

Prevalence and distribution of anopheline mosquitoes in malaria endemic areas of Asir region, Saudi Arabia

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معدل انتشار وتوزع بعوض الأنوفيل في المناطق الموطونة بالمalaria في ناحية عسير بالمملكة العربية السعودية

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الخلاصة: لدراسة معدل انتشار بعوض الأنوفيل أخذت عينات مما يزيد على 180 موقعا موطونا بالمalaria في ناحية عسير في المملكة العربية السعودية خلال السنوات 1999-2001، فجمعت 7085 يرقة و754 أنثى بالغة من الأنوفيلات، وتم التعرف على سبعة أنواع هي: الأنوفيلة الذائبة، والأنوفيلة الصلدة، والأنوفيلة السرجتية، والأنوفيلة العربية، والأنوفيلة الرقشاء، والأنوفيلة الطرخودية، والأنوفيلة الريتورية. ومن المعروف أن كلا من الأنوفيلة العربية والأنوفيلة السرجتية من نواقل المalaria في المنطقة. كما لوحظ وجود الأنوفيلة الذائبة في جميع المواقع وأنها الأكثر توافرا بين جميع الأنواع، أما الأنوفيلة الطرخودية فقد جمعت بأعداد قليلة على شكل يرقات. وسجلت الأنوفيلة الرقشاء والريتورية لأول مرة في ناحية عسير. وبعد فإن الأنوفيلة السرجتية هي من نوع يوجد في المناطق الشمالية لناحية عسير، أما الأنوفيلة العربية فتوجد أكثر ما توجد في المناطق الجنوبية منها.

ABSTRACT To study the prevalence of anopheline mosquitoes, over 180 sites were sampled in malaria-endemic areas of Asir region, Saudi Arabia, during June 1999–April 2001. A total of 7085 larval and 754 adult female *Anopheles* spp. specimens were collected. Seven species were identified: *An. dthali*, *An. rupicolus*, *An. sergentii*, *An. arabiensis*, *An. multicolor*, *An. turkhudi* and *An. pretoriensis*. Both *An. arabiensis* and *An. sergentii* are known vectors of malaria in the region. *An. dthali* occurred in all sites and was the most abundant species. *An. turkhudi* was collected in low numbers as larvae only. *An. multicolor* and *An. pretoriensis* were recorded for the first time in Asir region. *An. sergentii* is a species of the northern areas of the region, whereas *An. arabiensis* was more prevalent in the south.

Prévalence et distribution des moustiques anophèles dans les zones d'endémie palustre de la région d'Asir (Arabie saoudite)

RESUME Pour étudier la prévalence des moustiques anophèles, des prélèvements ont été effectués dans plus de 180 sites dans les zones d'endémie palustre de la région d'Asir (Arabie saoudite) de juin 1999 à avril 2001. Au total, 7085 échantillons de larves et 754 échantillons d'anophèles femelles adultes ont été prélevés. Sept espèces ont été identifiées : *An. dthali*, *An. rupicolus*, *An. sergentii*, *An. arabiensis*, *An. multicolor*, *An. turkhudi* et *An. pretoriensis*. *An. arabiensis* et *An. sergentii* sont tous deux des vecteurs connus du paludisme dans cette région. *An. dthali* se trouvait dans tous les sites et constituait l'espèce la plus abondante. *An. turkhudi* a été recueillie en petit nombre à l'état larvaire uniquement. *An. multicolor* et *An. pretoriensis* ont été recensées pour la première fois dans la région d'Asir. *An. sergentii* est une espèce des zones septentrionales de la région, tandis que *An. arabiensis* est plus répandue dans le sud.

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Introduction

The Tihama lowlands of Asir region the southwestern part of Saudi Arabia are known to be malaria endemic [1,2]. The disease is highly endemic on foothills and lowlands along the wadis, and meso- to hypoendemic on coastal plains along the Red Sea [3]. The malaria control programme that started in Asir as well as other regions in 1963 continues as one of the major preventive health activities in the region.

Mattingly and Knight [4] described about 46 species, subspecies and varieties of mosquito in the Arabian peninsula and about 12 species in Saudi Arabia. However, there was no specific reference to those occurring in Asir region. Warrell [5], reviewing the malarial diseases in Saudi Arabia, mentioned 3 *Anopheles* spp. in this region. According to the Malaria Control Programme in Saudi Arabia [3] and the annual reports of the Malaria Control Department of Asir region [6], only 5 species of *Anopheles* have been reported. These are *An. arabiensis*, *An. sergentii*, *An. dthali*, *An. rupicolus* and *An. turkhudi*. It is noteworthy that no reliable scientific work has ever been published to clarify the prevalent species of mosquito in the region.

The present study was conducted over 2 successive years to identify the prevalent species of *Anopheles* and to update our knowledge on their distribution pattern to assist in the planning and implementation of a malaria vector control in Asir region.

Methods

Study area and sampling sites

The Tihama lowlands of Asir region extend north-south along the foothills of the Hijaz-Asir mountain ranges and coastal plains along the Red Sea. The area is divided into

2 major sub-regions: Tihamat Asir and Tihamat Qahtan. The average rainfall in these lowlands is estimated to be 250 mm per annum with a year-to-year variability [3]. Owing to favourable climatic conditions, the area is relatively rich with plant growth of numerous trees species, bushes and perennial shrubs. More information about climate, zoogeography and plant ecology are available [7,8]. Many rocky valleys (wadis) at the Tihama foothills or crossing these plains have permanent or seasonally running water because of heavy rains or floods from the highlands range. These wadis provide mosquitoes with favourable breeding places and a wide range of hosts (people and domestic animals) living along the wadis.

Malaria is more endemic on foothills and lowlands along the wadis, but less endemic on the coastal plains. All sites sampled for this study lay within the malaria areas of the Tihama lowlands of Asir region. More than 70% of these sites received regular visits for collection of larvae and adult mosquitoes, while the rest were visited occasionally. Almost all the sites lie on or near the bank of the wadis.

The study was carried out in 2 successive years, from June 1999 to April 2001.

Sampling techniques

Adult female anopheline mosquitoes were collected by the following 2 methods.

Spray sheet method (room collection) [9]. This targeted indoor resting mosquitoes. Each locality received 2-4 collection visits every week. Maximum collection visits (4 visits per week) were made after the decline of rainfalls and floods and continued during the cold season (November-March). Collection visits took place between 06.00-10.30 hours and 6 to 10 rooms were examined on

each visit. Checked rooms were sprayed using insecticide (Raid®, SC Johnson). The majority of rooms visited during this study were made up of cement blocks or mud with wooden or thatched roofs. Wooden beds and cupboards as well as hanging materials on the walls provided resting places for mosquitoes.

Human landing catches [10]. This method was adopted to collect nocturnal human-biting mosquitoes. Direct catching from human baits was mainly done outdoors, since the indoor collection was not always welcomed by village residents. This method was performed once a week at each locality. The collection team comprising 4 people (2 baits and 2 collectors) were stationed at a selected village and started their activity between 18.00–02.00 hours. The same team was employed to perform room collections the next morning in the same village. Mosquitoes landing on baits were carefully sucked up using aspirators and then collected on paper cups on an hourly basis. Individuals acting as baits during the first half of the night exchanged positions during the second half. Temperature, relative humidity readings and wind velocity were recorded every hour.

Mosquitoes collected by both methods were kept in paper cups and transferred to the entomology laboratories at Abha, Muhayil or Al-Majarda for identification.

Anopheline larvae were collected by searching different types of larval breeding sites, e.g. various types of permanent water bodies along the edges or banks of the wadis, domestic water basins, small water pools, tins and tyres, man-made ditches, wells, etc. Using a dipping method described by the World Health Organization [10], all these potential breeding sites were surveyed for anopheline larvae. White plastic dippers (5 cm depth) were used for collection of larvae. More than 23 months

were spent, with a rate of 4 to 8 visits a week, employing 4 collection teams to collect larvae from frequently visited sites. Collected larvae were preserved in 30% alcohol and transferred to the laboratory where larvae specimens were mounted for identification.

Both adult and larva species were identified according to Mattingly and Knight [4], Glick [11] and Hopkins [12].

Results

A total of 134 sites in 5 localities (Al-Majarda, Muhayil, Al-Farsha, Rijal Alma'a and Maraba) were frequently visited during the period of the study. Occasional visits were made to 53 sites in the Al-Birk, Al-Gahma and Wadi Beni-Hashbal areas. All these sites lie within the malaria areas of Asir region and have variable degrees of malaria endemicity.

A total of 7085 larval and 754 adult female *Anopheles* specimens were collected from both frequently and occasionally visited sites by different methods. In general, the number of mosquito specimens collected was rather low over the period of study. This is due to the effective regular and extensive larvicide operations that take place in the region. In addition, occasional use of space and residual sprays assists in reducing the density of flying mosquitoes in the area.

Frequently visited sites

At frequently visited sites 552 adult female anopheline mosquitoes were collected with the room spray method. Four species of *Anopheles* were identified: *An. arabiensis*, *An. sergentii*, *An. dthali* and *An. rupicolus*. The night catch, using human baits, resulted in the collection of 77 specimens of 3 species of adult females only: *An. arabiensis*, *An. sergentii* and *An. dthali*.

The number of larvae collected at frequently visited sites using the dipping technique revealed a total collection of 7003 third and fourth instar larvae. Seven species of *Anopheles* were identified: *An. dthali*, *An. rupicolus*, *An. arabiensis*, *An. sergentii*, *An. multicolor*, *An. turkhudi* and *An. pretoriensis*.

Occasionally visited sites

Many sites in Al-Birk village were visited during the last 5 months of the survey, i.e. during the period December 2000 to April 2001 (Table 1). A total collection of 116 adult anophelines were obtained from inside rooms, using the spray sheet method. *An. dthali* was the most prevalent species (76.7% of adults collected) and *An. sergentii* and *An. rupicolus* were also identified. Although more than 280 breeding units were examined for larvae of anophelines, none were found.

Al-Gahma area received 3 visits with some intervals between each. Two species were detected in the area, *An. sergentii* and *An. dthali* (Table 1).

All sites

An. dthali was the most abundant species of anopheline in the region, comprising about 59.6% (4222) of the total collection of larvae (Figure 1). It was encountered in all sites visited; the frequency varied across locality but it was the most prevalent species in almost every locality (Table 2). *An. dthali* was most prevalent in Muhayil and Rijal Alma'a: 35.8% (1511/4222) and 22.7% (960/4222) respectively. In Maraba, *An. dthali* comprised 94.4% of the species collected from this locality. A high proportion (40.3%) of the total collection of adult females from indoor resting places were *An. dthali*. However, only 2 specimens were caught during the human landing catches.

An. rupicolus was relatively abundant compared with other species. Of the total collection of larvae, 15.6% (1104/7085) were of this species. It was encountered in all sites except Maraba and Al-Birk (Table 2). This species was apparently more prevalent in the northern areas of the region; 95.7% (1056/1104) of *An. rupi-*

Table 1 Frequency of *Anopheles* species (adult females and larvae) collected from occasionally visited sites in Asir region, Saudi Arabia

Locality		Total	<i>An. turk-</i>	<i>An. multi-</i>	<i>An. rupi-</i>	<i>An.</i>	<i>An. ser-</i>
		No.	hudi %	color %	colus %	dthali %	gentii %
Al-Birk	Larvae	0	0.0	0.0	0.0	0.0	0.0
	Adults	116	0.0	0.0	20.7	76.7	2.6
Wadi Beni-Hashbal	Larvae	45	95.6	4.4	0.0	0.0	0.0
	Adults	0	0.0	0.0	0.0	0.0	0.0
Al-Gahma	Larvae	37	0.0	0.0	0.0	94.6	5.4
	Adults	9	0.0	0.0	0.0	0.0	100.0
Total	Larvae	82	52.5	2.4	0.0	42.7	2.4
	Adults	125	0.0	0.0	19.2	71.2	9.6

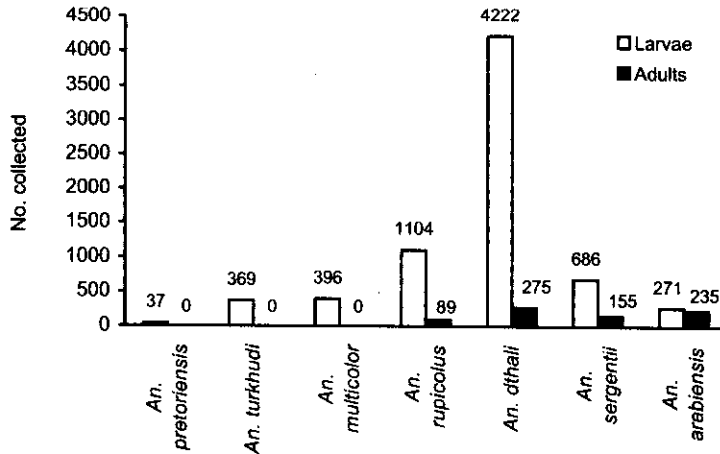


Figure 1 Frequency of *Anopheles* species (adult females and larvae) collected from all visited sites in Asir region, Saudi Arabia

colus larvae were collected from Muhayil and Al-Majarda. Very few female adults of *An. rupicolus* were found resting inside rooms and they did not attack any of the human baits during this study.

Most of the *An. sergentii* larvae (98.7%, 677/686) were collected from sites in Muhayil and Al-Majarda in Tihamat Asir. Only 7 larvae of *An. sergentii* were

collected from a single site in Al-Farsha in Tihamat Qahtan during the whole period of study. Room collection revealed a comparatively low percentage of *An. sergentii* adult females among indoor resting mosquitoes (16.9%, 93/552), whereas during the night catch using outdoor human baits *An. sergentii* was the most common of the species (64.9%, 50/77).

Table 2 Distribution of *Anopheles* species (larvae) collected from all visited sites in Asir region, Saudi Arabia

Locality	Total No.	<i>An. preto-</i>	<i>An. tur-</i>	<i>An. multi-</i>	<i>An. rupi-</i>	<i>An. dthali</i>	<i>An. ser-</i>	<i>An. arab-</i>
		<i>riensis</i>	<i>khudi</i>	<i>color</i>	<i>colus</i>		<i>gentii</i>	<i>iensis</i>
		%	%	%	%	%	%	%
Al-Farsha	1201	3.1	15.9	26.6	2.9	40.8	0.6	10.1
Al-Marjarda	1123	0.0	5.4	0.0	17.4	58.2	11.0	8.0
Muhayil	3020	0.0	2.0	0.0	28.5	50.0	18.3	1.2
Rijal Alma'a	1053	0.0	0.3	5.2	1.2	91.2	0.0	2.1
Maraba	606	0.0	2.0	3.3	0.0	94.4	0.0	0.3
Al-Birk	82	0.0	52.5	2.4	0.0	42.7	2.4	0.0

An. arabiensis is the principal vector of malaria in the region. It was encountered in all frequently surveyed localities but in low densities, comprising only 3.8% (271) of the total larvae collected (Figure 1). *An. arabiensis* was the most prevalence species in the Al-Farsha locality in Tihamat Qahtan (Table 2) and 45.0% (122/271) of *An. arabiensis* larvae were collected from this locality. It was also observed that their breeding places were difficult to find, especially at the foot of mountains. Of the total collection of adults obtained by the spray sheet method, the highest proportion (38.0%) were *An. arabiensis*, higher than *An. sergentii* and *An. dthali*. During the night catch, about 32.5% (25/77) of *An. arabiensis* were captured while attempting to feed on human baits outdoors.

An. multicolor was found only as larvae—no adults were caught during the whole study—and comprised 5.6% (396) of the total larvae collection (Figure 1). Although the annual reports of the Malaria Control Department in Asir region have not mentioned the existence of this species, *An. multicolor* larvae were collected from 3 sites of the region: Al-Farsha, Rijal Alma'a and Maraba (Table 2).

An. turkhudi is of minor importance from a health point of view since it does not play any role in disease transmission in the region. It was also detectable as larvae only and comprised 5.2% (369) of the total collection. *An. turkhudi* were collected in low densities from many sites visited and was most abundant in Al-Farsha.

An. pretoriensis was found in only 1 site: Wadi Al-Kahla near Al-Farsha at the Saudi-Yemeni border (Table 2). This is the first record of *An. pretoriensis* larvae in Asir region. This species was found only as larvae and attempts to collect adults from the same area were unsuccessful. A total of

37 larval specimens were collected and identified (Figure 1).

Wadi Beni-Hashbal was visited once. Three sites were investigated and revealed the presence of *An. multicolor* and *An. turkhudi* (Table 1).

Discussion

This comprehensive study was conducted to update our knowledge of the prevalent species of *Anopheles* mosquito and their distribution in the Asir region of Saudi Arabia. This region is part of the Afrotropical (Ethiopian) zone of malaria [13] and one of the malaria-endemic areas of Saudi Arabia [3]. Seven species of *Anopheles* were encountered during the study: *An. arabiensis*, *An. sergentii*, *An. dthali*, *An. rupicolus*, *An. turkhudi*, *An. multicolor* and *An. pretoriensis*. The first 5 species are annually reported by the Ministry of Health, Saudi Arabia. *An. multicolor* and *An. pretoriensis* have not been recorded in Asir region before.

Mattingly and Knight [4] did not find *An. multicolor* south of Jeddah. So it was considered as an exclusively northern species of the Arabian peninsula. They described it as a desert species as it required only a little rainfall. These findings contradict the fact that larvae in our study were obtained from several localities characterized by relatively heavy rainfall. However, we could not collect any adults of this species, despite the considerable number of larvae specimens obtained. This may be due to a difference in behaviour from other species in the area, which could mean that the collection methods were not suitable and that different methods are used in northern parts [3]. Further investigation of this issue is required.

As for *An. pretoriensis*, it has been reported in the neighbouring Jazan region [3] and further south in the Republic of Yemen [14], but this is the first record of this species in the Asir region. It was collected from a single place near the Saudi-Yemeni border.

An. sergentii and *An. rupicolus* were previously reported to have almost the same distribution as *An. multicolor* in the Arabian peninsula [4]. To an extent, this is true for their distribution in Asir region. Nevertheless, few larval specimens of these species were collected from Al-Farsha locality. The high yield of outdoor night catch from human baits and low room collection of *An. sergentii* are good indications of the outdoor feeding and resting habits of the species.

An. arabiensis, the main vector of malaria in the Asir region [3], was more abundant in the southern part of the region. It was more prevalent in Al-Farsha than any of the other locations (Table 2). This agrees with the findings of Al-Maktari and Bassiouny [14], who found *An. arabiensis* to be the most prevalent species in the extending Tihama plains in the Republic of Yemen. It appears that *An. arabiensis* behaves differently from *An. sergentii* and most likely it feeds and rests indoors.

It is evident that females of *An. turkhu-di* avoid any contact with man or his dwellings, as no adult mosquitoes were ever caught during the whole period of study.

An. dhali was the most dominant species in Asir region and showed a wide range of distribution within the region. Mattingly and Knight [4] referred to its occurrence in the Ethiopian, Palaearctic and Oriental zones. Thus, its role in disease dissemination in the region needs to be clarified.

Only 3 species of *Anopheles* were captured in the coastal areas of Al-Birk and Al-Gahmah; however, further work is needed to identify other prevalent species.

In our study, larval collection by itself was the most reliable method for capturing the species in the region. Other techniques used for adult collection are dependant on the biting and resting behaviour of these mosquitoes. Therefore, the spray sheet method and the human landing catches were successful in capturing anthropophilic (human biting) and endophilic (indoor resting) species. Of the 7 anopheline species encountered in the present study, *An. arabiensis* and *An. sergentii* had these characteristics. Both species are known to play major role in malaria transmission in the region [4]. Thus, during routine evaluation of malaria vector control operations in the region, the spray sheet and human bait techniques seem to be more reliable and easy to practice.

In conclusion, 7 species of *Anopheles* exist in Asir region. Among these *An. multicolor* and *An. pretoriensis* are reported for the first time in the region. *An. multicolor* is regarded as a secondary malaria vector in some localities of Saudi Arabia [3] and hence its role in disease transmission in Asir region needs further investigation.

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