the drop in environmental temperature.

From the rabbits obtained there is the suggestion that there was approximately an 8% recovery rate (92% mortality) which accords quite well with the recovery rate found with a virus giving a mean survival time somewhere between 16 and 28 days (Fenner & Marshall, 1957). We do not claim the sample of 124 rabbits to be random. It is more than likely that there was a higher percentage of recovered rabbits in the population, as it is much easier to catch or shoot a relatively immobile diseased rabbit. Making an approximate estimation of the recovery rate of 10% this means that a nucleus of 100 rabbits would be left out of the estimated population of 1000 plus any rabbits which the disease had by-passed. This nucleus would rapidly build up to the original numbers if undisturbed by ordinary control measures. In fact, on a day in June 1963, 293 rabbits were counted and the report added the comment that this number of rabbits obviously represented only a fraction of the total population.

Table 3. *Correlation of sex, infection and recovery rates of 106 rabbits where it was possible to determine sex*

Sex	Totals	Susceptible		Infected		
		Recovered (AB only)	(no AG or AB)	(AG or AG + AB)	AG only	AG + AB
M	47	11	14	22	7	15
F	59	7	7	45	15	30

The significance of the rabbit flea in myxomatosis has been the subject of some discussion (Rothschild, 1960; Andrewes, Thompson & Mansi, 1959) and much of this discussion has been based upon the supposition that the rabbit flea is a sedentary ectoparasite. However, recent work by Mead-Briggs (1964) has shown that this vector is more mobile than was hitherto supposed and we have concluded that the numbers of fleas found on the rabbits (av. 17 per rabbit) from this area would be quite sufficient to promote efficient spread of the disease.

An attempt was made to correlate sex, disease incidence and recovery rate and Table 3 shows the relationship of the presence or absence of antigen and antibody with sex. Although these figures suggest that males have less chance of infection and that if infected they have a greater chance of recovery, the numbers of rabbits involved are too small for any definite conclusions to be drawn.

The record of the weights showed a relatively normal distribution for the time of year, the type of site and method of collection. The sex ratio is also normal for such a sample.

Since the detailed records kept of the original outbreak in the Edenbridge area of Kent, no comparable records have been made of other outbreaks and so our knowledge of the behaviour of attenuated strains of myxoma virus in the field, in this country, is negligible.

Before any conclusions can be reached on the evolution of myxoma virus in Great Britain further detailed studies, combining proper field observations with full laboratory tests, must be made. Such studies are not as difficult as they were 5 years ago when the rabbit population was very low. Since that time certain areas,

especially those where rabbits are difficult to control (e.g. cliffs, quarries, War Department ranges, and those areas where scrub is maintained for game rearing purposes) have suffered a resurgence of the rabbit followed by periodic outbreaks of myxomatosis. It is these areas which are now the potential experimental sites.

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REFERENCES

- ANDREWES, C. H., THOMPSON, H. V. & MANSI, W. (1959). Myxomatosis: present position and future prospects in Great Britain. *Nature, Lond.*, **184**, 1179.
- ARMOUR, C. J. & THOMPSON, H. V. (1955). Spread of myxomatosis in the first outbreak in Great Britain. *Ann. appl. Biol.* **43**, 511.
- CHAPPLE, P. J. & BOWEN, E. T. W. (1963). A note on two attenuated strains of myxoma virus isolated in Great Britain. *J. Hyg., Camb.*, **61**, 161.
- CHAPPLE, P. J., BOWEN, E. T. W. & LEWIS, N. D. (1963). Some observations on the use of the Ouchterlony gel diffusion technique in the study of myxomatosis. *J. Hyg., Camb.*, **61**, 373.
- FENNER, F. & MARSHALL, I. D. (1957). A comparison of the virulence for European rabbits (*Oryctolagus cuniculus*) of strains of myxoma virus recovered in the field in Australia, Europe and America. *J. Hyg., Camb.*, **55**, 149.
- MEAD-BRIGGS, A. R. (1964). Some experiments concerning the interchange of rabbit fleas, *Spilopsyllus cuniculi* (Dale), between living rabbit hosts. *J. Anim. Ecol.* **33**, 13.
- MYKYTOWYCZ, R. (1956). The effect of season and mode of transmission on the severity of myxomatosis due to an attenuated strain of the virus. *Aust. J. exp. Biol. med. Sci.* **34**, 121.
- ROTHSCHILD, M. (1960). Observations and speculations concerning the flea vector of myxomatosis in Britain. *Ent. mon. Mag.* **96**, 106.
- WESTWOOD, J. C. N., PHIPPS, P. H. & BOULTER, E. A. (1957). The titration of vaccinia virus on the chorio-allantoic membrane of the developing chick embryo. *J. Hyg., Camb.*, **55**, 123.