Original Article

Blood Meal Preference of Some Anopheline Mosquitoes in Command and Non-command Areas of Rajasthan, India

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Abstract

Background: The present study was undertaken to compare the entomological situation by analyzing the blood meal of mosquitoes of canal irrigated and non-irrigated areas of Bikaner in order to explore scientific information on the vector biology and malaria burden profile and to plan proper strategies for malaria control and eradication.

Methods: Adult mosquitoes were collected and the abdomen of the blood fed females were crushed on a filter paper for blood meal analysis and subjected to precipitin test.

Results: The blood meal analysis showed that *Anopheles subpictus* had a preference towards cattle blood, *An. culicifacies* and *An. stephensi* preferred human blood, while, *An. annularis* was noted to feed only on bovine blood.

Conclusion: Although *An. annularis*, has been recently reported from the area, was found to feed exclusively on bovine blood, earlier reports suggest that this species is a vector of malaria and therefore preventive measures should be taken well in advance before this species gets established in the area.

Keywords: Malaria, Anopheline mosquitoes, Canal irrigated (Command area), non-command area, Thar Desert

Introduction

Malaria imposes great socio–economic burden on humanity. According to WHO estimates, 300–500 million malaria cases are reported annually (WHO 1998). In addition, the estimated annual mortality attributed to malaria ranges from 7 lakhs to 2.7 million globally (Kumar et al. 2007). In the South–Eastern Asian region, 1.2 billion are exposed to the risk of this infectious disease, most of who live in India (Kondrachine 1992). Of the 2.5 million reported cases in South–East Asia, India alone contributes about 70% and currently 80.5% of the 109 million population of the country live in malaria risk areas. Of this, 4.2%, 32.5% and 43.8% live in areas of high, moderate and low risk to malaria respectively (http://www.searo.who.int/).

Until recently, the states of Orissa, Jharkhand, West Bengal, North-Eastern states, Chattisgarh and Madhya Pradesh contributed to bulk of malaria (Dash et al. 2008), with the state of Rajasthan having no case in the list. Of late, malaria has become the most dangerous and debilitating vector borne disease of the Thar Desert, and has been witnessing a spate of epidemics since 1990 every alternate year, while, in 1994 and 1995 it assumed a continuous trend and the total number of malaria positive cases in the later year were the third largest ever reported (Tyagi 1997).

Bikaner, situated in the North-Western part of Rajasthan is characterized by extremes of temperature, both in summer as well as in winter and low and erratic rains. Loonkaransar Tehsil lying in Bikaner district is a canal irrigated area whose ecology has changed drastically due to the introduction of Indira Gandhi canal. There is an increase

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in vegetation leading to enhanced ambient relative humidity and availability of water logging at number of places resulting from the canal seepage. After the initiation of irrigation activities in the region, there has been a tremendous increase in the number of malaria cases. Kolayat Tehsil, lying about 51 km from Bikaner city has large water body in the form of lakes. The present study was undertaken to study the blood meal preference of different anopheline species.

Materials and Methods

Study area

District of Bikaner

Bikaner District is a part of the Great Indian Thar Desert. It lies in Northwestern part of Rajasthan located between 27°11' and 29°03' North latitudes and 71°54' and 74°22' East longitudes (www.indianetzone.com>Rajasthan> Districts of Rajasthan). The total area of the district is 30289 sq km with a general elevation lying between 154 to 429 m above the mean sea level, sloping towards North-West. Sand dunes range in height from 6 to 30 m.

The district has a dry climate with large variation in temperature and has scanty rainfall. The summer months are extremely hot with the day temperature sometime going up to 49 °C, June being the hottest month. During winter the minimum temperature sometimes drops up to 2 °C below freezing point, January being the coldest month. The average rainfall in the district is 259.6 mm and average relative humidity is of the order of 15 to 20 percent.

The present study was carried out in three Tehsils viz, Bikaner, Kolayat and Loonkaransar.

Bikaner Tehsil

Bikaner Tehsil occupies an area of 3192.52 sq km and lies between 27°15'–28°22' North latitude and 72°06'–73°46' East longitudes above mean sea level of 238 m (www.mapsofindia.com). The total population is 7, 25,810 and density of population per sq km is 235. In this Tehsil, the survey was carried out only in Bikaner City. Five sampling sites were selected in the city for study purpose.

Kolayat Tehsil

The main Tehsil headquarter of Kolayat lies approximately 51 km away from Bikaner city. The total area of the Tehsil is 7957. 62 sq km lying between 28°28' and 60″ N latitudes and 73°45'0″ E longitudes, 191 m above mean sea level (www.fallingrain. com). The total population of the Tehsil is 2, 07,749 with the density of population per sq km being 26. Five villages of this Tehsil were surveyed.

Loonkaransar Tehsil

The main Tehsil head quarter of Loonkaransar lies 70 km away from Bikaner. The Tehsil occupies an area of 5036.58 sq km and lies between 28°33'–29°03' North latitudes and 73°05'–74°12' East longitudes, and 191 m above sea level (www.collinsmaps. com). The total population of the Tehsil is 1, 74,293 and the density of population per sq km is 35. Five villages lying near the canal distributaries were selected for the study under irrigated area.

Canal irrigated and non–irrigated study area

The area under study was demarcated into two major heads viz., canal irrigated (command) and non-irrigated (non–command areas), based on distribution of water from the main canal (IGNP) and its distributaries. Indira Gandhi Nahar Pariyojna, formerly known as Rajasthan Nahar Pariyojna, measures 649 km long canal with a distributary system of 8776 km which draws water from the Sutlej and Beas Rivers and courses to Northwestern arid land of Rajasthan. The command area under this system is 0.49 lac hectare at 110- irrigation intensity. Therefore, the vil-

lages studied under Loonkaransar Tehsil were a part of irrigated area, while those surveyed in Kolayat Tehsil and the city of Bikaner were placed under non-irrigated area.

Period of survey and periodicity of collection

The survey was carried out over a period of two years from October 2006 to September 2008. Samples were collected every week from the selected locations. The anopheline species were collected from vicinities of different water bodies/breeding sites. The adult mosquitoes were collected during dawn and dusk from the field areas. Through surveys different breeding and resting sites of anopheline species were identified. The collection sites were puddles, ponds, lake, cattle water points, pipe leakages, construction sites, households, culverts/causeways, and fodder rooms.

Method of collection

Adult mosquitoes were collected with the help of aspirator tubes and torch light. These were then transferred into collection tubes plugged with cotton and brought live to the laboratory. The abdomen of the blood fed female were crushed on Whatman's filter paper No. 1 for blood meal analysis. It was then subjected to precipitin test using gel-diffusion method for identifying the blood meal as given by Collins et al. (1983). In this method the blood obtained by crushing the abdomen of the vector is tested against anti-sera for blood of man and other host animals, the reaction against a particular antiserum is presumptive evidence that the blood belongs to that host.

Reagents

Solution 1

This solution was prepared by dissolving 1.03 g sodium barbiturate and 0.81 g of barbituric acid in 100 ml of distilled water.

Solution 2

This solution was prepared by dissolving 0.45 g of Agarose in 50 ml of distilled water.

Procedure

First the dry blood obtained from the vector stomach was dissolved in normal saline (0.7 g/100 ml DW), 4.5 h before the experiment. 50 ml of solution 1 was added to 50 ml of solution 2. Microslides (glass) were prepared by spreading 3 ml of mixed solution on each slide, which on drying formed a gel. These slides were then kept in refrigerator for 12 to 15 h. Various test antisera and mosquito blood dissolved in normal saline were released in micro quantities onto the wells in the gel slides. When the serum of blood ingested by a mosquito comes in contact with a specific antiserum, a precipitation occurs at the place of contact through gel. The presence of turbidity through transparent gel layer between test sample and antisera is indicative of the blood source (host).

Results

During the study, three species including *Anopheles subpictus*, *An. stephensi* and *An. culicifacies* were documented from Bikaner, two species namely *An. subpictus* and *An. stephensi* were noted at Kolayat tehsil, while three species were observed in Loonkaransar tehsil which included *An. subpictus*, *An. stephensi* and *An. annularis*. Results of blood meal analysis have been presented in Table 1. The results of the blood meal analysis showed that *An. subpictus* had a preference towards cattle blood as compared to human, while *An. culicifacies* and *An. stephensi* preferred human blood and *An. annularis* gut content revealed the presence of only bovine blood. The results therefore indicate that *An. annularis* seems to be specifically zoophagic while rest of the three species are zoophagic as well as anthropophagic in nature.

Table 1. Blood meal preference of anopheline mosquito species (%) on different hosts

Species	Bovine	Mixed	Human
Anopheles subpictus	57.44	25.53	17.02
Anopheles stephensi	15.60	30.56	53.84
Anopheles culicifacies	–	28.00	72.00
Anopheles annularis	100	–	–

Discussion

The findings revealed that *An. subpictus* had a preference towards cattle blood as compared to human, while *An. culicifacies* and *An. stephensi* preferred human blood and *An. annularis* gut content showed the presence of only bovine blood. According to Bruce-Chwatt and Goeckel (1960) the information on the host preference of *An. culicifacies* which extends from south eastern Arabia and Pakistan to Indo-China and south across India to Ceylon was very confusing. In those parts of India where this species was considered the main local vector, the result of precipitin tests positive to man did not exceed to 2% of all, while Afridi and Puri (1940) showed that the ratio between the number of cattle and man influenced the feeding behavior while Senior White and Rao (1943) found that it varied in relation to the type of shelter in which the mosquitoes were collected. The overall anthropophilic index of *An. culicifacies* was reported to be 11.9% by Bhatt et al. (2008). According to Joshi et al. (1988), the blood meal results of *An. culicifacies* indicated the mosquito species to be predominantly zoophagic in nature. Roy et al. (1991) observed the percentage of mixed feeds i.e., mosquito feeding both on human and cattle hosts was only 3 to 4%. Further, they also found that mosquitoes collected from human dwellings did not show a higher percentage of human feeding than cattle feeding. *Anopheles dirus* was reported to be highly anthropophilic by Dutta et al. (1996). Thapar et al. (1998) reported a high proportion of *An. culicifacies* between 76–100% to feed on bovine hosts followed by 0.32–24.2 on human. According to them *An. culicifacies* and *An. stephensi* were zoophagic in nature. Grieco et al. (2002) documented *An. vestitipennis* to feed mostly on human blood but it also fed on cattle blood, while *An. albimanus* and *An. punctimacula* fed mostly on cattle blood and rarely on humans. Parida et al. (2006) reported the overall anthropophilic index of *An. culicifacies* to be 1.6 and of *An. annularis* to be 3.2%. Further, *An. culicifacies* was also noted to show seasonality in its anthropophilic index by them.

The final choice of host by a hungry mosquito may depend on extrinsic or intrinsic factors. Extrinsic factors such as availability of the host, its range of flight and resting habits, etc may influence the selection, but the mosquito is still able to exert a free choice when extrinsic factors lead it to a number of possible hosts (eg, in huts where humans and animals live together). Extrinsic factors such as the sleeping habits of the hosts, the odors given out by them, the large exposed area of warm skin, the accessibility of the capillary blood vessels, the palatability of the blood, or possibly simple random selection lead to the final choice. However, intrinsic factor, a genetic requirement of blood from a certain host or a particular capacity to respond to some impulses only-may override these tendencies. In addition to this, certain characteristics of behavior may be due to interplay of both intrinsic and extrinsic factors. This is the case of the difference between the indoor- and outdoor-biting behaviors. *Anopheles* which enters houses (endophilic species) will have better opportunities for feeding on man than those which

remain out of doors (exophilic species). Obviously, knowledge of the behavior of mosquitoes (dispersion, gono-trophic cycle, nightly turnover, resting-habits) is most useful for the study of feeding preferences in malaria vectors, and therefore of their epidemiological importance. It might be worth recalling here that females normally seek a blood meal within 24 hours of emergence. The frequency of subsequent feeds is determined by the duration of the gono-trophic cycle and by the rate of digestion, which is more rapid in hot, humid weather. The amount of blood that has been ingested by fully fed female *Anopheles* varies according to the size of the species, but there is also some variation between individual females (Bruce-Chwatt and Goeckel 1960).

It could therefore be concluded that although during the present study *An. annularis*, which has been recently reported from the area, was found to feed exclusively on bovine blood, earlier reports suggest by Guresekaran et al. (1989) and Amerasinghe et al. (1991) that this species is a vector of malaria and therefore preventive measures should be taken well in advance before this species gets established in the area.

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