

## Original Article

# Species Variety of the Calf and Human-Attracted Mosquitoes in Southwest Iran

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### Abstract

**Background:** Any mosquito control methods requires precise information about population dynamics, variety, biology and mosquito habitat. This research assessed Culicid mosquitoes' attraction to a human host and a calf to better understand their behavior.

**Methods:** Adult mosquitoes were sampled in 22 weeks in southwestern Iran's Nur Ali Village from May to October 2015. The mosquitoes were drawn to the person and calf as bait, while the unbaited trap was also used. A substantial statistical difference between attracted mosquitoes to the hosts was determined in the T-test.

**Results:** Within 22 weeks, 29821 mosquitoes were captured. Only 9% were collected from the human baited net trap, 89.1% from the calf baited net trap, and 1.9% from the unbaited net trap. The number of collected female mosquitoes was statistically significantly higher using the calf baited net trap of the total mosquitoes, 916 were randomly identified at the species level by local identification keys. Of these, 63 were *Anopheles stephensi* (human: 16%, calf: 75% and unbaited: 9%), 83 *An. pulcherrimus* (human: 27%, calf: 60% and unbaited: 13%), 118 *Aedes caspius* (human: 24%, calf: 69% and unbaited: 7%), 493 *Culex tritaeniorhynchus* (human: 52%, calf: 37% and unbaited: 11%), 153 *Cx. quinquefasciatus* (human: 44%, calf: 47% and unbaited: 9%), and 6 *Cx. theileri* (human: 33%, calf: 50% and unbaited: 17%).

**Conclusion:** The obtained results here provide useful insights into the mosquito population and the possibility of using this information as an essential part of integrated vector management regarding the reemergence of malaria or other mosquito-borne.

**Keywords:** Mosquitoes; Diversity; Iran

## Introduction

Blood feeding of female mosquitoes (Diptera: Culicidae) plays an essential role in transmitting diseases by mosquitoes, for instance, Rift Valley fever, yellow fever, West Nile, dengue and malaria. The above plus other transmitted diseases by vectors cause about one billion people infected and more than one million deaths worldwide every year (1). Parasitic and arboviral mosquito-borne diseases have been reported from Iran for a long time. Despite considerable progress recently in eliminating ma-

laria in Iran, 799 reported malaria (632 cases were imported) were registered in 2015 (2). The records of other mosquito-borne diseases in Iran, including dengue, West Nile fever, dirofilariasis and sinbis, were reported in the literature (3-9). Based on a retrospective cross-sectional study, 541 cases of malaria were reported in Khuzestan Province in the southwest of Iran between 2001 and 2014. The most prevalent malaria species was *Plasmodium vivax* with 478 (88.35%) cases compared with *P. falciparum*

with 63 (11.65%) cases (10). The Culicidae has 41 or 113 genera (depending on the classification of the tribe Aedini), which are classified in the number of 3583 mosquito species. These 41 (113) genera are divided into the following two subfamilies: Anophelinae and Culicinae (11). Based on the most recent Iranian mosquito studies, 70 species were reported in 8 to 12 genera in Iran (12-15). Several species of culicid mosquitoes are recorded in Khuzestan Province, including *An. stephensi*, *An. fluviatilis* s.l., *An. dthali*, *An. Superpictus*, *An. pulcherrimus*, *An. culicifacies*, *An. hyrcanus*, *An. sacharovi*, *Culex pipiens*, *Cx. perexiguus*, *Cx. sinaiticus*, *Cx. modestus*, *Cx. tritaeniorhynchus*, *Cx. theileri*, *Cx. pusillus*, *Cx. quinquefasciatus*, *Aedes caspius*, *Uranotaenia unguiculata* and a *Culiseta* species (14-21). Insecticide-treated nets and indoor residual spraying were used as the primary control activities of malaria vectors in the country (2). The explanation behind the progress made against malaria by Iran and other countries was that governments were using Insecticide-treated nets and indoor residual spraying to avoid malaria in significant transmission areas. It will aid little in managing malaria and zoonotic diseases with zophilic vectors (22, 23). Due to indoor residual spraying and treated mosquito nets by many societies, mosquitoes have adapted to remain alive by being more likely to feed on animal blood than ever. Hence, appropriate management steps will be required to eliminate malaria or other vector-borne diseases in some regions of the world where it is caused by zophilic disease vectors that are difficult to control (22, 24-26). The natural tendency of mosquitoes to feed from domestic animals, especially cattle reduces bites to nearby humans. Therefore, the possibility of diversion of mosquitoes to animals was proposed as a malaria control method (27-29). The above concept application has been presented as a part of the integrated vector management, such as applying insecticides on cattle in the areas under specific conditions (30). After insecticide

resistance and the harmful effects of certain insecticides in the environment were noted, this approach seemed like the best option. It is inexpensive, effective, and easy to encourage people to participate and contribute to disease vectors' control (31, 32). This research attempts to determine and compare the number of collected female mosquitoes in human baited, calf baited, and unbaited net traps to explain the obtained mosquito population's assortment traps toward providing information about the available potential control methods. Besides, it provided some basic knowledge into the vector population to help the health authorities make the right decisions in case of any mosquito-borne disease in the area.

## Materials and Methods

### Study area

This research was carried out in Nour Ali Village (31° 57' 17" N, 48° 55' 42"E, and 41M above sea level) within the central district in Shoushtar County, Khuzestan Province in the southwest of Iran (Fig. 1).

With 16 families, the village has 79 and an average of 4.9 persons per household. The village, situated along with one of the Karoun River branches called Gargar, is plain and flat. For several aquaculture farms in and around the village, this river supplies vital water, people farming crops, herds cows, sheep, goats, and ostrich.

### Adult sampling

The sampling of adult mosquitoes was conducted for 22 weeks period from May to October 2015. Mosquitoes were collected using three types of net traps: first, a big standing rectangle is 2m by 1.5m by 1.5m as a human baited (a volunteer female in her fifties, who weighs 65kg) net trap. A steel frame surrounded an open area with a mosquito net all around.

Second, a calf baited (four months with an average of 60kg calf) net trap (3m by 3m by

3m). The calf was held in the center of the net trap inside a steel frame. The third was the unbaited net trap (2 by one by 1.6m). Our experiment's unbaited net trap was used to describe and relate the findings to only a human and a calf to attract the mosquitoes. The netting sides were raised approximately 30 centimeters above the ground to enable host-seeking mosquitoes to enter the net trap inside (33). Traps were placed at the end of the day and stayed in position until dawn. The next morning, before entering the net trap to catch mosquitoes trapped overnight, the net trap sides were lowered to the floor. Mosquitoes were captured by an aspirator from the internal walls and roof of the net trap and placed into labeled paper cups for later identification (33).

### Field identification of mosquitoes

All female mosquitoes were sorted. For every night trapping, if sufficient samples were available, 20 specimens were randomly chosen and identified from each baited net trap and five specimens from unbaited net trap to species level according to the local keys available (34, 35).

### Statistics

The collected data during 22 weeks from all types of net traps were considered for analysis. The collected data were entered in Ms-Excel 2010 version. The calculations were done using the SPSS version 25.00. The means of

collected mosquitoes in the net traps were compared using a T-test.

## Results

Of the total collected female mosquitoes, 9 percent were trapped by human baited, 89.1 percent by calf baited and 1.9 percent by unbaited net trap (Fig. 2).

This research showed that statistically, lesser Culicid mosquitoes ( $25.73 \pm 6.57$ ) were attracted by Unbaited net trap relative to human baited net trap ( $122.36 \pm 39.44$ ),  $t(22.16) = 2.42$ ,  $p < 0.05$ . This analysis showed that the calf baited net trap was statistically stronger at attracting mosquitoes ( $1207.41 \pm 264.46$ ) than the Unbaited baited net ( $25.73 \pm 6.57$ ) ( $t(21.03) = 4.47$ ,  $p < 0.05$ ). This analysis showed that human baited net trap, relative to calf baited net trap ( $1207.41 \pm 264.46$ ),  $t(21.93) = 4.05$ ,  $p < 0.05$ , attracted fewer Culicid mosquitoes ( $122.36 \pm 39.44$ ) statistically (Fig. 2).

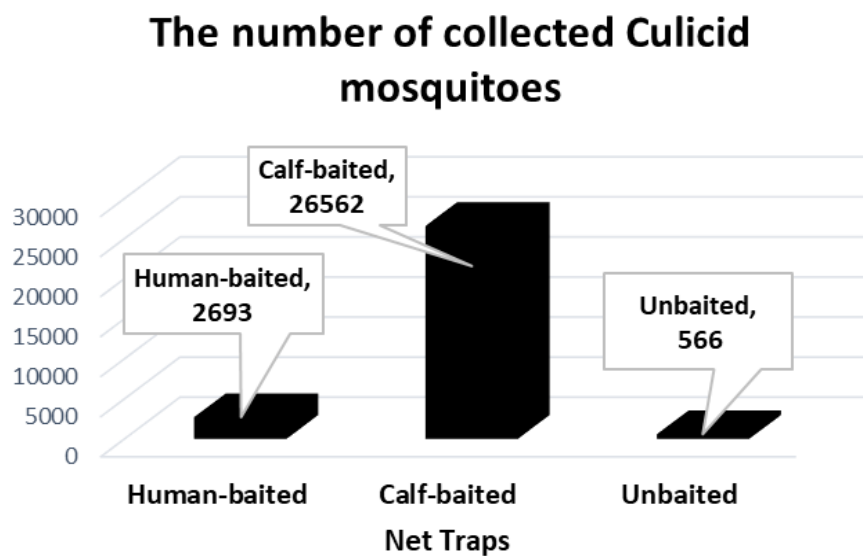
Of the total 916 identified female mosquitoes, they were 42.3% ( $n = 388$ ) from human baited net trap, 47.3% ( $n = 433$ ) from calf baited net trap and 10.4% ( $n = 95$ ) from unbaited net trap. The results of the species identification showed out of 916 identified female mosquitoes were 6.9% ( $n = 63$ ) *Anopheles stephensi*, 9.1% ( $n = 83$ ) *An. pulcherrimus*, 12.9% ( $n = 118$ ) *Aedes caspius*, 53.8% ( $n = 493$ ) *Culex tritaeniorhynchus*, 16.7% ( $n = 153$ ) *Cx. quinquefasciatus*, and 0.6% ( $n = 6$ ) *Cx. Theileri*.

**Table 1.** The mosquito's comparison in the trap with human bait against the trap containing a calf baited in Iran's southwest in 2015

Species	Mean±Std. Deviation calf baited	Mean ±Std. Deviation human baited	F	P
<i>Ae. caspius</i>	81(3.68 ± 4.05)	28(1.27 ± 1.42)	6.95	0.01
<i>Cx. tritaeniorhynchus</i>	180(8.18 ± 3.96)	258(11.73 ± 5.17)	8.03	0.00
<i>Cx. quinquefasciatus</i>	72(3.27 ± 2.51)	68(3.09 ± 2.38)	0.06	0.80
<i>Cx. theileri</i>	3(0.14 ± 0.46)	2(0.09 ± 0.29)	0.16	0.68
<i>An. stephensi</i>	47(2.14 ± 2.94)	10(0.45 ± 0.80)	7.47	0.00
<i>An. pulcherrimus</i>	50(2.27 ± 2.51)	22(1.00 ± 1.41)	5.17	0.02



**Fig. 1.** The area of this research is Shoushtar County (Khuzestan Province) in Iran's southwest 2015



**Fig. 2.** The comparison of human baited, calf baited and an unbaited net trap to collect mosquitoes in 22 weeks from May to October 2015 in southwestern Iran

## Discussion

The factors that control the distribution of diseases transmitted by vectors are still poorly known in many areas. The arrival of humans into new areas as part of their migration pattern increases exposure to vector and pathogen vectors. Determining the significance of mosquitoes as disease vectors requires knowledge of their biology and ecology (36).

This research's observations have shown that collected mosquitoes were *An. stephensi*, *An. pulcherrimus*, *Ae. caspius*, *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus* and *Cx. theileri*. *Anopheles stephensi* was believed to be the primary malaria vector in the south (37, 38), in similar ways, it was found that blood-feeding females stayed substantially in animal shel-

ters following blood-feeding (17). *Anopheles pulcherrimus* was recorded as a potential malaria vector in southeastern Iran (14). The primary Rift Valley fever vector in Saudi Arabia has been identified as *Cx. tritaeniorhynchus*, despite positive reports in Iran, potential vectors were not established (15, 39-40). Nevertheless, the ability to transmit the vector-borne diseases regarding these species' blood-feeding is considered nuisance and annoyance pests for inhabitants in the area. The findings of this analysis revealed that *Cx. tritaeniorhynchus* from Culicinae and *An. pulcherrimus* from Anophelinae had the most significant number of specimens among the collected samples (Table 1). While *Cx. theileri* was scarce in the traps. Due to the abundance of aquaculture farm-related breeding sites in the region, variations between the densities of collected species will occur due to some species' favor to these locations than others. Also, the difference in the attractiveness of the hosts for different species may cause different collection densities. The propensity of mosquitoes to have a particular host relies on genetic variations between their various populations. However, regardless of genetic traits, it depends on the environmental conditions, the hosts' presence, density and the interaction between the vector and the disease microorganisms (41). A strong positive relationship ( $p < 0.05$ ) was statistically demonstrated between the collected mosquitoes' densities in the net traps. The differences between the hosts' attractiveness in the current study may refer to the number of odors of different hosts and the olfaction, making the mosquitoes search for their hosts (42). The collected mosquitoes' significant results ( $1207.41 \pm 264.46$ ) were observed in the calf baited net trap against the human baited and unbaited net trap. The higher mean of mosquito density showed significantly in the human baited net trap ( $122.36 \pm 39.44$ ) compared to the unbaited net trap ( $25.73 \pm 6.57$ ). The baited traps' host attraction has been verified by the lowest number of trapped mosquitoes without bait in the trap ( $p < 0.05$ ). Factors

affecting the host preference for hematophagy include accessibility of hosts, their protective actions, and the suitability composites of their blood (43). Our data indicate that the calf body's relatively large size compared to the exposed human body parts can lead to a calf releasing higher carbon dioxide, attracting more hungry mosquitoes (44–46). As shown in Table 1, our results indicate that *An. pulcherrimus* and *An. stephensi* were particularly capable of feeding on the calf. This result was close to previous studies (25, 44). However, only the small number of specimens described may be biased as related. The *Culex* spp reported were *Cx. quinquefasciatus*, *Cx. theileri* and *Cx. tritaeniorhynchus*, which are considered effective vectors ((15). Many *Culex* mosquitoes have been affiliated with human populations that prepare an optimal environment for mosquito larvae, such as standing water (47). Our studies found no statistically important change in the attraction of *Cx. quinquefasciatus* and *Cx. theileri* to the calf or human. These results agree that previous studies that reported *Cx. quinquefasciatus* have different preferences, from 100 percent on humans to a high bird-likeness degree (41, 48). The vast range of hosts that *Cx. quinquefasciatus* can feed blood under many distinct conditions seems to have chosen the most prevalent host type (41). Our observation concerning *Cx. theileri* did not follow previous studies that considered much of animals' blood meals than humans (49, 50). In contrast (Table 1), *Cx. tritaeniorhynchus* was markedly attracted to humans than the calf. Previous experiments have proven this species to be zoophilic, although only a limited number of species reported here could be biased (51). *Aedes caspius*, as a potential vector of some vector-borne diseases (15), was drawn significantly to the calf. More mosquito studies are recommended to better understand and evaluate mosquito tendencies in the field, as a subset of collected mosquitoes has been identified. As Figure 2 shows, keeping a calf has been shown to attract ten times as many mos-

quitoes as we would expect the man to have. This protection can improve when implemented as an integrated control measure. Nevertheless, some reports have demonstrated that, when animals are kept near humans, it could lead to the person being attacked by mosquitoes (32, 52). Therefore, the number of zoophilic mosquitoes that can transmit mosquito vector-borne diseases to humans is increased by keeping an animal within a human settlement in rural areas (53). However, maintaining the different sheds outside the human residences helps eliminate the area's associated vector-borne disease (31, 53). Consequently, local studies are critical before the control method is applied. Some key factors, such as species variety of mosquitoes, the assortment of hosts, distance between animal shelters and residential areas, socio-economic factors, and host preferences, should be considered (32, 54).

## Conclusion

The obtained results here provide useful insights into the mosquito population regarding keeping animals in the right and a safe distance from their dwellings in the area and the possibility of using integrated vector management in case of the reemergence of malaria or other transmitted diseases by vectors in the southwest of Iran.

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