WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTE

a 63297

WHO/Mal/379
8 February 1963

ORIGINAL: ENGLISH

PRELIMINARY REPORT ON THE POSSIBILITY OF THE DETERMINATION OF PHYSIOLOGICAL AGE IN ANOPHELES GAMBIAE AND ANOPHELES FUNESTUS

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INTRODUCTION

Although Polovodova's method for the determination of the physiological age of mosquitos has been widely used in the Soviet Union for the study of Anopheles maculipennis and other species, relatively few applications of the method have been attempted in tropical countries. In Africa, in particular, few reports have appeared on the feasibility of the method for the study of the important vectors of malaria: Anopheles gambiae Giles and Anopheles funestus Giles. The results of these studies were of interest but the assessment of the applicability of this method for the main African vectors was not conclusive.

This paper records the results of preliminary observations on the physiological age of these two vectors in Tanganyika, together with notes on the difficulties encountered in the application of the technique and suggestions for overcoming them. The studies were made over a short period only, in October and the first 10 days of November 1962, at Muheza near the coast, and at Gonja in South Pare district further inland. In neither district were any mosquito control measures currently in force. At Gonja residual spraying with dieldrin has been carried out during the years 1955-1959, the last spraying cycle having been completed 3-1/2 years previous to the present study. At Muheza there are virtually no domestic animals and both species are known to be highly anthropophilic. But at Gonja many cattle are present, most of them being herded at night outside in the immediate vicinity of houses.

TECHNICAL POINTS

As a result of studying age changes in the ovaries of females of several species of African Anopheles, it has been found that the application of Polovodova's method is possible. However, it must be noted that the determination of the physiological age in A. gambiae and A. funestus is more difficult than in A. maculipennis and certain other African species such as A. coustani (s.l.) and A. machardyi.

Special difficulties were encountered in assessing the age of two categories of parous females:

1. In freshly-fed females (Sella's stages 2 and 3) in which ovulation has recently taken place. A. gambiae and A. funestus differ from A. maculipennis in the greater speed of development of the ovaries, although the rate of contraction of the ovariolar sacs appears to be of the same order. In this group of females three conditions are found. The first is in those in which the intimal sheath of each ovariole is large and sac-like. If, in such females, no ovarioles showing degenerating follicles are present, determination of age is not possible.

The second condition comprises those in which partial contraction of the sac has occurred, owing to a greater lapse of time since ovulation. Until contraction has reached a certain stage separation of the dilatations is not complete and accurate age determination is not possible. In such females the impression may be gained that a single large dilatation is present without a free terminal stalk. In these specimens individual ovarioles must be very carefully stretched and a diagnosis of age condition only made if the intact terminal stalk can be seen and at the same time dilatations become partially separated, and so distinguishable, even before full contraction has occurred. Also it often happens with strong stretching of ovarioles (over-stretching) that in some ovarioles one dilatation on the partially contracted sac remains attached to the calyx while the other part is seen to be detached and connected to the follicle. In such ovarioles age determination cannot be carried out. Because of these possible sources of error, it must be emphasized that when confronted with partially contracted sacs or broken ovariole tubes the observer should search for tubes with degenerating follicles; only if these are found can a diagnosis be made.

In the third condition, as commonly noted with all other species, the sacs are fully contracted and, although normal dilatations are present, some difficulty may be encountered in examining such females owing to the ease with which the ovariolar stalks are broken.

2. Also as observed with all other species, other difficulties may arise with fully gravid females with ovaries in Christophers' stage V, when the ovaries are fully developed. First, the intimal sheaths as well as the ovariolar stalks are very easily torn, rendering age diagnosis impossible. Secondly, the yolk from damaged eggs tends to obscure the field. In fully gravid females, therefore, only the ovarioles showing degeneration can be used for study.

A further point of difference between A. maculipennis and A. gambiae and A. funestus lies in the relative paucity of degenerating follicles seen in the latter two species. In A. maculipennis a well marked decline occurs in the number of eggs laid with each successive oviposition, with the result that old females show many degenerations. In the African species studied, although the number of egg batches counted was small (see Table 6), it appears that the decline of fertility with age is much less marked and degenerating follicles correspondingly scarce. This scarcity thus contributes to the difficulties encountered in determining the age of these species.

In view of some of these difficulties it was found that females with ovaries at Christophers' stage IV were the most suitable for dissection. In A. funestus, under the temperature conditions existing at the time of this study, the ovaries have reached stage IV on the second morning after the blood meal. By this time the ovariolar sacs are to a large extent contracted and normal dilatations can be seen. Moreover the elongation of the follicles facilitates the separation and examination of individual ovarioles. In A. gambiae, stage IV is usually passed during the night. However, owing to the more rapid rate of contraction of sacs in this species, females with ovaries at late stage III show similar advantages.

RESULTS OF DISSECTIONS

Mosquitos were caught in houses by spray-catching with pyrethrum. Dissection was either carried out on the same day or else the material was preserved in the refrigerator until the following day. In addition, a number of hand catches were made in houses and calf-huts at Gonja. Collections were only made on 13 days at Muheza and on six days at Gonja. Thus the results recorded here merely represent the age condition on a very limited number of days, and it is not possible to speak in terms of the age composition of the population.

The results of the dissections are shown in Tables 1 and 2. It will be seen that females of A. funestus were found at all ages with up to nine dilatations. About 25% of mosquitos had three or more dilatations. Since the duration of the gonotrophic cycle is known to be three days under local conditions, and since an additional one or two days are spent in the pre-gravid phase, it is evident that at least 10 to 13 days must have elapsed from the first blood meal in all these females. Further, since the extrinsic incubation period for Plasmodium falciparum is 12 to 13 days in the temperature conditions encountered, it follows that this group must be regarded as epidemiologically dangerous. Similarly, it appears that some females at least, i.e. those with nine dilatations, must have reached an age of approximately one month.

In Table 3 the age condition of sporozoite positive A. funestus is shown. Although the number dissected was small, it will be seen that most of the infective specimens had four or three dilatations. Since these age-groups are well represented in the catches, the results suggest that such females may be found to be the most important from the point of view of malaria transmission. Filarial larvae (fully developed) were found in two females with five dilatations, and thoracic (sausage) forms in two specimens with three and five dilatations.

Table 2 shows the results of dissections of A. gambiae. In both series of catches the proportion of nullipars was higher than in A. funestus, but individuals with up to six dilatations were found. The catches from Muheza were too small for further comment, but although those from Gonja are also based on a very short series of observations the proportion of nullipars did not differ greatly from that observed during the previous six months (see Table 4). Two specimens from Muheza with three and six dilatations respectively were found with sporozoites.

FERTILITY IN RELATION TO AGE

As recorded above it was found that relatively few degenerating follicles were found in the ovaries of both A. gambiae and A. funestus, even in older females. It was decided accordingly to count the number of developed eggs in females with ovaries at stages IV and V in order to check what effect, if any, age had on fertility. The results of these counts are shown in Tables 5 and 6. Although much longer series are required to establish the relationship on a firm basis, it is clear from the tables that there is no very obvious decline in fertility with age such as has been observed in A. maculipennis. Considerable variation in size of egg batch between different individuals of the same age was noticed, but observations on wing measurements (not yet analysed) suggest that these are primarily associated with differences in size of insect.

IDENTIFICATION OF BLOOD MEALS BY AGGLUTINATION TESTS

A small trial was made of a technique, adapted for malariological purposes in 1956 by Vlasenko for rapid detection of non-human blood in the stomachs of freshly-fed mosquitos. The test involves mixing the stomach contents with saline on a glass slide and the addition of a drop of Group AB serum. No agglutination occurs with human blood, but if any non-human cells are present agglutination is observed. Using this test at Gonja, 44% of 258 freshly-fed A. gambiae were found to contain non-human blood.

CONCLUSIONS

Polovodova's method for the determination of physiological age was found to be fully applicable to A. gambiae and A. funestus. However, attention must be drawn to several obstacles encountered:

(a) The greater difficulty of dissection in mosquitos of small size and with small ovarioles.

(b) The relatively smaller number of degenerating follicles seen in these species, as compared with A. maculipennis.

(c) The presence of sacs in many freshly-fed (Salla's stages 2 and 3) females makes the determination of age difficult, especially when degenerating follicles are scarce.

In view of these difficulties it was found that females with ovaries at stage IV were the most suitable for examination. However, determinations were possible on females at all stages, provided degenerating follicles could be found, or provided full contraction of ovariolar sacs had occurred.

TABLE 1. PHYSIOLOGICAL AGE OF FEMALE A. FUNESTUS FROM HOUSE CATCHES AT MUHEZA

Period	Total dissected	Unfed (Sella 1)	Pre-gravid	Nulli-parous	Number of dilatations									
					1	2	3	4	5	6	7	8	9	?
2- 5.10.62	101	3	11	30	16	21	8	7	2	-	-	-	-	3
10-12.10.62	162	8	14	40	26	38	19	9	2	2	-	-	-	4
16-19.10.62	264	5	27	44	23	57	35	31	13	3	4	-	-	22
5- 8.11.62	320	17	57	52	53	52	37	31	6	2	1	1	3	8
Total	847	33	109	166	118	168	99	78	23	7	5	1	3	37

TABLE 2. PHYSIOLOGICAL AGE OF FEMALE A. GAMBIAE FROM HOUSE CATCHES

Area	Total dissected	Unfed (Sella 1)	Pre-gravid	Nulli-parous	Number of dilatations						
					1	2	3	4	5	6	?
Muheza	142	4	23	43	26	22	6	5	2	1	10
Gonja	414	-	59	117	95	94	34	7	-	1	7

TABLE 3. RESULTS OF DISSECTIONS FOR SPOROZOITES IN A. FUNESTUS BY AGE-GROUPS

	Number of dilatations						
	3	4	5	6	7	8	9
Numbers + ve	1	4	3*	1	1	-	-
Dissected	95	64	23	7	5	1	3
Percentage + ve	1	6	9	14	20	-	-

* Includes one female from catches on which age determination was not otherwise carried out.

TABLE 4. PROPORTION OF NULLIPAROUS A. GAMBIAE IN S. PARE DISTRICT
MAY-OCTOBER 1962

Month	Number dissected	Percentage nulliparous*
1962:		
May	126	41.5
June	131	43.9
July	100	38.5
August	137	48.3
September	310	34.9
1-18 October	123	35.7
Present series 22-29 October	414	32.8

* Excluding pre-gravid

TABLE 5. A. FUNESTUS
NUMBER OF EGGS LAID IN RELATION TO PHYSIOLOGICAL AGE

Age	Number counted	Mean size egg batch	Standard error	Range
Nullipars	42	114.2	± 4.5	54-163
1-par	48	126.6	± 2.9	88-171
2-par	56	114.2	± 2.7	71-166
3-par	20	117	± 3.9	84-152
Over 3-par	16	116.1	± 5.6	77-163

TABLE 6. A. GAMBIAE
NUMBER OF EGGS LAID IN RELATION TO PHYSIOLOGICAL AGE

Age	Number counted	Mean size egg batch	Standard error	Range
Nullipars	51	159.7	± 6.2	66-275
1-par	21	152.9	± 6.6	107-210
2- and 3-par	11	140.7	± 7.8	101-190

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