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A double-blinded, placebo-controlled trial of garlic as a mosquito repellant: a preliminary study

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Abstract. The hypothesis that the ingestion of garlic provides protection against bloodsucking pests such as mosquitoes was investigated using a randomized, double-blinded, placebo-controlled crossover study. Subjects were asked to consume either garlic (one visit) or a placebo (the other visit). They were then exposed to laboratory-reared *Aedes aegypti* (Linnaeus) (Diptera: Culicidae). The numbers of mosquitoes that did not feed on the subjects, the number of mosquito bites, the weights of the mosquitoes after feeding and the amounts of blood ingested were determined. The data did not provide evidence of significant systemic mosquito repellence. A limitation of the study is that more prolonged ingestion of garlic may be needed to accomplish repellence.

Key words. *Aedes aegypti*, garlic, human serum albumin, mosquitoes, repellant.

Introduction

Blood-feeding arthropods are of great medical and veterinary importance, in part due to their nuisance value and in greater part due to their role as vectors of infectious agents that cause considerable morbidity and mortality (Gubler, 1998). Of the many arthropods that feed on humans, mosquitoes are of importance globally (Gibbons & Vaughn, 2002; Kager, 2002; Messer *et al.*, 2003). There are two possible approaches to the current and perhaps growing threat of mosquito-borne illnesses. One, which has been used quite extensively, is aerial spraying with pesticide(s) known to control mosquito populations. Indiscriminate spraying has profound ecological consequences (Carson, 1962). Although the public may be reassured by aerial spraying, one has to question whether this approach accomplishes the desired goals and whether it may have detrimental consequences in the long run.

Another strategy is the combination of protective clothing, mosquito avoidance and topical repellents such as permethrin (a synthetic pyrethroid) or diethyltoluamide. None is a universally practical solution. The use of topical repellents is also fraught with both logistical and potentially toxic side effects. It is unrealistic to apply repellents over every inch of exposed skin or to meticulously apply topical agents every time one ventures out of one's home. Too many topical applications have relatively short half-lives and are easily removed upon sweating or bathing. Thus, their efficacy is for a limited time period. There is the final consideration that although diethyltoluamide had a long documented history of benignity, the possibility of sensitization and long-term toxicity cannot be fully discounted.

These considerations highlight the need for systemically active mosquito repellents that could be used population wide. Such an agent must be of proven low (or no) toxicity and must be capable of consumption over long periods of time without the risk of causing untoward effects on the target population. A belief in the ability of garlic, a member of the lily family, to repel insects seems to be widespread in the lay community. References to the use of garlic sprays as insect repellents abound on the web (see for instance http://www.garlicbarrier.com/ and http://www.agnews.tamu.edu/stories/ENTO/Oct2897a.htm). A particularly widespread application of garlic appears to be for sprays on fruiting trees as an insect repellent. There is also widespread belief, particularly among pet owners, that blood-feeding insects avoid feeding on mammals that have ingested garlic. Horse lovers routinely feed their horses garlic, and claim that such feedings reduce the number of attacks by horseflies, black

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flies and mosquitoes. Web pages catering to equestrian interests recommend incorporation of garlic in feed to prevent horses from being troubled by these nuisance insects (see, for instance, http://www.garlicbarrier.com/).

There are very few publications documenting the efficacy of garlic. In one of the few existing trials, for example, a group of Swedish Army conscripts was randomized into two groups, one of which was provided with garlic capsules to be ingested daily and the other with a placebo (Stjernberg & Berglund, 2000). After 8 weeks, the groups were taken off the regimen. After a 2-week 'washout' phase, the groups were crossed and put on placebo (or garlic) for another 10 weeks. The authors report that there was a significant reduction in tick bites when consuming garlic compared with placebo in per protocol analysis. A greater number of the participants were bitten by ticks during placebo consumption. This study has been criticized on methodological grounds (McHugh, 2001; Ranstam, 2001; Tunon, 2001). It has been pointed out that the investigators took no caution to insure that the two groups did not consume garlic in the diet (thus confounding the division between the placebo and the non-placebo group), that the tick feeding was entirely self reported and therefore poorly documented, and that the difference between the two was only in the 'per-protocol' analysis and not in the 'intention to treat' analysis. These are valid criticisms, and although the study should be regarded as an interesting first attempt, it is clear that a more rigorous study is called for. This study describes a trial designed to determine if a short-term ingestion of garlic would make individuals less attractive as targets for mosquitoes.

Materials and methods

Mosquito rearing

The black-eyed Liverpool strain of *Aedes aegypti* (Linnaeus) was obtained from Dr B. Christensen, University of Wisconsin. They were reared using standard techniques, in an incubator maintained at 80% humidity, 27°C. To ensure that subjects enrolling in the study were not inadvertently exposed to an arthropod-borne infection, individual mosquitoes from the colony were tested for known arboviruses by Dr B. Beaty, Colorado State University (Fort Collins, Colorado, U.S.A.). The mosquitoes were negative for flaviviruses and α-orbunyaviruses by fluorescence. Extracts of the mosquitoes did not cause cytopathic changes in Vero cells.

Patient recruitment

Patients were recruited to the study by a combination of an advertisement placed in local newspapers as well as in bulletin boards. In addition, the principal investigator was interviewed on a local television news channel. This combination of advertisements drew a total of 81 responses. All respondents were screened by the nurse coordinator for the study. All subjects were interviewed to determine that they did not have evidence of allergic reactions to insects, such as dust mites, or known hyper-reactivity to mosquitoes or to garlic. Patients who had any chronic serious illness or were currently on medications for such illnesses were excluded from the study. Fifty-one subjects were recruited to the study on a first-come, first-served basis.

Blinding

Garlic tablets were purchased from General Nutrition Sciences (Pittsburgh, Pennsylvania, U.S.A.). Each capsule is described by the manufacturer as being equivalent to one clove of native garlic. In order to generate placebo capsules that could not be distinguished from the active ingredient, the caplets were placed in empty gelatin capsules and sealed by the research pharmacist at the University of Connecticut Health Center. For making up placebo capsules, empty gelatin capsules of identical appearance were filled with 250 mg of lactose. Subjects enrolled in the study were randomized such that they would receive either garlic or placebo at their first visit and then either placebo or garlic during their second visit. Only the research pharmacist retained access to the order in which the subjects received the garlic and the placebo. The code was not revealed to the subjects, the nurse practitioner, the research assistant, the principle investigator (PI) or co-PI until the study was completed, and the enzyme-linked immunosorbent assay (ELISA) data were available.

Procedure

The study was designed as a double-blinded, randomized, acute intervention crossover protocol. Each subject served as his/her own control. They were asked to consume two caplets of garlic or placebo the night before arrival at the Health Center and two more at lunch on the day of the study. They were asked to refrain from adding excess garlic (salt, powder or extract) to food over a 48-h period before the study and on the day of the study. They were asked not to use perfumes or colognes or apply any hand-creams or lotions on themselves on the day of the study.

Upon arrival at the Health Center, the subjects were met and briefly interviewed by one of the authors (M.H.). They were then brought over to the mosquito facility. Individuals were asked to bare their arms, and place their forearm on a container with an average of 10 (range 7–11) adult female mosquitoes within for 2 min. The containers were covered with a meshwork that prevented egress of mosquitoes from the container. At the end of the 2-min exposure, the area of skin overlying the container was examined and the number of bite marks noted and recorded. The subjects were asked to remain at the Health Center for 30 min, to monitor any unexpected systemic allergic or syncopal responses. Subject identification numbers but not names were marked on the containers. After the subjects had left the room, the adult female mosquitoes were examined to determine the number that were engorged with blood, and the completeness of the

bloodmeal. On a return visit 28 days later, subjects were provided either with garlic or placebo (crossover) and the exposure to mosquitoes repeated.

Outcomes

Two subjective and two objective outcomes were used to determine whether garlic had any impact on the attractiveness of individuals to mosquitoes. The two subjective parameters were the number of mosquito bites that were visible on the arms of the patients and the number of fully fed, partially fed or unfed mosquitoes at the end of each exposure. The two objective parameters were the weights of the mosquitoes at the end of the exposure, and the amount of human serum albumin (HSA) within the mosquitoes.

The amount of HSA within each batch of mosquitoes was determined using a standard sandwich ELISA, using the mouse monoclonal antibody HSA-11 (Sigma Chemical Company, St. Louis, MO, U.S.A.) for capture and purified horseradish peroxidase-conjugated immunoglobulin from rabbit anti-HSA (RDI, Flanders, NJ, U.S.A.). Plates were coated with 2 μg/mL solution of the monoclonal antibody HSA-11 in coating buffer (0.1 M Na_2HPO_4 pH 9.0). The plates were washed with phosphate-buffered saline containing 0.05% Tween-20. They were blocked with 3% Carnation instant milk in phosphate-buffered saline.

Purified HSA (Sigma) in amounts ranging from 250 μg to 0.1 μg, were used in the ELISA to obtain a standard curve of optical density (O.D.) vs. mass of HSA. Mosquitoes were homogenized using an IKA Labortechnik Ultra-Turrax T8.01 Netzgerät tissue homogenizer (D79219, Staufen, Germany), in 1.0 mL of phosphate-buffered saline. Large particles were removed by centrifugation at 9300 g for 5 min. The clear supernatants were used in the ELISA assay, using at least four dilutions. The O.D. values in the four dilutions were interpolated into the standard curve. The average of these four interpolated values was used as the HSA content of that batch of mosquitoes.

Data analysis

All data were entered into an Access® database maintained by the General Clinical Research Center (GCRC) Informatics core using a web-based, HTML front end. Data in the database were exported to Excel spreadsheets and analysed for statistical significance using SPSS and SAS.

Results

Demographics of the study population

The 51 individuals recruited to this study ranged in age from 21 to 75 years (mean = 42.2 years). One subject was Asian and four black; the rest were non-Hispanic Caucasians. Thirty-four individuals were female and 17 male.

Retention

Of the 51 subjects enrolled in the study, two signed the consent forms but did not participate further (one elected not to participate; the other was lost to follow-up). Thus, 49 individuals completed the study.

Effectiveness of blinding

To determine the effectiveness of subject blinding, we asked all subjects at the time of their exit interviews whether they were able to guess the visit on which they received the garlic. Of the 49 individuals who completed the study, 23 were not able to make a guess and felt that they had been unable to determine, by taste or odour, when they had received garlic. Twenty-six others hazarded a guess. In 20 instances, their guess was accurate and matched the visit on which they consumed garlic. In six instances, there was discordance between the guess and the true visit on which garlic had been provided. Thus, it appears that our blinding was reasonably effective at the level of the subjects. In terms of the principal investigator (T.V.R.), and coprincipal investigator (S.W.), blinding was complete in that they did not come face to face with the subjects and therefore were not in a position to determine when they may have consumed garlic.

Enumeration of visible bites

One subjective parameter to determine the efficacy of garlic was to count the number of visible mosquito-bite generated puncture wounds in the exposed area of the forearm. This determination was performed by the registered nurse, who was also blinded to the relationship of the visit and garlic or placebo consumed at the visit. These data are shown in Table 1. The subjects were found to fall into three groups. In one group ($n = 30$), there were more visible bite marks during the first visit than the second. In group two ($n = 15$), there more visible bite marks on the second visit than on the first. In the final group ($n = 4$), the number of bites were equivalent during the two visits.

After unblinding, the number of visible bites on each subject during the 'garlic visit' and the 'placebo visit' were determined. The range was 0–11; the mean for the garlic visit was 4.75 (SD ±1.99) and for the placebo visit 4.46 (SD ±2.58). These values were not significantly different ($P = 0.28$ by the non-parametric Sign test). The observations did not permit us to reject the null hypothesis.

Evaluation of extent of feeding by exposed mosquitoes

If garlic were acting as a mosquito repellent, then there should be more unfed mosquitoes on the visits when subjects had ingested garlic. The 10 female mosquitoes that were exposed to the arms at each visit were examined and enumerated as fed or unfed. As with the number of bites,

Table 1. Change in the number of visible mosquito bites on the arms of exposed subjects at two visits. Subjects were classified into three groups; those who had more bites at visit 1, those that had more bites at visit 2 or those that had equal numbers of bites at the two visits

Observation	*n*	Subject identification
More bites at visit 1	30	1, 2, 3, 5, 8, 10, 12, 13, 14, 16, 17, 18, 20, 21, 23, 24, 25, 26, 27, 30, 31, 33, 34, 36, 37, 38, 44, 45, 46, 50
More bites at visit 2	15	4, 6, 7, 11, 19, 22, 28, 29, 35, 40, 41, 42, 47, 49, 51
Equal at both visits	4	15, 39, 43, 48

subjects could be divided into three groups (Table 2). In one group ($n = 10$), there were more unfed mosquitoes on visit 1, in another group ($n = 33$), there were more unfed mosquitoes on visit 2, and there were a few individuals ($n = 6$) for whom there were equivalent numbers of unfed mosquitoes on both visits.

After unblinding, the number of unfed mosquitoes for each subject during the 'garlic visit' and the 'placebo visit' was determined. The range was 0–10; the mean for the garlic visit was 4.42 (SD ±2.1) and for the placebo visit 4.54 (SD ±2.4). These values were not significantly different ($P = 0.50$ by the non-parametric Sign test.) The observations did not permit us to reject the null hypothesis.

Weights of mosquitoes after feeding

If mosquitoes fed upon the exposed arm, we anticipated that there would be an increase in their weight, as mosquitoes ingest approximately 2–3 μL of blood at a feeding. Thus, upon exposure to the arm, if all the mosquitoes fed on the arm, a net increase in weight of 20–30 mg would be expected (10 mosquitoes × 2–3 μL = 20–30 μL of blood; each μL weighs about 1 mg, not 1 μg, despite the common misconception). If mosquitoes were inhibited from feeding because of ingestion of garlic, such an increase in weight might not be expected to take place.

The mosquitoes were weighed, therefore, at the end of the blood-feeding for all subjects at both visits and the weights at each visit were compared (Table 3). We found that there were three groups of patients. In one ($n = 25$), the cohort of mosquitoes weighed more after feeding on the subjects on the first visit; in the second ($n = 20$), they weighed more after the second visit; in the third ($n = 3$), they weighed the same after the two visits. In the case of one subject, the weight of the mosquitoes after one of the visits was misplaced, and we were unable to make the determination.

After unblinding, we determined the weights of the mosquitoes for each subject during the 'garlic visit' and the 'placebo visit'. The range was 2.7–4.6 mg; the mean for the garlic visit was 3.6 mg (SD ±0.49) and for the placebo visit 3.8 mg (SD ±0.57). These values were not significantly different ($P = 0.45$ by the non-parametric Sign test.) The observations did not permit us to reject the null hypothesis.

Human serum albumin content in fed mosquitoes

Serum albumin is a reasonably stable parameter in blood in most human subjects, and remains stable within a subject at various times, as long as the nutritional status is not profoundly changed. We therefore used the amount of serum albumin within a cohort of mosquitoes as a sensitive parameter of the amount of blood ingested by the group of mosquitoes during exposure. Mosquitoes were ground as described in the Materials and Methods section, and homogenates centrifuged to obtain a clear supernatant.

As with the other three parameters we evaluated, we found that subjects fell into three groups (Table 4). In group 1 ($n = 27$), there was more HSA in the mosquitoes after visit 1; in group two ($n = 20$), there was more HSA after visit 2; in one subject, the amount of HSA was identical after the two visits. In one subject, the data for HSA after one visit was misplaced and a determination could not be made.

After unblinding, the amount of HSA in the mosquitoes for each subject during the 'garlic visit' and the 'placebo visit' was determined. The range was 0–22 μg; the mean for the garlic visit was 4.92 (SD ± 5.4) and for the placebo visit 4.60 (SD ± 5.9). These values were significant by the non-parametric Sign test ($P = 0.03$). The null hypothesis should be rejected. It is worth noting (as detailed below in the discussion) that the placebo visit was associated with the lower HSA content in the mosquitoes.

Table 2. Change in the number of unfed mosquitoes at two visits. The number unfed was determined by chilling mosquitoes immediately after exposure to subjects; they were then examined by three independent observers to determine whether they were engorged or not. The averages of these three observations were used to determine the number of unfed mosquitoes. Subjects were classified into three groups, those who had more unfed mosquitoes at visit 1, those that had more unfed mosquitoes at visit 2 or those that had equal numbers of unfed mosquitoes at the two visits

Observation	*n*	Subject identification
More unfed at visit 1	33	1, 3, 4, 8, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, 31, 33, 34, 36, 37, 41, 42, 44, 45, 48, 50
More unfed at visit 2	10	5, 6, 28, 30, 35, 38, 39, 40, 46, 51
Equal at both visits	6	2, 7, 11, 43, 47, 49

Table 3. Change in the weights of mosquitoes at two visits. After mosquitoes had been examined visually for their fed or unfed status, they were weighed. Subjects were classified into three groups. Those for whom the mosquitoes weighed more at visit 1, those for whom mosquitoes weighed more at visit 2 and those for whom the mosquito weights were similar at the two visits

Observation	*n*	Subject identification
Combined mosquito weight greater at visit 1	25	2, 3, 4, 8, 12, 14, 15, 16, 17, 18, 20, 21, 23, 24, 25, 31, 33, 34, 38, 39, 41, 44, 45, 46, 50
Combined mosquito weight greater at visit 2	20	5, 6, 7, 13, 22, 26, 27, 28, 29, 30, 35, 36, 37, 40, 42, 43, 47, 48, 49, 51
Equal weights at the two visits	4	10, 11, 19

Discussion

This study describes a placebo-controlled, double-blinded trial to determine whether there is any validity to the widely held belief that the ingestion of garlic provides some protection against blood-feeding mosquitoes in humans. A previous study, to determine whether chronic ingestion of garlic provides protection against ticks, has been criticized on methodological grounds (Stjernberg & Berglund, 2000; McHugh, 2001; Ranstam, 2001; Tunon, 2001). The study design here sought to address some of those flaws. The major change instituted was an acute exposure design. This obviates some of the major problems in the previous study, most notably the possibility that subjects may be ingesting garlic with their foods. Because of the short duration of this trial, it was possible to require that subjects refrain from consuming garlic-containing foods. An additional problem that was noted in the previous study was that the subjects were not specifically instructed to avoid other pest repellents during the study. During the short-term course of this current study, subjects were instructed not to apply any topical mosquito repellents on their bodies. Again, the use of personal odourants such as perfumes, colognes and scented soaps was not controlled for. In order to avoid these confounding variables from the study, the subjects in the present study were asked to refrain from using colognes or perfumes on the day of the study and further not to apply any hand creams or lotions on their arms. Finally, the use of a crossover design permitted the use of every individual as his or her own control. In order to allow a sufficient 'washout time' between studies, a period of 28 days was allowed before the subjects were asked to return. Part of the reason for this design was that it has been described that women are variably attractive to mosquitoes depending upon the menstrual cycle. Although this is conceivably not of relevance to male subjects, it was felt that this would help standardize the design by having the same approach to male and female subjects.

The use of four independent parameters, two slightly subjective and two others objective, also helped alleviate one of the other perceived problems in the previous study, namely the possibility that self-reporting could bias the interpretation of the data.

Results from the exit interviews of the subjects as to which visit on which they received the garlic indicated that our blinding procedure was reasonably robust. A majority of the subjects could either not guess which visit they ingested garlic, or guessed incorrectly. Blinding was also robust at the level of the interpretation, as the PI and Co-PI did not know the visit on which the subjects received garlic, nor did they come face-to-face with the subjects in order to make an informed judgement based on odour.

Three of the four parameters that were used generated data that failed to reject the null hypothesis, namely that garlic would not affect the attractiveness of the subject to mosquito biting. In one case (HSA content in the mosquitoes), the data would reject the null hypothesis using the non-parametric Sign test. It is interesting to note that the alternative hypothesis suggested by the data is that garlic *increases* the attractiveness to mosquitoes, the opposite of what is suggested in the lay literature. However, although statistically significant, this result would not appear to be biologically meaningful. Thus the mean HSA content for the garlic visit was 4.92 μg and for the placebo visit was 4.6 μg. This amounts to a 6% difference. It seems highly unlikely that such a minor difference would either increase the nuisance factor on garlic ingestion or increase the chance of vector transmitted disease.

Thus, at least for acute avoidance of mosquitoes, loading up on garlic-containing foods may not help as an avoidance manoeuvre. This study may be criticized on the grounds that garlic tablets were used rather than raw garlic.

Table 4. Change in human serum albumin (HAS) in mosquitoes at two visits. Mosquitoes were homogenized using a hand-held homogenizer. The insoluble material was removed by centrifugation at 16 000 g in an Eppendorf centrifuge for 5 min. The HSA content in the supernatant was determined by enzyme-linked immunosorbent assay. Subjects were divided into three groups: individuals for whom the mosquitoes contained more HSA at visit 1; individuals for whom the HSA content was greater at visit 2; and individuals for whom the amount of HSA was comparable at the two visits

Observation	*n*	Subject identification
More HSA in mosquitoes at visit 1	27	2, 3, 4, 6, 10, 11, 14, 16, 18, 19, 20, 21, 23, 24, 25, 30, 33, 34, 37, 38, 41, 44, 45, 47, 48, 50, 51
More HAS in mosquitoes at visit 2	20	5, 7, 8, 12, 13, 15, 17, 22, 26, 27, 28, 29, 31, 35, 36, 39, 40, 42, 43, 46
Equal HSA at both visits	1	49

However, it is important to point out that dog and horse owners and fanciers, who use garlic routinely and with apparent success as a mosquito repellent, employ garlic powder rather than raw garlic. In this field, it is widely reported on the Web that a considerable period of 'loading up' on garlic is required for horses and dogs to become resistant to mosquito biting. Chronic ingestion might have a different effect on mosquito feeding. Although this may be true, such a chronic indulgence in large doses of garlic may be socially unacceptable. It is much more realistic that in an acute situation, such as the day before a picnic, loading on garlic may be a potential way to avoid being bitten extensively by mosquitoes. This study suggests that the ingestion of garlic for such an acute planned exposure may not be a feasible or effective strategy.

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