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Evaluation of the feeding patterns of important mosquito vector species using molecular techniques

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Abstract: This study investigated the feeding patterns of populations of *Anopheles sacharovi, Culex tritaeniorhynchus,* and *Culex pipiens* in the Mediterranean and Aegean regions of Turkey. Blood-fed females resting inside barns, houses, and chicken coops in the rural areas were collected between May 2017 and September 2019, and the genomic DNA from each female was isolated to determine the blood source after amplification of the mitochondrial *cytochrome b* gene region. *Cytb* results showed that out of 445 blood-fed *An. sacharovi* females 2 blood-fed from dogs, 9 from birds, and 434 from cows. The results also showed that all 216 blood-fed females *Cx. tritaeniorhynchus* captured fed from cows, 6 out of 97 *Cx. pipiens* females fed from horses and 91 from birds. The feeding pattern of mosquito populations can be affected by numerous factors such as sampling method, sample size, and host abundance. Although field studies on mosquito feeding patterns do not reveal the feeding preference of such vectors, they provide vital information to understanding of vector and host interactions in the research area. Moreover, these data can give an idea of possible human contact in an environment where various hosts are readily accessible to a given vector mosquito; this is important for the prevention of various human diseases caused by vector mosquitoes.

Keywords: Multiplex PCR, Blood-feeding, *Anopheles sacharovi, Culex pipiens, Culex tritaeniorhynchus,* Turkey

1. Introduction

Certain mosquito species in the genera *Culex*, *Anopheles*, and *Aedes* are vectors of various important virus, protozoa, and nematode pathogens (Rueda 2007; Benelli 2015; Benelli et al., 2016). Malaria, mostly caused by *Plasmodium vivax,* has been effective in Southeastern Anatolia and the Mediterranean regions of Turkey and has affected thousands of people for decades (Özbilgin et al., 2011). The main vector of *P. vivax* in Turkey is *An. sacharovi* (Ramsdale and Haas, 1978, Alten et al., 2000; Ramsdale et al., 2001). In 2005, with the Tashkent Declaration and the intensive malaria control programs implemented by the Turkish Ministry of Health, malaria cases were significantly reduced, and since 2014, endemic malaria cases have not been detected in Turkey (Özbilgin et al., 2011; WHO, 2015, 2019). *Cx. pipiens* and *Cx. tritaeniorhynchus* are cosmopolitan species that transmit viral pathogens such as St Louis, Japanese, Rift valley, West Nile, Tembusu viruses as well as filarial nematodes (Hubálek and Halouzka, 1999; Vinogradova, 2000; Fonseca et al., 2004; Kilpatrick et al., 2005; Hamer et al., 2008). These mosquitoes are widely prevalent in various European and Middle Eastern countries including Turkey (Becker et al., 2010; Gunay et al., 2015, Akıner et al., 2019).

An. sacharovi, *Cx. pipiens*, *Cx. tritaeniorhynchus* species have been the focus of research such as mosquito control, insecticide resistance, population genetics, feeding behaviors and vector host interactions as they are important vectors found in many countries. Furthermore, studies on mosquito host choice which is epidemiologically important have also increased considerably in recent years. Mosquito host choice is a mixed phenomenon, influenced by the interaction of genetic and environmental factors (Takken and Verhulst, 2013). Mosquito species can be generalists that display opportunistic feeding behavior or specialists that feed on selected hosts (Takken and Verhulst 2013; Fikrig and Harrington 2021; Melgarejo-Colmenares et al., 2022) and feeding pattern differences might vary depending on mosquito species and populations, season, fly starvation, host behavior, and the mosquito's ability to learn over time (Kilpatrick et al., 2006; Hamer et al., 2011; Thiemann et al., 2012; Burkett-Cadena et al., 2008; Janousek et al., 2014). Analysis of mosquito host feeding patterns provides vital information for understanding vector-host interactions and pathogen transmission dynamics (Yan and Stone, 2021). The choice of host has been studied both in the laboratory and in nature using preference trials including olfactometers, indoor

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observation rooms, traps, and experimental poultry. Also, host feeding patterns in samples collected from nature can be determined using molecular methods like multiplex PCR, microsatellite analysis, ELISA, or precipitation tests (Kent and Norris, 2005; Kent, 2009).

This study investigated the blood feeding patterns of *An. sacharovi, Cx. pipiens* and *Cx. tritaeniorhynchus* populations in the Aegean and Mediterranean regions of Turkey based on the blood found in female mosquitoes using PCR-based molecular methods. This is the first study on feeding patterns of mosquito populations obtained from Turkey. The study show that the results on the feeding patterns of these species are directly related to the host diversity, abundance, sampling methods and sampling points in the region.

2. Material and Method

2.1. Research area and blood fed females sampling and identification

Between May 2017 and September 2019, blood fed females were collected from Kadirli, Düziçi, Türkoğlu, Dörtyol, Kırıkhan, Kozan, Ceyhan, Yumurtalık, Karataş, Tuzla, Tarsus, Huzurkent, Manavgat, Isparta, Burdur, Sandıklı, Akköy, Dalaman, Söke and Manisa localities in the Aegean and Mediterranean regions of Turkey (Figure 1).

Selected sampling localities are villages where livestock and agricultural activities are carried out. Cows, sheep and goats are maintained and traded; horses, donkeys, dogs and chickens are common animals. Pigs are not reared in any of these localities. From each sampling locality, 3 houses actively occupied by people, 3 barns sheltering animals and chicken coops were selected. Blood fed females were caught by a person for 20 minutes using a mouth aspirator and a flashlight and captured females were transferred to paper cups covered with mosquito netting using an aspirator. It was aimed to collect an average of 30 blood fed females for each locality and each species. The cups with mosquitoes were labeled according to the sampling locality, and transported to the laboratory under cold conditions where they were divided into groups according to sampling areas, blood fed status, and coarse species distinctions, and preserved in a liquid nitrogen tank in 20 ml Falcon tubes. Afterward, the samples were transported to Aydın Adnan Menderes University Vector

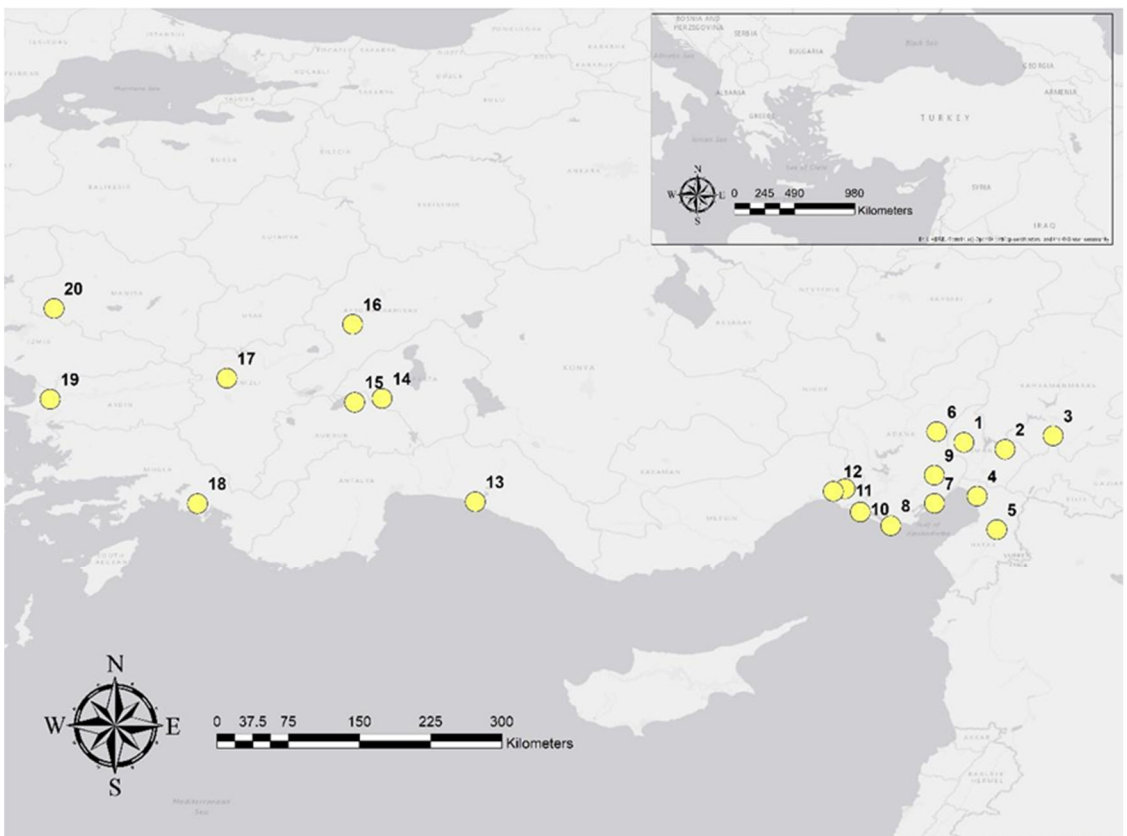

Figure 1. Sampling localities of *Anopheles sacharovi, Culex pipiens, Culex tritaeniorhynchus* populations (1. Kadirli, 2. Düziçi, 3. Türkoğlu, 4. Dörtyol, 5. Kırıkhan, 6. Kozan, 7. Yumurtalık, 8. Karataş, 9. Ceyhan, 10. Tuzla, 11. Tarsus, 12 .Huzurkent, 13. Manavgat, 14. Isparta, 15. Burdur, 16. Sandıklı, 17. Akköy, 18. Dalaman, 19. Söke, 20. Manisa).

Insects Research laboratory as soon as possible and stored at -80 ^{0}C until molecular analysis of the relevant samples. Blood fed females were examined under a Leica S8 Apo Binocular microscope and species identifications were made using the species identification key (Becker et al., 2003).

2.2. Determination of blood-feeding preferences

In total, DNA was extracted from 758 blood fed female mosquitoes using Invitrogen PureLink genomic DNA isolation kit. To determine the blood feeding patterns of *An. sacharovi, Cx. tritaeniorhynchus* and *Cx. pipiens* species, the mitochondrial *cytochrome b* (*cytb*) gene region of the samples were amplified by PCR using an Applied Biosystems Veriti thermocycler and a variety of specially designed primers by Kent and Norris, 2005; Pitzer et al., 2014; Lee et al., 2002 (Table 1).

The PCR was done using 50 ng DNA, 1.5 U Taq DNA polymerase, 2X Master mix red, 20 µl each of primers totaled to a volume of 25 µL. PCR reaction conditions were 95 °C for 5 min, 40 denaturation cycles at 95 °C for 30 s, annealing at 57 °C for 1 min, extension at 72 °C for 1 min, and the final extension step of 72 °C for 5 min. Each PCR product obtained was stored at 4 °C until the next process. Negative control was included to check for any contamination in the reaction while preparing each PCR reaction. PCR products were visualized under UV light on 2% agarose gel. The band size of our potential hosts is 334 bp for humans, 132 bp for goats, 680 bp for dogs, 561 bp for cows, 500 bp for horses, and 383 bp for birds (Figure 2).

2.3. Sequence analysis

To verify the detected hosts with the band sizes, samples were selected from the bands showing each detected host type and a total of 10 samples were sequenced. After the sequencing, the band profiles were checked, evaluated and the blood meal host was verified. Sequencing studies of *cytb* gene region PCR products of 10 selected samples were performed by Macrogen INC., (South Korea). The resulting sequences were saved in FASTA (http://www.ebi.ac.uk/fasta33/) format and are accessible from GenBank via the http://www.ncbi.nlm.nih.gov website with CLUSTAL in BioEdit 7.2.5. They were compared with the W program and the host animals were determined according to the sequences with closest compatibility to 100%.

3. Results

A total of 877 blood fed female mosquitoes were caught from the sampling locations: for *An. sacharovi* (511 samples in total) 430 were captured from barns, 61 from houses, and 20 chicken coops whereas 262 (235 from barns, 19 from houses and 8 from chicken coops) *Cx. tritaeniorhychus* and 104 (11 from barns, 14 from houses and 79 from chicken coops) *Cx. pipiens* were captured. Out of the 877 samples including all three species, DNA was isolated from 445 *An. sacharovi*, 216 *Cx. tritaeniorhychus* and 97 *Cx. pipiens*. In total 758 DNA were used in the amplification of the *cytb* gene region to determine their feeding patterns.

The results showed that 445 *An. sacharovi* females fed mostly from cows (97.5%) compared to dogs (0.44%) and birds (2%) in the sampling localities. Of the 43 females sampled from Söke locality, 9 females (6 females from the chicken coop, 1 female from the house and 2 females from the barn) fed from birds, and 2 out of the 26 female samples sampled from chicken coops in the Tarsus locality fed from dogs. *Cytb* gene region analyses revealed that all of the 189 blood fed *Cx. tritaeniorhynchus* females from barns, 19 from houses and 8 from chicken coops blood fed from cows (100%). Lastly, DNA obtained from 11 *Cx. pipiens* females sampled from barns, 14 from houses and 72 from chicken coops showed that 93.8% (91 females) fed from birds and 6.1% (6 females) from horses. All these specimens that blood fed from horse were captured in barns from Tarsus locality.

The localities where the sampling studies were carried out, numbers of captured samples and isolated

Table 1. Specific primers designed to determine blood meal preferences in *Anopheles sacharovi, Culex pipiens, Culex tritaeniorhynchus* populations

Primer	Sequence	Band size
Human741F	GGCTTACTTCTCTTCATTCTCTCCT	334
Goat894F	CCTAATCTTAGTACTTGTACCCTTCCT	132
Dog368F	GGAATTGTACTATTATTCCGCAACCAT	680
Cow121F	CATCGGCACAAATTTAGTCG	561
HorseF	CCCTACATCGGTACTACCC	500
BirdF	CCCCTCAGAATGATATTTGTCCTCA	383
BirdR	CCATCCAACATCTCAGCATGATGAAA	383
UNREV1025	GGTTGTCCTCCAATTCATGTTA	623

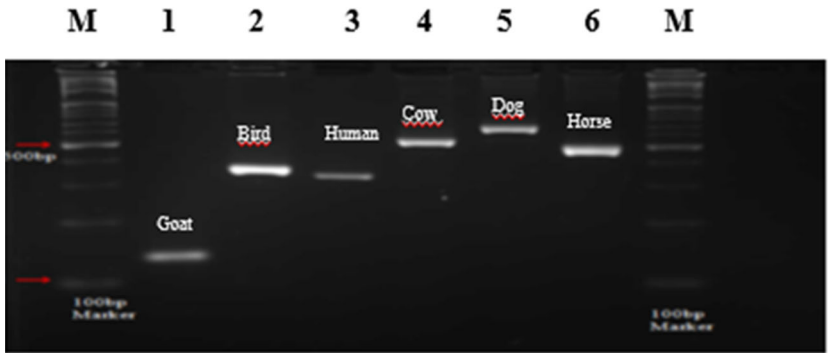

Figure 2. Agarose-gel image of *cytb* gene region of possible hosts (M: marker (100-1000 bp) 1. Goat; 2. Bird; 3. Human; 4. Cow; 5. Dog; 6. Horse)

DNAs and blood feeding patterns of *An. sacharovi, Cx. tritaeniorhychus* and *Cx. pipiens* are shown in Table 2.

4. Discussion

Anopheles species are zoophilic, anthropophilic or generalist mosquitoes that feed on a variety of animals ranging from mammals to birds but some species are selective, only feeding on one species (Lyimo and Ferguson, 2009). Despite being considered to be one of the most important malaria vector in Turkey (Kasap, 1990; Özbilgin et al., 2011), studies assessing the blood feeding pattern or preference of *An. sacharovi* using different techniques both in Turkey and in other countries have identified that females exhibit a high rate of blood feeding from animals, especially cows (Garret-Jones et al., 1964; Boreham and Garret-Jones, 1973; Demirhan and Kasap, 1995; Yaghoobi-Ershadi et al., 2001; Tavşanoğlu and Çağlar, 2008). Demirhan and Kasap (1995) determined the host preferences of females collected from 3 feeding rooms created under natural conditions, as well as from barns, houses and empty buildings in the Çukurova region of Turkey using the gel diffusion technique among different hosts (human, cow, horse, donkey, sheep and chicken). Although the hosts of *An. sacharovi* females changed proportionally according to the host variation in the rooms, the rate of blood-feeding from cows, horses and donkeys was found to be higher than from human hosts. Also samples collected from barns, houses and empty buildings significantly preferred cows as blood source host. In our study, the majority of *An. sacharovi* specimens (445 blood fed females) were caught from barn areas where cows were present, and it was found that 434 of these fed from cows, 2 from dogs, 9 from birds. None of the 61 females caught in the houses blood fed from humans. Tavşanoğlu and Çağlar (2008) analyzed 416 blood fed *An. sacharovi* females from 4 different villages in Şanlıurfa, Turkey, using the precipitin method. They found that 15.8% of the samples collected from the barns fed from humans, 84.2% from cows/sheep, 25.2% of samples collected from houses fed from humans, and 74.8% from cows/sheep. Similarly, using the precipitin test, female *An. sacharovi* captured from barns, houses and shelters in a village in Greece were evaluated for their feeding pattern among sheep/goat, pig, horse, bird, dog, cow, rabbit hosts. The results showed that females captured from barns fed from sheep/goats (the most common animal group in the village), pigs and horses, while those captured from homes fed from humans (Boreham and Garret-Jones, 1973). ELISA analyzes of *An. sacharovi* females captured from barns, chicken coops and bedrooms in Iran showed that 7.52% of barn samples, 14.1% of chicken coops samples, and 38% of bedroom samples fed blood from humans (Yaghoobi-Ershadi et al., 2001). Our results and the results of other studies demonstrate that *An. sacharovi* females have a higher tendency to blood fed more from animals than humans, however the density of the animal host species in the research area and the sampling areas can change the blood feeding pattern. In our study, most of the *An. sacharovi* females were sampled from barns with cows hence the higher rate of blood feeding from cows compared to other hosts. Also females captured from houses blood fed from cows instead of humans. This result shows that some females fed from cows in the barns spend their resting time in houses after blood sucking. It also reveals that the blood feeding pattern may be changing due to various factors such as the use of repellent solutions or mosquito nets in houses to ward off mosquitoes.

97 of blood fed *Cx. pipiens* females we caught from barns, house and chicken coops in different localities were analyzed and it was determined 91 females (72 from chicken coops, 5 from barns, 14 from houses) blood fed from birds and 6 females from horses. All chicken coops captured females blood fed from birds, whereas 6 females sampled from the barns in Tarsus locality blood fed from horses. Host detection of 148 blood fed *Cx. pipiens* females sampled from natural populations in Kayseri province of Turkey was performed using *cytb* analysis and it was determined that 98% of females fed from birds, 11.4%

Table 2. Number of captured samples, number of isolated DNA and *Cytb* results of blood fed females of *An. sacharovi, Cx. tritaeniorhychus* and *Cx. pipiens* from different localities in Turkey.

Region	Province	Localities	*Anopheles sacharovi*									*Culex tritaeniorhychus*									*Culex pipiens*								
			Barn			House			Chicken coop			Barn			House			Chicken coop			Barn			House			Chicken coop		
			No.	DNA	*Cyt b*	No.	DNA	*Cyt b*	No.	DNA	*Cyt b*	No.	DNA	*Cyt b*	No.	DNA	*Cyt b*	No.	DNA	*Cyt b*	No.	DNA	*Cyt b*	No.	DNA	*Cyt b*	No.	DNA	*Cyt b*
Mediterranean	Osmaniye	Kadirli	20	15	15 cows	6	6	6 cows	2	2	2 cows	24	21	21 cows														
		Düziçi	22	15	15 cows	5	5	5 cows	2	2	2 cows	28	20	20 cows														
	Kahramanmaraş	Türkoğlu	20	17	17 cows	6	6	6 cows																				
	Hatay	Dörtyol	20	20	20 cows																							
		Kırıkhan	25	18	18 cows	5	5	5 cows	2	2	2 cows	26	20	20 cows	4	4	4 cows	3	3	3 cows								
	Adana	Kozan	40	25	25 cows	5	5	5 cows																				
		Yumurtalık	22	17	17 cows	4	4	4 cows	2	2	2 cows	29	20	20 cows														
		Karataş	28	25	25 cows	2	2	2 cows	1	1	1 cows	26	20	20 cows	3	3	3 cows	1	1	1 cow								
		Tuzla										27	20	20 cows	2	2	2 cows											
		Ceyhan	22	22	22 cows																							
	Mersin	Tarsus	20	17	17 cows	4	4	4 cows	2	2	2 dogs	25	23	23 cows	4	4	4 cows	1	1	1 cow	6	6	6 horses					
		Huzurkent	26	21	21 cows	4	4	4 cows	1	1	1 cow	25	23	23 cows	2	2	2 cows											
	Antalya	Manavgat	20	17	17 cows	6	6	6 cows				25	22	22 cows	4	4	4 cows	3	3	3 cows				3	3	3 birds	12	12	12 birds
	Isparta	Center	26	26	26 cows																							
	Burdur	Center	22	22	22 cows																			3	3	3 birds	14	14	14 birds
Aegean	Afyon	Sandıklı	24	24	24 cows																							
	Denizli	Akköy	24	24	24 cows																							
	Muğla	Dalaman	22	16	16 cows	4	4	4 cows	2	2	2 cows										4	4	4 birds	5	5	5 birds	18	18	18 birds
	Aydın	Söke	27	23	2 birds 21 cows	10	10	1 birds 9 cows	6	6	6 birds																12	12	12 birds
	Manisa	Center																			1	1	1 bird	3	3	3 birds	23	16	16 birds
Total			**430**	**364**	**2 birds 362 cows**	**61**	**61**	**1 birds 60 cows**	**20**	**20**	**2 dogs, 6 birds 12 cows**	**235**	**189**	**189 cows**	**19**	**19**	**19 cows**	**8**	**8**	**8 cows**	**11**	**11**	**6 horses 5 birds**	**14**	**14**	**14 birds**	**79**	**72**	**72 birds**

from mammals (human: 6, dog: 2, sheep: 3, cow: 4) 1.9% from bird + mammal (Korkmaz et al., 2016). *COI* gene region analyzes performed on 330 *Cx. pipiens* females caught from various artificial resting areas such as under bridges and public toilets in California, USA indicated that 98% of the females blood fed from birds (Montgomery et al., 2011). In a study conducted in Portugal, *Cx. pipiens* females were caught from the barns and the host detection of 170 females was determined by ELISA method; 159 of the analyzed females blood fed from birds and 3 from humans (Gomes et al., 2013). Molaei et al. (2006) evaluated the blood meal of *Cx. restuans* and *Cx. pipiens* by amplifying the mtDNA *cytb* gene by PCR method and stated that 93% of these species obtained blood from birds. In various studies conducted in America and Europe, have shown that, *Cx. pipiens* host spectrum was determined at a rate of 64-97% from birds (Gómez-Díaz and Figuerola, 2010; Figuerola et al., 2007; Vazquez et al., 2010).

In our study 216 of the 262 blood fed *Cx. tritaeniorhychus* females captured in barns, houses and chicken coops in different localities were analyzed and it was determined that all blood fed from cows. Because cows are maintained in all sampling barns, there may not have been a tendency to suck blood from people, sheep, dogs, or chickens that could be potential hosts near the barns. The fact that the females caught from the houses also had blood fed from cows shows that some of the females rested in the houses after feeding. Analysis using the precipitin test of 20,522 blood fed *Cx. tritaeniorhychus* females caught with light traps on the Ryukyu Islands of Okinowa using the precipitin test showed that females sucked blood from cows, pigs, chickens, dogs and goats. In addition, human blood was detected in only 4 females, whereas cow and pig blood was detected in more than 80% of the females (Pennington and Phelps, 1968). A study conducted in Kuttanadu region of India analyzed 2553 *Cx. tritaeniorhychus* females using the agarose gel diffusion method and determined that 56.6% of females fed from cows, 6.3% from pigs and less than 2% from humans, poultry and goats (Arunachalam et al., 2005) In another study, blood fed *Cx. tritaeniorhychus* captured by dropnet method from open fields in Dibrugarh region of India were analyzed using the gel diffusion method; it was found that out of 276 females, 164 (59.6%) blood fed from cows, 110 from pigs and only one from humans (Bhattacharyya et al., 1994).

Studies of the blood-feeding behavior of many mosquito species have shown a temporary shift from birds to mammals or to different bird species (Tempelis et al., 1975, 1967; Edman and Taylor, 1968). Although most of the feeding behavior is heavily dependent on the animal's innate feeding tendencies and observable characteristics (Ulloa García et al., 2004, Lyimo and Ferguson, 2009; Takken and Verhulst, 2013; Melgarejo-Colmenares et al., 2022) all results show that the feeding behavior of field-collected mosquitoes can be influenced by environmental factors such as host abundance, host availability, or host behavior (Takken and Verhulst, 2013, Stephenson et al., 2019).

There are also some behavioral characteristics that enable many mosquito species to choose their hosts and to gravitate towards certain hosts over other alternatives, or to change their blood feeding tendencies between available hosts at different times. In particular endophagic or exophagic characteristics of the mosquito and the accessibility of the indoor or outdoor hosts are also important factors (Roiz et al., 2012; Takken and Verhulst, 2013; Rizzoli et al., 2015). Our research has revealed that all three species enter barns and chicken coops from outside and tend to suck blood from the animals present there. Our results may be biased as the most common host in any region may be more preferred and this may not indicate host choice. For this reason, feeding preference trials under laboratory conditions together with field studies may be a more objective approach to determining the host preference of mosquito species.

Availability of Data and Materials

All data sets collected and analyzed during the current study are available from the corresponding author (FB) on reasonable request.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

References

Akıner MM, Öztürk M, Başer, AB, Günay F, Hacıoğlu S et al. (2019). Arboviral screening of invasive *Aedes* species in northeastern Turkey: West Nile virus circulation and detection of insect-only viruses. PLoS neglected tropical diseases 13 (5): e0007334.

Alten B, Bellini R, Caglar S, Simsek FM Kaynas S (2000). Species composition and seasonal dynamics of mosquitoes in the Belek region of Turkey. Journal of Vector Ecology 25: 146-154.

Arunachalam N, Samuel PP, Hiriyan J, Rajendran R, Dash A (2005). Observations on the multiple feeding behavior of *Culex tritaeniorhynchus* (Diptera: Culicidae), the vector of Japanese encephalitis in Kerala in southern India. The American journal of tropical medicine and hygiene 72 (2): 198-200.

Becker N, Petric D, Boase C, Lane J, Zgomba M et al. (2003). Mosquitoes and their Control. Kluwer Academic / Plenum Publishers, New York.

Becker N, Petric D, Zgomba M, Boase C, Madon M et al. (2010). Mosquitoes and their control, Springer Science & Business Media.

Benelli G (2015). Research in mosquito control: current challenges for a brighter future. Parasitology research 114 (8): 2801-2805.

Benelli G, Lo Iacono A, Canale A, Mehlhorn H (2016). Mosquito vectors and the spread of cancer: an overlooked connection? Parasitology Research 115(6): 2131-2137.

Bhattacharyya D, Handique R, Dutta L, Dutta P, Doloi P et al. (1994). Host feeding patterns of Culex vishnui sub group of mosquitoes in Dibrugarh district of Assam. Journal of communicable diseases 26: 133-133.

Boreham PFL, Garrett-Jones C. (1973). Prevalence of mixed blood meals and double feeding in a malaria vector (*Anopheles sacharovi* Favre) Bulletin of the World Health Organization, 48 (5): 605-614.

Burkett-Cadena ND, Graham SP, Hassan HK, Guyer C, Eubanks MD et al. (2008). Blood feeding patterns of potential arbovirus vectors of the genus *Culex* targeting ectothermic hosts. The American journal of tropical medicine and hygiene 79: 809.

Demirhan O, Kasap M (1995). Blood feeding behavior of *Anopheles sacharovi* in Turkey. Journal of the American Mosquito Control Association-Mosquito News 11: 11-14.

Edman JD, Taylor DJ (1968). *Culex nigripalpus*: seasonal shift in the bird-mammal feeding ratio in a mosquito vector of human encephalitis. Science 161:67-68.

Figuerola J, Soriguer R, Rojo G, Tejedor CG, Jimenez-Clavero MA (2007). Seroconversion in wild birds and local circulation of West Nile virus, Spain. Emerging infectious diseases 13: 1915-1917.

Fikrig K, Harrington LC (2021). Understanding and interpreting mosquito blood feeding studies: the case of *Aedes albopictus*. Trends in Parasitology 37 (11): 959-975.

Fonseca DM, Keyghobadi N, Malcolm CA, Mehmet C, Schaffner F et al. (2004). Emerging vectors in the *Culex pipiens* complex. Science 303: 1535-1538.

Garrett-Jones C, Alves J, Ferreira N, World Health Organization (1964.) The prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. No. WHO/Mal/470.64. World Health Organization,

Gómez-Díaz E, Figuerola J (2010). New perspectives in tracing vector-borne interaction networks. Trends in parasitology 26: 470-76.

Gunay F, Alten B, Simsek F, Aldemir A, Linton YM (2015). Barcoding Turkish *Culex* mosquitoes to facilitate arbovirus vector incrimination studies reveals hidden diversity and new potential vectors. Acta tropica 143: 112-120.

Hamer GL, Chaves LF, Anderson TK, Kitron UD, Brawn JD et al. (2011). Fine-scale variation in vector host use and force of infection drive localized patterns of West Nile virus transmission. PloS One 6(8): e23767

Hamer GL, Kitron UD, Brawn JD, Loss SR, Ruiz MO et al. (2008). *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile virus to humans. Journal of Medical Entomology 45: 125-28.

Hubálek Z, Halouzka J (1999). West Nile fever--a reemerging mosquito-borne viral disease in Europe. Emerging infectious diseases 5(5): 643–650.

Janousek WM, Marra PP, Kilpatrick AM (2014). Avian roosting behavior influences vector-host interactions for West Nile virus hosts. Parasites & vectors 7: 1-11.

Kasap H (1990). Comparison of experimental in fectivity and development of Plas-modium vivax in *Anopheles sacharovi* and *An. superpictus* in Turkey. The American Journal of Tropical Medicine and Hygiene 42: 111-117.

Kent RJ (2009). Molecular methods for arthropod blood meal identification and applications to ecological and vector-borne disease studies. Molecular ecology resources 9: 4-18.

Kent RJ, Norris DE (2005). Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome B. The American journal of tropical medicine and hygiene 73(2): 336-342.

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P (2006b). West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biology 4:82.

Kilpatrick AM, Kramer LD, Campbell SR, Alleyne EO, Dobson AP et al. (2005). West Nile virus risk assessment and the bridge vector paradigm. Emerging infectious diseases 11 (3): 425-429.

Korkmaz S, Yıldırım A, Düzlü Ö, Çiloğlu A, Önder Z et al. (2016). Kayseri yöresinden toplanmış *Culex pipiens* komplekse ait sivrisinek (Diptera: Culicidae) örneklerinin kan beslenme identifikasyonu. Türkiye Parazitoloji Dergisi 40: 199-204.

Lee JH, Hassan HK, Hill G, Cupp EW, Higazi TB et al. (2002). Identification of mosquito avian-derived blood meals by polymerase chain reaction-heteroduplex analysis. The American journal of tropical medicine and hygiene 66 (5): 599-604.

Lyimo IN, Ferguson HM (2009). Ecological and evolutionary determinants of host species choice in mosquito vectors. Trends in parasitology 25: 189-196.

Melgarejo-Colmenares K, Cardo MV, Vezzani D (2022). Blood feeding habits of mosquitoes: hardly a bite in South America. Parasitology Research 121: 1829-1852.

Molaei G, Andreadis TG, Armstrong PM, Anderson JF, Vossbrinck CR (2006). Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, northeastern United States. Emerging infectious diseases 12 (3): 468-474.

Montgomery MJ, Thiemann T, Macedo P, Brown DA, Scott TW (2011). Blood-feeding patterns of the *Culex pipiens* complex in Sacramento and Yolo Counties, California. Journal of medical entomology 48 (2): 398-404.

Özbilgin AS, Topluoglu S, Islek E, Mollahaliloglu S, Erkoc Y (2011). Malaria in Turkey: successful control and strategies for achieving elimination. Acta tropica 120 (1-2): 15-23.

Pennington NE, Phelps CA (1968). Identification of the host range of *Culex tritaeniorhynchus* mosquitoes on Okinawa, Ryukyu Islands. Journal of Medical Entomology 5 (4): 483-487.

Pitzer JB, Kaufman PE, Tenbroeck SH, Maruniak JE (2014). Host blood meal identification by multiplex polymerase chain reaction for dispersal evidence of stable flies (Diptera: Muscidae) between livestock facilities. Journal of Medical Entomology 48: 53-60.

Ramsdale C, Haas E (1978). Some aspects of epidemiology of resurgent malaria in Turkey. Transactions of the Royal Society of Tropical Medicine and Hygiene 72 (6): 570-580.

Ramsdale CD, Alten B, Caglar SS, Ozer N (2001). A revised, annotated checklist of the mosquitoes (Diptera, Culicidae) of Turkey. European Mosquito Bulletin 9: 18-28.

Rizzoli, A, Jimenez-Clavero M., Barzon L, Cordioli P, Figuerola J et al. (2015). The challenge of West Nile Virüs In Europe: Knowledge Gaps And Research Priorities. Euro Surveillance**,** 20 (4):11-15.

Roiz D, Roussel M, Muñoz J, Ruiz S, Soriguer R et al. (2012). Efficacy of mosquito traps for collecting potential West Nile mosquito vectors in a natural Mediterranean wetland. The American journal of tropical medicine and hygiene 86 (4): 642-648.

Rueda LM (2007). Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in freshwater. Freshwater animal diversity assessment, Springer**:** 477-487.

Stephenson EB, Murphy AK, Jansen CC, Peel AJ McCallum H (2019). Interpreting mosquito feeding patterns in Australia through an ecological lens: an analysis of blood meal studies. Parasites & vectors 12 (1): 1-11.

Takken W, Verhulst NO (2013). Host preferences of blood-feeding mosquitoes. Annual review of entomology 58 (1): 433-453.

Tavşanoğlu N, Çağlar SS (2008). The vectorial capacity of *Anopheles sacharovi* in the malaria endemic area of Şanlıurfa, Turkey. European Mosquito Bulletin 26: 18-23.

Tempelis CH, Washino RK (1967). Host-Feeding patterns of *Culex tarsalis* in sacramento valley California with Notes on Other Species. Journal of Medical Entomology, 4: 315-318.

Tempelis CH (1975). Host-feeding patterns of mosquitoes, with a review of advances in analysis of blood meals by serology. Journal of Medical Entomology 11: 635- 653.

Thiemann TC, Lemenager DA, Kluh S, Carroll BD, Lothrop HD et al. (2012). Spatial variation in host feeding patterns of *Culex tarsalis* and the *Culex pipiens* complex (Diptera: Culicidae) in California. Journal of Medical Entomology 49: 903-16.

Ulloa A, Arredondo Jiménez JI, Fernández Salas I, Rodríguez MH, González Cerón L (2004). Innate host selection in *Anopheles vestitipennis* from southern Mexico. Journal of the American Mosquito Control Association 20(4): 337-341.

Vazquez A, Sánchez-Seco MP, Ruiz S, Molero F, Hernandez L et al. (2010). Putative new lineage of West Nile virus, Spain. Emerging infectious diseases 16 (3): 549-552.

Vinogradova EB (2000). *Culex pipiens pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control (Pensoft Publishers).

Yaghoobi-Ershadi MR, Namazi J, Piazak N (2001). Bionomics of *Anopheles sacharovi* in Ardebil province, northwestern Iran during a larval control program. Acta tropica 78 (3): 207-215.

Yan J, Kibech R, Stone CM (2021). Differential effects of larval and adult nutrition on female survival, fecundity, and size of the yellow fever mosquito, *Aedes aegypti*. Frontiers in zoology 18 (1): 1-9.

World Health Organization (2015). World Malaria Report 2014: Summary, World Health Organization.

World Health Organization (2019). World Malaria Report 2019, Geneva.