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Evidence of infection by viruses in small British field rodents

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SUMMARY

Four populations of small wild British rodents were studied by capture–re-capture methods over a period of three years. Samples of blood were taken from these animals and tested for antibodies to nine viruses. Animals were removed from another 11 sites around the UK, and immunosuppressed. Samples of tissue from these animals were tested for the presence of viruses by passage in laboratory mice and serum samples from some of them were tested for antibody to the nine viruses. Indications were found of the possible influence of epizootic outbreaks of certain diseases on animal populations.

INTRODUCTION

The role of communicable disease in the control of wild animal populations is not clear. Elton, Davis & Findlay (1935) undertook an investigation of virus infections in voles (*Microtus agrestis*) but were unable to report positively on their findings after several years. In the forty years that have elapsed since their pioneering work, the sensitivity of virological and serological methods has been greatly increased. We thought it opportune, therefore, to reopen the investigation, since although much epidemiological work has been done on animal reservoirs and carriage of individual viruses of public health importance, the role of infection in the population dynamics of natural wild populations has been neglected.

In addition, because of reports from Central Europe of the presence of rabies-like viruses in field rodents (Sodja, Lim & Matouch, 1971; Schneider, 1972) we took the opportunity to test neural tissues of British field rodents for the presence of neurotropic viruses including rabies and rabies-like viruses.

METHODS

Rodent species studied

The species studied were the Woodmouse (*Apodemus sylvaticus*), the Bank Vole (*Clethrionomys glareolus*), the Skomer Vole (*Clethrionomys glareolus Skomerensis*)

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Table 1. *Sites visited once only during the project*

Banchory, Kincardineshire	July	1977
Tregaron, Dyfed	May	1978
Marlborough, Wilts.	May	1977
Thatcham, Berks.	March	1978
Woodley, Berks.	—	1977 (various dates)
Bagnor, Berks.	July	1977
Alton, Hants.	February	1978
Rye, Sussex	March	1978
Babraham, Cambs.	May	1978
Coxtie Green, Essex	May	1978
Sheffield, Yorks.	April	1978

and the Short-tailed Field Vole (*Microtus agrestis*). A few examples were obtained of Yellow-necked mice (*Apodemus flavicollis*).

Study areas

Four areas were studied throughout the project, Laurieston, Kirkcudbrightshire; Llanerchyrfa, Powys; Oakfield, Berkshire; and Skomer Island, Dyfed. In addition, a site at Alice Holt, Hampshire was studied for one year (1976) and eleven other sites (the subsites) (Table 1) were visited once only during the project.

Field work

Squared trapping grids of 0·49 Ha with 10 m interstation distances were established at the four main sites and visited at approximately six-weekly intervals, except for Skomer which was visited once a year. The animals were caught in Longworth traps (Chitty & Kempson, 1949), hay being provided for bedding and oats for food. After capture the animals were removed from the traps, identified, weighed, sexed, and assigned to an age class – they were individually marked by means of toe clipping. Samples of blood were taken for the detection of antibodies. The blood samples were taken from the tail, ethyl chloride being used to stimulate a reactive hyperaemia. The blood was then prepared for serological study by the method described by Healing (1978). In order to prevent the transmission of pathogens from one study area to another, the traps were autoclaved before each trapping session and the trap and animal carriers were washed in a 5% solution of 'Tegodor' (Th. Goldschmidt Ag. Essen).

The rodent populations were calculated using Jolly's stochastic population model (Jolly, 1965). These results will be published elsewhere (Healing & Kaplan, in preparation).

Any animals found dead in the traps and animals caught during the single visits to the subsites were brought back to the laboratory for analysis.

Antibodies

The blood samples were analysed for antibodies to the various viruses by Complement Fixation (CF) and Haemagglutination Inhibition (HAI). Micro-methods were employed using 'Microtiter' mechanical diluting apparatus and

Table 2. *Viruses studied with the provenance of the antigens and antisera*

Ectromelia (Mouse pox) virus (Professor K. R. Dumbell, St Mary's Hospital Medical School, London W2).
Mouse adenovirus (Professor J. van der Veen, University of Nijmegen, Netherlands).
Sendai virus (Parainfluenza virus 1) (University of Reading).
Pneumonia virus of mice (PVM) (Central Veterinary Laboratories, Weybridge, Surrey, England).
Louping ill virus (Mordun Institute, Edinburgh).
Lymphocytic choriomeningitis virus (LCM) (Virus Reference Laboratory, Central Public Health Laboratories, Colindale, London NW9).
Reovirus III (Department of Pathology, Royal Berkshire Hospital, Reading, England).
Encephalomyocarditis virus (EMC) (Dr F. Brown, Animal Virus Research Institute, Pirbright).
Theiler's mouse encephalomyelitis virus (GD VII) (Dr R. Shope, Department of Epidemiology, Yale University, New Haven Commune, USA).

'Microtiter' 96-well plastic plates (Flow Laboratories Ltd). For each antibody investigated a single batch of antigen was used. Antigen potencies were checked regularly.

Removed animals

Sixty-eight animals removed from the subsites were brought back to the laboratory and treated with ACTH as an immunosuppressant. Ten doses, each of 5 i.u., were given intramuscularly over a period of 14 days. The ACTH used was Synacthen Depot (CIBA Laboratories). In view of the small numbers of animals that could be obtained at any time, it was not possible to run controls. Animals which died following this treatment were dissected aseptically and 10% (w/v) suspensions of brain, lung, and spleen tissues were prepared in phosphate buffered saline using TenBroeck grinders. Similar suspensions were prepared from tissue taken from animals that died in the traps. Penicillin, streptomycin, and fungizone were added to these suspensions and they were then passaged in suckling mice by intracerebral inoculation. Mice that died following this procedure were dissected aseptically, tissue suspensions prepared as above and further passage undertaken.

Passage animals that did not die were killed by exsanguination three weeks after inoculation and their blood tested for antibodies to the various viruses. Immunosuppressed animals that did not die were killed by exsanguination at the end of the treatment and their blood tested for antibodies.

Viruses studied

The viruses studied are listed in Table 2, with the provenance of the antisera and antigens used.

RESULTS

Blood samples from the field

Four hundred and thirty-six blood samples were taken at Laurieston, Llanerchyrfa, Oakfield and Skomer and tested for antibodies to the viruses (Table 2).

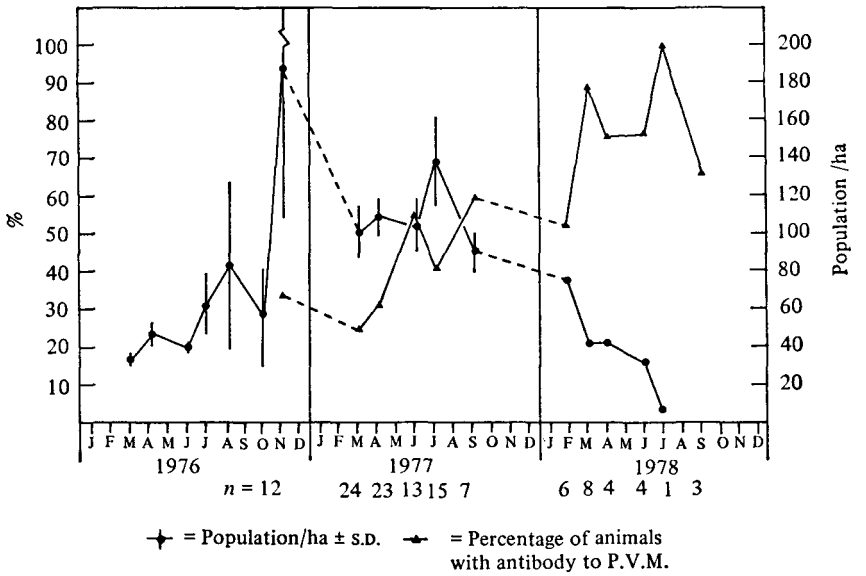

Fig. 1. Percentage of *Apodemus sylvaticus* at Oakfield with antibody to pneumonia virus of mice, related to calculated population size. *n*, Number of blood samples tested.

Some of these samples were very small and were only tested for antibody to PVM. Antibody to this virus cross-reacts slightly with Sendai virus and vice versa; some of the PVM positives may, therefore, have been due to antibody to Sendai virus. In the larger samples and the bloods from the animals that were immunosuppressed but did not die it was possible to distinguish clearly between these two infectious viruses.

Rodent populations and virus antibodies

Only for PVM and the two species, *Apodemus sylvaticus* and *Clethrionomys glareolus* from Oakfield, was there enough information to allow the percentage of individuals with antibody to the virus to be plotted against the populations (Figs. 1 and 2). The population data are drawn from Healing & Kaplan (in preparation). The PVM antibody data from Oakfield and from Skomer were examined for differences in occurrence of antibody to this virus between the two sexes (Table 3).

Removed animals

Fifty-two per cent of the animals treated with ACTH died during the treatment and dissection revealed gross pathological changes of the lungs consistent with pneumonia. Eighty-one animals were found dead in the traps and showed similar changes in the lungs on dissection. The suspensions made from the lungs of these animals were shown to be free of bacteria (before the addition of antibiotics). Of the animals brought in from the sub-sites, 22 which did not die during immuno-

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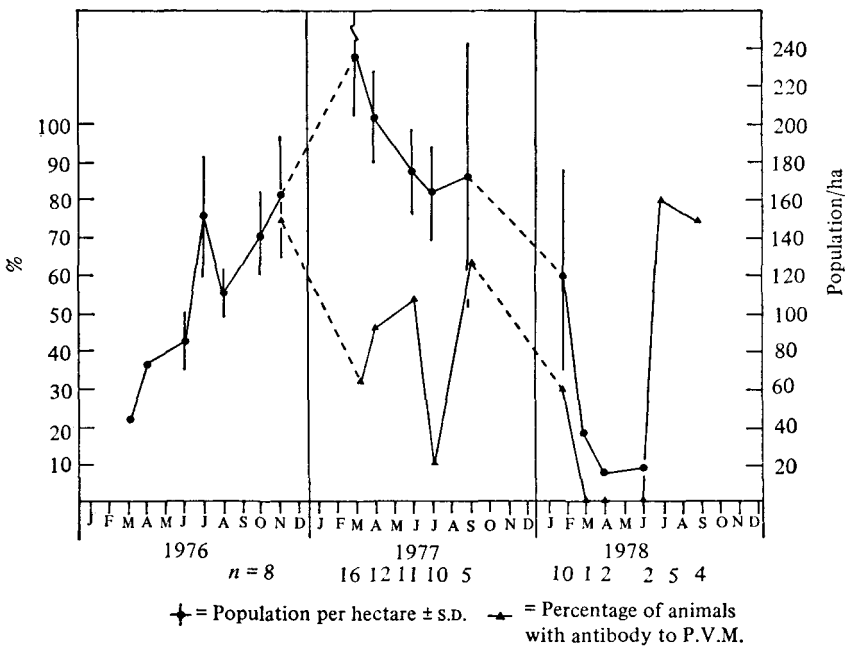

Fig. 2. Percentage of *Clethrionomys glareolus* at Oakfield with antibody to pneumonia virus of mice, related to calculated population size. *n*, Number of blood samples tested.

Table 3. *Antibody found in animal house stock after inoculation with tissue extracts from field rodents*

Tissue extract	Antibody in recipient mice
Spleen, A.f.*	LCM
Spleen, M.A.†	Reovirus III
Kidney, C.g.‡	Reovirus III; GDVII (at 2nd passage)
? A.s.§	GDVII (at 3rd passage)

* *Apodemus flavicollis*, Oakfield.
† *Microtus agrestis*, Alice Holt.
‡ *Clethrionomys glareolus*, Oakfield.
§ *Apodemus sylvaticus*, Thatcham.

suppression, were subsequently killed by exsanguination and their blood tested for antibodies to the various viruses by CF and HAI (Table 4).

Animal passage of tissue suspensions

Two hundred and sixty-nine tissue suspensions were prepared from field material and injected into suckling mice. The intracerebral route was used for all suspensions other than lung, which were inoculated intranasally. Twenty tissue suspensions were prepared from the 26 mice that died following inoculation and passaged again. In total, 5400 mice were used for passage. The sucklings were killed by exsanguination 21 days after inoculation and the bloods from each litter inoculated with a single suspension were pooled and CF and HAI tests performed. In total, 448

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Table 4. *Serum samples with antibody*

	Oakfield		Laurieston		Llanerchyrfa		Skomer	Subsites			Total			
	As	Cg	As	Cg	As	Ma	CgS	As	Cg	Ma	As	Cg*	Ma	All†
Ectro	—	0/1	1/11	0/3	0/9	7/20	2/18	1/8	0/5	—	2/28	0/9	7/20	11/75
M. Adeno	1/4	0/1	—	—	—	—	0/4	0/7	0/4	0/2	1/11	0/5	0/2	1/22
Sendai	6/13	3/7	1/1	0/1	0/1	1/2	28/33	6/8	3/7	2/2	13/23	6/15	3/4	50/75
PVM	48/108	30/78	1/9	1/1	1/3	2/10	28/67	8/9	5/9	2/3	58/129	36/88	4/13	126/297
L. Ill	0/7	0/3	1/42	1/13	—	0/2	0/23	0/8	0/5	0/1	1/57	1/21	0/3	2/104
LCM	0/4	0/2	—	0/1	0/1	1/2	4/10	2/8	0/5	—	2/13	0/8	1/2	7/33
REO III	0/11	0/5	0/3	0/2	0/1	0/2	4/72	0/8	1/8	—	0/23	1/15	0/2	5/112
EMC	1/4	0/1	—	—	—	0/1	0/13	0/7	2/4	1/2	1/11	2/5	1/3	4/32
GD VII	1/5	0/2	—	0/1	—	0/1	11/46	0/6	0/5	0/2	1/11	0/8	0/3	12/68

–, Not tested; As, *Apodemus sylvaticus*; Cg, *Clethrionomys glareolus*; CgS, *Clethrionomys glareolus Skomerensis*; Ma, *Microtus agrestis*. * Excluding CgS. † Including CgS.

blood samples were tested. Antibodies to PVM and Sendai virus were present in many of these bloods but were also present in the animal house controls. A few positive reactions to other viruses were obtained (Table 3).

Rabies virus. No animal inoculated intracerebrally with suspension of neural tissue showed any signs of neurotropic infection.

Ectromelia virus. The orthopox viruses cross-react quite extensively; the positive results for the presence of complement-fixing antibody can thus only be taken as indicating the presence of antibody to one or more orthopox viruses in the blood of the animals studied.

Ectromelia itself is a highly contagious and often fatal infection which frequently occurs in laboratory mice. It can be carried in latent form by individual mice and activated by stress (Andrewes, Pereira & Wildy, 1978).

Antibody to pox viruses was demonstrated in Skomer voles but not in the (rather small) group of mainland bank voles. Such antibody was also found in a small number of woodmice and a rather large number of short-tailed voles. Although 7/13 short-tailed voles from Llanerchyrfa were positive for pox-virus antibody, none of the nine woodmice examined was (Table 4).

Murine adenovirus. Like the pox viruses, the adenoviruses cross react, and any field results can only be considered as indicating the presence of adenoviruses in the wild animals. In laboratory stocks, murine adenovirus can fatally infect both suckling mice and adults, or cause inapparent infections (Andrewes *et al.* 1978). Antibody to adenovirus was found only in one woodmouse (from Oakfield) but the groups examined were small.

Sendai virus (*Parainfluenza type* I). Antibodies to Sendai virus were present in a large proportion of the animals examined. The slight cross reaction of Sendai with PVM has already been mentioned, but in all the positive sera (Table 4) it was possible to distinguish between the two by testing against both viruses; the titre against Sendai virus was always higher.

Pneumonia virus of mice. The results of tests for antibodies to PVM must be treated with caution owing to the possibility of cross reaction with Sendai virus. A large proportion of the samples were indeed tested for both, but in the case of the smaller samples it was only possible to test for PVM. These small samples were, however, mostly diluted 1/10 and cross-reactions occurred only occasionally at this dilution.

The presence of antibody to both PVM and Sendai virus in the animal-house stock at Reading University rendered abortive much of the passage work undertaken.

Louping ill virus. Smith, Varma & McMahon (1964) found louping ill in woodmice in Ayrshire. The present study demonstrated the presence of antibodies to louping ill in one woodmouse and one bank vole at the Kirkcudbrightshire site. Samples taken elsewhere in the United Kingdom (Table 4) were negative.

Lymphocytic choriomeningitis virus. Although house mice (*Mus musculus*) are probably the principal natural reservoir of LCM (Lehmann-Grube, 1971) a number of other rodent species have been implicated. According to Lehmann-Grube (1971) several authors have tested a number of species of small mammals both for the

virus itself and for antibody in their sera. Of 24 named species only 6 species of rodent were positive. The rodents were *Mus musculus*, *Microtus arvalis*, *Clethrionomys glareolus*, *Apodemus sylvaticus*, *A. flavicollis*, and *A. microps*. We examined a small sample of rodents for antibody to LCM and positive results were only obtained from Skomer (the Skomer vole), Llanerchyrfa (*M. agrestis*), Babraham (*A. sylvaticus*) and Rye (*A. sylvaticus*). A patchy distribution of LCM is not uncommon (Lehmann-Grube, 1971).

Reovirus III. Reoviruses cross react and cannot be distinguished by CFT. During the present study antibodies to Reovirus were found in one bank vole (from Essex) and in four Skomer voles.

EMC virus. Antibody to EMC virus was found in all three species studies on the mainland but not in the few Skomer voles tested. Andrewes *et al.* (1978) suggested that infection in wild rodents is probably inapparent, but its role in wild populations is not known.

GD VII. Antibody reacting with GD VII was detected in a single woodmouse at Oakfield and in several Skomer voles. In laboratory mice, infection with this virus usually causes inapparent intestinal infection (Andrewes *et al.* 1978).

DISCUSSION

Sodja *et al.* (1971) and Schneider (1972) have reported an isolation rate for rabies-like viruses from European field rodents of between 2% and 3%. Steck (1972) who has also isolated such viruses believed he could not exclude the possibility of a laboratory contamination. We were particularly careful to prepare all tissues for intracerebral inoculation in a class-3 safety cabinet which was sterilized frequently by exposure to formaldehyde and water vapour. Our failure to isolate rabies virus probably indicates its absence from the populations we investigated.

Although some of the groups in which particular antibodies were found were rather small, the results are nevertheless interesting since to the best of our knowledge no other survey has been made in the United Kingdom which covers so wide a geographical range. Antibody to all nine of the viruses used as test antigens was found in the blood of wild rodents. Each species of rodent studied lacked antibody to one or more of the viruses. Although the number of animals was usually small this may not have been a significant factor, since antibody to other viruses was found in equally small groups of animals. Assuming the presence of antibody to indicate a previous infection, woodmice were infected by the largest number of viruses (8/9), followed by Skomer voles (6/9), and mainland bank voles and short-tailed voles (5/9).

Some of the viruses in which we were interested are able to cause severe epizooties in mice (*Mus musculus*) housed in animal houses, e.g. ectromelia. The proportion of wild rodents with antibody to this virus was small except in *Microtus*, where 35% of the animals tested had antibody. All of these *Microtus* were caught at Llanerchyrfa, Powys during 1977 and 1978 when the population was small (Healing & Kaplan, in preparation). The coincidence of a relatively high incidence

Table 5. *Percentage of male and female animals with antibody to PVM*

		Males			Females		
Species	Date	No. tested	No. +ve	% +ve	No. tested	No. +ve	% +ve
Cg*	1977	24	8	33	30	12	40
	1978	14	5	36	10	5	50
CgS†	1977	13	0	0	15	2	13
	1978	20	14	70	19	12	63
As‡	1977	51	18	35	31	11	35
	1978	20	15	75	6	4	67

* *Clethrionomys glareolus*. Oakfield. All of 1977 and 1978 data.
† *Clethrionomys glareolus Skomerensis*. Skomer. August 1977 and 1978.
‡ *Apodemus sylvaticus*. Oakfield. All of 1977 and 1978 data.

of antibody to ectromelia virus and a small population may be significant of a recently antecedent epizooty of mousepox, but there is, of course, no way of confirming such a speculation.

The problem of cross-reacting antibodies has already been mentioned. The number of sera tested for antibodies to both PVM and Sendai virus was large and the results indicate that infections by both viruses were common in the populations studied as well as at the sub-sites sampled occasionally. The percentage of animals at Oakfield with antibodies to PVM was plotted against time for two rodent species (Figs. 1 and 2). For *Apodemus sylvaticus* there was an approximately inverse relationship between the population size and the percentage of animals with circulating antibody; for *Clethrionomys glareolus* the relationship was more direct. The results for 1978, when the rodent populations were low, are based on rather few animals and are difficult to interpret with certainty but suggest (Fig. 1 and Table 5) that there was an epizootic outbreak of PVM in the population of woodmice at Oakfield coinciding with a decline in the population. The data for the bank voles (Fig. 2 and Table 5) are less easy to interpret. Either there was no epizooty of PVM in this species or else bank voles are more susceptible than woodmice to respiratory infection and succumb more readily, which might explain reports (e.g. Ashby, 1967) that bank voles are particularly prone to death in trap. The results in Table 5 also suggest that there may have been an epizooty of PVM in the population of voles on Skomer Island in 1978. The population, which is rather stable (T. D. Healing, unpublished data), has been sampled in early August for several years and averaged $236{\cdot}5 \pm 14{\cdot}1$ animals per hectare from 1975 to 1977 inclusive. The numbers declined to 168·7/Ha in 1978 (population calculated using the capture–recapture model proposed by Hayne, 1949). Unfortunately no data on circulating antibodies are available for 1975 and 1976.

When rodents brought in from the field were immunosuppressed 52% died with severe pneumonia. Animals that were immunosuppressed but survived were subsequently killed by exsanguination; they had no gross post-mortem signs of lung involvement. Tests on tissue suspensions made from the lungs of those animals that did die showed that the aetiology of the pneumonia was not bacterial.

The immunosuppressant was therefore either activating a latent infection, making apparent an inapparent infection, or turning a chronic infection into an acute infection. Of these three alternatives the last is less likely than the others, since the animals showed no signs of being in poor condition before treatment; their coats were sleek, their eyes clear, and their appetite good. In addition, obviously ill animals are rarely captured in small-mammal traps which have to be searched for actively; any animal that is unfit is unlikely to move far. We conclude, therefore, that the infections activated were either latent or previously inapparent. There is little chance that the animals contracted the infections in the animal house despite the existence of both PVM and Sendai virus there. It was not possible to run controls, but not all the immunosuppressed animals died of pneumonia and not all the surviving animals had antibody to PVM and/or Sendai virus.

The presence of latent or inapparent viral infections in wild rodent populations could be important in the control of such populations, particularly if the infections could be activated by stress. This is a topic we hope to study experimentally in managed populations.

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