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Original article

Effects of blood meal sources on the biological characteristics of *Aedes aegypti* and *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

Mosquitoes transmit many diseases to humans and animals e.g., malaria, yellow fever, dengue, filariasis and encephalitis. The fundamental target of this search was to study the effect of three different blood meal sources (human; rabbit and pigeon) on some biological and behavioral properties of *Aedes aegypti* and *Culex pipiens*. The results have assured that the females of the mosquito *Ae. aegypti* that were fed on human blood meal has registered the highest feeding activity from feeding on the blood meal whereas the females of the other mosquito *Cx. pipiens* have shown the highest feeding activity after being fed on pigeons when compared with its feeding on other factors. The results have shown non-significant variation in the average time necessary to digest the blood meal on both mosquito species *Ae. aegypti* and *Cx. pipiens* that were fed on vertebrate hosts under laboratory conditions. Furthermore, results assured that the difference in blood meal sources has yielded distinct variation in the reproductive capacity and efficiency of both female mosquitoes under investigation where both species *Ae. aegypti* and *Cx. pipiens* already fed on human blood meal have yielded a pronounced distinctive increase in egg production (oviposition) when compared with females that were fed on pigeon or rabbit blood meal respectively. Moreover, feeding of the female mosquitoes under lab conditions on different blood meal sources did not affect the level of the hatching eggs that were laid by both mosquito females.

1. Introduction

The female mosquitoes are classified as notorious, vicious and dangerous external parasites which feed on the blood meals of a wide range of vertebrate animals including mammals Phylum: Chordata, Class: Mammalia, birds (Class: Aves), reptiles (crawlers and creepers (Reptilia: Herpetology), amphibians (clade: Batrachomorpha) and fishes (phylum*:* Chordata, subphylum*:* Vertebrata, class: *Pisces*) where the blood meals are considered the basic meals to provide and supply the mosquito females with needed amounts of dietary proteins and amino acids necessary for the formation and ripening of eggs (Roiberg and Gordon, 2005; O'Meara, 2020). Moreover, the process and the mechanism of the response of mosquitoes to their respective hosts and the degree of attraction required three important stages, starting with the activation phase, then followed by the orientation phase, and finally finished by the alighting phase on their hosts (Takken and Verhulst, 2013; Lacey et al., 2014). Hence it is noteworthy to mention that the new trend is changing and adopting the new agricultural practices in Europe with the goals of improving the human rural comfortable housing and the establishment of separate animal holdings i.e., pens and barns for sheltering livestock and pigs separated away from the buildings of human habitation and residential quarters. This has led and contributed to the movement of the mosquito vectors that prefer feeding on human blood as the sole vectors active in the transmission of the causal agents of malaria to feeding on these domesticated animals (Sota and Mogi, 1989). Therefore this situation of course and assuredly has reduced both parameters in the degree of human nuisance by the notorious female mosquitoes and evidently the rate of the transmission of human diseases

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which is defined and described as animalism prophylaxis or scientifically termed *Zooprophylaxix* (Hassanala et al., 2008; Laurent et al., 2017). Furthermore, Takken and Verhulst (2013) have mentioned that the preference of female mosquito vectors to a specific host for continued feeding blood meals would definitely and without a doubt would affect the mosquitoes abilities in the transmission of these disease-causing agents. Their study has assured that the preference of mosquitoes to their respective specific animal hosts did primarily rest on a genetic basis, although it has a distinctive degree of flexibility on the choice of the host especially when the host presence has high density and its blood would be more palatable source for feeding. Moreover (Richards et al., 2012) conducted research on the effects of the diversity of blood meal sources from different vertebrate hosts on the rate of feeding and the reproductive capacity of the mosquito *Cx. quinquefasciatus. Their study has recorded an increase in the average* number of eggs laid by each female and an increase in the rate of egg hatch of the mosquito females that were fed on chicken blood meal when compared with those fed on the cow blood meal. Moreover, the research work conducted by (Phasomkusolsil et al., 2013) have recorded variation in the feeding rate and the reproductive efficiency of the mosquito *Ae. aegypti* and other four species that belong to the genus *Anopheles* when they were fed on different vertebrate hosts. And their study has assured the reduction in the rate of feeding on blood by *Aedes* and also by the *Anopheles* mosquitoes under investigation when they were fed on a sheep blood meal. However, on the other hand, their studies have shown the delay in some mosquito species *An. sawadwongporni* and *An. minimus* in the length of time that is required to digest the blood meal and the completion of egg laying and oviposition till the 7th day from the beginning of feeding on blood. Moreover, the other mosquito species *An. dirus* and *An. Cracens* gave a low number of eggs with an evident reduction in the rate of egg hatch as was noticed when the females were fed on blood meals from different animal sources. The study of different mosquito species and their feeding preference on different hosts could most probably determine the contact rates of the hosts that feed on them and the transmission of the causal disease organisms which is considered one of the goals and objectives of this current study.

Generally, studying the effect of different blood meal sources on feeding behavior and reproductive potential of mosquito vectors will undoubtedly be very helpful in understanding the relationship between mosquitoes as ectoparasites feed on blood and its ability to transmit the diseases. The main goal of this study was conducted to evaluate the evaluating the effect of different blood meal sources (human, rabbit, pigeon) on the same biological and behavioral aspects of two culicine mosquitoes *Ae. aegypti* and *Cx. pipiens*.

2. Materials and methods

Tests were performed on a field strain of *Aedes aegypti* and *Culex pipiens* raised from wild larvae, collected from Jeddah, Saudi Arabia, and had been maintained in the laboratory under controlled conditions of 27 ± 1 °C and 70 ± 5% R.H., with a 14:10 (L:D) photoperiod according to (Mahyoub, 2018).

2.1. Feeding activity and time required for laying eggs

The experiment was conducted on 300 adult females, 3–6 days old, distributed in six cages, where 150 females of *Ae. aegypti* were distributed in three cages, and also150 females of *Cx. pipiens* were distributed in three cages, where each cage contained 50 adult females. The females were starved for 12 h by withholding the sugar solution from them, after that they were fed three different blood sources using Hemotek Membrane Feeding System. Where the females of *Ae. aegypti* in the first cage were fed on human blood, while the second cage was fed on rabbit blood and the last cage on pigeon blood, the experiment was repeated on females of *Cx. pipiens*. After an hour, the feeding activity on the blood for both species was calculated.

On the other hand, 20 blood-fed females were withdrawn from each cage and distributed individually to small plastic cups covered from the top with a piece of transparent tissue and tight with a rubber band. At the center of the cup, there was an opened hole and it was blocked by a small piece of cotton containing a sugar solution of 10% concentration. In the case of *Cx. pipiens* females, the cup contains tap water up to its middle, to receive the egg mass, while *Ae. aegypti* females the cup contains water and it is lined internally with two filter papers to lay the eggs.

2.2. Study the reproductive female capacity

The study of mosquito females' reproductive capacity (the average number of eggs laid (oviposition) and the percentage of eggs hatch) using the method of (Saleh and Wright, 1990).

1200 larva at the beginning of their fourth age were used, with 600 larvae of each species. The larvae were distributed in six Petri dishes, three dishes for each species (200 larva / Petri dishes).

The larvae of both mosquito species were reared till the pupation stage then the pupae of each group were transferred separately inside the rearing cages that were supplied with 10% sugar solution and all pupae were left till the emergence of the mosquito adults. After 2–3 days post-emergence of adult insects, all females were in each of the (first, second and third) cages fed on three different sources of vertebrate host blood, which were (human blood; rabbit blood, and pigeon blood), respectively.

Then, 20 blood-fed females were taken from each cage and put with 20 males in small plastic cups that were covered from the top with a piece of transparent tissue and tight with a rubber band. At the center of the cup, there was an opened hole and it was blocked by a small piece of cotton containing a sugar solution of 10% concentration. In the case of *Cx. pipiens* females, the cup contains tap water up to its middle, to receive the egg mass, while *Ae. aegypti* females the cup contains water and it is lined internally with two filter papers to lay the eggs.

The total number of laid eggs by the females of both mosquito species *Cx. pipiens* and *Ae. aegypti* that were fed on blood meals from the different hosts were recorded. The percentage of eggs hatched was determined by calculating the average number of eggs for each female was, and the number of larvae resulting from hatching, then the results were statistically analyzed according to the equation given by Almadiy and Mohammed (2014; Prasadini et al.,2019) as follows:

$$\text{Blood feeding activity} = \frac{\text{No. of engored mosquito females}}{\text{Total female mosquitoes}} \times 100$$

3. Results

3.1. The feeding activity on the blood meal of both mosquito species Ae. aegypti and Cx. pipiens fed on different blood hosts

Table 1 showed the result obtained from feeding two species of mosquito *Ae. aegypti* and *Cx. pipiens* on blood meal of three different vertebrate hosts (human, rabbit and pigeon) and their feeding activity (Fig. 1). The results showed that *Ae. aegypti* females have recorded the highest activity percentage rate of 95% when fed on a human blood meal, followed by those fed on pigeon blood meal 82%, then by those fed on rabbit blood meal 56%. These results

Table 1
Blood feeding activity of *Cx. pipiens* and *Ae. aegypti* fed on three different vertebrate hosts.

Mosquito sp.	Blood meal source	No. of engorged mosquito females*	Blood feeding activity (%) Mean ± SE**
Ae. aegypti	Human	45	90 **± 0.5**a
	Rabbit	28	56 **±** 0.15c
	Pigeon	41	82 **±** 0.2b
	LSD = 8.9		**P = 0.0001**
Cx. pipiens	Human	35	70 **±** 0.25b
	Rabbit	24	48 **±** 0.16c
	Pigeon	46	92 **±** 0.3a
	LSD = 12.5		**P = 0.0001**

*50 unfed mosquito females were used.
**Means followed by the same letter are not significantly different according to LSD at $\alpha = 0.05$.

Table 2
The duration time needed for the digestion of blood meal by *Cx. pipiens* and *Ae. aegypti* fed on three different vertebrate hosts.

Mosquito sp.	Mosquito sp.	Blood meal digestion (in days)	
		Range	Mean* ± S.E
Ae. aegypti	Human	4–5	4.6 ± 0.68a
	Rabbit	4–7	4.9 ± 0.70a
	Pigeon	3–5	4.4 ± 0.81a
	LSD = 0.9		**P = 0.156**
Cx. pipiens	Human	4–6	4.5 ± 0.61a
	Rabbit	4–7	4.7 ± 0.52a
	Pigeon	4–5	4.3 ± 0.48a
	LSD = 1.5		**P = 0.966**

* Mean of 20 blood-fed mosquito females; differences between means were not signification.

assured that the activity rate of *Ae. aegypti* females by feeding on a human blood meal gave the highest activity feeding rate than they feeding on the other host's blood (pigeon and the rabbit) by approximately 8.9% and 37.8% respectively.

Furthermore, the study indicated the rate of feeding activity on the blood of different hosts for *Cx. pipiens* was different from that reported in *Ae. aegypti*. Where the results showed that *Cx. pipiens* females recorded the highest activity when feeding on pigeon blood by 92% compared to feeding on human and rabbit blood by 70% and 48% respectively. These results confirm the feeding activity of *Cx pipiens* females on pigeon's blood was higher than their activity rate for feeding on the other host blood such as humans and rabbits by about 23.9%, and 47.8%, respectively.

3.2. The period of time required for the digestion of a blood meal by both mosquito species Cx. pipiens and Ae. aegypti fed on different blood hosts

On the other hand, as shown in Table 2 & Fig. 2, the period of time required for digesting blood meal (the second feeding stage and oviposition "Gonotrophic cycle") for both mosquito females *Cx. pipiens* and *Ae. aegypti* after feeding on a blood meal from three vertebrate hosts (human, rabbits and pigeons), results showed that the duration time needed by Ae. *aegypti* females to digest human blood meal ranged between (4–5) days with an average of 4.6 days, however the average duration time for digestion the blood meal of rabbit and pigeon were 4.9 days (4–7 days) and 4.4 days (3–5 day) respectively.

On the other side, results showed variation in the duration of the time span necessary for the digestion of blood meal and the completion of egg-laying by *Cx. pipiens* females with difference blood sources. Results showed the difference in the range of the duration time needed for the digestion of human blood meal which ranged between (4–6) days with an average of 4.5, whereas, the duration time ranged between (4–7) and (4–5) days with average 4.7 and 4.3 when females were fed on a blood meal from rabbit and pigeons, respectively. The statistical analysis showed the difference between the averages of duration time for digestion of blood meals from different hosts for both mosquito species *Cx. pipiens* and *Ae. aegypti* was not significant.

3.3. The reproductive capacity (egg production "oviposition") and the level of egg hatch(hatchability) on both mosquito species Ae. aegypti and Cx. pipiens that were fed on different blood hosts

As a result, shown in Table 3 and Fig. 3 the egg production of both mosquito species *Cx. pipiens* and *Ae. aegypti* after feeding their females on three different vertebrates (human, rabbit and pigeon) was recorded during the first cycle of feeding and egg-laying, gen-

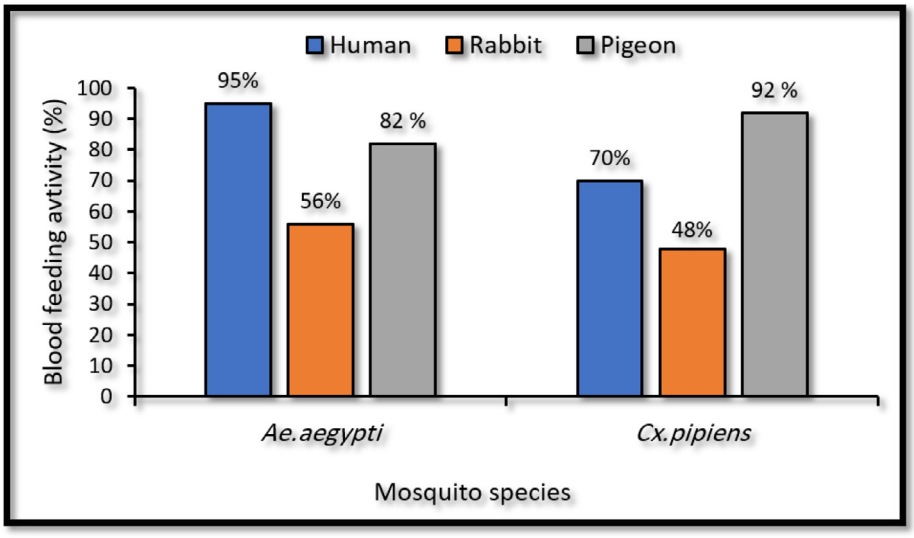

Fig. 1. The activity feeding on blood by females of *Ae. aegypti* and *Cx. pipiens* fed on three vertebrate hosts.

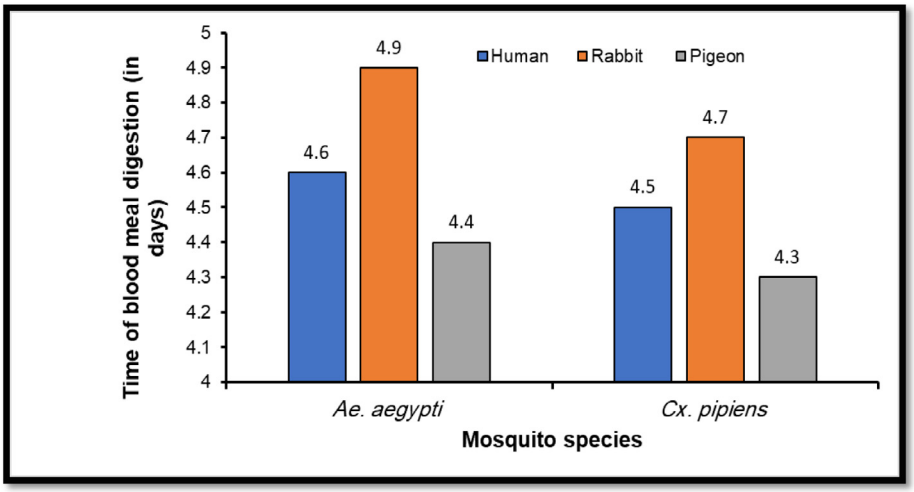

Fig. 2. The duration time needed for the digestion of blood meal by females of *Ae. aegypti* and *Cx. pipiens* fed on three different vertebrate hosts.

Table 3
Egg production of *Ae. egypti* and *Cx. pipiens* fed on blood meal of three different vertebrate hosts.

Mosquito sp.	Blood meal source	Total No. of eggs	No. of eggs/female	
			Range	(%) Mean ± SE*
Ae. aegypti	Human	1167	39–83	50.4a ± 2.7a
	Rabbit	664	22–48	33.2 ± 1.8b
	Pigeon	926	37–51	46.3 ± 3.6a
	LSD = 5.2			**P = 0.0001**
Cx. pipiens	Human	3225	113–188	161.3 ± 6.4a
	Rabbit	2043	68–117	102.2 ± 5.8c
	Pigeon	2921	112–162	146.1 ± 4.7b
	LSD = 19.4			**P = 0.0001**

* Mean of 20 engorged mosquito females; mean followed by the same liter are not significantly different (p = 0.05).

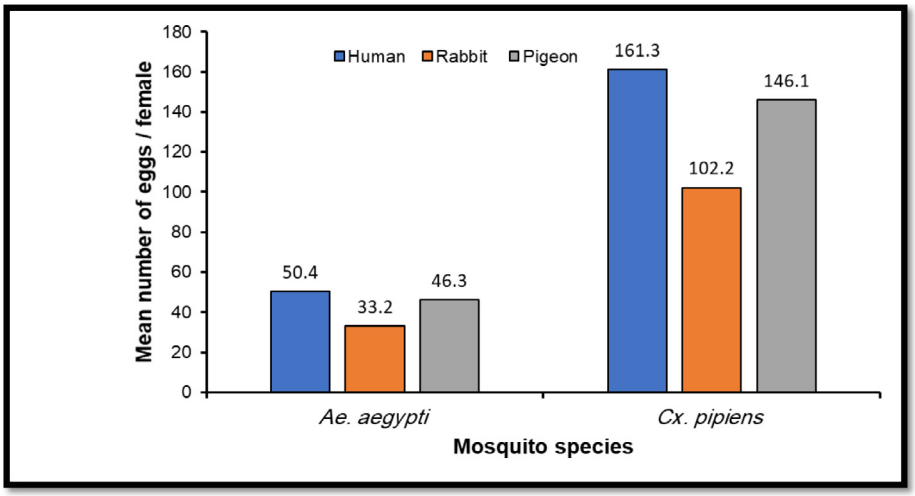

Fig. 3. The average number of egg production by females of *Ae. aegypti* and *Cx. pipiens* fed on three different vertebrate hosts.

erally, the result showed that feeding mosquito females on human blood meal have led to an increase in the number of laid eggs by females of both mosquito species when compared with feeding them with blood meals from other hosts.

Results showed the average number of eggs laid by females of *Ae. aegypti* that were fed on human blood was 50.4 eggs, whereas the average number of eggs/females which was fed on blood meals from rabbits and pigeons were 33.2 and 46.3 eggs, respectively. These results assured that feeding females of mosquito *Ae. aegypti* on human blood meal has increased an average number of egg production/female by rate 34.1% and 8.1% when compared with females that were fed on rabbit and pigeon respectively.

On the other hand, results showed an average number of eggs laid by females of *Cx. pipiens* that were fed on a blood meal from humans, rabbits and pigeons were 161.3; 102.2 and146.1 eggs, respectively.

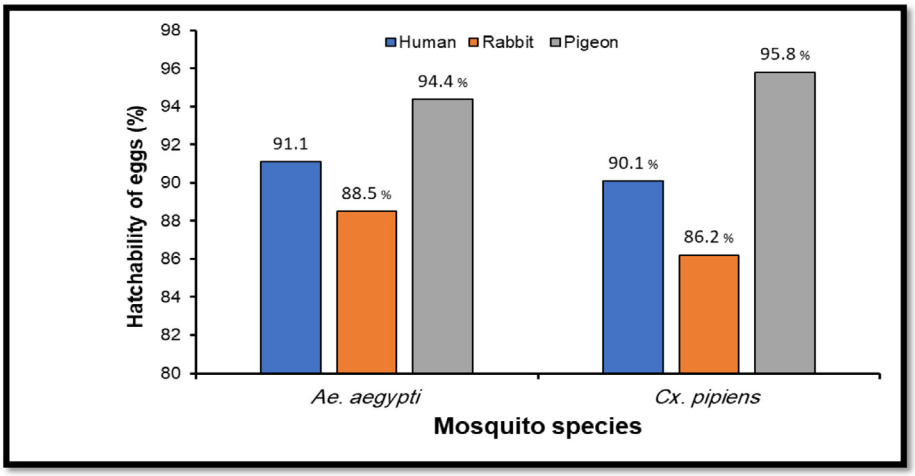

Fig. 4. The percentage of egg hatch of females of *Ae. aegypti* and *Cx. pipiens* fed on three vertebrate hosts.

These results also showed that feeding females of *Cx. pipiens* on human blood meal has led to an increase in the average number of eggs/females by approximately 36.6% when compared with female mosquitoes that were fed on rabbit blood meal and with an approximately 9.4% in case of females that were fed on a pigeon blood meal.

The statistical analysis showed that the increase in the average number of eggs/females of both mosquito species that were fed on human blood was significant when compared with the females that were fed on rabbit blood and not significant for the females that were fed on pigeon blood.

Results in Table 4 showed the level of egg hatch on both mosquito species *Ae. aegypti* and *Cx. pipiens* after fed on different sources of blood. The percentage rate of egg hatch of female mosquito *Ae. aegypti* were about 91.1%; 88.5% and 94.4% when they were fed on blood meals of human, rabbits, and pigeons, respectively. However, the rate of egg hatching recorded was approximately 90.1%; 86.2%. and 95.8% in the case of *Cx. pipiens* females that were fed on blood from three vertebrate hosts, respectively Fig. 4.

4. Discussion

Many contemporary research studies were conducted on the activity of feeding female mosquitoes with different sources of blood meal with the objective of explaining the relationship between the role of mosquitoes in the transmission of the causal disease organisms and their contact with the different hosts. Camison et al. (2020) reported that the activity feeding on human blood was most commonly preferred by the mosquito *Ae. aegypti* with the rate of 93% but this activity feeding has recorded a clear reduction at the rate of 36% for the species *Ae. japonicus*, however Shehata (2018); Chikwendu et al (2019) reported that *Cx. pipiens* gave the highest activity rates on feeding on pigeon blood 82.3% followed by feeding on guinea pig blood 79.3% then followed by humans 73.2% whereas the females of the mosquito *Anopheles sergentii* has recorded the highest rates of activity feeding on human blood 88.1% followed by guinea pig 77.1% then pigeon 76.9%.

Moreover, Kim et al. (2017) have recorded activity feeding for *Ae. albopictus* females on mammalian and bird blood with rates of 71% and 26%, respectively. Generally, it could be implied that the differences in mosquito activity feeding as an external parasite for obtaining a blood meal from different hosts may be attributed to the type of mosquito and its behavior during feeding on a specific host and the degree of body temperature of the host (Villareal et al., 2017; Escobat et al., 2020; Gulia-Nuss et al., 2015; Gunathilaka et al., 2017). The host smell and natural scent or aroma could play a stimulating or motivating role during the activation of the mosquitoes towards the specific host for obtaining the blood meal as reported by (Raji and De Gennaro, 2017; Gonzales and Hansen 2016).

It is noteworthy to infer that future studies of the feeding cycle or the feed system or the nutrient cycle and egg-laying or oviposition would be urgent and an indispensable information rational asset for the estimation of the range of repetition, reoccurrence and frequency of the mosquitoes contact with the host. It could also be considered pivotal in its effect on the transmission of cross – infection which was proved and assured by the World Health Organization (WHO, 1975). Thus it is highly an esteemed proposition to reiterate that the contemporary studies by scientists of WHO which supposed that if one female mosquito is carrying the

Table 4
The percentage of egg hatch of *Ae. egypti* and *Cx. pipiens* fed on three different vertebrate hosts.

Mosquito sp.	Blood meal source	Total No. of eggs*	Total No. of larvae hatched	Hatchability (%) Mean ± SE**
Ae. aegypti	Human	1167	1063	91.1 ± 3.1a
	Rabbit	664	588	88.5 ± 4.6a
	Pigeon	926	874	44.4 ± 6.7a
	LSD = 48.2			**P = 0.0001**
Cx. pipiens	Human	3225	2905	90.1 ± 5.8a
	Rabbit	2043	1761	86.2 ± 2.2a
	Pigeon	2921	2798	95.8 ± 7.7a
	LSD = 40.4			**P = 0.0001**

* Total of 20 engorged mosquito females.

infection and lived for 12 days and the nutrient cycle and egg–laying is completed every 4 days, this means that this female mosquito will be able to feed for three times during its life cycle or by other means would have the opportunity for transmission of cross infection to 3 persons. Therefore, the calculation of the time scale of the nutrient system and egg laying is considered the utmost demand as a biological feature and characteristic for understanding the possibilities and abilities of mosquitoes on the transmission of the causal disease agents which was termed the Vectorial capacity (Mayton et al., 2020; Harrison et al., 2021).

5. Conclusion and recommendations

The study of the relationship between the mosquitoes as external virulent parasites and their feeding on the blood of different patterns of vertebrate hosts, without doubt, is an urgent important necessity of grave importance for the understanding, cognizance, discernment and acquisition of vital indispensable information about the abilities of mosquitoes as vectors in the transmission of the causal disease organisms in the environment. Therefore, more studies are needed on the relationship between the number of feeding occurrences and the disease cycle and the widespread dispersal and surveillance of mosquitoes on humans or other related animals in the Kingdom of Saudi Arabia.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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