

LEAGUE OF NATIONS

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At its meeting in October 1937 the Reporting Committee of the Malaria Commission recommended that the Commission should collect and publish from time to time information on the subject of the bionomics of anophelines with special reference to differences shown by varieties of different species, and that the studies begun in England and Roumania upon the longevity of carrier species of anopheline should be continued.

The Secretariat of the Commission therefore has the honour to communicate a

PRELIMINARY REPORT ON THE LONGEVITY OF THE RACES OF ANOPHELES
MACULIPENNIS

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In 1938, the Malaria Commission of the League of Nations provided us with financial assistance to investigate the longevity of the races of A. maculipennis. One of us (E.U.) stayed for two months at the Horton Malaria-Therapy Centre and, on returning to Roumania, continued, and is continuing, his investigations at a village, (Osoiu), in northern Roumania. One of us (P.G.S.) spent a month at this village in June 1938, and has continued the investigations in the laboratory at Epsom since that time. Although it is too early to draw any final conclusions, we believe that our results to date are of sufficient interest to justify a preliminary note on this subject. We hope that we shall be able to continue these and other enquiries for a further period and that sufficient material will accumulate to enable us to arrive at some definite conclusions.

Origin of the enquiry.

In 1933, 1935, 1937 and 1938, one of us (P.G.S.) visited Roumania and, on each occasion, took with him large numbers of malaria-infected mosquitoes from England. Even earlier, in 1930, a batch of infected mosquitoes was taken from England to Rome. On all four journeys the mosquitoes taken were A. maculipennis var. atroparvus. It was observed that, providing a blood meal was available for the insects when required, during the three or four days in the train, the death rate was very low. On the other hand, when mosquitoes were brought to England from Roumania the death rate was extremely high despite great care taken of them. The insects brought from Roumania to England were a mixture of vars.. typicus and messeae.

A further observation which has a bearing on this subject was that in the laboratory at Horton where atroparvus only is used, it was often found that 80% survived long enough for sporozoites to appear in the salivary glands; usually a period of about 10 days at 24.C. On the other hand the Malaria Therapy Centre at the Hospital Socola, Roumania, Dr. Vieru-Chelarescu found it very difficult to keep alive for about 2 weeks even 10% of the infected mosquitoes. These findings were recorded at all seasons of the year. She was working with a mixture of typicus and messeae. As far as we could ascertain the conditions prevailing in our two laboratories, the one at Jași and the one at Epsom, were similar and the laboratory technique was identical.

Methods employed for longevity experiments.

The village of Osoiu was selected for these studies at the suggestion of Professor Ciuca. At Osoiu malaria is endemic, mosquitoes are numerous and three varieties, typicus, messeae and atroparvus are present in varying proportions. There is a well-equipped dispensary in the village and Dr. Alexa placed the necessary apparatus at our disposal and gave us every possible assistance. In the dispensary grounds there is a room occupied at night by a boy who is employed at the dispensary. A series of temperature and humidity readings were recorded in this room, while at the same time similar readings were carried out in the living rooms of the peasants in order to see that they were comparable. This was found to be the case. The adults used were freshly bred from eggs which had been identified when first laid by adults collected in nature. The larvae were bred in earthenware pans containing tufts of grass as described by Shute (1936). For the purpose of this enquiry it was thought to be essential that the mosquitoes should have ample opportunities of blood meals either human or animal so that there should not be an artificial death rate brought about by the lack of opportunities to feed. The pupae were collected and transferred to a suitable cage and after the adults had emerged for one or two days they were given a meal of blood, either human or animal, and then a given number were transferred to a large cage. The survivals were counted every three days; all the insects had a meal of blood at the beginning of the experiment. Every night a rabbit was put into the cage and remained there until about 6 a.m. the following morning. Each insect was then carefully examined and those which had not fed on the rabbit during the previous night were given the opportunity to feed from one of us or from a pony on the estate. The survivals were counted every three days. In order to get as good a picture as possible it was considered necessary to keep as a control some specimens of mosquitoes which had been infected with malaria on the same day as each longevity experiment was started. The blood of a number of children was examined and when one was found whose blood contained P.vivax gametocytes some specimens of atroparvus were fed on it. These infected insects were kept in a separate cage in the same room as the longevity mosquitoes but

they were fed only on sugar solution so that we could examine the mid-guts of one or more each day until their glands became infected. Only atroparvus were used for infecting purposes in these experiments and it has been assumed for the purposes of this experiment that the parasite developed at the same rate in all three varieties, an assumption which may require verification. We decided that as far as possible, one experiment should be carried out each month of the year. It would, of course, have been much better if we could have had three or four experiments each month but this was not possible with the facilities available.

RESULTS OF EXPERIMENTS AT OSOIU.

SERIES A.

Experiment No. 1 (June 10th to June 25th).

(1) Conditions of experiment.

Minimum dry bulb reading 15°C. Maximum dry bulb reading 27°C. The percentage of saturation varied between 55 and 81.

(2) Insects used.

Eighty specimens each of atroparvus, typicus and messeae.

(3) Results.

Survivals on the 15th day.

<u>Atroparvus</u>	90%
<u>Typicus</u>	30%
<u>Messeae</u>	4%

Mosquitoes, (atroparvus) infected with P.vivax on the day this experiment began, showed sporozoites in the glands on the 15th day.

(4) Conclusions.

On the assumption that the rate of development of P.vivax is the same in all three varieties of maculipennis, the percentages of each variety which would survive long enough to become infective and therefore dangerous, would be:

<u>Atroparvus</u>	72 out of 80 = 90%
<u>Typicus</u>	24 " " 80 = 30%
<u>Messeae</u>	3 " " 80 = 4%

SERIES A.

Experiment No.2 (June 22nd to July 3rd.)

(1) Conditions of experiment.

Minimum dry bulb reading 20°C. Maximum dry bulb reading 34°C. The percentage of saturation varied between 60 and 80.

In this experiment, no atroparvus being available, only messeae and typicus were compared.

(2) Insects used.

Twenty-five specimens each of messeae and typicus.

(3) Results.

Survivals on the 12th day.

<u>Typicus</u>	38%
<u>Messeae</u>	22%

Mosquitoes, (atroparvus), infected with P. vivax on the day this experiment began, showed sporozoites in the glands on the 12th day.

(4) Conclusions.

On the assumption that the rate of development of P. vivax is the same in all three varieties of maculipennis the percentages of each variety which would survive long enough to become infective and therefore dangerous, would be:-

<u>Typicus</u>	19 out of 50	=	38%
<u>Messeae</u>	11 " " 50	=	22%

SERIES A.

Experiment No.3. (July 19th to July 30th).

(1) Conditions of experiment.

Minimum dry bulb reading 22°C. Maximum dry bulb reading 30°C. The percentage of saturation varied between 63 and 82.

(2) Insects used.

Fifty specimens of atroparvus, typicus and messeae.

(3) Results.

Survivals on the 12th day.

<u>Atroparvus</u>	90%
<u>Typicus</u>	68%
<u>Messeae</u>	44%

Mosquitoes, (atroparvus) infected with P. vivax on the day this experiment began, showed sporozoites in the glands on the 12th day.

(4) Conclusions.

On the assumption that the rate of development of P. vivax is the same in all three varieties of maculipennis,

the percentages of each variety which would survive long enough to become infective and therefore dangerous, would be:-

<u>Atroparvus</u>	45 out of 50	=	90%
<u>Typicus</u>	34 out of 50	=	68%
<u>Messeae</u>	22 out of 50	=	44%

SERIES A

Experiment No.4. (August 12th to August 24th).

(1) Conditions of experiment.

Minimum dry bulb reading 22°C. Maximum dry bulb reading 30°C. The percentage of saturation varied between 64 and 94.

(2) Insects used.

Fifty specimens each of atroparvus, typicus and messeae.

(3) Results.

Survivals on the 13th day.

<u>Atroparvus</u>	100%
<u>Typicus</u>	85%
<u>Messeae</u>	65%

Mosquitoes, (atroparvus) infected with P. vivax on the day this experiment began showed sporozoites in the glands on the 13th day.

(4) Conclusions.

On the assumption that the rate of development of P. vivax is the same in all three varieties of maculipennis, the percentages of each variety which would survive long enough to become infective and therefore dangerous, would be:-

<u>Atroparvus</u>	50 out of 50	=	100%
<u>Typicus</u>	42 out of 50	=	85%
<u>Messeae</u>	32 out of 50	=	65%

SERIES A

Experiment No.5. (August 27th to September 9th).

(1) Conditions of experiment.

Minimum dry bulb reading 18°C. Maximum dry bulb reading 29°C. The percentage of saturation varied between 63 and 82.

(2) Insects used.

Fifty specimens each of atroparvus, typicus and messeae.

(3) Results.

Survivals on the 14th day.

<u>Atroparvus</u>	88%
<u>Typicus</u>	52%
<u>Messeae</u>	6%

Mosquitoes, (atroparvus) infected with P. vivax on the day this experiment began, showed sporozoites in the glands on the 14th day.

(4) Conclusions.

On the assumption that the rate of development of P. vivax is the same in all three varieties of maculipennis, the percentages of each variety which would survive long enough to become infective and therefore dangerous, would be:-

<u>Atroparvus</u>	44 out of 50	=	88%
<u>Typicus</u>	26 out of 50	=	52%
<u>Messeae</u>	3 out of 50	=	6%

SERIES B.

Experiment No. 6. (July 17th to August 3rd).

(1) Conditions of experiment.

In this experiment all the insects were kept in our hot room at a constant temperature of 24°C. The percentage of saturation varied between 70 and 90.

(2) Insects used.

Twenty specimens each of atroparvus, labranchiae, typicus and messeae.

(3) Results.

Survivals on the 11th day.

<u>Atroparvus</u>	95%
<u>Labranchiae</u>	70%
<u>Typicus</u>	70%
<u>Messeae</u>	50%

Mosquitoes, (atroparvus), infected with P. vivax on the day this experiment began, showed sporozoites in the glands on the 11th day.

(4) Conclusions.

On the assumption that the rate of development of P. vivax is the same in all four varieties of maculipennis, the percentages of each variety which would survive long enough to become infective and therefore dangerous, would be:

<u>Atroparvus</u>	19 out of 20	=	95%
<u>Labranchiae</u>	14 " " 20	=	70%
<u>Typicus</u>	14 " " 20	=	70%
<u>Messeae</u>	10 " " 20	=	50%

SERIES B.

Experiment No.7. (July 17th to August 3rd).

(1) Conditions of experiment.

In this experiment the only difference from the previous one was that the mosquitoes were kept in an out-house in the hospital grounds under more or less natural conditions and where normal atmospheric temperatures prevailed.

Minimum dry bulb reading, which represents night or early morning temperatures was 16°C. The maximum dry bulb reading was 26°C. The percentage of saturation varied between 60 and 80.

(2) Insects used.

Twenty specimens each of atroparvus, labranchiae, typicus and messeae.

(3) Results.

Survivals on the 21st day.

<u>Atroparvus</u>	60%
<u>Labranchiae</u>	80%
<u>Typicus</u>	45%
<u>Messeae</u>	30%

Mosquitoes, (atroparvus) infected with P. vivax on the day this experiment began, showed sporozoites in the glands on the 21st day.

(4) Conclusions.

On the assumption that the rate of development of P. vivax is the same in all four varieties of maculipennis, the percentages of each variety which would survive long enough to become infective and therefore dangerous, would be:-

<u>Atroparvus</u>	12	out of	20	=	60%
<u>Labranchiae</u>	16	"	"	20	= 80%
<u>Typicus</u>	9	"	"	20	= 45%
<u>Messeae</u>	6	"	"	20	= 30%

DISCUSSION.

In all, 7 longevity experiments were carried out, 5 in Roumania and 2 in England. The average number of each of the varieties which survived long enough for the malaria parasite (P. vivax) to complete its development was

as follows:-

<u>Atroparvus</u>	87%
<u>Labranchiae</u>	75%
<u>Typicus</u>	55%
<u>Messeae</u>	32%

It is important to note that, in the case of atroparvus, except for the one experiment in nature in England, the survival rate was always 88% or over.

The labranchiae material available for studies was insufficient to enable even a tentative conclusion to be reached.

With messeae, too, there was considerable variation in the survival rates, in fact, even more so that in the case of typicus. The highest was during the month of August when it was 65% and the lowest in June when it was only 4%. It will be noted that the lowest percentages surviving of both typicus and messeae were during the month of June and the highest for these two varieties were during the month of August. If the survival rates of atroparvus, typicus and messeae be compared, it will be seen that whereas about 90% of the first variety survives during the transmission season (June to August) the rates are much lower with the other two. Typicus appears to live longer than messeae but not nearly so long as atroparvus. The variations in the survival rates occurring among the three varieties are greatest in messeae.

These figures are the results of the trials in Roumania and also include the English experiments. A comparison of the August experiments in Roumania and in England reveal that there is very little difference between the two, if anything, the survival rate of typicus and messeae is slightly higher in Roumania than was the case in England, except for atroparvus, but the differences are slight.

In June, the number of days necessary for the parasite to complete its cycle in atroparvus was 15, in nature in Roumania. As only 4% of the messeae survived for this time it suggests that messeae cannot play any important role in the spread of malaria at this season of the year. Typicus, however, would have been more important than messeae while atroparvus would be many times more dangerous than both the other varieties, if these findings are comparable with what happens in nature.

It is interesting to note that in the August experiment (the hottest season of the year in northern Roumania), the survival rate was at its highest in all three varieties.

Another important factor is that none of the typicus or messeae were fertilized while all the atroparvus were, and batches of eggs appeared in the cage of this variety almost daily. The developing ovaries and subsequent egg laying could have been expected to have had some adverse effect on the length of life of these insects and therefore typicus and messeae should have survived longer than atroparvus if ovary development played an important part in the death rate.

The labranchiae material available for studies was insufficient to enable even a tentative conclusion to be made. In typicus there was considerable variation in the longevity results which may possibly be due to the variations of temperature, humidity and food, according to the seasons of the year. The fact remains, however, that on a single occasion the survival rate of typicus was fairly high 85%. This, we think, may possibly indicate that the season of the year (August) is the one most favourable to typicus in this district and if this is so this variety would probably play an important part in the transmission of malaria, whereas at other seasons of the year it would be relatively unimportant. The results of a single experiment do not, however, justify any definite conclusions.

PROVISIONAL SUMMARY.

Atroparvus usually feeds very readily either on man or animals, but typicus, while feeding freely on animals, is often reluctant to bite man. Messeae is more reluctant to bite man than typicus is, even when partly starved.

The longevity of the varieties of A. maculipennis studied appears to depend not only upon external conditions such as temperature and humidity, but also upon some constitutional factor inherent in the variety. Under the conditions of our experiments evidence has been obtained which strongly suggests that atroparvus lives longer than any of the other varieties, and that typicus lives longer than messeae. The two last species show great variations in their mortality rates at different seasons of the year under conditions which would appear to be optimum for the survival of a high percentage of atroparvus. It is still uncertain what parts are played by external differences by varietal peculiarities and by feeding preferences in determining the length of life of these insects under different conditions. The results obtained suggest, however, that in Roumania atroparvus may be a more dangerous insect than either messeae or typicus, but that at certain seasons messeae may also be an important carrier of malaria for a short time. Many more observations will, however, need to be made before these tentative conclusions can be accepted.

BRIEF NOTE ON THE RELATIVE CARRYING POWERS OF THE VARIETIES.

One or two experiments concerning the relative carrying powers of the varieties suggest that there are considerable differences in them. Only a few experiments have so far been carried out but it is rather significant that the

percentage of atroparvus infected was always higher than typicus or messeae. Not only was it the case that the percentage infected was higher but also that the average number of oocysts per insect was greater. One experiment may be quoted.

Messeae and atroparvus.

The adults used were bred from eggs laid in the laboratory. The atroparvus were from our pure strain which have been breeding in the Horton laboratory continuously since 1933. The messeae used were obtained from adults collected from a farm near Woking, Surrey. They were brought to the laboratory and adults were reared from the eggs which these messeae laid.

The species of parasite was P. vivax. The mosquitoes were fed on the same patient at the same time and were kept in separate cages in the laboratory at a constant temperature of 25°C throughout the experiment. The mosquitoes were all dissected on the 8th day. The oocysts were nearly mature, having reached the sporoblast formation stage but none had ruptured. The patient on whom the mosquitoes fed showed 50 ripe male gametocytes per 500 leucocytes, approximately 500 per c.m.m.

In this experiment 28 messeae and 28 atroparvus were used.

<u>Atroparvus</u>		<u>Messeae</u>	
Number dissected	28	Number dissected	28
Number positive	22	Number positive	17
Number negative	6	Number negative	11
Percentage positive	79	Percentage positive	61

<u>Atroparvus</u>	<u>Messeae</u>
Number of oocysts per stomach.	Number of oocysts per stomach.
5	1
6	1
8	2
9	3
10	3
17	4
18	4
25	4
26	4
36	5
37	8
82	10
98	10
98	13
98	20
103	20
126	20
127	22
146	44
148	105
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1223	303

Average per stomach 61.

Average per stomach 15.

Of the seventeen infected messeae, eleven showed less than ten oocysts and only one specimen had more than fifty, while of the twenty-two infected atroparvus, only four had less than ten while nine had more than fifty. This means that more than 50% of the messeae would lose the majority of their sporozoites much more quickly than the atroparvus, probably after two, or perhaps three, feeds. On the other hand, the atroparvus would have continued to inject many sporozoites even after a greater number of feeds and those which were heavily infected would not have become negative even after many feeds, probably for the rest of their lives.

This is an example of the relative carrying powers of two of the varieties of A. maculipennis and although many more experiments are necessary before any conclusions can be reached, it appears to indicate that there are important differences in the relative carrying powers of the different varieties of A. maculipennis studied.