FIELD STUDIES OF THE ROLE OF *ANOPHELES ATROPARVUS* IN THE TRANSMISSION OF MYXOMATOSIS IN ENGLAND

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INTRODUCTION

The first reported case of myxomatosis among wild rabbits in England occurred in October 1953 (Andrewes, 1954; Ritchie, Hudson & Thompson, 1954), and early in 1954 an investigation on possible insect vectors was initiated. Work on myxomatosis in Australia has stressed the important part played by mosquitoes—*Anopheles annulipes* in particular—in the spread of the disease. Although conditions there are very different from those in Britain, there are parts of England where mosquitoes are locally abundant and where their possible role in the spread of the disease could not be ignored. In the spring and summer of 1954 studies were concentrated on the original outbreak centre near Edenbridge in Kent, an inland area where anophelines (*A. maculipennis messeae*, *A. claviger* and *A. plumbeus*) were only present in small numbers, and where the dominant mosquitoes were woodland *Aëdes* of the *cantans-annulipes* group. The role of these woodland *Aëdes* is discussed in a separate paper (Muirhead-Thomson, 1956). During the summer of 1954 the disease was also active in many coastal districts of south-east England, including many low-lying areas which had long been recognized as specially suitable for *Anopheles atroparvus*,† a species whose dense breeding is mainly confined to coastal marshes (Edwards, Oldroyd & Smart, 1934; Marshall, 1938; Shute, 1954).

THE OUTBREAK AT NEWHAVEN

An unusual opportunity for investigating the part played by *A. atroparvus* in the transmission of myxomatosis occurred in the late summer of 1954 when heavy losses among domestic rabbits were reported from several different parts of Newhaven, on the Sussex coast. Later investigation showed that in addition to the reported cases in the four or five larger rabbitries, there were many other unreported cases, particularly in those places where only two or three rabbits were kept as pets. In the larger rabbitries one keeper reported that he had lost forty; another reported losing fifty out of his 150, while a third owner lost so many of his prize Chinchilla gigantas that he eventually gave up rabbit-keeping.

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† This insect has been generally known as *A. maculipennis atroparvus*; according to Mattingly (1950) the correct name is *A. labranchiae* subsp. *atroparvus* van Thiel.

THE IMPLICATION OF *ANOPHELES ATROPARVUS* AT NEWHAVEN

From the end of September 1954 onwards attention was concentrated on two of the larger rabbitries, in which cases of myxomatosis were still occurring, and in which rabbits, often in an advanced stage of the disease, were still present.

Both these rabbitries yielded *A. atroparvus*, one of them in large numbers with a single collection producing eighty-three females, of which eighty contained blood, fifteen of these being freshly gorged. Some of these mosquitoes were induced to bite healthy rabbits while others were sent to the National Institute for Medical Research, Mill Hill, for virus titration by grinding and inoculation.* Both methods confirmed the existence of natural infections with myxoma virus in *A. atroparvus*, as follows:

(1) *By direct bite:*

Date of collection	No. of *atroparvus*	Date of exposure to healthy host	No. feeding	Results
27. ix. 54–1. x. 54	11	5–6. x. 54	2 or 3 probed, but no blood taken	First symptoms on 12. x. 54, rabbit died 19. x. 54

(2) *By grinding and inoculation:*

(*a*) The eleven *atroparvus* in the biting experiment above were sent to Mill Hill on 7. x. 54. Virus was isolated from them.

(*b*) Eighty-three *atroparvus* caught in rabbit hutches, Newhaven, on 12. x. 54 were sent to Mill Hill on 14. x. 54. Virus was isolated from them.

After the first collection of mosquitoes in these rabbitries the owners started using an insecticide regularly and no more *atroparvus* were found.

Both of these rabbitries were in the built-up part of the town, and there were no other animal shelters in the immediate neighbourhood which might have yielded more mosquitoes. However, from that time onwards *atroparvus* was taken regularly in cow-sheds, pig-sheds, hay sheds and other farm buildings in several farms on the outskirts of Newhaven, and it appeared possible that some of those mosquitoes, before they went into semi-hibernation, may have been exposed to infection either from diseased wild rabbits or from domestic stock. Batches of wild-caught *atroparvus* from Newhaven, and also from Sheppey in Kent (another area where *atroparvus* could have been exposed to infection) were fed on anaesthetized healthy rabbits, while other batches were tested for virus by inoculation of ground insects. Out of about 1000 *atroparvus* tested in one or other of these ways, no infected mosquitoes were found.

To return to the naturally infected *atroparvus* found in the Newhaven rabbitries, there were two possible sources of infection. At that time the disease was active among wild rabbits along the cliff tops in the immediate vicinity of the rabbit houses, and dying myxomatous rabbits were seen close at hand. The mosquitoes therefore could have picked up the infection either from the wild rabbits or from the diseased domestic rabbits in their hutches. Although the disease must have come from wild rabbits in the first instance, it seems likely that the increasing

* For his assistance in this matter I am greatly indebted to Dr C. H. Andrewes.

number of diseased domestic rabbits in hutches provided a much more potent source of infection, particularly at a time when *atroparvus* was seeking blood meals indoors before going into semi-hibernation in an occupied animal shelter. This applies especially to some of the prize rabbits which had been inoculated 2–3 weeks before the outbreak, but which contracted the disease and were kept alive as long as possible in the hope that they might recover.

These findings demonstrate fairly clearly the important part *atroparvus* can play in spreading the disease among domestic rabbits, but nothing is yet known about the possible part played by this mosquito in spreading the disease among populations of wild rabbits, or even in conveying the infection from diseased wild rabbits to healthy domestic ones.

Laboratory experiments show that *atroparvus* is an extremely efficient vector of myxoma virus, being capable of retaining its infection for long periods. But before discussing these experiments it would be more fitting to review some aspects of the general behaviour of *atroparvus*, particularly in relation to the present problem.

THE HABITS OF *ANOPHELES ATROPARVUS*

A. atroparvus is the main vector of malaria in northern Europe, and a great deal of work has been done on its habits, particularly in the Netherlands where malaria is still endemic. There and in Britain (Shute, 1954) *atroparvus* is mainly confined to low-lying estuarine areas where suitable brackish water breeding places are available. In Britain the main *atroparvus* centres are along the south and east coasts of England, particularly in Kent, Essex and Norfolk, but there are lesser centres on parts of the west and south-west coasts. In Britain the adult mosquitoes are locally abundant in suitable dark stables, cow-sheds, pig-sheds, etc., throughout most of the year. The winter is spent in occupied animal shelters and other suitable farm buildings where the mosquito lives in semi-hibernation, blood meals being taken at intervals throughout the cold months from November to April. The increased mechanization of British farms and the progressive disappearance of horses and stables have had a great effect on the habits and possibly the numbers of this mosquito. Shute has drawn attention also to the effect on the mosquito of the great decrease in the number of pigs kept by smallholders and farm-workers in recent years; and increasing use of light, well-ventilated cow-sheds, and of insecticides have combined to make this mosquito surprisingly difficult to find in places where we would expect large numbers. Findings in the present investigation which conflict a little with previous ideas are probably due to these rapidly changing conditions.

During the winter large collections of *atroparvus* were made regularly at one or two farms in Newhaven, and in Sheppey. Although many mosquitoes were found in occupied, dark, ill-ventilated cow and calf-sheds, as we might expect, large numbers were also taken in hay sheds, and in the dark spaces between the top of the hay and the underside of the roof. One specially suitable hay shed was part of a long divided barn in another part of which calves were housed throughout the winter, but regular high catches were also taken in hay sheds some distance from

animal shelters. From the condition of these semi-hibernating females, the ease with which they would take a blood meal without subsequent development of fat-body, and from later identification of egg samples, there is no doubt that they were *atroparvus*, although the hay shed as a hibernating site is more usually associated with the completely hibernating *Anopheles maculipennis messeae.*

The temperature in unoccupied hay sheds was undoubtedly much cooler than in occupied barns and stables, often near that outside. This may be why *atroparvus* in such places fed very little on blood throughout the winter, as shown by the following samples taken in December 1954:

Conditions of female *atroparvus*	Newhaven 13. xii. 54, hay shed adjoining cow-shed	Sheppey 17. xii. 54, open type of occupied calf-shed
Unfed, abdomen completely flat, no trace of blood	83	91
Traces of blood	13	17
Old blood meal digesting	4	9
Fresh blood	0	0
	100	117

In this arbitrary classification the 'old blood meal digesting' indicated a blood feed taken at least 3–4 days previously. Both samples agree in showing an unexpectedly high proportion of unfed females—over 80 %—and no indication that any mosquitoes had fed within the previous 2 or 3 days. Later observations on semi-hibernating *atroparvus* kept in a cage under conditions resembling those in hay sheds showed that a single blood meal every 3–4 weeks was quite adequate for survival through the winter, and that many mosquitoes survived for 2 months between blood meals. Except that fatty tissue was not deposited, mosquitoes so surviving seemed to be truly hibernating.

In protected cages many overwintering *atroparvus* caught in October and November were still alive the following May, and one survived till mid-July, being then at least 8 months old. The exact conditions under which *atroparvus* passes the winter, and its length of life under those conditions, are of interest because *atroparvus* infected with myxoma virus in October and November can carry the infection until the following spring, at least in the laboratory and possibly also in nature.

There were no opportunities for studying the relation between *atroparvus* and wild rabbits in Britain. By the end of the summer of 1954 the rabbit population in many of the most suitable *atroparvus* areas was much reduced, and by the spring of 1955 the whole south-east coastal area of England had been practically cleared of wild rabbits. Even under the best conditions an investigation into this problem could have been successful only where a suitable colony of wild rabbits was combined with a high mosquito population. Such places were discovered too late. There is no evidence that *atroparvus* played an important part in the spread of the disease among wild rabbits, as *Anopheles annulipes* did in Australia. The rate of spread of myxomatosis in flat coastal areas with a high *atroparvus* population did not differ obviously from that in mosquito-free areas inland. One of the earliest reported outbreaks was at Alciston, about 5 miles from Newhaven, in East Sussex in

November 1953. By June and July 1954 most of the farm lands round Newhaven had been affected by the disease. In Newhaven itself, however, an isolated colony of wild rabbits on the cliff top remained free from the disease until the end of September 1954, when the first diseased rabbits were seen. This locality was separated by a little over 1 mile of low-lying estuarine swamps from *atroparvus* areas where the disease had first appeared several months previously. The remarkably slow progress of the disease in this area of unusually abundant *atroparvus* suggests that this mosquito played very little part.

There is little more information about feeding habits on domestic rabbits. Reference has already been made to the high catches of blood-fed *atroparvus* in rabbitries in Newhaven. None of this material could be spared for more exact precipitin tests, but the absence of farms and farm animals nearby, when the batches contained mosquitoes infected with myxoma virus, certainly indicates blood feeding on domestic rabbits. A close association between *atroparvus* and domestic rabbits has previously been recorded by other workers, but there is no convincing record of precipitin tests.

A rather more exact observation under very different conditions was made in the summer of 1955. At that time large numbers of *atroparvus* were taken regularly in dark pig-sheds and adjacent dark shelters in a farm in the Newhaven area. Three or four yards from these shelters was another shed in which three domestic rabbits were kept in separate dark hutches. Preliminary crude observations were made as follows:

	Day-time collections	
Date	*atroparvus* in pig-sheds	*atroparvus* in adjacent rabbit-hutches
17. vi. 55	15	0
11. vii. 55	25	0
22. vii. 55	150 approx.	2
5. viii. 55	400 approx.	4
24. viii. 55	250 approx.	0

Blood smears taken from 170 freshly engorged mosquitoes in the final sample were sent to Dr Weitz for precipitin testing with the following results:

111 had fed on pig
58 had fed on ox
0 had fed on rabbit

169

The high proportion of ox blood clearly indicates that mosquitoes feeding on other hosts some distance away (the calf-sheds and byres were at the other end of the farm) would still shelter in the pig-sheds, and that the absence of rabbit blood therefore truly indicates negligible feeding on rabbits. The low preference of *atroparvus* for rabbit blood might appear difficult to reconcile with the implication of this mosquito in the outbreak of myxomatosis among domestic rabbits referred to above. In that outbreak many *atroparvus* were taken in the affected rabbitries, and virus was recovered from two separate batches of these wild-caught mosquitoes. However, the rabbitries in which this outbreak was particularly marked were

situated mainly in built-up areas some distance from farms and farm animals. This, combined with the additional attraction of many rabbits concentrated in one place, may have been sufficient to attract *atroparvus*. But this is only a preliminary and very tentative interpretation; obviously much more work would have to be done to explain satisfactorily the wide difference in attraction of *atroparvus* to rabbits in these two series of observations.

DISCUSSION

The observations now reported indicate that in some localities in England *A. atroparvus* can play an important part in the transmission of myxomatosis among domestic rabbits. There is no evidence, however, to show that this mosquito plays any major role in spreading the disease among wild rabbits. Laboratory experiments on transmission have shown that *atroparvus* is an efficient vector of myxoma virus and is capable of carrying the infection over winter from autumn to the following spring, infectivity still being retained after several intermediate blood meals. Further discussion follows presentation of the laboratory data in the accompanying paper (Andrewes, Muirhead-Thomson & Stevenson, 1956).

SUMMARY

Evidence is presented that outbreaks of myxomatosis among domestic rabbits at Newhaven in Sussex were spread through the agency of *Anopheles atroparvus*. The habits of this insect are discussed in relation to its possible importance as a vector of myxomatosis.

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