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Variation in glucose-6-phosphate dehydrogenase activity in the adults and different immature stages of *Aedes aegypti*

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Abstract

In India, *Aedes aegypti* is the principal vector of the dengue viruses. Dengue viruses require a stipulated time to replicate and disseminate inside the mosquito's body before transmission to nai¨ve hosts can occur. Glucose-6-phospahte dehydrogenase (g6pd) is a NADP generating enzyme and support many biosynthetic reactions in *Ae. aegypti*. The study was aimed to record the variation in g6pd activity with increasing age and to monitor the impact of environmental conditions on the g6pd activity. The g6pd activity in newly emerged *Ae. aegypti* adults was higher than larvae, pupae and elder mosquitoes. The activity of the g6pd was decreased with increasing age of adults and was consistent for all the cases upto 20 days. There was a significant higher g6pd activity recorded in the field collected larvae, pupae and females than the laboratory reared mosquitoes. The enhanced g6pd activity in field collected mosquitoes may be due to higher rate of metabolism and this leads to more risk of disease transmission. Therefore, larval control strategies will be more effective for the control of the vector borne disease in the desert region. The g6pd activity may be used as a tool for the prediction of age of adults for a particular period.

Key words : Age, dengue, longevity, mosquito, vector

INTRODUCTION

Dengue fever and associated DHF is an emerging global disease and arguably the most important arboviral disease threatening human population. Approximately 2.5 billion people are at risk of the disease and each year an estimated 50-100 million cases occur [1]. Rajasthan, is one of the dengue endemic States in India [2-3]. *Aedes aegypti,* the vector of dengue fever, is widely present in India [4], including the Thar desert in Rajasthan [5]. The extrinsic incubation period (EIP) of dengue virus is the interval between when a female Aedes blood feeds on an infected host and when it is able to transmit the virus. The EIP is estimated to be between 10 and 14 days for dengue virus [6-7]. The age of female Aedes mosquito must have longer than the duration of the initial non-feeding period plus EIP of the virus to contribute to dengue transmission. Hence, accurate estimation of the age of disease transmitting mosquito vectors is of prime importance for the measurement of transmission and control success. Therefore, study on physiology and biochemistry of vectors with development is one of the important elements for the development of much-needed new approaches for age determination.

The changes in proportions of cuticular hydrocarbons (CHC) from legs to predict adult age has been recently used in *Ae. aegypti* [8]. This method has been shown to be capable of estimating adult *Ae. aegypti* age upto 15 days in field evaluations [9]. Moreover, many individuals live >15 days in field and that dengue transmission occurs in mosquitoes 12 days and older, therefore this approach has a limited utility in epidemiological investigations. The methods mostly used for the age determination of female mosquitoes depend on variation in ovarian structure. The technique based on changes in tracheal skeins are capable of distinguishing parous from non-porous mosquitoes, but are not capable of any finer age determinations within those categories [10]. Age determination of Aedes mosquitoes using the ovarian oil injection techniques was not proper because ovariolar sac do not form gonotrophic dialations and can not be used for physiological age determination [11].

Understanding environmental determinants of disease transmission is one of the most pressing challenges faced by research and public health scientists, especially with the recent emergence and re-emergence of infectious diseases [12]. Energy homeostasis is an essential process in mosquitoes to counter the environmental determinants by physiological modifications [13]. Carbon source conversion in living tissues involves tight regulation of enzymes of the glycolytic pathway [14]. The activities of NADP linked enzymes which generate NADPH play key role in metabolic regulation [15]. The metabolic changes that occur during different periods of life span are dependent upon the concentration of co-enzymes NADPH [16]. Glucose-6-phospahte dehydrogenase (g6pd) is a NADP generating enzyme and support many biosynthetic reactions in *Ae. aegypti*. The present study was undertaken to estimate the variation in g6pd activity in different stages of male and female *Ae. aegypti*. The study was also aimed to monitor the impact of environmental conditions on activity of g6pd.

MATERIALS AND METHODS

Rearing of field collected *Ae. aegypti*

Source of Ae. aegypti

Egg, larvae and pupae of *Ae. aegypti* were collected from the containers of the houses. Mosquito collection places were situated in urban areas of Jodhpur City of Rajasthan state, India. They were transported to the insectary in ambient condition and were reared as described by Cook et al. [9].

Collection and maintenance of field collected *Ae. aegypti*

Immature stages of *Ae. aegypti* were collected from the field from the month of July to March. They were collected in enamel bowl along with the water where they were inhabiting and brought in ambient condition to the insectary. In the insectary they were transferred to the enamel trays and were kept in the

same water collected from the breeding habitats. They were not provided any supplementary food. Field collected eggs, larvae and pupae were reared in the insectary at an optimum photoperiod (10:14 [L/D] h), temperature (28±1°C) and relative humidity (75±5%). Adults emerged from the field collected pupae were not put in cyclic cages and kept in a separate rack of insectary to avoid mixing.

Colonization of laboratory reared *Ae. aegypti*

The F-1 progeny of the field collected mosquitoes were reared for colonization. The laboratory reared *Ae. aegypti* colony was established from collections made from the Bomba Mahala areas of Jodhpur. *Ae. aegypti* larvae were colonized under a standard insectarium condition at 28±1°C, 75±5% relative humidity and a 10L:14D photoperiod. To maintain a uniform adult size at emergence, larvae were reared at a fixed density of 200 per/tray. Larvae were fed on a mixture of yeast powder and dog biscuit (1:2) in 24 hours interval. Adults that emerged were fed on 10% glucose solution and soaked raisins. Adults of *Ae. aegypti* were deprived of glucose for 12 hours before blood fed. Females were provided blood meal at weekly intervals. Adults were allowed to mate freely and kept in cyclic cages ($0.6m^3$).

Longevity

Hundred laboratory reared *Ae. aegypti* pupae were randomly taken from the rearing trays and kept in cages for adult emergence. Same number of field collected pupae along with the field water were kept in separate cages for the adult emergence. Both types of pupae were kept in the cages for adult emergence and un-emerged pupae were discarded on day-3. Males and females emerged from the laboratory reared and field collected pupae were maintained in the insectary in separate cages and were continuously provided with 10% glucose solution and soaked raisins. Emerged adults were maintained for one month to conduct the g6pd assays at five days interval. Each of ten males and females in triplicate were taken for the g6pd assays. Blood meal was not given to the adults maintained for the g6pd assays.

Glucose-6-phosphate dehydrogenase (g6pd) assay

Ten fourth instars *Ae. aegypti* were taken from the field collected and laboratory reared colonies and each of them were placed separately in 1.5ml microcentrifuge tubes. Each larva was homogenized in 200 µL of 5mM glycine (pH-8) with a grinder. Similarly ten numbers of field collected and laboratory reared pupae were homogenized. Within 24 hours of emergence 10 males and 10 females were taken from the cage and placed in test tubes covered with mesh. The mosquitoes in the tubes were placed in freezer for 5 minutes for anesthetizing. Each anesthetized mosquito was transferred to 1.5ml microfuge tube and homogenized in 200 µL of 5mM glycine (pH-8) with a grinder. 100ul homogenized tissue of larvae, pupae and adult mosquitoes was transferred separately to the spectrophotometer cuvettes maintained at 30°C in a dry bath containing substrates and co-factors. The reaction mixture of 3ml containing 2.7ml of 55mM TrisHcl containing 3.3mM $MgCl_2$ (pH-7.8), 100µl of 6mM NADP, 100µl of 0.1M glucose-6-phophate and 100µl of mosquito tissue. The optical density was read at 340 nm for 10 min at interval of 1 min using UV-Vis spectrophotometer. Each experiment was conducted in triplicates on different days. The g6pd activity was measured as described by Glock and McLean [17].

Protein estimation

Protein analysis was carried out using the Bradford dye-binding microtitre plate assay procedure [18]. For this purpose, 10uL of mosquito tissue was added to microtitre plate to which 300uL diluted BioRad reagent was added. Samples were read at 590 nm after 5 minutes of incubation at room temperature. In each micro-titer plate, eight blanks were prepared with 10uL distilled water and 300uL BioRad solution. Protein concentrations were obtained from a standard curve based on bovine serum albumin.

Statistical analysis: Differences among mean values were analysed with the Microsoft Excel statistical packages.

RESULTS

A comparative g6pd activity in the laboratory reared and field collected larvae, pupae and adults of *Ae. aegypti* are shown in figure 1 & 2. The g6pd activity in both males and females from day-0 to day-30 at the interval of 5 days are shown in figure 1 & 2.

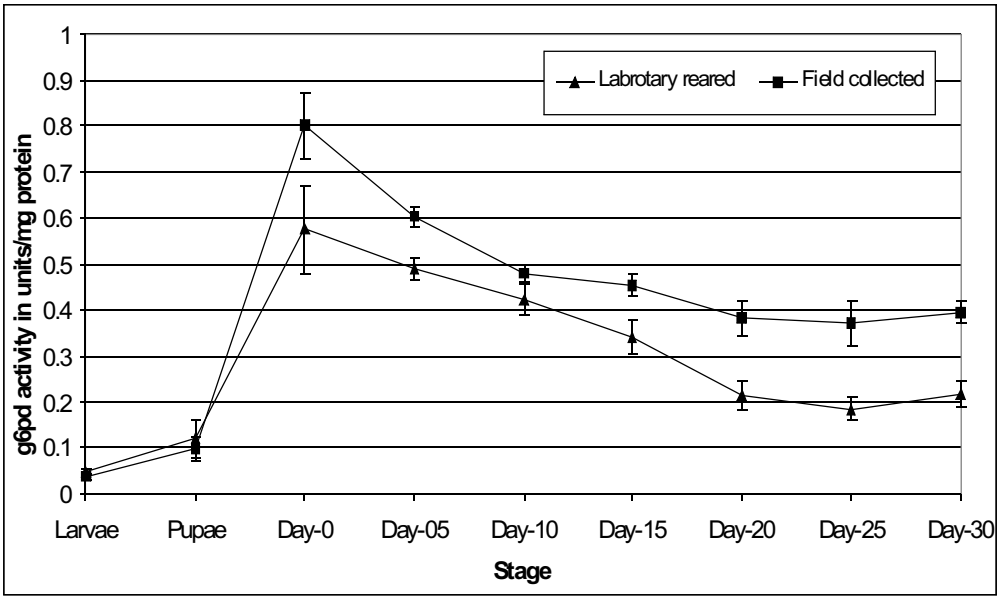

Fig 1: Glucose-6-phosphate dehydrogenase activity in larvae, pupae and females of laboratory reared and field collected *Aedes aegypti.*

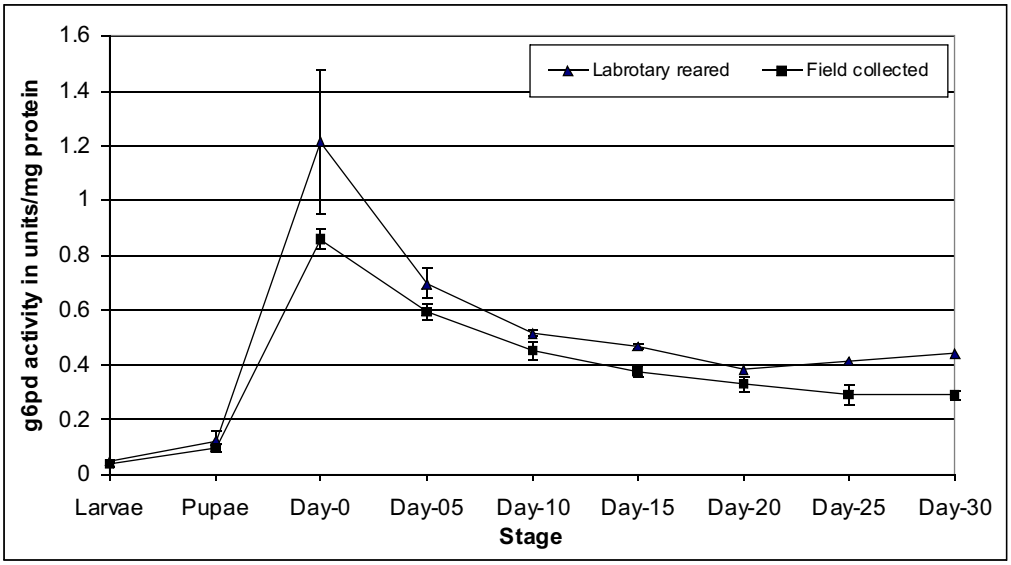

Fig 2: Glucose-6-phosphate dehydrogenase activity in larvae, pupae and males of laboratory reared and field collected *Aedes aegypti.*

Laboratory reared and field collected mosquitoes were consistently showed that (1) g6pd activity in newly emerged adults was higher than larvae, pupae and elder mosquitoes (2) higher g6pd activity was in pupae than larvae (3) the g6pd activity in the females was decreased upto 25 days (Fig. 1). Furthermore, the g6pd activity in the 25 days old laboratory reared and field collected females were recorded to be 68% and 54% lower than the newly emerged females respectively. However, the activity was found to be higher in 30 days old mosquitoes than the 25 days old one. Significant (*t test*, P=0.002) higher g6pd activity found in field collected females than the laboratory reared females.

The g6pd activity of the laboratory reared males was decreased upto 20 days and thereafter it was increased. When the g6pd activity was compared among field collected males, the activity in 25 days old was recorded 67% lower than the newly emerged ones. However, the activity in 30 days old mosquitoes was found to be nearly equal to the 25 days old mosquitoes. When the g6pd activity was compared between the laboratory reared and field collected males, the g6pd activity in laboratory reared males was found to be significantly higher than the field collected males (*t test*, P=0.006).

DISCUSSION

Age composition, parity and vector competence are crucial factors for understanding the transmission dynamics of *dengue fever and associated DHF*. Age composition of vectors has significance role in epidemiological investigations and evaluation of operational research. Larval rearing temperature, competitions and chemical contaminants have also been associated with alterations in adult longevity [19-20] and susceptible to pathogen. In the present study, higher g6pd activity was recorded in early emerged adults than the pupae and larvae. Lang and Stephan [15] matched the g6pd activity with the period of cellular growth and supporting the hypothesis that NADPH concentration is high during the periods of greatest biosynthetic activity. However, in this study a decreased activity of g6pd was recorded in the elder males and females *Ae. aegypti*, which may be due to the decreased ability of the aged cell to synthesize ribose from glucose. The g6pd activity in the males and females *Anopheles stephensi* was also recorded to be inversely proportional to their age [21]. These observations are similar to the present study. One of the other reasons for the decreased g6pd activity in elder mosquitoes may be due to the sugar feeding frequency decline with age as reported by Foster [22]. The g6pd activity was obtained from the samples of the whole mosquito, which is advantageous in growth and aging investigations because the results reflect a general phenomenon of all cells rather than of an isolated and perhaps atypical cell type [15].

Mosquitoes in the desert part can survive in the extremes of temperature and low humidity as well [23]. Free living insects are under much harsher environmental conditions and may generally be smaller in size and have lower levels of energetic reserves than those in standard controlled environments [24]. Climate warming affects mosquito physiology because metabolic rate increases exponentially rather than linearly with temperature in ectotherms [25]. When temperature of habitat rises, the larvae take a shorter time to mature [26] and consequently, there is a greater capacity to produce more offspring. An increase in ambient temperature will accelerate the digestion of blood meals taken by females, leading to increased human biting rate, faster parasite sporogonic development [27], translating to an increased disease transmission efficiency [28]. The g6pd activity in the males *An. stephensi* was also found to be significantly higher than the females [20]. The sugar metabolism of males metabolize was faster than females [21].

CONCLUSION

The g6pd activity of the field collected larvae, pupae and females was higher than the laboratory reared mosquitoes. Therefore, it could be concluded that the enhanced g6pd activity may due to higher rate of metabolism. A higher metabolic activity may be due to the influence of the desert climate. The g6pd activity in the laboratory reared and field collected male mosquitoes were found to be higher than their counter part females. Males metabolize sugar faster than females and this may be the reason for the higher g6pd activity in males.

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