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Entomological Survey in Kalibar, A Resurgent Malaria Focus in East-Azerbaijan, Iran

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Abstract: An entomological survey was carried out on the bionomics of Anophelines species in Kalibar, East-Azerbaijan during the year 2003-2004. The result showed that five Anophelines species comprising *A. sacharovi*, *A. maculipennis*, *A. superpictus*, *A. hyrcanus* and *A. claviger* were present in the study area. Based on the species density rate it was assumed that *A. sacharovi* could play an important role in malaria transmission and *A. maculipennis* and *A. superpictus* could be considered as secondary vectors. *A. sacharovi* was active from May to September which peaks in July and August in outdoor and indoor, respectively. This species was mainly endophil and anthropophil. Biting activity of *A. sacharovi* took place in the second half of night. Susceptibility tests using the WHO-recommended discriminative doses of insecticides revealed that this species is resistant to DDT, tolerant to dieldrin and susceptible to malathion, fenitrothion, permethrin, and deltamethrin. *A. maculipennis* had high density at the beginning of summer, then its population fell down slowly and in early autumn reached to the lowest. The population of this species was found frequently in human shelters and most of its bites took place in the second half of night. Its larvae occurred in slow flowing water and channels with water plants and were more abundant in August.

Key words: Anopheles, malaria, bionomics, insecticide, Kalibar, Iran

INTRODUCTION

Despite several years campaign against malaria, around 300-500 million clinical cases occur every year with over 1.2 to 1.7 million deaths that contain over 1 million deaths among under 5 year-old African children, over 90% of the cases (generally *falciparum* infections) occur in Sub Sahara district of Africa[1]. Malaria is endemic in southern parts of Iran. According to recent information, a total of 18,000 cases were reported from these areas. Iran bordering line with Azerbaijan and Armenia countries was accounted as a malaria free region. However, in 1991, after collapse of former Soviet Union, a new threat of malaria importation emerged from those countries into the north of Iran including West-Azerbaijan, East-Azerbaijan, Ardebile and Gillan provinces. East-Azerbaijan province is located in northeastern Iran in borderline with Republic of Azerbaijan and Armenia countries. Little is known about the bionomics and vector potential of the anophelines of the province. In the northern part of Iran, limited numbers of imported cases of malaria have been reported[2]. In 1991, after collapse of former Soviet Union, a new threat of malaria importation emerged from those countries into some parts of West-Azerbaijan, East-1.04 Azerbaijan, Ardebile and Gillan provinces of Iran. Various factors can affect malaria resurgence in this region[3,4].

Anopheles sacharovi Favre is widespread in Austria, Corsica, Cyprus, Greece, Italy, Iraq, Iran, Jordan, Lebanon, Palestine, Sardinia, Syria, Turkey, former USSR and former Yugoslavia[5]. It is a potential vector of malaria in southeastern Europe and an important vector in the Syrian Arab Republic and in northern Iraq[6]. This species is also one of the malaria vectors in Iran and has a more localized distribution in the central, northwest, west, southwestern and Fars province in the south[7].

Recently, Yaghoobi *et al.*[8] have studied ecology of *An. sacharovi* over a period of 12 months in Parsabad and Germi counties, Ardebil province. ELISA testing of 210 blood meals of the species showed that few of those found in cowsheds or chicken coops had fed on humans, but of those found in bedrooms 38.5% had fed on humans. Insecticide susceptibility tests showed that the species is susceptible to malathion and propoxur but resistant to DDT and dieldrin[8]. The aim of this study

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was to assess the bionomics of main malaria vectors in the region.

MATERIALS AND METHODS

Study area: East-Azerbaijan province is located in north-western Iran (between 39°, 26'-36°, 45'N latitude and 45°, 5'-48°,22'E longitude). The province is bounded by Armenia an Azerbaijan in the north, borderline of 235 km, by West-Azerbaijan province in the south and by East-Azerbaijan and Ardebil and Zanjan provinces in the east. In August 2004, localities with recent autochthonous malaria cases were visited to determine the types of dwellings, their accessibility and their proximity to potential mosquito breeding sites. Based on preliminary surveys, borderline villages with relatively high numbers of anophelines were selected for continued sampling. The investigation was conducted each 15 days between June through October 2004 in four villages of Larijan, Garoujeh, Jaafarabad, Mahmoudabad. The main occupation of the people in selected villages is husbandry of domesticated animals, business and agriculture.

Larval and water collections: Larvae were sampled using standard dippers (500 mL) from breeding places close to adult collection sites. The mean number of larvae was calculated per 10 dips. Third and fourth instars larvae were immediately preserved for later identification. The larvae were counted during the period June to October. Water was sampled for chemical analysis from breeding places of *A. sacharovi* in one village (Larijan-Garmadooz) of Kalibar counties. Samples were sent to the environmental chemistry laboratory, Department of Environmental Health Engineering in the School of Public Health, Tehran University of Medical Sciences for chemical analysis.

Adult collections: Pyrethrum Space Spray Catches (PSSC) were performed biweekly in eight fixed shelters (four human and four animal shelters) located in different parts of the village in each county by standard methods[9] using 0.2% pyrethrum spray. The density was calculated as the number of mosquitoes per shelter. Attempts were also made to catch Anophelines outdoors using mouth aspirators. *A. sacharovi* and *A. maculipennis* s.s. are identifiable at adult stage. In *A. sacharovi* all fringing hairs of the wing are dark and dark spots on the wings are less conspicuous but in *A. maculipennis* s.s. the fringing hairs are light at the tip of the wing and form a distinct spot and dark spots on the wings are conspicuous[10]. Females were sorted according to abdominal condition[11].

CDC light trap collection: Light-traps enable collection of nocturnal, phototactic mosquitoes with minimal collection effort[12]. CDC miniature light-traps were used for collecting mosquitoes from houses and cattle sheds at night. The traps were suspend from the hatched roofs of animal shelters and human dwellings.

Blood meal identification: To determine host preference pattern, blood fed mosquitoes were collected from human dwellings, stables, light-traps, storerooms and outdoor resting places around villages. The blood meals of the identified anophelines were smeared on circles of Watman No.1 filter paper. They were interleaved with non-absorbent 'onionskin' paper and sent with the necessary information to the Parasitology Department in the Iran Pasteur Institute for Enzyme-Linked Immunosorbent Assay (ELISA)[13].

Dissection of mosquitoes: To determine the age composition and epidemiological potential of the population of anophelines, female specimens were collected and their physiological age was recorded by the method of Detinova[14].

Insecticide susceptibility test: The field collection of *An. sacharovi* for susceptibility test was between July and August 2004 in Jafarabad (Khodafarin) of Kalibar counties. Adult mosquito susceptibility tests were carried out following the standard WHO method[15], where 4-5 individual replicates of 20-25 adults were tested. Engorged *A. sacharovi* females from indoor resting sites were used. The exposure tubes were held in a vertical position during the tests. The exposure time for each insecticide was 1 h. The mortality rate was scored after a 24 h recovery period. Insecticide exposure took place in a room with temperature of 27±2°C and relative humidity 55-60%. The following insecticide impregnated papers were supplied by WHO: DDT (4%), malathion (5%), fenitrothion(1%), deltamethrin (0.05%),permethrin (0.75%).

RESULTS

Breeding places and their chemical analysis: The density of *A. sacharovi* and *A. hyrcanus* larvae was very low in most breeding sites through the year, probably due to the extensive distribution of larvivorous fish, *Gambussia affinis* and environmental management. At the breeding sites, immature stages were present in clear samples along the edges of slowing rear-ends of water supply channels and roadside ditches. Perennial temporal and transient pools were the most rice field hence breeding place was from rice field. Larvae of *A. sacharovi* and *A. maculipennis* were found in small numbers (6/10 dips) by the end of June. The maximum larval density (20/10 dips) was in August. No larvae sampling was done from late October to the April because of high rainfall and cold whether. The result of water chemical analyses of larval habitats belong to the plains of Larijan showed that pH is equal to 7.1, the concentrations of Ca, Mg and Na was 410, 121 and 102 mg L^{-1} respectively. Salinity is one of the important factors for breeding site, which is calculated 86 mg L^{-1}. Permanent and temporary hardness was 1810 and 125.2.

Abundance and parity: Out of 1631 collected mosquitoes during the study, the majority were *A. sacharovi* (83.32%) and other species were *A. maculipennis* (13.12%), *A. hyrcanus* (3.61%) and *A. superpictus* (0.12%). Light trap, total and hand catch methods revealed that *A. sacharovi* is active from May to September with one peak of activity, which occurs in August. However, *A. maculipennis* started to appear in late May and disappeared in early October. The maximum density of *A. maculipennis* was in dwellings and shelters of villages and its maximum density was twice of other two species, *A. superpictus* and *A. hyrcanus*. These two species were present in study villages from May through October. *A. superpictus* was found only in September. Dissection of collected specimens revealed that the parity was 74, 80, and 72% in *A. sacharovi, A. maculipennis* and *A. hyrcanus* respectively. Maximum level parity in *A. sacharovi, A. maculipennis* was 81 and 90% in September.

Biting behavior and biting cycle: Catches began at about 30 min prior to sunset and ended at 30 minutes after sunrise. Human biting started at 20 pm and continued until 07 am. More bites of *A. sacharovi* took place in the second half of the night from human (01:00 to 03:00), cow and donkey. Peak of *A. maculipennis* was in the second half of night on human (03:00 to 04:00) and cow, but we were not able to catch any *A. maculipennis* from donkey. Peak of bites of *A. hyrcanus* from human and cow was in the second half of night but catching from donkey was in the first half of night (three hours after sunset). The biting pattern on the cow was more or less the same as on human, but the number of bites on cow was much greater than the number of bites received by human night.

CDC light trap catches: Using the CDC light traps, only 26 female adult *Anopheles* were collected during the study periods. They included *A. hyrcanus* (73.07%) and *A. sacharovi* (26.92 %). Other species that were obtained included *Culex* sp. All female *Anopheles* were dissected and the result revealed that *A. sacharovi* was unfed.

Table 1: Mortality rate of adult females of *Anopheles sacharovi* against different insecticides in the study area.

Insecticides	No. of tested mosquitoes	No of dead mosquitoes	Mortality rate	Exposure time (h)
DDT4%	102	66	65	1
Dieldrin 0.4%	99	89	90	1
Malathion 5%	102	102	100	1
Fenitrothion 1 %	100	91	91	1
Deltamethrin 0.05%	100	96	96	1
Permethrin 0.75%	100	100	100	1
Control	100	2	1	1

Host preference pattern: Result of ELISA on 109 blood meals of *A. sacharovi, A. maculipennis* and *A. hyrcanus* showed a varied proportion of positive reaction with alkaline phosphatase anti-human conjugate in different species. 75% of samples were *A. sacharovi*, 18.5% *A. maculipennis* and 6.5% *A. hyrcanus*. Of those collected *A. sacharovi* 21% had fed on human alone, 22.2% on cow, 19.7% on donkey and 37.1% on other animals.

In *A. maculipennis* 5% had fed on human alone, 25% on cow, 20% on donkey, and 50% on other animals. *A. hyrcanus* had no fed on human, but fed 71.4% on cow, 14.3% on donkey and 14.3% on other animals. These data indicated high antropophilicity in *A. sacharovi* and high zoophilicity habits in *A. hyrcanus*.

Susceptibility status: By applying WHO criteria (98-100% mortality indicates susceptibility (Table 1), 80-97% mortality requires confirmation of resistance with other methods and <80% mortality suggests resistance), results revealed that this species is resistant to DDT, tolerant to dieldrin, but susceptible to malathion, permethrin and possible sensitivity to fenitrothion and deltamethrin.

DISCUSSION

This study was the first report on the ecology of malaria vectors in this part of East-Azerbaijan province, where malaria has been present for a very long time. This species has a wide distribution in Kalibar counties and was collected in inspected rural districts. Results revealed that the main prevalent malaria vectors may be *A. sacharovi* and *A. maculipennis*. The remaining includes *A. superpictus* and *A. hyrcanus*, which may play a role as secondary vectors of malaria. This hypothesis needs more investigation. This study led to first report of occurrence of *A. claviger* in the region. Vatandoost *et al.*[4] and Yaghoobi-Ershadi *et al.*[8] had found these species in the vicinity of study area, including *A. sacharovi, A. maculipennis, A. superpictus* and *A. hyrcanus*[4,8]. Larvae were found in small numbers (6/10 dips) by the end of June. The maximum larval density (20/10 dips) was in August. In present study, thesalinity of the breeding places ranged 86 mg L^{-1}. Vatandoost *et al.*[4] found that water salinity between 0.05-0.22 is mg L^{-1} appropriate for *A. sacharovi* in other parts of Iran[4]. In the villages of Bogorodica, formerly Yugoslavia, the salinity in a breeding place of *A. sacharovi* was reported as 74 mg L^{-1}[16]. In central Asia, high salinity is better tolerated by the larvae of *A. sacharovi* than any other member of the *A. maculipennis* complex. Its larvae can develop in water having salinities up to 200 mgL^{-1}[17]. Gokberk[18] reported that breeding places of larvae of *A. sacharovi* and *A. maculipennis* vary in different areas of Turkey. Present results showed that *A. sacharovi* was active from August and *A. maculipennis* peak was June. In West-Azerbaijan it was active from May to October, with two peaks of activity in August and October. However, *A. maculipennis* started to appear in early May and disappeared in late June[4]. According to Yaghoobi-Ershadi[8], *A. sacharovi* was active from June to late November with one peak of activity in early August on the plain of Parsabad and from late June to late November with one peak of activity in late August in the mountainous area of Germi. CDC light traps are able to attract *A. hyrcanus* and less *A. sacharovi* and cannot catch any *A. maculipennis*. Vatandoost *et al.*[4] found CDC light trap for collection of *A. hyrcanus* (69.2%), *A. maculipennis* (7.7%) and *A. sacharovi* (23.1 %).

Most of the mosquitoes caught from different places for anthropophilicity was *A. sacharovi* 21%, *A. maculipennis* 5% and nill for *A. hyrcanus*. The figure in West-Azerbaijan for *An. sacharovi* was recorded as 5%[4]. In 1935, precipitin tests of *A. sacharovi* blood meals in Greece demonstrated positive human reactions from 61.5% of specimens taken from human dwellings and from 7.5% of those from animal shelters. Studies in Greece reported positive human reactions in 38.5% of mosquitoes from human dwellings and 1.1% from animal shelters. In 1966, human positive reactions of *An. sacharovi* taken from a variety of shelters were 5.6% in Greece and 30.5% in Syria[19]. Values of 26.4% from human dwellings and 9.4% from animal shelters were obtained in Iran in 1985[2].

These findings suggest that the feeding preferences of *A. sacharovi* vary from year to year and place to place. No comparative figures from Turkey were found, so this study was undertaken to provide comparative data. The feeding habits of *A. sacharovi* in Adana Province, Turkey, were investigated by use of the gel diffusion technique. Mosquitoes were collected from various villages of Cukurova, Turkey and from feeding rooms especially prepared for these experiments. A human, cow, sheep, chicken, horse and donkey were used as hosts in these rooms. The results showed that *A. sacharovi* is a zoophilic species[20].

The females preferred donkey when human, cow, sheep, chicken and horse were equally available. Their preference changed to horse, cow and sheep in the absence of donkey. The Host Preference Index (HPI) was always smaller than the one for humans in habitats offering a choice of hosts. The human blood index was high only in human dwellings. In other habitats, the numbers of mosquitoes feeding on animals were higher than on humans. Although the human blood index was low, *A. sacharovi* is the principal vector of human malaria in Turkey, partly because a significant proportion of those resting in human dwellings have fed upon the occupants and partly because of the uneven distribution of human and animal hosts[20].

Results of the susceptibility tests of *An. sacharovi* to several insecticides showed that this species is resistant to DDT and tolerant to dieldrin, but susceptible to malathion, permethrin and possible sensitivity to fenitrothion and deltamethrin. Another study in West-Azerbaijan showed DDT resistance, tolerant to dieldrin and susceptible to malathion, fenitrothion, bendiocarb, propoxur, lambdacyhalothrin, deltamethrin, permethrin , cyfluthrin, in the *A. sacharovi*[4]. From the results of susceptibility tests it can be concluded that *A. sacharovi* exhibits resistance to DDT and is tolerant to dieldrin. In 1957, for the first time, *A. sacharovi* were tested against DDT 4% in Fars Province. The results showed that this species was susceptible to DDT 4%. In 1957, DDT was used for malaria vector control in the region. In 1959, the mortality of *A. sacharovi* to DDT 4% decreased to 35-40%, indicating resistance in this population. Due to DDT resistance, dieldrin was replaced for vector control in 1961. Subsequently, after 2 years of dieldrin application, resistance to this insecticide was reported[21]. The first report of *A. sacharovi* resistance to DDT occurred in 1959, from Kazeroon and subsequently from Izeh and Meshkinshahr[7]. Several studies have been done on the ecology of malaria vectors in Iran. In 1967, malathion was used in the region and until 1973 there was no report of malathion resistance in this population. Ghavami[22] showed that the *A. sacharovi* collected from Kazerun region were susceptible to DDT. It is often asserted that insecticide resistance in a mosquito population is gradually lost and reversion to susceptibility occurs after withdrawal of insecticide pressure[22-25] Yaghoobi-Ershadi *et al.*[8] showed that this species in Parsabad and Germi counties in Ardebil province of Iran is resistant to DDT and dieldrin[8]. Kasap *et al.*[20], reported resistance to 12 insecticides by specimens of *A. sacharovi*, both in the laboratory and those collected in the malarious areas located in the southern part of Turkey. In Adana, Adiyaman and

Antalya, *A. sacharovi* was susceptible only to malathion and pirimiphos-methyl[20]. In this study, pirimiphos-methyl was not used. In other parts of Turkey this species was susceptible to dieldrin, fenitrothion, lambdacyhalothrin, cyfluthrin, etofenprox, malathion and pirimiphos-methy[20]. It should be noted that both vertical and horizontal positions were used during the exposure times. Hemingway[26] reported that DDT resistance in *A. sacharovi* was scattered in the population in 1984, despite the replacement of DDT by malathion for malaria control 13 years earlier. It was also reported that populations of this species in Cukurova had an altered acetyl cholinesterase resistance mechanism, conferring broad-spectrum resistance against organophosphates and carbamates. Specimens of *A. sacharovi* collected in the field in 1989-1990 were still resistant to DDT, organophosphate and carbamates, although at lower frequencies than in 1984[26]. In addition to the acetyl cholinesterase resistance mechanism, there is evidence of an increased level of glutathione S-transferase in some of the *A. sacharovi* population tested. This is known to be correlated with DDT resistance in other anophelines. In Iraq, Manouchehri *et al.*[7] reported that *A. sacharovi* was resistant to DDT, but susceptible to malathion. In this study, DDT resistance and dieldrin tolerance was investigated[27]. Selection for resistance in malaria vectors can result from the agricultural application of insecticides[28-30]. According to the Department of Agriculture in provinces of Iran, 9 types of organophosphates and 3 types of pyrethroid insecticides are distributed among farmers for agricultural pest control, due to the extensive use of permethrin for agricultural pest control and the occurrence of cross-resistance between permethrin and DDT in malaria vectors[31].

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