Epidem. Inf. (1989), **103**, 333–357
Printed in Great Britain

Myxomatosis in farmland rabbit populations in England and Wales

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(*Accepted 24 April 1989*)

SUMMARY

The overall pattern and consequences of myxomatosis in wild rabbit populations were studied at three farmland sites in lowland southern England and upland central Wales between 1971 and 1978. When results from all years were combined, the disease showed a clear two-peaked annual cycle, with a main autumn peak between August and January, and a subsidiary spring peak during February to April.

Rabbit fleas, the main vectors of myxomatosis in Britain, were present on full-grown rabbits in sufficient numbers for transmission to occur throughout the year, but the observed seasonal pattern of the disease appeared to be influenced by seasonal mass movements of these fleas. However other factors were also important including the timing and success of the main rabbit breeding season, the proportion of rabbits which had recovered from the disease and the timing and extent of autumn rabbit mortality from other causes.

Significantly more males than females, and more adults and immatures than juveniles, were observed to be infected by myxomatosis. Only 25–27% of the total populations were seen to be infected during outbreaks. Using two independent methods of calculation, it was estimated that between 47 and 69% of infected rabbits died from the disease (much lower than the expected 90–95% for fully susceptible rabbits with the partly attenuated virus strains that predominated). Thus it was estimated that 12–19% of the total rabbit populations were known to have died directly or indirectly from myxomatosis.

Although the effects of myxomatosis were much less than during the 1950s and 1960s, it continued to be an important mortality factor. It may still have a regulatory effect on rabbit numbers, with autumn/winter peaks of disease reducing the numbers of rabbits present at the start of the breeding season.

INTRODUCTION

Myxomatosis is a disease of wild European and domestic rabbits, *Oryctolagus cuniculus*, caused by infection with myxoma viruses which are found naturally in some *Sylvilagus* rabbit species of South America and California. In the natural hosts infection causes only mild symptoms, but in *O. cuniculus* mortality can be high. Myxomatosis is normally transmitted between host rabbits when virus

particles adhere to the piercing mouthparts of a biting insect vector. In Britain the rabbit flea, *Spilopsyllus cuniculi*, is the most important vector of the disease (1) and its role in transmitting different myxoma virus strains has been investigated (2, 3); other blood sucking insects may play a minor role as vectors in some circumstances.

Myxomatosis first appeared in Britain amongst wild rabbits in Kent during September 1953 and in just over 2 years had spread throughout the country (4, 5). It is estimated that during this period over 99% of rabbits were killed nationally (6–8) as virtually every rabbit which became infected succumbed to the disease. Rabbits, however, were not exterminated by myxomatosis and, despite the continued presence of the disease in rabbit populations, their abundance has increased slowly but steadily in Britain during the ensuing 30 years (9), though there has been great instability in numbers within local populations (10, 11). During the initial outbreak a few rabbits were known to have recovered from infection, as indicated by the presence of antibodies in their serum (6) and subsequently increasing numbers of rabbits survived infection by less virulent strains of virus (12, 13). The proportion of such recovered (antibody-positive) rabbits in a population can influence the timing and subsequent severity of disease outbreaks. In Britain there is evidence for a seasonal cycle of myxomatosis occurrence, governed by the breeding cycle of the rabbit and with the resulting increase in numbers of young susceptible rabbits leading to peak incidence of the disease in late summer and autumn, though it is present for most of the year (13, 14).

The changing relationship between the myxoma virus, its vectors and its rabbit host in Britain has been reviewed (1, 15). Most recently, Fenner (16) has reviewed the evolutionary changes in myxomatosis – the attenuation of virus strains in the field and the development of resistance in rabbit populations.

Thus there is much published information about changes in the virulence of myxoma virus strains, the development of enhanced genetic resistance, and the effects of these factors on mortality in the laboratory. However, there is little published information on the pattern of individual myxomatosis outbreaks in Britain, the actual mortality of infected rabbits *in the field*, and the resulting effects on rabbit populations. Armour & Thompson (4) who described the original 1953/4 outbreak in southern England caused by a fully virulent virus strain, assumed that the observed reduction in rabbit numbers during the outbreak (close to 100%) was entirely caused by the disease.

Later, Chapple & Lewis (17), who described an outbreak during 1962 in Yorkshire, caused by a moderately attenuated virus strain, estimated mortality as 92% from the proportion of recovered (antibody-positive) rabbits in shot samples taken during the outbreak. However, as these authors pointed out, this is likely to be an over-estimate as it is biased in favour of the less mobile infected rabbits. Despite, this, these methods probably gave reasonably accurate estimates of the undoubtedly drastic effects of myxomatosis. A more reliable method was required to assess the situation during the 1970s in Britain, where a much lower kill rate was expected than for the original outbreaks. The method adopted in detailed studies of myxomatosis in Australia (18, 19) was considered appropriate, combining estimates of the proportion of rabbits infected during individual

outbreaks and the proportion of these infected rabbits that died from the disease. The characteristics of myxomatosis outbreaks and the effects of the disease were investigated as part of a wider study of the population dynamics of rabbits on three farmland sites between 1971 and 1978 in the UK.

METHODS

Study areas

Three study sites were chosen for detailed study representing varied farming and habitat conditions, with the rabbit populations on them subjected to differing external influences. The two lowland sites (50–200 m altitude) in southern England, Bow Hill in West Sussex (188 ha; 8 km north-west of Chichester) and Bridgets Farm in Hampshire (435 ha; 6 km north-east of Winchester) were intensively cultivated, mixed arable and pasture on basic, chalk substrates; they had mild and relatively dry climates, with moderately high rabbit populations. The contrasting upland site (250–500 m) in central Wales, Bylchau Farm on the Powys/Dyfed border (206 ha; 5 km south-west of Llantwrtyd Wells) was upland sheep pasture on acidic, shale substrate; it had a colder and wetter climate, with a low density rabbit population.

Field methods

Rabbit counts. Standardized counts, conducted on foot along set transect routes were used to monitor rabbit population fluctuations at the three sites. Monthly series of at least four twilight counts using binoculars were carried out at Bow Hill and Bridgets Farm, lasting 1 h after dawn or before dusk. Less regular night counts using a spotlight were carried out at Bylchau Farm, at variable times after dark, supplemented in winter by estimates based on tracks in snow. All rabbits seen during counts or any other casual sightings were classified approximately by size into full-grown (> 4 months) and juveniles (< 4 months). Records were kept of infected rabbits seen during counts and from other casual sightings of rabbits. As such rabbits were usually seen at a distance, only those with advanced symptoms could be detected, when their changed behaviour (slow and uncharacteristic reactions to disturbance) often drew attention to them, so that the presence of swollen eyelids could be confirmed through binoculars.

Trapping. A continuous programme of live-trapping and examination of rabbits was undertaken mainly using unbaited cage-traps set on rabbit runs during April to September, and ferrets run through rabbit burrows with the entrances purse-netted during October to March. Cage-trapping was biased towards catching juvenile rabbits, and ferreting biased towards full-grown rabbits. Other catching methods were occasionally used; stopped-snares, long-nets, drop-nets, permanent wooden 'smeuse' boxes set in otherwise rabbit-proof fences, dogs and by hand. The age-group (as defined below), sex and weight were recorded for all rabbits caught. Every rabbit was individually marked before release.

Rabbits were examined for symptoms of myxomatosis which were classified as in Table 1. Samples for virus isolation were taken on cotton wool swabs from the eyelid lesions of some rabbits showing advanced symptoms, and frozen at -20 °C prior to laboratory testing for virulence. A blood sample was also taken from a

Table 1. *Classification of myxomatosis disease stages, with an approximate timetable for the most frequent Grade IIIA virus strains*

Stage	Timetable (days)	Field symptoms on rabbits	Mean duration (days)
Incubating	0–5	No external symptoms visible No detectable serum antigens	5
Antigen	5–7	Slight lump at site of infection Virus antigens detectable in serum	2
Initial	7–10	Slightly swollen eyelids Watery discharge from eyes	3
Advanced	10–40	Full range of external symptoms*	20†
Recovery	30–60‡	Obvious nodules and scars§	30

* Usually very swollen eyelids with large oedematous lumps (sometimes becoming temporarily blind), swollen ear bases and ano-genital area; often additional swellings on nose, along flanks, down limbs and on feet.

† Death occurs at 17–23 days in some rabbits, hence lower mean duration.

‡ Bare patches and slight scars may persist many weeks beyond this in some cases.

§ Usually nodules and scars on eyelids, ear bases and ano-genital area; often additional nodules/scars on nose, along flanks, down limbs and on feet.

prominent ear vein from most rabbits on first capture and again if caught more than 3 months later, and stored in a refrigerator prior to testing for presence of antibodies to myxoma virus.

Rabbit age determination. Live-trapped rabbits were classified as juveniles (< 4 months), immatures (4–8 months) and adults (> 8 months). Separation of adults from young was on the basis of (i) a closed versus part-closed or open fusion line (notch) between diaphysis and proximal epiphysis of the tibia (20) and (ii) an absence versus presence of a slight or prominent cartilage (lump) at the fusion line between diaphysis and distal epiphysis of the ulna (21). The tibial notch and ulnar lump were detected on live rabbits up to about 8 months old when felt through the skin, and these young were further separated into juveniles based on whether live body weight at first capture was below or above 950 g which corresponds approximately with 4 months old (Cowan & Tittensor, unpublished data).

Mortalities. Regular searches were made for rabbit carcases resulting from natural deaths, while rabbits killed by normal control methods were also examined whenever possible. At Bow Hill restrictions imposed because of game bird interests prevented the use of dogs to aid searching and so the mortality sample was biased by a high proportion of deaths due to control, mainly by night-shooting. At Bridgets Farm trained dogs were used to search the whole site monthly, so the mortality sample contained a much higher proportion of natural deaths. At Bylchau Farm relatively few carcases survived long enough to be found, even with dogs, because of the presence of many scavening predators. All carcases were examined for ear-tags, age-class and sex as well as for cause of death where this was attributable from external signs or internal examination; any symptoms of myxomatosis were classified as in Table 1. Eyelid lesions were taken from some infected carcases and frozen at -20 °C prior to testing for virulence.

Vectors. No continuous assessments were made of potential vectors of myxomatosis at the three sites, but a small representative sample of ectoparasites

was taken at Bridgets Farm. Fifty-five rabbits (31 full-grown and 24 juvenile) were live-trapped between July and September 1977 and examined for any ectoparasites.

Laboratory techniques

Myxoma antibodies. Blood or serum sample samples were tested for the presence of antibodies to soluble myxoma viral antigens by one or more of the following methods; Ouchterlony gel diffusion test (22), crossed-over immuno-electrophoresis (23), Sobey gel diffusion test (24) and serum neutralization test (25).

Myxoma viral antigens. Viral antigens were occasionally detected in blood or serum samples tested by the gel diffusion tests.

Virulence grading. Virus strains were extracted from swabs using 0·5 ml of phosphate buffered saline, pH 7·2 (PBS), or from lesions using 5 ml of PBS in a Colworth Stomacher (27), and were then passaged once in domestic rabbits to obtain fresh samples of virus which were titrated by intradermal injection on the shaved flank of domestic rabbits. The testing procedure of Fenner & Marshall (28) was then used, and the virus strains recovered from the field were allocated to one of six virulence grades according to the mean survival time (MST) of a test group of five or six domestic rabbits each injected with 10–100 ID_{50}. Where some rabbits recovered from infection, the MST was calculated by the method suggested by Sampford (29).

RESULTS

Monthly myxomatosis occurrence

Figs. 1–3 give monthly totals of all disease cases at the three study sites over the observation period. Cases showing antigen/initial and recovery stages have been adjusted to the most probable month for showing advanced symptoms (using Table 1). Two measures of total recorded occurrence each month are presented, representing maximum and minimum estimates of observed on-site cases; these are (i) total known occurrences of myxomatosis from all surveillance sources, where the same rabbit may have been recorded several times, and (ii) known occurrences of myxomatosis in different individuals (mainly from marked rabbits), where all uncertain or repeat records have been excluded. Off-site records of diseased rabbits have been indicated in months where these locations were close to the relevant site. In months where no advanced stages of myxomatosis were recorded, other evidence for the active presence of the disease was sought, either by back projection to likely infection dates or from changes in the known antibody history of marked rabbits. An outbreak was defined as extending over a period during which there was continuous evidence of the presence of myxomatosis. However, at Bridgets Farm, where the disease was present continuously for long periods, summer months with minimum evidence of disease were considered to be intervals between outbreaks.

Fig. 1 present the monthly numbers of myxomatosis cases on the Bow Hill site; between July 1971 and June 1977 myxomatosis was present on-site in 50 out of the 72 months observed, and just off-site in a further 2 months. Intervals between successive outbreaks varied from 1–4 months, apart from summer 1972 when much lower rabbit numbers were present, probably causing the 6-month interval observed. Outbreak lengths varied considerably, from 2–11 months duration.

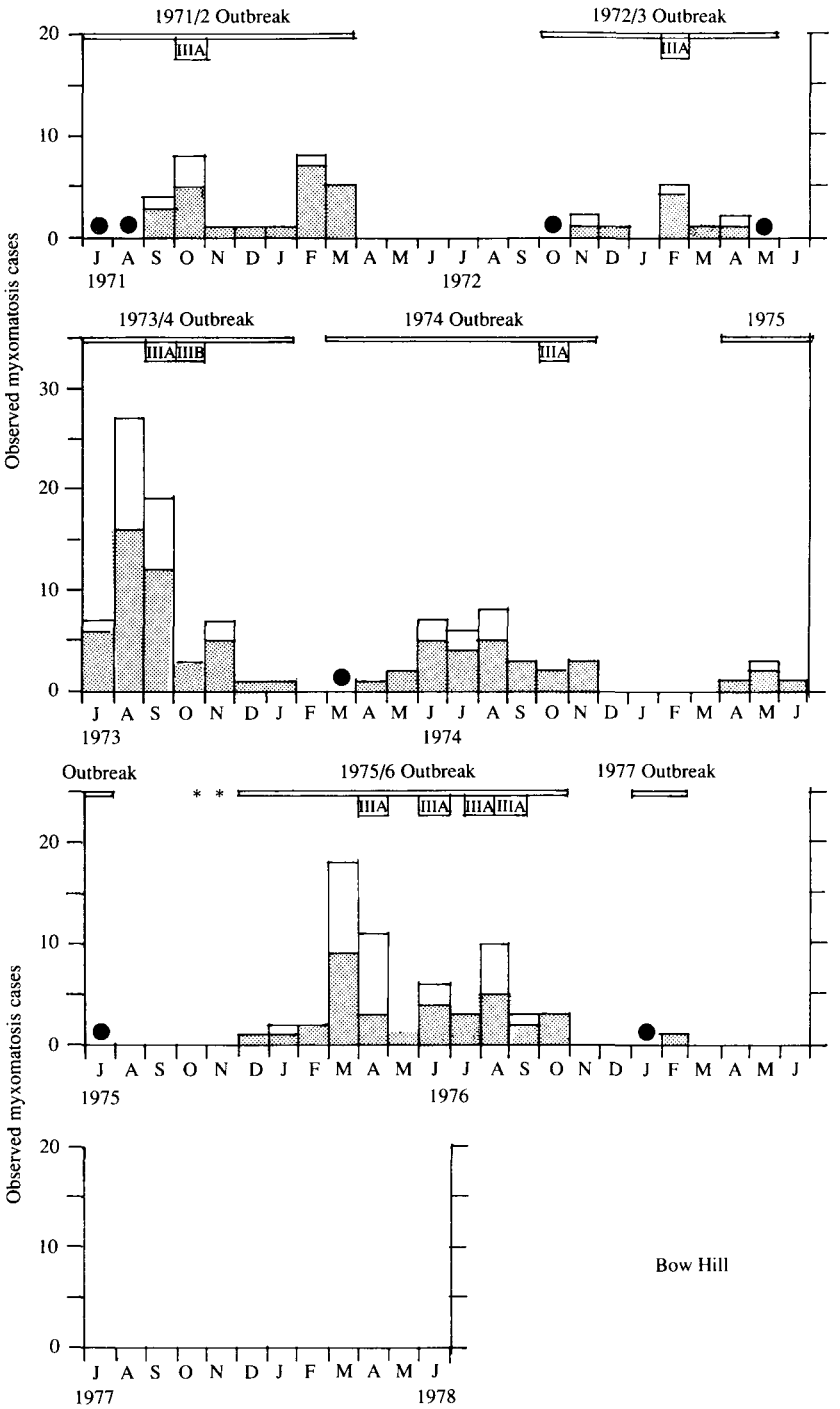

Fig. 1. Number of observed cases of myxomatosis in each month (July 1971–June 1978) at Bow Hill. Maximum (unshaded) numbers are total known occurrences of myxomatosis from all sources: minimum (shaded) numbers are known occurrences in different individuals, excluding all uncertain or repeat records. (*) indicates months when there was evidence of the disease just off-site (●) indicates months when the only evidence of the disease was indirect (see text). The length of each outbreak is indicated as is the virulence grade of each virus strain tested.

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Intensive trapping and mortality searching had ceased by July 1977 at this site, and no further disease cases were detected during regular counts and casual sightings in the period to June 1978, despite relatively high rabbit numbers.

In most years peaks and troughs of numbers of disease cases were observed; there were usually two distinct peaks per outbreak, during late summer/autumn (notably around August) and during late winter/spring (often February to March), followed by a summer trough (between May and July). However in the shorter 1973/4 outbreak there was a suggestion of a double autumn peak, with a particularly rapid build up to a peak in August and a less distinct rise in November, while in the 1974 and 1975/6 outbreaks there were small mid-summer peaks (June to August). Field strains of virus present were almost exclusively Grade IIIA for the five outbreaks for which samples were obtained; the only exception was the 1973/4 outbreak, when a mixture of Grade IIIA and IIIB strains was found.

Fig. 2 presents the monthly numbers of myxomatosis cases on the Bridgets Farm site; between May 1971 and July 1978 the disease was present on-site in 79 out of the 87 months observed, and just off-site in 1 further month. Intervals between successive outbreaks varied from 1–3 months, with active myxomatosis present for continuous periods of up to 34 months (August 1972 to May 1975) and 23 months (July 1976 to May 1978), in spite of the much lower overall population density of rabbits in the latter period. Outbreak length varied little, being between 10 and 12 months duration.

Peaks and troughs of numbers of disease cases were clear in most years; again there were usually two peaks per outbreak, during late summer/autumn (often around October) and during spring (notably March or April), followed by a summer trough (normally June or July). However the autumn peak was as early as August in the 1977/8 outbreak and as late as January in the 1972/3 outbreak, and there was a distinct double autumn peak (October and January) in the 1973/4 outbreak. Field strains of virus present were predominantly Grade IIIA for all but one of the seven outbreaks observed; however, there was more variation at this site, with Grades II, IIIB and IV strains found during individual outbreaks.

Fig. 3 presents the monthly numbers of myxomatosis cases on the Bylchau Farm site; between March 1973 and August 1978 the disease was present on-site in 25 out of the 66 months observed, and in the vicinity off-site for 1 further month. Intervals between successive outbreaks were extremely variable, ranging from 4 up to 17 months (the latter coincided with an exceptionally low density of rabbits). Outbreak length was also very variable, with three short outbreaks apparently lasting only 2 months, while the two longer ones lasted 8 and 11 months (both coincided with rabbit population peaks). Field strains of virus present were again predominantly Grade IIIA for the three outbreaks sampled; a Grade IV strain was also found in the 1973/4 outbreak.

If known disease cases from the three main study areas are reduced to a common standard for comparison, by allowing for variations in the length of observation period and in the size of the sites, myxomatosis was equally common at Bow Hill (11–16 observed cases per year per 100 ha) and at Bridgets Farm (9–11 cases, but much less common by Bylchau Farm (1–2 cases).

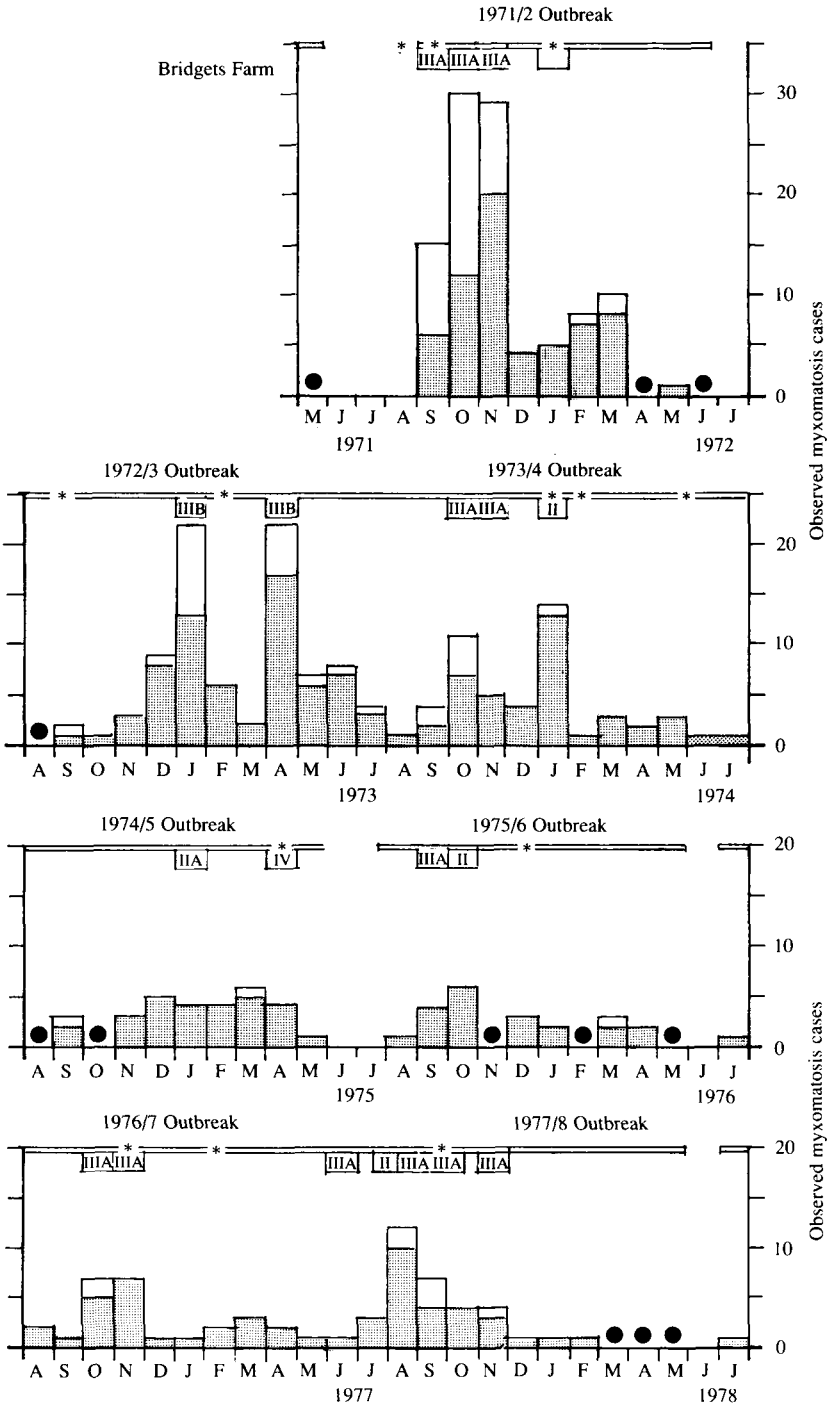

Fig. 2. Number of observed cases of myxomatosis in each month (May 1971–June 1978) at Bridgets Farm. Symbols are explained in the legend to Fig. 1.

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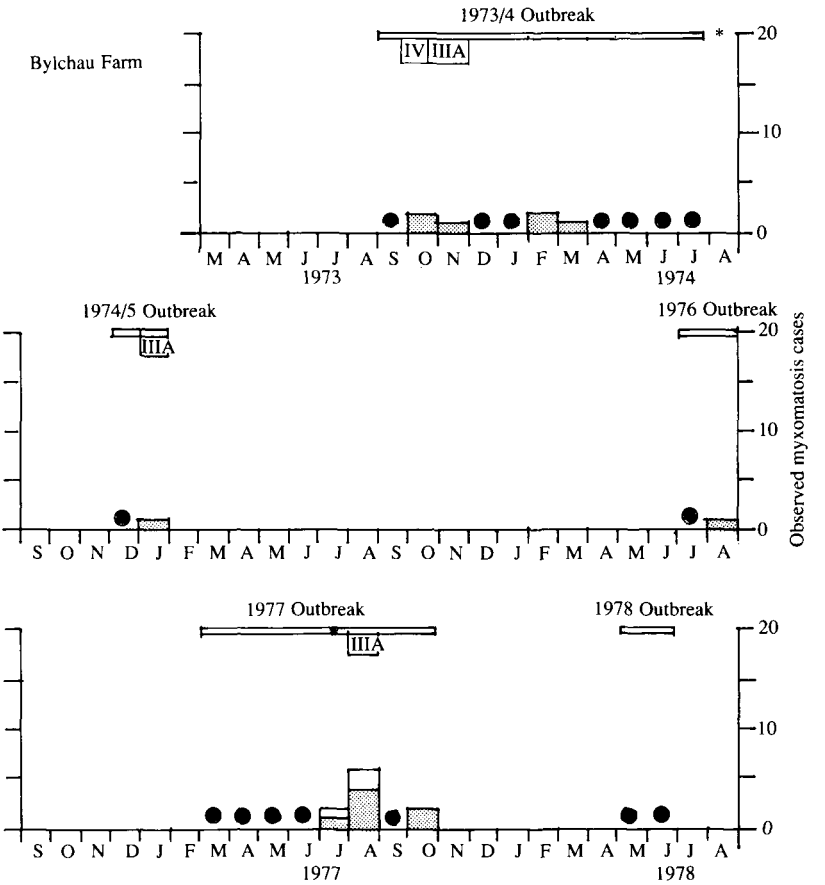

Fig. 3. Number of observed cases of myxomatosis in each month (March 1973–August 1978) at Bylchau Farm. Symbols are explained in the legend to Fig. 1.

Proportion of rabbits with detectable antibodies

Fig. 4 summarizes the results of tests for the presence of antibodies to myxoma virus in blood samples of rabbits captured at Bow Hill and Bridgets Farm. The proportions of adult, immature and juvenile rabbits with detectable antibodies are presented for each month of the year along with the mean proportion of rabbits infected for each month. At Bow Hill, the proportion of adults with detectable antibodies was relatively high throughout the year, with peaks in March and August, corresponding to the height of the spring and the late summer outbreaks. The proportions of antibody-positive immature and juvenile rabbits were low until July–August (during the summer outbreak). At Bridgets Farm, the proportion of antibody-positive adults was again relatively high most of the year with increases in April and October/November, corresponding to spring and autumn outbreaks. The proportion of antibody-positive juveniles increased in April/May after the spring outbreak and the proportion of antibody-positive immature rabbits rose in July as antibody-positive juveniles became immature. (By November, the numbers of juvenile and immature rabbits sampled were very low.)

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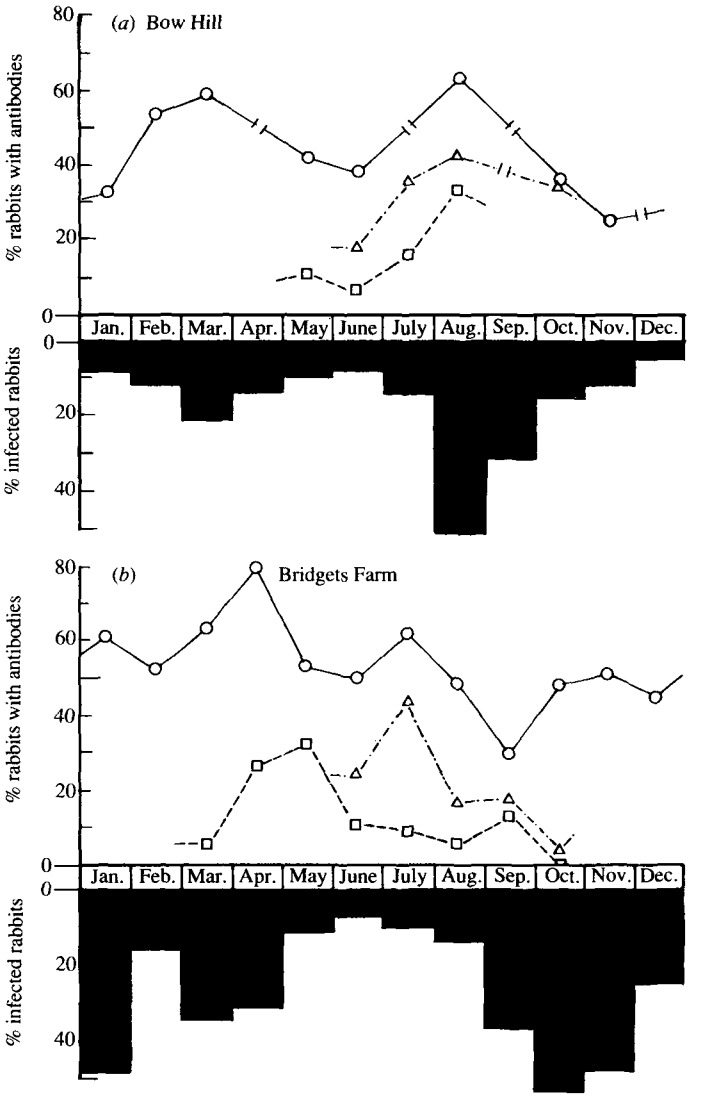

Fig. 4. Monthly percentage of rabbits (in each of three age classes) with detectable antibodies to myxoma virus and percentage of infected rabbits in the live-trapped sample at (*a*) Bow Hill and (*b*) Bridgets Farm. ○, Adults (> 8 months); △, immatures (4–8 months old); □, juveniles (< 4 months).

Rabbit abundance

Fig. 5 shows the population fluctuations of rabbits at the three sites over the period of myxomatosis observations, based upon count indices of rabbits visible above ground (i.e. nestlings are excluded). These indices have been smoothed (to remove short-term variations in observability of rabbits due to changing crop heights) and corrected for seasonal variations in the proportion of rabbits counted, since a much lower proportion of the total population was visible during the winter months. The figures for Bylchau Farm are not directly comparable with those for the other sites, because of differences in the methods of collecting the indices.

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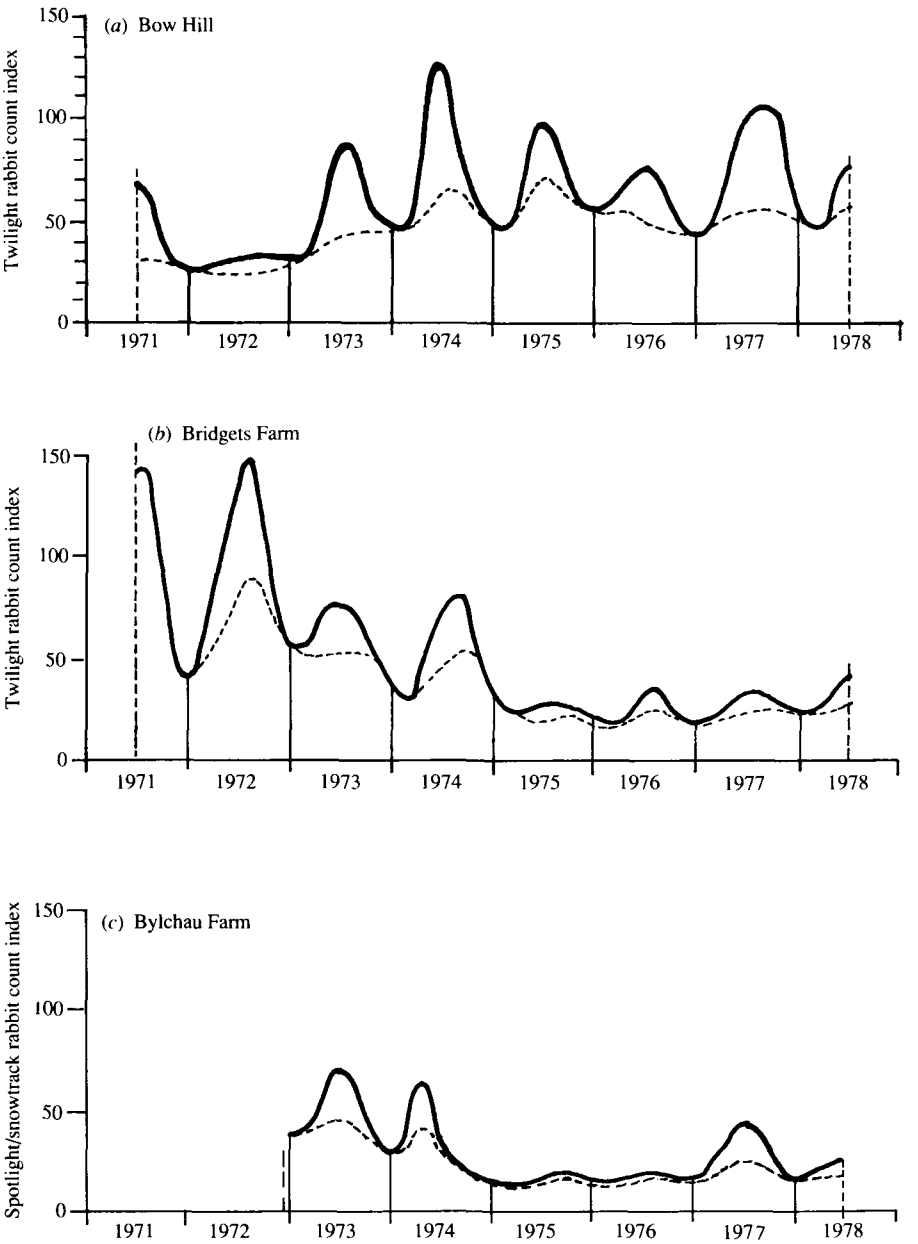

Fig. 5. Fluctuations in rabbit count indices during the study at (*a*) Bow Hill, (*b*) Bridgets Farm and (*c*) Bylchau Farm. ——, Total rabbits (> 1 month); – – – – full grown only (> 4 months).

At Bow Hill (Fig. 5*a*), apart from the unusually low population during 1972, numbers of rabbits were moderately high; winter trough numbers (estimated at $1{\cdot}2 \pm 0{\cdot}3$ rabbits per ha) were relatively similar from year to year, with most variation occurring between summer peak numbers as a result of variable breeding success (cf. the numbers of 1- to 4-month-old young in different years). At Bridgets

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Farm (Fig. 5*b*), there was an obvious decline in both winter and summer numbers of rabbits during the course of the study; winter trough numbers were exceptionally high in 1972/3, but subsequently were similar from year to year (estimated at $0{\cdot}6 \pm 0{\cdot}2$ rabbits per ha), while summer breeding success was again variable (cf. numbers of 1- to 4-month-old young in different years). The rabbit population at Bylchau Farm (Fig. 5*c*) was at much lower density (average overwinter numbers estimated at $0{\cdot}2 \pm 0{\cdot}1$ rabbits per ha); from an initial minor peak of abundance, there was a population crash in the second half of 1974, followed by 2 years of extremely low density before partial recovery.

At each site there was a substantial annual drop in overall numbers of adult and particularly of young rabbits in the autumn/early winter period, normally between August and December but the exact timing of this main autumn mortality was variable between years.

Virus virulence

Results (Table 2) from 33 field samples tested for mean survival time and thus virulence grade show that most myxoma viruses were partially attenuated Grade IIIA strains; mean survival times for susceptible laboratory rabbits ranged from 15·3 to 32·6 days.

The susceptibility of the laboratory rabbits used for virulence tests did not change over the period 1971-8, since rabbits from the same source were used as control animals in tests for resistance to myxomatosis (30, 31). In those tests, groups of domestic rabbits were injected with a standard dose of a Grade IIIA virus strain (Brecon) and mean survival times did not change significantly.

Vectors of myxomatosis

In the sample of ectoparasites taken from Bridgets Farm during summer 1977, only rabbit fleas were found. Apparent differences between mean infestation levels on full-grown female rabbits (36·1 fleas per host) and full-grown male rabbits (21·9 fleas per host) were not statistically significant: however there were significantly ($P < 0{\cdot}005$) more fleas on full-grown rabbits (28·3 fleas per host) than on juveniles (8·7 fleas per host).

Prevalence rates

Estimates of the proportions of rabbits known to be infected during individual outbreaks (*outbreak prevalence rates*) were made from the proportion of marked rabbits with evidence of having been infected (either showing symptoms or changing from antibody-negative to antibody-positive). Outbreak prevalence rates ranged from < 6 to 55% (mean 27%) in seven outbreaks at Bow Hill, from 15–34% (mean 25%) in seven outbreaks at Bridgets Farm, and from 14–38% (mean 26%) in five outbreaks at Bylchau Farm (Table 3).

Prevalence rates of different sexes and age classes of the rabbit population at each site were calculated for the whole study period, using the total sample of live-trapped and dead rabbits examined (Tables 4, 5). In order to examine prevalence rates during spring and autumn outbreaks, the months January–June were taken as the spring period and July–December as the autumn period.

Table 2. *Virulence grade distribution of 33 myxoma strains sampled at the three study sites, 1971–8*

	Virus strains					
Grades*...	I	II	IIIA	IIIB	IV	V
MST*...	< 12·5	12·6–16·5	16·6–22·5	22·6–28·5	28·6–50·5	> 50·6
& Kill†...	> 99%	95–99%	90–95%	70–90%	50–70%	< 50%
Bow Hill	—	—	8	1	—	—
Bridgets Farm	—	3	13	3	1	—
Bylchau Farm	—	—	3	—	1	—
Combined sample	0%	9%	73%	12%	6%	0%

* Virulence grades are based upon the mean survival time (MST) in days of test groups of infected laboratory rabbits.

† Expected per cent kill figures are for fully susceptible laboratory rabbits under captive conditions.

Table 3. *Prevalence rates for individual myxomatosis outbreaks at the three study sites, 1971–8*

Site/outbreak	Marked rabbit sample	Outbreak prevalence rate %
Bow Hill		
1971/2	81	37
1972/3	122	20
1973/4	95	55
1974	206	28
1975	115	10
1975/6	65	31
1977	16	< 6
Mean	700	27
Bridgets farm		
1971/2	204	34
1972/3	321	23
1973/4	249	28
1974/5	159	23
1975/6	132	21
1976/7	145	15
1977/8	153	31
Mean	1363	25
Bylchau farm		
1973/4	104	22
1974/5. 1976*	10	30
1977	42	38
1978	14	14
Mean	170	26

* Two outbreaks with small sample sizes are combined here for analysis.

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Table 4. *Relative prevalence of myxomatosis in all male and female rabbits examined at the three study sites, 1971–8*

	Autumn period (July/December)			Spring period (January/June)			Total period (all months)		
Study site	Male	Female	χ^2	Male	Female	χ^2	Male	Female	χ^2
Bow Hill	17·9%	17·6%	0·0	7·0%	4·9%	0·9	11·4%	9·7%	0·5
(sample size)	(151)	(148)		(228)	(243)		(379)	(391)	
Bridgets Farm	16·8%	12·9%	2·4	18·0%	12·0%	5·0*	17·3%	12·5%	7·1†
(sample size)	(375)	(418)		(317)	(392)		(692)	(810)	
Bylchau Farm	9·3%	10·9%	0·1	2·4%	2·2%	0·0	4·7%	5·5%	0·1
(sample size)	(43)	(55)		(85)	(90)		(128)	(145)	
Total sample	16·5%	13·9%	1·7	11·9%	8·4%	4·6†	14·1%	10·9%	5·0*
(sample size)	(560)	(621)		(630)	(725)		(1199)	(1346)	

*$P < 0{\cdot}01$; †$P < 0{\cdot}05$.

Combining results for all three study sites over the entire study period, a significantly ($P < 0{\cdot}05$) higher proportion of males than of females was seen to be infected (Table 4), particularly during the spring period. The results for the Bridgets Farm site on their own were significant, while those for the Bow Hill site showed a similar trend in spring.

The results from individual sites showed that significantly ($P < 0{\cdot}001$) smaller proportions of juvenile rabbits than of adult plus immature rabbits were seen to be infected (Table 5). Combining the data from the three sites, this was also true for both spring and autumn periods. The variation in prevalence rate with age class was most apparent at Bridgets Farm where higher proportions of adults than of juveniles were infected in both spring and autumn periods. At Bow Hill and Bylchau Farm, the highest prevalence rates were in immature rabbits.

Deaths due to myxomatosis

Estimates of the proportion of infected rabbits that died from the disease (*case fatality rate*) were made using two independent methods, both combining information from all the outbreaks studied at all three main sites.

Method one was based on the subsequent fate of marked rabbits that were observed with disease symptoms, but using *only* those known to have died or recovered from infection within 1 month of showing active symptoms; thus, by combining results from successive outbreaks the sample size was sufficiently large to give useful results, despite excluding all rabbits of unknown fate.

In *Method two* a different approach was adopted to give an independent estimate of case fatality rate based upon the proportion of rabbits in the total trapped sample, whether marked or not, which showed the four stages of myxomatosis development; antigen, initial, advanced and recovery. If no deaths occurred from the disease and if sampling was random in relation to the different infection stages the proportion of each stage found (observed) should be equal to the relative length of time that each stage lasts (expected), ie. its relative availability for sampling. The timetable used for calculating the length of each stage (which was based upon the most frequent Grade IIIA virus strain) is given in Table 1. Where

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Table 5. *Relative prevalence of myxomatosis in all adult, immature and juvenile rabbits examined at the three study sites, 1971–8*

	Autumn period (July/December)				Spring period (January/June)				Total period (all months)			
Study site	AD	IM	JV	χ^2	AD	IM	JV	χ^2	AD	IM	JV	χ^2
Bow Hill	11%	21%	22%	5·2	8%	4%	3%	5·7	9%	18%	7%	14·7†
(sample size)	(111)	(131)	(79)		(225)	(24)	(278)		(336)	(155)	(357)	
Bridgets Farm	18%	15%	4%	30·9†	17%	18%	7%	20·5†	18%	16%	5%	50·6†
(sample size)	(490)	(162)	(304)		(556)	(28)	(321)		(1046)	(190)	(625)	
Bylchau Farm	16%	18%	7%	8·9*	5%	0%	0%	—	5%	20%	0%	19·7†
(sample size)	(50)	(27)	(25)		(86)	(8)	(102)		(136)	(35)	(127)	
Total sample	16%	18%	7%	21·2†	14%	10%	4%	39·4†	15%	17%	5%	64·7†
(sample size)	(651)	(320)	(408)		(867)	(60)	(701)		(1518)	(380)	(1109)	

Age classes were Adult, AD (> 8 months), Immature, IM (4–8 months), Juvenile, JV (< 4 months).
*$P < 0{\cdot}01$; †$P < 0{\cdot}001$.

Table 6. *Overall case fatality from myxomatosis infection at the three study sites, 1971/8, by two methods of estimation*

	Study site			
	Bow Hill	Bridgets Farm	Bylchau Farm	Total sample
	Method one*			
No. infected	79	146	12	237
Died	4 (5%)	33 (23%)	2 (17%)	39 (16%)
Fate				
Recovered	8 (10%)	34 (23%)	2 (17%)	44 (19%)
Unknown	67 (85%)	79 (54%)	8 (66%)	154 (65%)
Estimated fatality rate	> 33·8%	> 49·3%	> 50·0%	> 47·0%
	Method two†			
No. infected	79	145	12	236
Disease stage				
Antigen	1 (1%)	5 (4%)	0 (0%)	6 (3%)
Initial	7 (9%)	25 (17%)	2 (17%)	34 (14%)
Advanced	43 (954%)	83 (57%)	6 (50%)	132 (56%)
Recovery	28 (36%)	32 (22%)	4 (33%)	64 (27%)
Estimated fatality rate	< 54·2%	< 76·4%	< 58·3%	< 69·0%

* Method one estimates mortality from the known subsequent fate of marked rabbits within 1 month of showing disease symptoms (including repeat infections); see text for assumptions.

† Method two estimates mortality from the proportion of myxomatosis disease stages observed in the trapped sample (excluding known repeat infections); see text for assumptions.

deaths from the disease occurred, however, deviations of the observed from the expected ratio should be in proportion to the losses caused by the disease and case fatality rate can be estimated using a formula which allows for the mean length in days of each stage:

$$\text{case fatality rate} = \left(\frac{30x - 25y}{30x}\right) \times 100\%,$$

where x = number of diseased rabbits showing *antigen*, *initial* and *advanced* symptoms, and y = number of diseased rabbits showing *recovery* symptoms.

Case fatality rate estimates for each site over the whole period (Table 6) varied from 33–76% and the estimates for all sites combined varied from 47–69%.

Estimates of the proportion of the total rabbit population within a site that died from myxomatosis during an outbreak (*field mortality rate*) can be obtained from the relationship:

$$\text{field mortality rate} = \text{outbreak prevalence rate} \times \text{case fatality rate}.$$

Using the total sample estimates of case fatality rate from the three sites combined (47–69%), and the mean estimates of prevalence rate for individual outbreaks from each of these sites (25–27%) (Table 3), field mortality rate was estimated to average 12–19% per outbreak.

Further information on the mortality resulting from myxomatosis was obtained from the regular searches for rabbit carcases at the Bridgets Farm site. Of 912

https://doi.org/10.1017/S0950268800030703 Published online by Cambridge University Press

Table 7. *Primary causes of death attributed to 130 rabbits found dead with myxomatosis symptoms at Bridgets Farm, 1971/8*

Primary cause of death	Rabbit deaths with myxomatosis symptoms* (% of $n = 130$)	Proportion of all known deaths from that primary cause†
Myxomatosis	64 (49%)	100% (of 64)
Predation	25 (19%)	10% (of 245)
Trapping accident	18 (14%)	24% (of 74)
Road casualty	11 (9%)	15% (of 72)
Farm machinery	3 (2%)	17% (of 18)
Rabbit control	0 (0%)	0% (of 226)
Other known causes	2 (2%)	15% (of 13)
Cause unknown	7 (5%)	4% (of 200)

* Myxomatosis was considered to be the direct cause of 49% of the 130 known deaths associated with myxomatosis symptoms, but only a contributory factor to the other 51% of these.

† A total of 912 rabbits were found and examined at Bridgets Farm, distributed amongst the primary causes of death as shown in parentheses.

dead rabbits examined, the cause of death was considered identifiable in 712 carcases; of these, myxomatosis was considered to be the direct cause of 64 deaths (9%), while in another 59 cases (8%) the disease was also present and thus probably a contributory factor. Table 7 shows the *primary cause* of death for all 130 rabbit carcases found with obvious symptoms of myxomatosis, and also the relative importance of the disease as a *contributory factor* to the other main causes of death.

The monthly distribution of deaths caused directly or indirectly by myxomatosis at Bridgets Farm followed a very similar pattern to that of the proportion of rabbits infected at that site (Fig. 2*b*), suggesting that case fatality rate was constant throughout the year.

Proportion of juvenile rabbits with detectable antibodies

Fig. 6, using combined data from the three sites, shows that significantly more 2- to 4-week-old rabbits had detectable antibodies than did 4- to 11-week-old rabbits ($\chi^2 = 6{\cdot}5$, $P < 0{\cdot}01$). The proportion with antibodies increased thereafter only gradually with age.

Effects on reproductive status

In a sample, from all three sites, of 24 infected female rabbits (including 11 rabbits aged 4–8 months) examined internally, follicle production, pregnancy and lactation appeared to follow the normal seasonal pattern. There was, however, some partial loss of litters *in utero*, though the sample was too small for comparison of this rate of embryo loss with that of healthy females. In a sample of 26 infected males (including 10 rabbits aged 4–8 months) there was some indication of reduced sperm production, but the majority of testicular and epididymal weights followed the normal seasonal pattern. However, only 2 (15%)

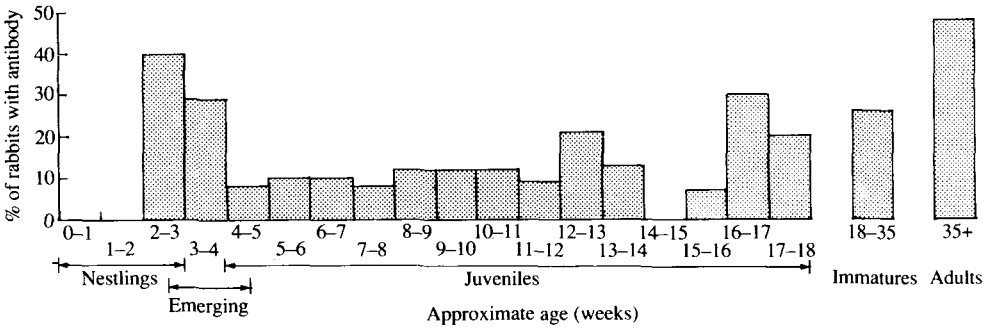

Fig. 6. Variation with age (from live body weight) in the proportion of rabbits detectable antibodies to myxoma virus. (Very few rabbits under 2 weeks old were sampled.)

of the 13 diseased adult males examined between December and May had abundant sperm in epididymal smears, at a time when over half would be expected to have such active sperm (32).

DISCUSSION

Pattern of myxomatosis occurrence

A general pattern of the occurrence of myxomatosis during the 1970s in Britain can be constructed from the recorded occurrence of disease at the three main sites. Over the whole study period, myxomatosis could be found in any calendar month at the two lowland sites, but a two-peaked annual cycle was usually apparent, with a major peak in autumn (occurring some time between August and January, but most often in October) and a minor one in spring (almost always between February and April), followed by a trough period in early summer (normally May–July) when the disease was often apparently absent from a site. In a few outbreaks, a double autumn peak was observed, separated by 2 or 3 months, while in others there was a minor mid-summer peak, but these were exceptions. Less detailed observations were also made at 96 widely separated sites in England and Wales during the 1970s (33), which produced a similar result of distinct peaks in September/October and March/April, and a trough around June, suggesting that this general pattern was typical of most rabbit populations in Britain.

At the two lowland study sites, with moderate to high rabbit densities for most of the period observed, separate outbreaks were recognized in most years (although the disease was present almost continuously). Typically, these outbreaks lasted between 8 and 12 months, with intervals of 1–4 months between them. At Bridgets Farm in particular, a regular pattern of annual outbreaks was apparent, and this was considered to be a function of the size of the site and of the abundance of the rabbits; the larger the area examined or the denser the rabbit population, the greater the chance of finding disease present continuously with a regular annual pattern.

At the upland site, there was no clear pattern though myxomatosis tended to occur most often in spring and autumn. Here the rabbits were at much lower density and more isolated from adjoining populations, and therefore observations

over a far larger area would be needed to demonstrate the presence of a general pattern.

There were clear differences between the timing and duration of the main autumn peaks at the two lowland sites, which can be explained by differences in the timing and success of their respective rabbit breeding seasons. Bow Hill demonstrated particularly sharp late summer peaks in most years, though the length of outbreaks varied considerably; this site had a rabbit population with on average proportionately more young of the year surviving beyond the nestling stage, and consequently higher numbers of young available for infection in July/August. If it is assumed that rabbits with detectable antibodies to myxoma viral antigens have recovered from infection, there was little increase in the proportion of recovered juveniles following the minor spring outbreaks and proportions of recovered immature and adult rabbits were also low in June. This may explain why the main outbreaks of myxomatosis often occurred early at Bow Hill. Peaks of disease occurrence there built up rapidly, and higher proportions of juveniles and immatures than of adults were infected during autumn. Bridgets Farm, in contrast, normally showed more gradual autumn or early winter peaks and more regular outbreaks. Fewer young of the year survived beyond the nestling stage in most years and the relatively high proportion of recovered juvenile rabbits in April and May confirm the substantial involvement of juveniles in the spring outbreaks, so smaller numbers of young were available for infection in summer at Bridgets Farm. Disease prevalence increased more slowly until September, by which time the proportion of recovered immature rabbits had declined probably due to young born after the spring outbreaks. Consequently, a higher proportion of adults than juveniles were infected during autumn outbreaks. Thus the density of and proportion of recovered rabbits in the summer/early autumn host population probably determine whether the myxomatosis peak is early or late. Variations in the timing of the end of the breeding season and in the proportion of young does breeding late may also influence the timing of early autumn peaks, while differences in the timing of dispersal and mortality of the young influence later peaks.

Importance of the rabbit flea as a vector

In the brief survey of sedentary ectoparasites at Bridgets Farm only rabbit fleas were found, though other possible vectors present at the sites such as several mosquito species (34–36), and sheep ticks (37, 38) may contribute seasonally to transmission. However, rabbit fleas were considered to be the main vectors of myxomatosis throughout the year at all three sites as in Britain generally (1).

Allan (39) and Mead-Briggs, Vaughan & Rennison (40) showed that rabbit flea infestations were lowest from June to October. The mean numbers of fleas on adult rabbit hosts (36·1 on does: 21·9 on bucks) found at Bridgets Farm between July and September in this study were consistent with those reported by these authors, and suggested that numbers did not drop below an average of 20 fleas per adult at any time during the year. A simple calculation shows that there were sufficient fleas to ensure continuation of an outbreak once initiated. Mead-Briggs & Vaughan (2) found that, on average, 42% of fleas fed on rabbits infected with intermediate Grade IIIA/B virus strains were capable of transmitting infection to

another rabbit; Mead-Briggs (41) found that there was considerable interchange of fleas between rabbits and that, on pasture, about 45% of fleas leaving a rabbit are likely to find a new host. Thus, from an infestation of 20 fleas on an infected rabbit, at least three infective fleas are likely to find a new host. In addition, virus can still be transmitted by fleas living in burrows on rabbit nests for up to 3 months after last feeding on infected hosts (7, 42, 43). Therefore, it is unlikely that myxomatosis outbreaks would be limited by lack of fleas to act as vectors. However, seasonal changes in the relative numbers of fleas per host and in their rate of transference between hosts could explain the changes in the prevalence and rate of spread of myxomatosis.

Disease prevalence in spring peaked between February and April, soon after the start of the main rabbit breeding season in January or February, when fleas become more active in seeking pregnant females (40). Any observed differences in timing of spring peaks between years or sites could have been due to variations in the onset of pregnancies (in turn affected by prevailing food and weather conditions) and to the relative density of and proportions of recovered rabbits in the spring host population.

Disease prevalence was more varied in the autumn, peaking once or occasionally twice between August and January. Early peaks followed the dispersal of fleas from reproductive females at the end of the main breeding season in June or July, whereas later peaks followed the progressive transference of fleas from dead rabbits to live survivors during heavy autumn mortality due to predation, etc. (40). This mortality occurred mainly between August and December, and is likely to have been a more gradual process than the sharper start and finish of the breeding season, making the later peaks of disease prevalence less well defined than earlier peaks.

Disease prevalence

The method used here for estimating the prevalence of myxomatosis assumes that the marked rabbit samples were representative of the total rabbit populations, and that the detected immunological changes (from antibody-negative to antibody-positive) were due to infection and recovery rather than to any shortcomings of the immunological tests used. In addition, any nestlings becoming infected and dying from myxomatosis before emergence from the nest will have been missed, though the presence of maternal antibody may have improved their chances of survival (44). Even after emergence very young rabbits die more quickly from myxomatosis than older ones (19, 44) and so were less likely to be detected while infected.

Male rabbits were more likely to be infected than females particularly in spring; this is consistent with bucks ranging more widely than does, and coming into contact with more rabbits outside their social groups (45).

A higher proportion of adults than juveniles were seen to be infected in the spring period (Table 5), suggesting that mature hosts contribute substantially to the spring disease peak. This difference was most marked at Bridgets Farm, where from 1975 onwards there were numerous predators killing juveniles as they emerged from the nests at weaning, making it impossible to examine such juveniles for infection. In addition it was not usually possible to examine nestlings

for infection below ground, though several litters in breeding stops were handled at Bylchau Farm without symptoms being observed. Since young rabbits can die much more quickly from myxomatosis than older rabbits (19, 46), there could thus have been considerable undetected nestling as well as juvenile mortality from infection at the study sites. Williams & Parer (47) also found proportionately fewer juvenile rabbits (< 3 months old) had contracted the disease in their more detailed Australian study, but concluded that this was because most died before exposure to myxomatosis. However, Shepherd & Edmonds (48), reporting on changes in the epidemiology of myxomatosis following the introduction of rabbit fleas to Victoria in Australia, indicate that during some outbreaks 'mortality (from myxomatosis) was most severe in kittens before they appeared above ground'. Considerably fewer fleas were found on juvenile than on full-grown rabbits at Bridgets Farm, in agreement with the observations of Allan (39) and Soriguer (49). Thus juveniles should be relatively less at risk of infection, until they progressively acquire fleas as immatures (4-10 months old) during autumn (40); this is another factor responsible for the low numbers of infected juveniles recorded, and may also influence the seasonal prevalence of myxomatosis.

Mortality due to myxomatosis

A number of factors are important in estimating the mortality due to myxomatosis on the study sites; the virulence of the virus strains present, the presence of resistance, the prevalence rate and the case fatality rate of the disease. The virulence of field strains of myxoma virus sampled at the three sites parallelled the results obtained in national surveys (15, 50). Most strains were of intermediate Grade IIIA virulence, while fully virulent Grade I and highly attenuated Grade V strains were absent. There were no obvious differences in virulence between the sites to account for the observed variations in prevalence and case fatality rates.

It seems likely that some degree of enhanced genetic resistance was present in the rabbit populations on all three sites. Ross & Sanders (30, 31) found such resistance wherever they have looked in Britain since 1970, including one population at Micheldever – 10 km from Bridgets Farm. Further indirect evidence of resistance is provided by the lower than expected case fatality rates found at each site (see below).

Myxomatosis was almost equally common on the two lowland sites, with relatively high rabbit densities, on which there were roughly ten times as many cases per year per 100 ha, as on Bylchau Farm, where rabbit densities were low throughout the period of study. However, a positive correlation between initial rabbit density and numbers of cases seen for individual outbreaks, at the three sites combined, fell just short of significance, so clearly other modifying factors are involved. Despite this difference, the mean outbreak prevalence rates were similar for all three sites (25–27 %). These prevalence rates, even accepting the limitations of the data, proved to be unexpectedly low because of slow and inefficient transmission during all outbreaks observed (51).

The effect of myxomatosis as a direct or indirect mortality factor was investigated using two independent methods of estimating case fatality rate, based upon different assumptions about the data. For *method one*, all rabbits that

died within 1 month of being seen with symptoms were assumed to have died (directly or indirectly) from the disease; certainly some will have died from other primary causes while infected, and the extent to which myxomatosis contributed to such deaths is a matter for conjecture. It was also assumed that there were equal chances of detection of death or recovery from myxomatosis; in fact there was a greater chance of finding recovered rabbits, since a proportion of these would have been identifiable and available for sampling over several subsequent months, while dead rabbits were only available for up to 1 month after exhibiting advanced symptoms. Thus this method probably somewhat *underestimated* mortality.

The accuracy of *method two* depended on the accuracy of the mean durations of the four stages of infection, based upon numerous observations of infected wild rabbits in the laboratory (30, 31). The validity of the method can be tested, using data for the active disease stages only (before any consequential deaths can affect the expected ratio), by comparing the observed ratio of *antigen/initial* to *advanced* symptoms (in Table 5) with the expected ratio based on their relative estimated durations. These ratios for each of the three sites were not significantly different from the expected 5:20 ratio; for the combined sample, the observed ratio was 40:132 ($\chi^2 = 1{\cdot}14$, $P > 0{\cdot}25$). This method assumes equal chances of detection of the different disease stages; though recovery symptoms were more easily overlooked than other active disease stages, this bias was counteracted to some extent by the greater persistence of recovery symptoms in some individual rabbits. Therefore, this method may have slightly *overestimated* mortality.

The actual case fatality rates are thus likely to lie somewhere between the estimates from the two different methods presented in Table 5; the apparent differences between sites were unlikely to be significant. The total sample estimates of between 47 and 69% were considerably lower than the expected 90–95% fatality in fully susceptible rabbits infected with Grade IIIA virus strains.

There was no evidence for any seasonal changes in case fatality rate from myxomatosis, unlike the situation reported in the more extreme conditions of Australia; Dunsmore & Price (52) and Williams & Parer (47) found under 50% mortality during summer outbreaks, whereas Dunsmore, Williams & Price (18), Williams *et al.* (53, 54) and Fullagar (19) found at least 85% mortality in winter outbreaks.

Using the mean estimates of minimum outbreak prevalence rate (25–27%) and of overall case fatality rate (47–69%), it is calculated that at least 12–19% of the total rabbit population present during an outbreak die directly or indirectly due to myxomatosis (field mortality rate).

Although field mortality rates in the 1970s were much lower than those reported in previous decades, myxomatosis remained a significant cause of death. This was confirmed by information from searches for rabbit carcases at Bridgets Farm, where 17% of all carcase deaths were attributable directly or indirectly to myxomatosis. The agreement with the estimates of field mortality rate is surprisingly good; if anything, the proportion of deaths attributed directly to the disease is underestimated, because the probability of collecting rabbits that die

from some other causes (such as rabbit control, road casualty or trapping accident) was greater than for deaths due to myxomatosis alone.

What effects do these field mortality rates have on rabbit population trends? Since the major outbreaks of myxomatosis occur most commonly in autumn/early winter, after the heavy autumn mortality of rabbits has already begun, the hypothesis is that the disease reinforces the effects of the other causes of mortality, resulting in lower numbers of rabbits surviving to the start of the next breeding season. If this hypothesis is correct, myxomatosis continues to have a regulatory effect in reducing the rate of increase in rabbit numbers. The less marked spring peak of disease occurs when the rabbit breeding season is well under way, and a second (more tentative) hypothesis is that myxomatosis mortality in spring is also additive, decreasing survival of adults and early young and consequently affecting the size of the subsequent summer population levels of rabbits. This may not have any long-term effect, but could reduce damage to agricultural crops in summer/autumn. These hypotheses are being tested in a controlled field experiment, but if correct they suggest that increases in both winter and summer rabbit numbers would result if the observed decline in field mortality continues. It seems likely that case fatality rates from myxomatosis soon may be reduced substantially, since resistance has continued to increase (30, 31) but the compensating increase in virus virulence may not continue (because of the current absence of fully virulent field strains (50)). Thus a dramatic increase in rabbit abundance seems a real possibility and such an upsurge in numbers is likely to have serious economic consequences for agriculture and other rural industries in Britain.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of many Laboratory and Regional colleagues in conducting this study, particularly Mr J. A. Woods, for local supervision of the Welsh site, and Miss L. M. Lelliott for laboratory analysis of samples. We thank Mr M. W. Renwick, the West Dean Estate and the Nature Conservancy Council for use of the Bow Hill site; the Director of the MAFF Experimental Husbandry Farm, Mr H. Gray and British Rail for use of the Bridgets Farm site; Mr W. R. L. Jones and the Forestry Commission for use of the Bylchau Farm site. We also thank Dr D. P. Sleeman for use of his unpublished data on flea distribution. Mr H. G. Lloyd, Dr A. R. Mead-Briggs, Dr W. A. Rees, Dr R. C. Trout and Mr J. A. Vaughan gave much useful comment during the study and on drafts of this text.

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