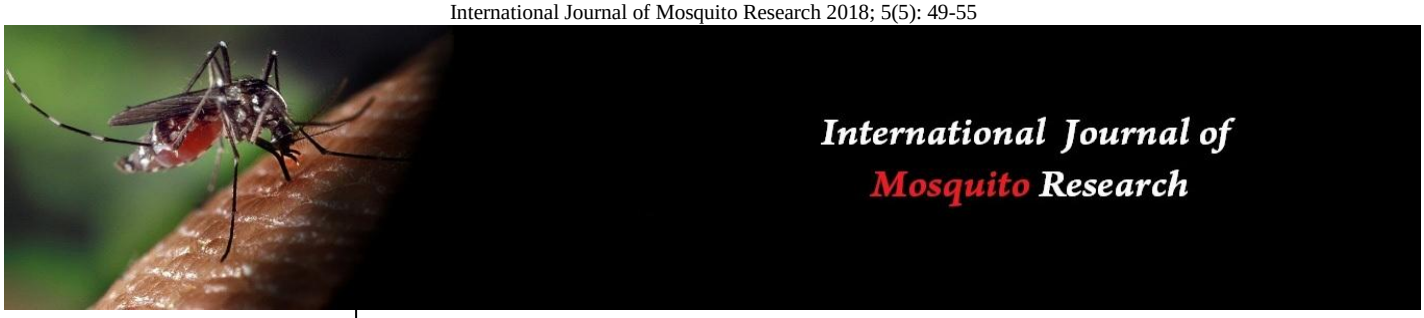

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Vectorial role of *An. dthali* (Diptera: Culicidae) as a malaria vector in Jazan region / Kingdom of Saudi Arabia

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Abstract
Jazan region is known to be the most malarious region in Saudi Arabia accounting for an average of >50% of all local malaria cases recorded in the country. *Anopheles dthali* Patton (Diptera: Culicidae) is suspected to be a secondary vector of malaria in Jazan region. The aim of this study was to investigate the role of *A. dthali* as a secondary malaria vector in the region**.** A cross sectional study was conducted in the Harob and Eledabi malarious areas from Oct. 2014 – Sept. 2015. Adults collected from the study area were subjected to investigation about their vectorial capacity (VC) and other entomological parameters. Polymerase Chain Reaction (PCR) technique was used to determine the sporozoite rate (SR) and blood meal source (BMS)**.** *A. dthali* was found to be the most predominant Anopheline species in Jazan region followed by *A. arabiensis* and *A. pretoriensis. A. multicolor* was collected only as larvae in the study areas. Laboratory analysis of adult *A. dthali* for other entomological parameters showed the following findings for Harob and Eledabi, respectively; the anthropophagic index (AI) was 11% and 15%, SR 0.01 and 0.02, entomological inoculation rate (EIR) 0.73 and 1.8, low VC of 0.0001 and 0.0004.

Keywords: A. *dthali, Malaria vector,* Vectorial capacity, Jazan, KSA

1. Introduction
Malaria is one of the important endemic diseases in the South-western KSA and is one of the country's foremost health problems. Jazan region is known to be the most malarious region in KSA, accounting an average of >50% of all locally malaria cases recorded in KSA [1].
The following species of *Anopheles* mosquitoes recorded as primary malaria vectors in KSA: *An. gambiae s. l* (Southern part), *A. sergentii* (Western region), *A. stephensi* (Eastern region) and *A. superpictus* (Northern region) [2, 3]. In addition to the four mentioned species, the following *Anopheles* have been reported in the country: *A. cinereus* Illiger (Abha, Asir Regions), *A. dthali* (Western and Madinah region), *A. fluviatilis* James (Eastern Region), *A. multicolor* Cambouliu (Eastern, Western and Madinah Regions), *A. pharoensis* Theobald (Tabuk region), *A. pretoriensis* Theobald (Jazan Region), *A. pulcherrimus* Theobald (Eastern Region), *A. rhodesiensis rupicola* Lewis (Yanbu in Madinah Regions), *A. turkhudi* Theobald (Western and Madinah regions), *A. coustani* Laveran and *A. tenebrosus* Donitz (Eastern Region) [2, 4, 5]. None of these species have yet been incriminated in malaria transmission in KSA [5].
Previous studies only stated the importance of *A. gambiae s.l.* as a potential malaria vector in the Tihama [3-6]. Also recent studies revealed that *A. arabiensis* is the most important malaria vector in this region [1].
Studies of *A. dthali* showed that a number of *A. dthali* have been incriminated as secondary Malaria vector from the Southern part of Iran [7]. Also in Iran gland infection of *A. dthali* was reported [8, 9], this species was repeatedly found infected during 1965-1967. In north of Somali dissection of 14 *A. dthali* showed only one mosquito has sporozoites in the salivary gland [10]. The precipitin tests in *A. dthali* of specimens from Morocco and KSA showed 4 - 18.7% were positive for human blood [11], this species has been suspected as a vector of malaria in KSA [7, 12]. The aim of this study was to assess the incrimination and vectorial capacity (VC) of *A. Dthali* as a Malaria vector in Jazan region-KSA.

2. Materials and Methods

2.1 Study Area

Jazan area is about 11,670 km^2 located in south-western part of KSA with a coastal boundary 250 km along the Red Sea and a 120 km border with the Republic of Yemen [13] (Fig.1). The study was carried out in two different locations in Jazan Region, *viz.* Harob and Eledabi. These areas have different altitudes and geographical characteristics. In these sites, the population lives mostly in villages widely scattered in the plain and foothill areas. Nearly all the villages in the plain are situated along valleys and rarely lie further than 1km from the valleys [1].

Fig 1: Jazan region of Saudi Arabia

2.2 Adult collection

A. dthali specimens were collected from indoor human dwellings of 26 villages distributed in two Malaria Control Stations (Eledabi and Harob) from Oct. 2014 to Sept. 2015. The direct catch of mosquitoes using human baits is not generally recommended, because of ethical concerns over the exposure of collectors to malaria and other viral infection. In this case, the collection of specimens was performed using Pyrethroid Spray Catches (PSC) as described by WHO [14]. The PSC collection was done every week between 6.30 and 10.00 a.m. from all stations. The sample of house coverage was about 25 rooms weekly, and the total coverage of the rooms (about 100/month) was rotated every month. Observations about the home, the number of occupants during the night before collection, temperature and relative humidity (R.H. %), were recorded. Collected mosquitoes were brought to the National Center for Vector-Borne Diseases (VBDs) in Jazan for morphological identification, sporozoite rate (SR) and determination of host -preference.

2.3 Dissection of Adult Females Mosquitoes

Adult females ovarian dissection was done according to that described in the Entomological Laboratory Techniques for Malaria Control [15], while parity rate (PR) was similar to those described in the Manual of Practical Entomology in Malaria [16].

2.4 Identification Blood–Meal and Detection of the sporozoite

The collected blood–fed *A. dthali* was taken to determine the source of their blood meals. After removing the legs, wings, thorax and head, the abdomens were preserved individually in 1.5 ml plastic tube, labeled, capped, and stored at -86 until further investigation to determine blood meal and sporozoite using the PCR technique. Head and thorax of nulliparous females do not need to be tested for detection of sporozoite, because they are not infected.

2.5 Estimation of Vectorial capacity (VC) for *A. dthali*

The VC is defined as the "daily rate at which future inoculations arise from a currently infective case".

It is directly related to the:

- number of bites / person / day (or man-biting rate, Ma)
- feeding habits (anthropohilic *vs*. zoophilic)
- life expectancy of the mosquito
- VC is expressed by the following formula [17,18]:-

$$VC = \frac{ma^2 p^n}{-\log_e p}$$

Where

VC = Vectorial Capacity, Ma = man- biting rate, A = man-biting habit, p^n = probability of vector survival through the sporogonic period of parasite, n = sporogonic period of parasite.

To estimate longevity of females *A. dthali*, in particular, the survival rate (probability of mathematical expression of the likelihood of female Anophelines remaining alive for a specified period), Detinova method was used [19]. From the formula $p^n = M$, the daily survival rate was computed, where n = duration of gonotraphic cycle; M = proportion of parous

females; and, p = daily survival rate.
From the known value of n and M, the survival rate was calculated using $p = \sqrt[n]{M}$

2.6 Statistical Analysis
The statistical analysis was carried out using SPSS v.21.

3. Results
A total of 1,384 adult female of *A. dthali* mosquitoes were collected from the two study areas throughout the study period. The mean *A. dthali* population density was 1.9 mosquito/house. *A. dthali* mosquitoes caught from Eledabi area accounted for 731 (52.8%) and from Harob the total collected was 653 (47.2%) (Tables 1).

Table 1: Abundance of adult *A. dthali* mosquitoes in Eledabi and Harob (Oct. 2014-Sept. 2015)

Station / Month	*Adult of A. dthali* No. (%)*		Total
	Eledabi	Harob	
Oct. 2014	98 (13.41)	9(15.16)	197 (14.23)
Nov.	97(13.27)	80 (12.25)	177 (12.79)
Dec.	83 (11.35)	63 (9.65)	146 (10.47)
Jan. 2015	73 (9.99)	60 (9.19)	133 (9.6)
Feb.	55 (7.52)	44 (6.74)	99 (7.15)
Mar.	41 (5.61)	37 (5.67)	78 (5.64)
Apr.	13 (1.78)	16 (2.45)	29 (2.11)
May	18 (2.46)	9 (1.38)	27 (1.95)
June	23 (3.15)	17 (2.60)	40 (2.89)
July	66 (9.03)	56 (8.58)	122 (8.82)
Aug.	74 (10.12)	77 (11.79)	151 (10.91)
Sept.	90 (12.31)	95 (14.55)	185 (13.37)
Total	731	653	1,384
Mean	60.9	54.4	115.3
SE±	8.9	8.8	17.5
C.V.%	50.4	56.1	52.6

3.1 Resting Preference of *A. dthali*
The outdoor collection was significantly higher than the indoor collection with a noticeable preference to animal shelter ($P < 0.01$). *A. dthali* in the two study areas was highly exophilic with some degree of tendency to being endophilic. The highest number was found resting outdoors in animal shelters and animal's food storage room (Fig. 2)

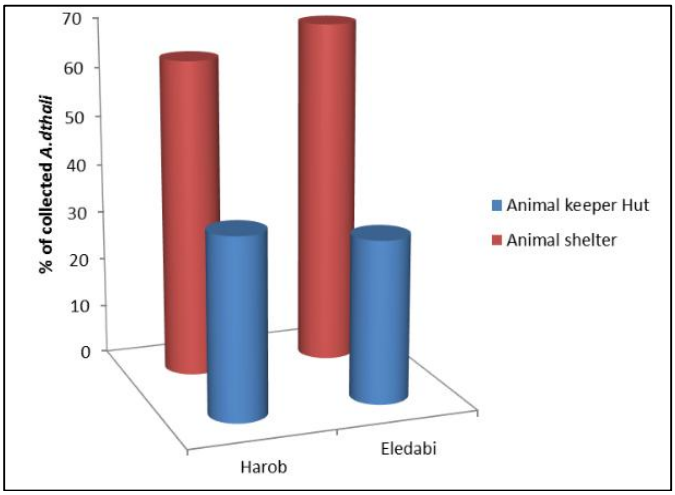

Fig 2: Resting habit of *A. dthali* in Harob and Eledabi

3.2 Examination of the Abdominal State
Percentage of fed *A. dthali* collected from Harob and Eledabi was 47.5% (657 adults) in the two areas; the mean was 18.4 and 19.9, respectively (Table 2). On other hand, the total number of unfed (UF) mosquitoes collected from the two stations was 460 adults (33.2%); the mean was 25.3 and 29.4 at Harob and Eledabi, respectively. There was highly significant difference ($p < 0.001$) between fed (F) and unfed (UF) mosquitoes in the two study areas. While, no significant differences in the abdominal status across the two study areas were found (P values of UF, FF, HG and G were 0.751, 0.623, 0.977 and 0.409, respectively).

Table 2: Gonotrophic cycle of *A. dthali* collected from Harob and Eledabi area (Oct. 2014-Sept. 2015)

Status / Area	U	F	HG	G	Total
Harob	221(33.8%)	304(46.6%)	77(11.8%)	51(7.8%)	653
Eledabi	239(32.7%)	353(48.3%)	74(10.1%)	65(8.9%)	731
Total	460 (33.2%)	657(47.5%)	151(10.9%)	116(8.4)	1384

3.3 Feeding Preferences of the *A. dthali* Mosquitoes in Jazan
Results of the PCR on blood meal analysis as shown on table (3) indicate that 147 (73.5%) of *A. dthali* were positive for animal (sheep) blood and only 26 (13%) of had taken human blood (Plate 1). The Human Blood Index (HBI) was 0.13 indicating low anthropophilic behaviors.

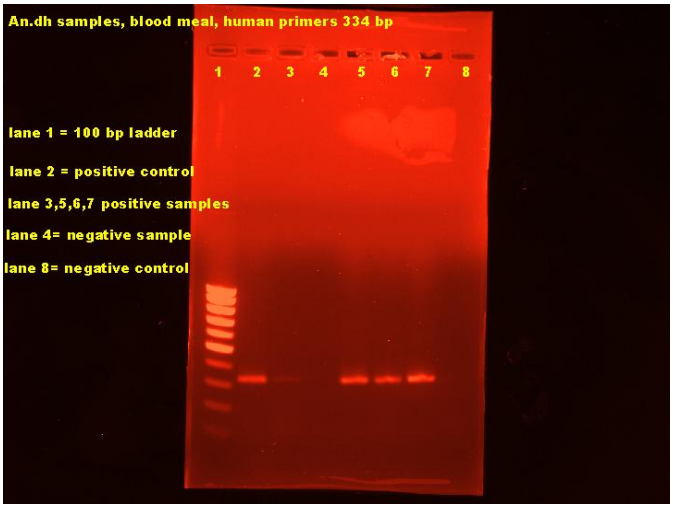

Plate 1: A typical example of a gel with positive samples for the human blood in *A. dthali*

Lane 1 represents the 100bp Ladder, lane 2 positive control, lanes 8 negative controls, lanes 3, 5, 6 and 7 samples were identified as positive for human blood

Table 3: PCR identification of feeding preference (Blood meal) of *A. dthali* collected from Harob and Eledabi areas, Jazan Region, KSA.

Area	Total examined	Human blood	Animal (Sheep blood)	Mixed	HBI
Harob	100	11(11%)	74(74%)	15(15%)	0.11
Eledabi	100	15(15%)	73(73%)	12(12%)	0.15
Total	200	26 (13%)	147(73.5%)	27(13.5%)	0.13

3.4 PCR Test for Detection of Plasmodium (Sporozoite) Species and EIR

Plasmodium species in the infected mosquito was determined using the PCR method. Out of 300 randomly selected *A. dthali* mosquitoes, only four (1.3%) were positive for *P. falciparum* sporozoite; three from Eledabi (SR = 0.02) and one from Harob (SR = 0.01). The results are shown in table (4) and plate (2).

Table 4: Detection of Sporozoite and annual EIR in *A. dthali* collected from Eledabi and Harob area of Jazan Region, KSA.

Station	No. examined	No. (+) (Sporozoite)	SR	Annual EIR
Eledabi	150	3	0.02	1.8
Harob	150	1	0.01	0.7
Total	300	4	0.013	1.1

SR=Sporozoite rate. EIR = Entomological Inoculation Rate

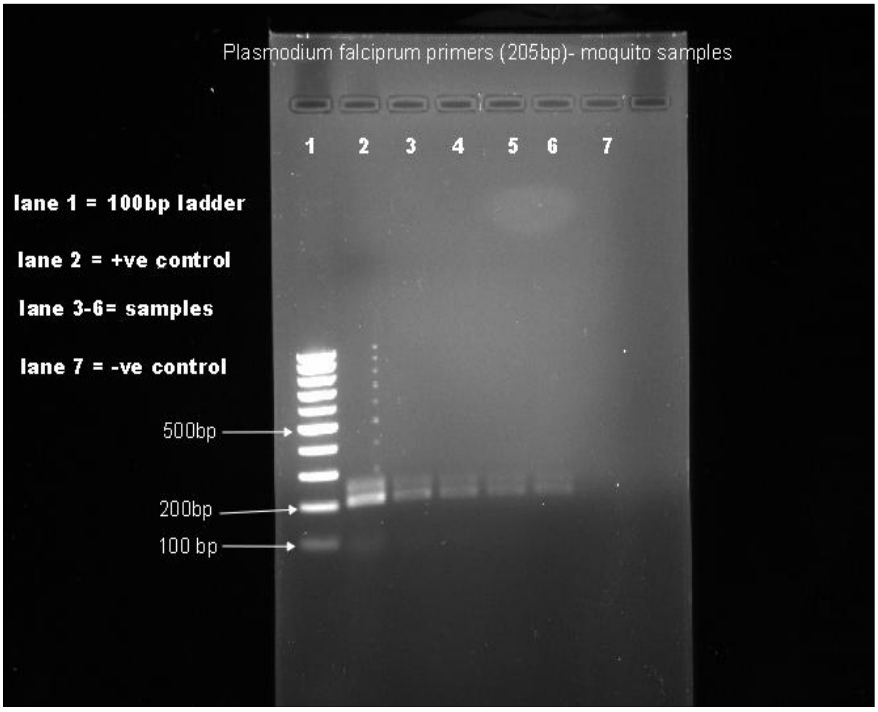

Plate 2: Stained agarose gel for the detection of sporozoite in *A. dthali*.

Lane 1 represents the 100bp Ladder, lane 2 positive control, lanes 7 negative controls, lanes 3, 4, 5, and 6 samples were identified as *plasmodium falciparum sporozoite*

3.5 Vectorial Capacity Parameters of *A. dthali*

The result of ovarian dissections of *A. dthali* in Eledabi and Harob station was as follows: Eledabi (82/250) = 0.328, and Harob (68/250) = 0.272. Given an interval of 2 days between blood meals, the probability of surviving one day (denoted as ***p***) can be estimated as:

$$p = \sqrt{\text{Proportion parous}}$$

Thus, $p = \sqrt{0.328} = 0.573$ in Eledabi and $\sqrt{0.272} = 0.522$ in Harob.

As p is the probability of surviving one day, p^n is the probability of surviving n days (At an average daily temperature of 27° C, it would take about 10 days for *P. falciparum* to complete the sporogonic cycle in the vector) so the probability of *P. falciparum* that can be transmitted by *A. dthali* in Harob = 0.522^{10} (0.002) and in Eledabi= 0.573^{10} (0.004). VC was found as 0.0004 and 0.0001 in Eledabi and Harob, respectively.

4. Discussion

Entomological investigation of Adult Anopheline in the two study areas revealed the presence of three Anopheline species; *A. dthali* (62.2%), *A. arabiensis* (32.2%) and *An. pretoriensis* (5.6%). This result is relevant to the finding of Alsheikh[1] who reported the presence of the adult *A. dthali* and *A. pretoriensis* in the Jazan region. Results indicated that *A. dthali* is the predominant species in the two study areas where it comprised 63.9% and 60.4% of the total adult Anopheline mosquitoes collected from Eledabi and Harob, respectively. *A. dthali* spread in plain and mountain areas of the two study areas throughout the year and peaked in Oct.

A. dthali is widespread in semi-arid parts of the WHO Eastern Mediterranean region. It is found in north Ethiopia and Somalia, Socotra, north Africa to north west of Pakistan, Southwest of Saudi Arabia, around the Red sea, Adan Gulf [20-23] in Iran [9], Eritrea [24] and Sudan [25].

The percentage of the fed *A. dthali* females collected from the two study areas was higher (47.5%) than unfed ones (33.2%), which is mainly due to the availability of the host both indoor and outdoor without any protecting barriers**.** It has been reported that the regular use of IBN which acts as a biting barrier was an effective means of reducing mosquito bite on sleeping persons [26, 27].

The proportion of gravid female to the fed female (116/657) in the two study areas was 1:5.7 (>1/5th of the fed females). This may be due to the fact that the female avoiding mechanism for the impact of indoor residual spraying (IRS) by changing their behavior to outdoor feeding (exophagy) and

outdoor resting (exophily) and /or due to the killing effect of IRS and space spraying targeting the resting places of mosquitoes. This result agree with the findings of Russell [28], Reddy [29], and Bugoro [30].

Under natural circumstances where the majority of the hosts (Human and animal) is domestics and kept in or near human dwellings at night, a high proportion of *A. dthali* females feed and remains there to rest for whole gonotraphic period [9].

In this study, outdoor collection was significantly higher than the indoor collection with a noticeable preference to animal shelter ($P<0.01$). These results indicate that the *A. dthali* was highly exophilic *and* zoophilic. It prefers to feed outdoor on animal and human (animal keepers used to sleep out their hut near the sheep shelter) thus mosquitoes feeding behavior may be governed by human sleeping behavior. This finding is in line with Service [31] who stated that the behavior of both people and mosquitoes is relevant in malaria transmission. It was reported that in southern Arabia, Mauritania and Somalia the adults are not uncommon indoors [23].

Although *A. dthali* adults in the two study areas were highly exophilic, there was some degree of tendency to being endophilic. This phenomenon of the *A. dthali* in the two study areas exhibited the highest number of mosquitoes resting outdoors in animal shelters and animal's food store room. This finding is in agreement with the work of Manouchehri and Rohani [9] who found *A. dthali* to be more prevalent in animal shelters, tent and human dwellings. No mosquitoes were collected from the moderate building with Freon air conditioners.

The identification of the blood meal source of freshly fed female mosquitoes remains important to understand their host-preference and vectorial role [16]. Many female *Anopheles* mosquitoes bite humans to obtain a blood meal, and a few feed on humans in preference to animals [32, 33]. According to Takken and Verhulst [34], host-preference is defined as the trait to preferentially select certain host species above others. This selective behavior has a great influence on disease transmission.

Results of the PCR for identification of the *A. dthali* blood meal sources indicated that 73.5% had taken sheep blood (high zoophilic) and the human blood meal was only 13% (Low anthropophilic behavior). The mixed meal (human and sheep blood) was 13.5% of the tested *A. dthali*.

The human blood index (HBI) in Harob and Eledabi is 11% and 15%, respectively. This result showed a low anthropophilic behavior of *A. dthali* in the two study areas, a result which agreed with the findings of Bruce-Chwatt [11] who reported 4 - 18.7% HBI of *A. dthali* from Morocco and Saudi Arabia. The results were also relevant with those reported from Iran; for instance Edrissian [35] reported 12.5% HBI in south of Iran and Manouchehri [8] reported 20.8% HBI from north Bandar Abbas. This result confirmed that the host-preference in the two study areas depends not only on the innate host-preference of the mosquito species, but also is determined by other factors including; the human outdoor sleeping behavior in the vicinity to the sheep shelters, favorable climatic conditions in the two study areas, and vector control interventions. This result was in agreement with the work of Takken and Verhulst [34] and Smallegange [36], who stated that host-preference resulting from selective behaviour may be attributed to extrinsic and intrinsic factors.

The results showed that the annual Entomological Inoculation Rate (EIR) was higher in Eledabi (1.8 ib/p/yr) by 2.6 folds than in Harob (0.7 ib/p/yr). The calculation of the annual EIR in the two study areas was 1.1. It is worthy to mention that only the annual EIR of less than one could reduce parasite rates to a level that could interrupt malaria transmission [37]. Based on that, it can be speculated that Eledabi area is more at risk of malaria transmission than do Harob area. This necessitates the prompt preventive measures by the public health authorities in Eledabi to avoid high malaria transmission and hyper-endemicity in the area.

The tracheation method distinguishes between nulliparous (tightly coiled tracheols) and parous (stretched tracheols)[38], which is relatively faster and easier was used in this study. The proportion of parous mosquito was expressed as noted by MacDonald [17].

In Eledabi, the proportion of parous *A. dthali* females was found as 1.2 fold more than in Harob, and the probability of the survival in Eledabi was found double that of Harob area. One of the entomological determinants in the transmission of malaria in an area is the age status of individual females in the population of the vector. Age also indicates the efficacy level of VC interventions in an area [39].

The results of the sporozoite rate (SR) showed that only four mosquitoes were found infected with sporozoite (3 from Eledabi and only one from Harob) giving SR of 0.02 and 0.01 in Eledabi and Harob, respectively. The potentiality of *A. dthali* to complete the extrinsic cycle of malaria parasite was confirmed by several studies, *i.e.* in Somalia [10], it was found one specimen of *A. dthali* with sporozoite-positive glands among 14 dissected females. In Bander Abbas reported two positive females of *A. dthali* to sporozoites infection [40]. *A. dthali* was repeatedly found infected during 1965-1967, with SR reported in three different areas as 1%, 2.1% and 7.7% [8, 9]. Also one specimen with sprozoite-positive was reported in each of Anseba and Gash-Barka (SR = 0.45) areas of Eretria [24, 41].

This is the first study done on the vectorial capacity of *A. dthali* in Saudi Arabia as well as over the entire world. The previous studies were concentrated on the presence of the sporozoite in the salivary gland of the *A. dthali*. The vectorial capacity for *A. dthali* in this study was found to be 0.0004 and 0.0001 for Eledabi and Harob, respectively. This low vectorial capacity of *A. dthali* in the two study areas could be attributed to the extensive VC activities using IRS and ITNs as recommended by the WHO.

Considering the paucity of work ever done to determine the vectorial capacity of anopheles species in the world, especially *A. dthali*, these findings could serve as a reference point for any further researches.

5. Conclusion

A. dthali was found to be the most predominant Anopheline species in the two study areas. This species proved to be a secondary vector of malaria for the first time in Jazan region and the KSA. Thus, the incriminating of the *A. dthali* as a secondary vector of malaria, along with its high density in vast areas of the region would create a burden to the health authorities and necessitate the periodical surveillance of the *A. dthali* and prompted preventive and control measures. The vectorial capacity was found to be very low in the two study

areas, nonetheless, and considering the paucity of work done to determine the vectorial capacity of *Anopheles* species in the world, this finding could serve as a reference for the future researches.

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