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Resurgence of *Anopheles sacharovi* following malaria eradication

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In the Lamia Plain, Greece, 12 years after the cessation of DDT spraying, Anopheles sacharovi *is still present in very high densities and appears to have the same biting habits and host preferences as previously. Mosquitos of this species are still resistant to DDT and dieldrin.*

Intensive indoor spraying of DDT and other residual insecticides was discontinued in Greece almost 12 years ago and malaria, although not eradicated *sensu stricto*, is no longer considered to be a public health problem of any importance. According to the latest information from the Ministry of Social Services (D. Avramides, personal communication) 26 malaria cases were detected in the whole of Greece during the 1970 transmission season, 8 of them being classified as indigenous (6 of these were concentrated in one particular area, 51 km west of Salonica), 1 as a relapsing case, 8 as induced, and 9 as imported cases.

The dramatic effect of indoor residual spraying on domestic anopheline populations (particularly *Anopheles sacharovi*, the principal malaria vector in Greece) is well known. An obvious change in the behaviour of this species, at least as regards its resting habits, was noted from the onset of spraying operations and this change has been interpreted in different ways (Hadjinicolaou, 1954). Not very long after the initial application of residual insecticides, the phenomenon of resistance of *A. sacharovi* to DDT and dieldrin was observed and confirmed, at first in the District of Laconia (S. Peloponessus) and later in several other districts.

In routine field surveys it has been found that the densities of *A. sacharovi* are now as high as ever in most areas where it was known to breed profusely before DDT was used. This mosquito is now being found in abundance in houses, stables, and animal sheds and is biting man freely indoors and in the open. Since extensive data are available on the biology of *A. sacharovi* and its behaviour in Greece prior to the introduction of DDT, it was thought that it would be of some value to investigate the present density, biting habits, and host preferences of this mosquito.

The investigations began late in the 1968 season and continued through the 1969 and 1970 seasons.

THE LOCALITY

Lamia plain, situated 190–250 km north-west of Athens, was formerly an area with a high degree of malaria endemicity; the malaria vectors *A. sacharovi*, *A. superpictus*, and *A. maculipennis* and the nonvector rural species *A. hyrcanus*, *A. claviger*, *A. algeriensis*, *A. plumbeus*, and *A. marteri* were all well represented (Livadas-Sphangos, 1940). The area of the plain is estimated to be about 400 km^2. The river Sperchios flows through it and its estuary is about 4 km from the village of Thermopylae.

The population, according to the March 1971 census, is 160 000 but this figure includes the inhabitants of the mountainous Phthiotis District. This area is mainly agricultural and the livestock is composed mainly of sheep and goats, although a considerable number of dairy cattle and other domestic animals are kept in certain areas of the plain.

The Lamia plain was subjected to intensive DDT treatment from 1946 to 1959. Since then, no residual insecticide of any kind has been used as an antimalarial measure.

The small village of Thermopylae is situated on the main highway at a distance of 2 km from the Thermopylae sulfur springs, 20 km south-east of

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Lamia and 15 km north-west of Kamena Vourla on the sea shore. In the village there are 75 houses, 70 stables, 40 warehouses, and 9 animal sheds. There are 350 inhabitants, but during July, August, and September the population increases considerably as visitors come to stay in the village. The animal population of the village consists of about 85 cows, 75 horses, mules, and donkeys, and 3100 sheep and goats. There are also a few pigs and a number of chickens, rabbits, cats, and dogs.

Most of the houses of the village are protected by wire screening, but the main doors are usually not screened; the screening in some houses is badly maintained. Many of the inhabitants use aerosol sprays in their bedrooms. During the hottest period of the summer (July–August) a few people may sleep under bednets in the open.

A field laboratory was established in one of the typical village houses and entomological observations were carried out there from August 1968 until the end of the 1970 season.

RESULTS

Throughout the period of this investigation hand-catching of mosquitos was practised regularly in and around the observation village. Periodic catches were made from human habitations, stables, sheep sheds, artificial pit shelters, and also from our field station during the course of observations of the nocturnal activities and the behaviour of anophelines in the presence of the insecticide. A considerable proportion of the catch was classified according to species and the results are given in Table 1.

A. sacharovi appeared to be the main malaria-vector species in all types of catch and shelter. *A. superpictus* and *A. maculipennis* were present only in small numbers. Of the nonvector species, only *A. hyrcanus* was observed biting indoors and outdoors, in addition to *A. sacharovi*.

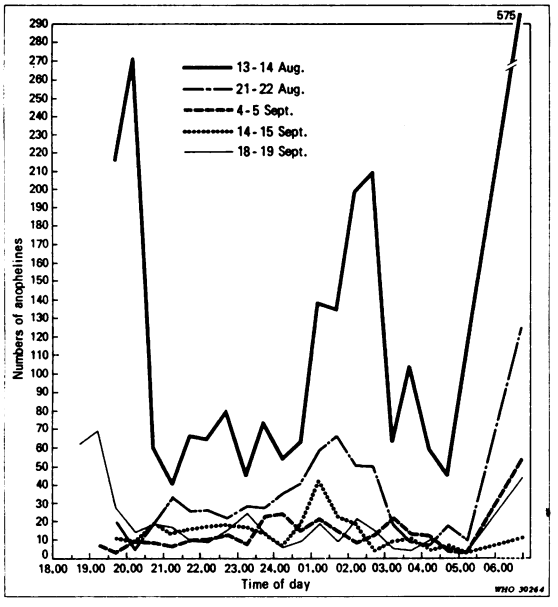
Before DDT was used, *A. sacharovi* was the predominant species in the lower part of the Lamia plain, but *A. superpictus* and *A. maculipennis* were then represented in considerably higher proportions, ranging from 4% to 17% of the total catches of anopheline malaria vectors for *A. superpictus*, and from 1% to 8% for *A. maculipennis* (Sphangos, 1943).

Since the Second World War a lot of drainage work has been done in the plain, particularly in the freshwater marshy areas, and thousands of hectares have been reclaimed for agriculture. This has led to the virtual disappearance of *A. maculi-*

Table 1. Anophelines caught in Thermopylae village during 1968–70 classified according to species

	1968					1969					1970				
	Total no. of anopheles examined	Species				Total no. of anopheles examined	Species				Total no. of anopheles examined	Species			
		A. sacharovi	*A. hyrcanus*	*A. superpictus*	*A. maculipennis*		*A. sacharovi*	*A. hyrcanus*	*A. superpictus*	*A. maculipennis*		*A. sacharovi*	*A. hyrcanus*	*A. superpictus*	*A. maculipennis*
from houses	1 940	1 932 99.59 %	7 0.36 %	7 0.05 %	0	326	325 99.69 %	1 0.31 %	0	0	1 563	1 553 99.36 %	10 0.64 %	0	0
from a stable	—	—	—	—	—	8 857	8 851 99.93 %	0	6 0.07 %	0	5 266	5 263 99.94 %	2 0.04 %	0	1 0.02 %
from artificial pit shelters	1 905	1 903 99.90 %	1 0.05 %	0	1 0.05 %	—	—	—	—	—	709	709 100 %	0	0	0
from a sheep shed	2 762	2 758 99.86 %	2 0.07 %	2 0.07 %	0	—	—	—	—	—	2 960	2 959 99.97 %	0	1 0.03 %	0
during all night observations	2 046	1 734 84.75 %	312 15.25 %	0	0	347	347 100 %	0	0	0	—	—	—	—	—
from the two observation rooms	—	—	—	—	—	6 128	5 854 95.53 %	274 4.47 %	0	0	6 717	6 290 93.64 %	427 6.36 %		0
total	8 653	8 327 (96.23 %)	322 (3.72 %)	3 (0.03 %)	1 (0.01 %)	15 658	15 377 (98.21 %)	275 (1.76 %)	6 (0.04 %)	0	17 215	16 774 (97.44 %)	439 (2.55 %)	1 (0.01 %)	1 (0.01 %)

Fig. 1. Cycle of nocturnal activity of anopheline mosquitos.[a]



[a] Numbers of mosquitos entering a baited bedroom on 5 different nights: Thermopylae, 1968.

pennis from the area. In addition, a stream that flowed from the hills, through the plain, to the sea has been diverted for irrigation purposes, and no other suitable breeding places exist for *A. superpictus* elsewhere in that part of the plain.

The high proportion of *A. sacharovi* in the total catches at the Thermopylae field station are reflected in the data given in Table 1. The proportion of *A. hyrcanus* caught during the night was higher than the proportion during the day from human habitations, stables and pit shelters, indicating that this species may enter a room at night, feed, rest for some time, then leave at dawn to shelter elsewhere (Table 1). The cycle of nocturnal activity of anopheline mosquitos is shown in Fig. 1.

Host preferences as determined by precipitin tests

With the object of obtaining information on the present-day host preferences of *A. sacharovi*, and of comparing these with the data available from the pre-DDT days, a number of blood-meal smears were collected from mosquitos in human habitations, stables, and artificial pit shelters in the 1968 and 1970 seasons in the village of Thermopylae. The smears were tested at the Imperial College of Science and Technology in London, England, and the results are tabulated in Table 2.

Table 2. Results of precipitin tests of *A. sacharovi* blood meals from Thermopylae

Host	1968			1970						
	Human habitations	Animal sheds	Artificial pit shelters	Human habitations	Animal sheds			Artificial pit shelters		
					S_1[a]	S_2[a]	Total	P_1[a]	P_2[a]	Total
man	187 (61.5 %)	3 (1.3 %)	9 (5.1 %)	100 (38.5 %)	1 (0.3 %)	4 (1.1 %)	5 (0.7 %)	0	1 (2.2 %)	1 (1.8 %)
animal	114 (37.5 %)	232 (98.3 %)	164 (93.7 %)	136 (52.3 %)	309 (93.1 %)	320 (84.9 %)	629 (88.7 %)	10 (100.0 %)	42 (93.3 %)	52 (94.5 %)
mammal	1 (0.3 %)	0	0	1 (0.4 %)	1 (0.3 %)	4 (1.1 %)	5 (0.7 %)	0	1 (2.2 %)	1 (1.8 %)
mixed feeds (man + animal)	2 (0.7 %)	0	0	10 (3.8 %)	1 (0.3 %)	3 (0.8 %)	4 (0.6 %)	0	0	0
mixed feeds (animal + animal)	0	1 (0.4 %)	1 (0.6 %)	13 (5.0 %)	19 (5.7 %)	44 (11.7 %)	63 (8.9 %)	0	0	0
negative	0	0	1 (0.6 %)	0	1 (0.3 %)	2 (0.5 %)	3 (0.4 %)	0	1 (2.2 %)	1 (1.8 %)
total	304	236	175	260	332	377	709	10	45	55

[a] S_1 = sheep shed in the periphery of the village
S_2 = stable in the village
P_1 = pit shelter in the periphery
P_2 = pit shelter in the village.

Table 3. Host preferences of *A. sacharovi* collected from houses, stables and artificial pit shelters in 1932–34, in 1968, and in 1970

Anopheline species	Year	Locality	From houses			From stables			From artificial pit shelters		
			Total positive reactions	Man positives (%)	Animal positives (%)	Total positive reactions	Man positives (%)	Animal positives (%)	Total positive reactions	Man positives (%)	Animal positives (%)
A. sacharovi	1932–34	Kavalla, Macedonia[a]	3 980	61.3	38.7	2 855	7.5	92.5	—	—	—
A. sacharovi	1968	Lamia	304	61.5	38.5	236	1.3	98.7	175	5.1	94.9
A. sacharovi	1970	Lamia	260	38.5	61.5	709	0.7	99.3	55	1.8	98.2

[a] Data from Barber & Rice (1935)

A. sacharovi has been described as an anthropophilic species in Greece (Lividas & Sphangos, 1941). The term may not be strictly appropriate, but the species is indeed anthropophilic in the sense that it bites man to a high degree in the presence of large numbers of animals. The results of precipitin tests of samples in 1968 from Thermopylae show that roughly 61% of the mosquitos from human habitations had fed on man, a very high proportion considering that the man–animal ratio of that village was about 1 : 9, or 1 : 7.2 at the peak of the summer season.

Table 3 presents the results of a large number of precipitin tests of *A. sacharovi* blood smears of specimens collected from houses and stables in the Kavalla area during 1932–34 (Barber & Rice, 1935). If the degree of anthropophily of this species at that time is compared with that in 1968 in the Lamia plain (included in the same table) it will be noted that there is hardly any difference. The results of 1970, however, present a somewhat different picture.

Genetic status of A. sacharovi populations

In September 1968, a few individual egg batches of *A. sacharovi* taken from specimens collected separately from bedrooms and artificial pit shelters at Thermopylae were sent to the Ross Institute so that they might be crossed with colonies of *A. sacharovi* from other sources or countries. Dr G. Davidson (personal communication) reported that the material sent from Lamia was found to be the same species as the Turkey strain of *A. sacharovi* maintained at the Institute. Four families from artificial pit shelters and 5 families from bedrooms were found to be conspecific.

In Turkey, and more specifically in Turkish Thrace on the other side of the river Evros, *A. sacharovi* is found in large numbers and a considerable number of malaria cases have been recorded in recent years (C. Ramsdale, personal communication). Hence the danger of malaria gaining a foothold on the Greek side of the river should not be underestimated. In many formerly malarious countries, where the disease is no longer considered a major public health problem, there is a tendency to ignore or to minimize the potential danger of malaria reappearing and, under favourable circumstances, leading to local or widespread outbreaks. In Greece, *A. sacharovi* exists in very high densities and the species appears to have the same habits as in the days before DDT was used. It is highly anthropophilic, as it always has been, and certainly there is no reason to think that its potentiality as a vector is now lower than that it has been in the past.

Susceptibility tests

A. sacharovi in Greece has been for a number of years resista tont both DDT and dieldrin. This has been reconfirmed by susceptibility tests carried out at Thermopylae in August–September 1969. The mortality after exposure to 4% DDT for 1 h was 33.8%, and after similar exposure to 4% dieldrin was 5.6%.

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RÉSUMÉ

RÉAPPARITION D'*ANOPHELES SACHAROVI* APRÈS ÉRADICATION DU PALUDISME

Une étude a été entreprise dans la plaine de Lamia (Grèce) sur le comportement actuel d'*Anopheles sacharovi* après plusieurs années d'interruption des pulvérisations de DDT, le paludisme ayant été complètement éliminé de cette région.

A. sacharovi semble être à l'heure actuelle le seul vecteur du paludisme rencontré dans la partie basse de la plaine qui s'étend vers la mer. Sa densité est élevée, comme le montrent les récoltes effectuées dans les habitations, les étables et les fosses-abris naturelles ou artificielles. Les moustiques appartenant à cette espèce piquent spontanément l'homme tant à l'extérieur qu'à l'intérieur des habitations où ils pénètrent en grand nombre, et constituent maintenant un véritable fléau aux alentours du village des Thermopyles.

A. sacharovi ne semble pas avoir changé ses habitudes depuis l'époque où le traitement au DDT n'existait pas encore. Ses hôtes préférés sont les mêmes que dans les années 30. L'espèce est hautement anthropophile, c'est-à-dire que, même en présence d'un grand nombre d'animaux, elle est attirée par l'homme. De plus, elle est résistante au DDT et à la dieldrine.

D'après des renseignements fournis par le Ross Institute of Tropical Hygiene de Londres, *A. sacharovi* rencontré dans la plaine de Lamia est bien la même espèce que *A. sacharovi* que l'on trouve en Syrie et dans certaines régions de Turquie.

REFERENCES

Barber, M. A. & Rice, J. B. (1935) *Ann. trop. Med. Parasit.*, **29**, 3

Hadjinicolaou, J. (1954) *Observations on the control of some insects of medical importance.* In: *Proceedings of the First International Symposium on the Control of Insect Vectors of Disease, Rome, 1953*, pp. 160-183

Livadas, G. A. & Sphangos, J. C. (1941) *Malaria in Greece* Athens, Pyrsos Press, vol. 1, pp. 69-107

Sphangos, J. C. (1943) *The use of* Gambusia *in malaria control in Greece.* Thesis, Akadimaiki Iatriki, Athens