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Identification of blood meals in field collected *Culex pipiens, Anopheles sacharovi* and *Culex tritaeniorhynchus* (Diptera: Culicidae) using the ELISA method

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Abstract: Information obtained about the blood feeding behavior of mosquito populations in nature is important for understanding their vectorial capacity and evaluating their potential role in the transmission of vector-borne diseases. This study investigated the feeding patterns of *Culex pipiens, Anopheles sacharovi*, and *Cx. tritaeniorhynchus* by using the ELISA method. A total of 435 blood-fed females were collected from the Aegean and Mediterranean regions of Türkiye between May 2021 and September 2023. The types and numbers of potential domestic animal hosts were recorded in each village. Of the 372 blood meals tested, 358 gave a single host positive result mainly on cow and human, chicken, respectively, in both villages. On the other hand, the host tendency for chicken was found in a very low degree in both villages. The human and other hosts were underselected, highlighting a marked zoophily for the vectors. Overall, these hosts were mainly selected in proportion to their abundance. These results highlight the zoophilic and exophagic behavior of these vectors. In conclusion, understanding the blood feeding behavior of mosquito populations is important for vector biology of these vectors. More research is needed to study the feeding behavior of outdoor mosquito populations and the influence of domestic animals on disease epidemiology.

Key words: Blood feeding preferences, ELISA, *Culex pipiens, Anopheles sacharovi, Culex tritaeniorhynchus*

1. Introduction

Mosquitoes are one of the most important vectors of insect-borne diseases. Some *Anopheles, Aedes* and *Culex* species are major vectors of numerous diseases of global importance, including malaria, lymphatic filariasis, Japanese encephalitis, West Nile fever, Zika, and Chikungunya fever (Deng et al., 2023). These infections threaten >50% of the world's population (Franklinos et al., 2019) and cause thousands of deaths every year (Takken and Verhulst, 2013; WHO, 2022). Malaria stands out as a significant public health concern among these vector-borne diseases with nearly half of the global population at risk of malaria infection (WHO, 2021). In Türkiye, *Plasmodium vivax* is the predominant parasite responsible for malaria transmission, with *An. sacharovi* serving as the primary vector (Özbilgin et al., 2011). Notably, a collaborative effort to eliminate malaria was undertaken in 1957 through the Tashkent Declaration, signed by Türkiye and eight Central Asian countries (Tajikistan, Uzbekistan, Azerbaijan, Kyrgyzstan, Armenia, Georgia, Turkmenistan, Kazakhstan). However, despite such initiatives, the global burden of vector-borne diseases has shown a concerning rise, with reemergence in previously controlled areas and

expansion into new territories (Stanaway et al., 2016; Paixão et al., 2018). Consequently, mosquito control efforts are mainly focused on these vectors (Becker et al., 2020). West Nile virus (WNV) is another mosquito-borne disease of concern, with birds serving as the primary reservoir and *Culex pipiens* mosquitoes acting as the principal vector (Touray et al., 2023). In Türkiye, WNV incidence exhibited fluctuations, with 7, 23, and 10 cases reported in 2017, 2018, and 2019, respectively (ECDC, 2020). Conversely, Japanese encephalitis virus (JEV) is transmitted by *Cx. tritaeniorhynchus* mosquitoes, which are geographically restricted to certain Aegean, Mediterranean, and Thrace provinces in Türkiye (Alten et al., 2000; Gunay et al., 2015; Bursali and Simsek, 2024). Unlike WNV, JEV is endemic to rural agricultural areas of East Asia, and no JEV cases have been reported in Türkiye.

Mosquito blood feeding is crucial for their survival and reproduction. While the need for blood to reproduce eggs is universal among mosquito females, their blood sucking habits are intricately linked to the spread of disease and host preference can dramatically impact disease transmission (Santos et al., 2019). Identifying the blood feeding preferences of these vectors is important for

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understanding the dynamics of disease transmission to humans. It has been shown that some mosquito species are generalists and will feed on any available host like humans, farm and wild animals, or birds (Stone and Gross, 2018). Other species such as *Aedes aegypti, Cx. pipiens*, and *Anopheles gambiae* prefer certain types of animals. Host seeking females use a variety of cues to find their preferred hosts, including carbon dioxide, body heat, sweat and visual cues (Takken and Verhulst, 2013). Understanding the two key components of mosquito feeding 'host preference and selection' is crucial. Preference dictates which species the mosquito prioritizes; this can vary during host seeking for different species and among different populations of the same species (Stone and Gross, 2018). Selection involves the specific choices made within that preference based on environmental cues like scent and warmth (Talebzadeh et al., 2023).

Numerous factors such as mosquito genetics, environment, host characteristics, and accessibility can influence feeding (Takken and Verhulst, 2013; Batista et al., 2018). This interplay of genetic and environmental factors shows a complex picture of mosquito behavior, one that requires continuous research and adaptation of control strategies to effectively combat the spread of disease. Various methods have been used to determine the source of a blood meal from collected wild mosquitoes, aiding in the understanding of vector-host interactions within the research area (McKinney, 1972; Kent, 2009, Baum et al., 2013; Afonso et al., 2012; Bursalı and Şimşek, 2022). Serological assays like ELISA (Beier et al., 1988), the precipitin test (Bull and King, 1923), and DNA analysis (Bursalı, 2020; Bursalı and Şimşek, 2022) are essential for studying the feeding behavior of wild mosquitoes. Even though the methods are not enough to explain the exact preference of vectors, recent studies can provide an estimate of the potential interaction of a particular vector mosquito with humans that may lead to a promising insight for the prevention of numerous human diseases carried by vector mosquitoes (Lyimo et al., 2009).

There is limited data on the host preference of *Cx. pipiens, An. sacharovi*, and *Cx. tritaeniorhynchus* in Türkiye. This study aimed to investigate the natural feeding patterns of *Cx. pipiens, An. sacharovi* and *Cx. tritaeniorhynchus* mosquito populations from the Aegean and Mediterranean regions of Türkiye using the ELISA method.

## 2. Materials and methods

2.1. Mosquito sampling
Blood-fed female mosquitoes sampling was conducted from 27 different locations in the Aegean and Mediterranean regions of Türkiye (Figure 1) between May 2021 and September 2023. The selected sampling locations were rural villages, where the main livelihood activities are livestock farming and agriculture. Besides horses, donkeys, and dogs, common animals include chickens, cows, sheep, and goats are kept and traded. Pigs are not raised in any of these locations. Since *Cx. pipiens, An. sacharovi*, and *Cx. tritaeniorhynchus* prefer to rest and feed indoors (endophilic and endophagic) (Becker et al., 2020), villages with structures like chicken coops and barns were the prime targets. These structures often provide ideal resting and breeding grounds for the mosquitoes. Additionally, human dwellings are typically nearby, and people primarily sleep indoors. Given the endophilic nature of these mosquitoes, there is a higher chance of them entering houses after feeding on livestock, potentially transmitting diseases. Three inhabited houses, three barns with livestock, and poultry coops were chosen from each sampling location. Domestic animals are typically housed in open-air enclosures, except for a small number of households utilizing separate, roofed structures for cows. Notably, the practice of keeping animals within dwellings is absent within the local customs.

As a result of the Mediterranean climate, it was hot and dry when the sampling had been performed. A mouth aspirator and a flashlight were used to catch blood-fed females resting on walls for thirty minutes and thereupon, the females were moved to paper cups with mosquito netting. On average, 25 mosquitoes were collected from each locality including 10 blood-fed females from every species. The cups were labelled the sampling locality and transported under cold conditions (4 °C) to the laboratory where they were sorted into groups based on the sample areas. Information on geographic coordinates, the number of residents in houses, the type of house material (cement walls or banco walls), and whether domestic animals were present were noted during a single interview with all household heads. Afterward, the samples were stored at –80 °C at Aydın Adnan Menderes University Vector Insects Research laboratory 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 the origin of blood meals
This study employed direct enzyme-linked immunosorbent assay (ELISA) technique (Beier et al., 1988) to identify the blood source of field-collected female mosquitoes exhibiting either fresh blood meals or evidence of partial digestion. The method relies on the reaction between antibodies specific to potential host blood (human, cow, dog, horse, chicken) and the ingested blood meal within the mosquito. Each blood-fed female mosquito was homogenized in phosphate-buffered saline (PBS/Sigma). Duplicate aliquots of the homogenate were added to wells
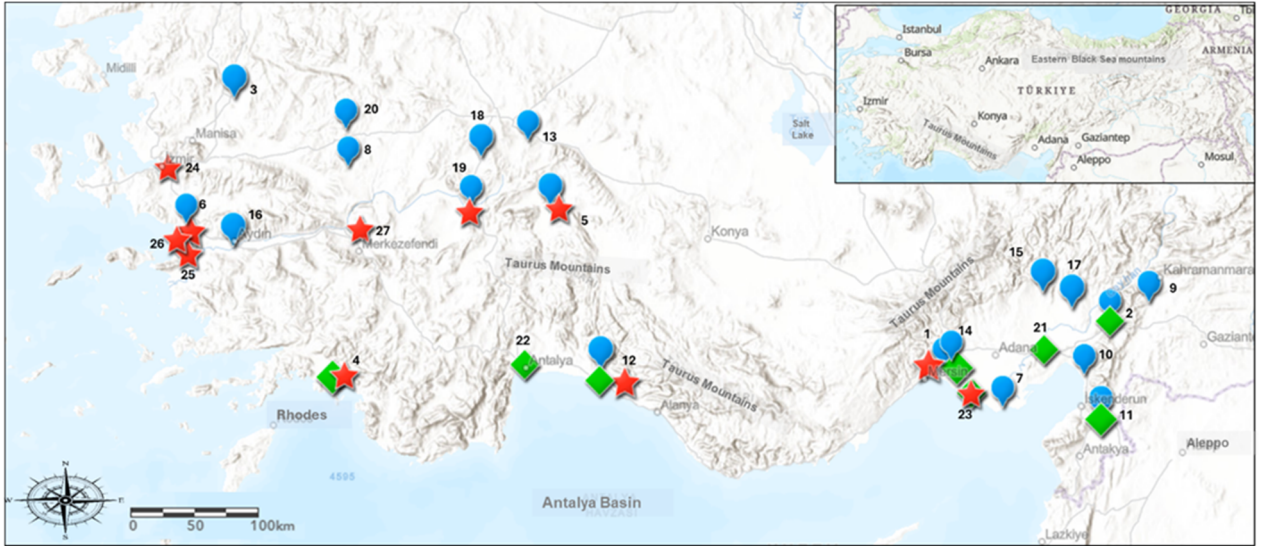
Figure 1. Sampling localities of *Anopheles sacharovi, Culex pipiens, Culex tritaeniorhynchus* populations (1. Huzurkent, 2. Düziçi, 3. Akhisar, 4. Dalaman, 5. Gelendost, 6. Selçuk, 7. Karataş, 8. Eşme, 9. Türkoğlu, 10. Dörtyol, 11. Kırıkhan, 12. Manavgat, 13. Afyon, 14. Tarsus, 15. Kadirli, 16. Aydın, 17. Kozan, 18. Sandıklı, 19. Dinar, 20. Uşak, 21. Ceyhan, 22. Antalya, 23. Tuzla 24. İzmir, 25. Söke, 26. Kuşadası, 27. Akköy). Red stars indicate locations where *Cx. pipiens* was sampled, the green diamond shape indicates locations of *Cx. tritaeniorhynchus* and the blue pins indicate the locations of *An. sacharovi*.

of 96-well plates (Corning 96-well Clear Round Bottom Polystyrene Not Treated Sterile Microplate) and incubated at room temperature. Wells were then washed with PBS containing Tween 20. Peroxidase-conjugated antihuman IgG (Sigma-Aldrich A0170) was added and incubated, followed by further washing. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid/Thermo) peroxidase substrate was added, and the development of a green color was observed visually. Absorbance of each was measured at 405 nm using a microplate reader (BioTek ELx808 Absorbance Plate Reader). Multiple detection approach was used for human and bovine blood. For mosquitoes initially screened for human blood, a second step identified bovine blood meals within the same well. This involved adding a phosphatase-conjugated antibovine IgG (Sigma) conjugate and following similar incubation and washing steps. Finally, a different substrate was used to detect the presence of bovine blood through a colorimetric change. All blood meal sources were identified simultaneously with the five antibodies used (antihuman IgG, antihorse IgG, anticow(bovine) IgG, antidog IgG, antichicken IgG Sigma-Aldrich).

The choice of antibodies targeted the most likely hosts prevalent in the study villages based on field observations. Negative controls consisted of unfed laboratory mosquitoes and were used to establish a cut-off value for positive identification of each blood source. Positive controls containing blood from each potential host species were included for validation.

Ethical approval for this study was obtained from the relevant ethics committee Aydın Adnan Menderes University (approval number: 64583101/2024/10).

2.3. Data analysis

For each village, information about the number of households, their geographic coordinates, the presence or absence of domestic animals, and the number of occupants in sampled rooms were entered into an Excel workbook. According to the results obtained from spectrophotometric measurements, the blank absorbance (only PBS) was subtracted from the absorbance of each sample. Then, the values of mosquito blood samples were calculated as a percentage relative to each positive control (such as direct human blood, bovine (cow) blood, etc.). These data were statistically analyzed using GraphPad software to determine interhost selectivity for each sample. Multiple variable analysis was performed using the select and transform program, where values greater than 1 on the selected Z score among 5 different hosts were identified. Accordingly, single, or multiple host blood meal data were obtained for each mosquito sample. To investigate potential associations between mosquito species, locality, and host preference, a chi-square test was employed. Corresponding p-values were calculated to assess the statistical significance of the observed relationships. Importantly, all analyses were conducted on weighted data to account for potential biases or unequal variances within the dataset.

3. Results

A total of 435 freshly female mosquitoes were collected in 27 different locations between May 2021 and September 2023. *An. sacharovi*, *Cx. tritaeniorhychus*, and *Cx. pipiens* were captured from barns, houses, and chicken coops (Table 1).

A total of 372 blood meals were tested by ELISA for host identification successfully. In all localities, cows were the most common hosts, followed by human and chicken hosts. Most of the blood meals were taken from only one of the five vertebrate hosts tested (human or animal). Mixed blood meals were observed for *An. sacharovi* in

Table 1. Sampling locality and abdominal status information of *Culex pipiens, Anopheles sacharovi*, and *Culex tritaeniorhynchus* species.

Sites	Locality number	Coordinates	Species	Fed	Unfed	Total
Mersin-Huzurkent	1	36°53'01.0"N 34°49'32.3"E	*Anopheles sacharovi*	12	0	12
			Culex tritaeniorhynchus	10	0	10
			Culex pipiens	10	0	10
Osmaniye-Düziçi	2	37°14'53.0"N 36°27'12.8"E	*Anopheles sacharovi*	10	2	12
			Culex tritaeniorhynchus	10	4	14
Manisa-Akhisar	3	38°55'17.5"N 27°50'14.9"E	*Anopheles sacharovi*	8	0	8
Muğla-Dalaman	4	36°49'04.6"N 28°48'40.8"E	*Anopheles sacharovi*	5	0	5
			Culex tritaeniorhynchus	10	0	10
			Culex pipiens	10	2	12
Isparta-Gelendost	5	38°06'33.4"N 30°56'48.6"E	*Anopheles sacharovi*	10	2	12
			Culex pipiens	10	0	10
İzmir-Selçuk	6	37°56'22.3"N 27°22'27.3"E	*Anopheles sacharovi*	5	0	5
			Culex pipiens	10	3	13
Adana-Karataş	7	36°34'15.8"N 35°23'49.4"E	*Anopheles sacharovi*	8	0	8
Uşak-Eşme	8	38°24'21.0"N 28°57'45.9"E		10	5	15
Kahramanmaraş-Türkoğlu	9	37°22'40.7"N 36°50'23.6"E		10	0	10
Hatay-Dörtyol	10	36°49'26.0"N 36°12'23.7"E		10	4	14
Hatay-Kırıkhan	11	36°29'03.4"N 36°21'57.8"E	*Anopheles sacharovi*	10	5	15
			Culex tritaeniorhynchus	10	5	15
Antalya-Manavgat	12	36°47'25.8"N 31°26'31.6"E	*Anopheles sacharovi*	8	2	10
			Culex tritaeniorhynchus	10	2	12
			Culex pipiens	10	0	10
Afyon-Merkez	13	38°36'14.1"N 30°43'40.1"E		10	3	13
Mersin-Tarsus	14	36°56'04.4"N 34°53'33.7"E		10	0	10
Osmaniye-Kadirli	15	37°21'19.0"N 36°04'37.2"E	*Anopheles sacharovi*	10	1	11
Aydın-Merkez	16	37°48'47.3"N 27°50'05.6"E		10	2	12
Adana-Kozan	17	37°26'39.1"N 35°47'58.7"E		10	0	10
Afyon-Sandıklı	18	38°27'48.1"N 30°16'28.7"E		10	2	12

Table 1. (Continued)

Afyon-Dinar	19	38°04'18.0"N 30°10'12.4"E	*Anopheles sacharovi*	10	4	14
			Culex pipiens	8	0	8
Uşak-Merkez	20	38°40'49.6"N 28°56'10.2"E	*Anopheles sacharovi*	10	0	10
Adana-Ceyhan	21	37°01'27.4"N 35°48'32.4"E		10	3	13
Antalya-Merkez	22	36°54'34.2"N 30°41'41.1"E		10	4	14
Adana-Tuzla	23	36°41'39.8"N 35°05'22.3"E	*Culex tritaeniorhynchus*	10	0	10
			Culex pipiens	10	0	10
İzmir-Merkez	24	38°25'00.8"N 27°11'44.5"E		10	2	12
Aydın-Söke	25	37°45'37.5"N 27°23'25.5"E		10	0	10
Aydın-Kuşadası	26	37°52'09.2"N 27°16'49.6"E	*Culex pipiens*	10	0	10
Denizli-Akköy	27	37°57'02.0"N 29°04'47.9"E		10	4	14

Aydın, Osmaniye, Uşak, İzmir, Muğla, Hatay; for *Cx. pipiens* in Aydın and for *Cx. tritaeniorhychus* in Mersin and Muğla populations; primarily involving human/animal combinations for all mosquito species (Table 2).

The results showed that 186 *An. sacharovi* females were fed mostly from cows (86.6%) compared to humans (6.5%) in the sampling localities. No blood meal was taken from other potential hosts. For *Cx. pipiens* females fed mostly from cows (84%) comparing to humans (10.4%); no feeding on equine, or dogs was observed in all villages. For *Cx. tritaeniorhychus* females mostly from cows (98.3%) (Figure 2).

The feeding rates were statistically different between the localities. Comparative analysis of host preference across various locations revealed cow blood as the primary target for the mosquito species studied (χ^2 = 120.935, p < 0.001). This trend was consistent within each locality, with all collected mosquitoes exhibiting a preference for cow hosts compared to other available options in the surrounding area (χ^2 = 24.401, p < 0.001). Due to the predominance of humans, bovine, ovine, equine and chickens, the following analyses are carried out on these five vertebrate hosts (Figure 3).

Table 2. Origin of blood meals of mosquitoes from all studied localities.

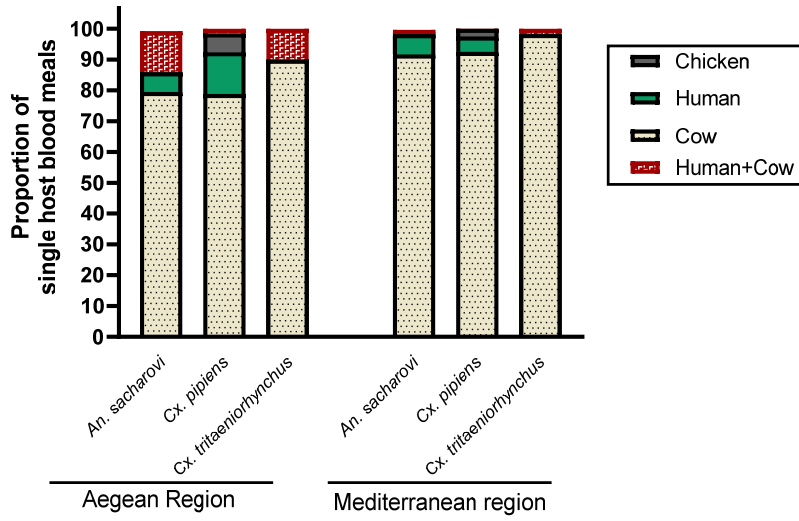| Host	*An. sacharovi*	*Cx. pipiens*	*Cx. tritaeniorhychus*
Human	13 (6.5%)	11 (10.4%)	0
Cow	161 (86.6%)	89 (84.0%)	79 (98.3%)
Chicken	0	5 (4.7%)	0
Dog	0	0	0
Horse	0	0	0
Total single host blood meals	174	105	79
% of single host blood meals	93.0	94.3	98.3
Human + cow	12	1	1
% of multiple host bloodmeals	6.5	0.94	1.7
	Total (n = 186)	Total (n = 106)	Total (n = 80)

Figure 2. Percentage distributions of single and multiple host meal choices for three mosquito species collected in the Aegean and Mediterranean regions.
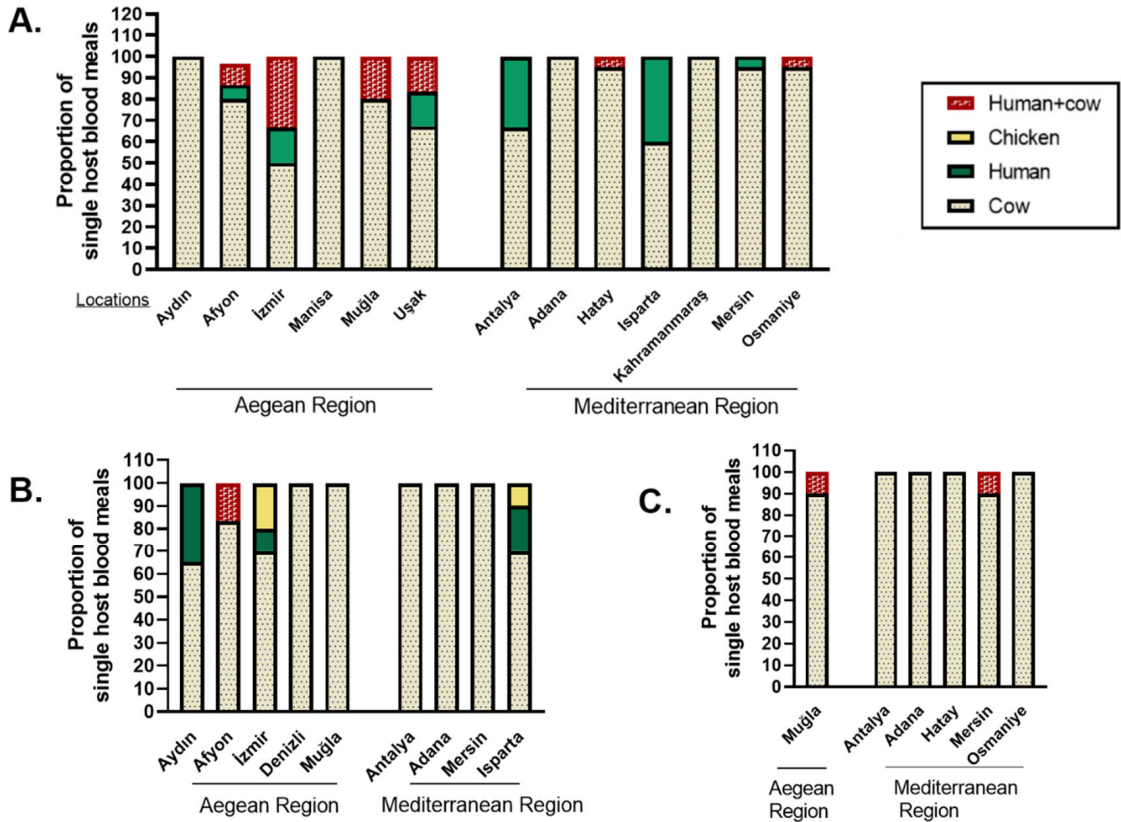

Figure 3. The percentage distribution of single and multiple host meal choices of A) *An. sacharovi*, B) *Cx. pipiens*, C) *Cx. tritaeniorhynchus* between humans and cows in both the Aegean and Mediterranean regions.

4. Discussion

Identifying host preferences is crucial for understanding how diseases spread to humans. Mosquito species exhibit varying feeding preferences. Some species are generalists and others opportunistically feed on any available host, including humans, livestock, wildlife, or birds (Stone and Gross, 2018). Certain species including *Ae. aegypti, Cx pipiens, An. funestus* and *An. gambiae*, demonstrate a more defined preference for specific host types (Yan et al., 2021). Analyzing a mosquito's blood meal offers a valuable approach to identifying potential and preferred hosts. Feeding preferences are a key factor influencing mosquito host selection and, the likelihood of a host encountering a mosquito (Guta et al., 2021). While mosquito host selection has a genetic basis, with species responding to specific cues (Clements, 1999), real-world expression of these preferences is also influenced by numerous environmental factors including the selection of sampling sites (influenced by host availability, both indoors and outdoors) and the trapping methods employed (Fikrig and Harrington, 2021). Kramer and Ciota (2015) and Keven et al. (2017) emphasize that the transmission potential of vector-borne pathogens by mosquitoes depends heavily on three key factors: host availability, density, and accessibility. Moreover, recent studies showed that when preferred hosts are scarce, mosquitoes may turn to alternative animal hosts for survival (Mbewe et al., 2022).

Over the years, detection of blood meal using PCR (polymerase chain reaction) and ELISA-based methods have been the two of the most used methods as they are both highly sensitive, specific and can be used to simultaneous to test multiple samples (Bursalı and Şimşek, 2022). However, ELISA is a simple and easily automated technique that utilizes the specific bond between a foreign antigen and antibody to detect target molecules in biological samples. Unlike PCR, it can be used to indicate previous exposure to molecules even after they are no longer present in the sample. It is time efficient and can be standardized and quantified (Shah and Maghsoudlou, 2016; Perestam et al., 2017; Alhajj et al., 2023). Despite these advantages, few studies have used the ELISA method to determine the blood meals of mosquitoes (Beier et al., 1988; Mbewe et al., 2022; Gueye et al., 2023).

This study determined the natural feeding patterns of *Cx. pipiens*, *An. sacharovi*, and *Cx. tritaeniorhynchus* mosquito populations from the Aegean and Mediterranean regions of Türkiye using the ELISA method. A total of 435 blood-fed mosquitoes were collected from various locations between May 2021 and September 2023. Mosquitoes are analyzed to identify the blood source of these wild type populations. Our results showed that cow blood meals were found as the most common feed for all tested mosquito species (*An. sacharovi:* 86.6%; *Cx. pipiens*:

84%; *Cx. tritaeniorhynchus*: 98.3%). Additionally, human blood meals were identified in all three species at much lower frequencies (<10.4) compared to the preference ratio for cow blood. Mixed blood meals from two different hosts (human and cow blood) were observed in a small percentage (4%). Our data is similar to the results obtained in our previous study investigating the feeding patterns of the populations of the same mosquito species using multiplex PCR method (Bursalı and Şimşek, 2022). Blood-fed females were collected from rural barns, houses, and chicken coops between May 2017 and September 2019. Mitochondrial cytochrome b gene analysis of 445 blood-fed *An. sacharovi* revealed the host selection for cow was the primary host (434), followed by birds (9) and dogs (2). Among 216 captured blood-fed samples of *Cx. tritaeniorhynchus* was detected as fed on cows. Among 97 blood-fed *Cx. pipiens*, 91 of them was fed on birds while 6 fed on horses.

Based on the results, *An. sacharovi* revealed a predominance of single blood meals, derived from either humans or animals, compared to mixed meals containing blood from both sources. These findings concur with observations reported by Ngom et al. (2013) and Gueye et al. (2023) in other Senegalese locations. However, these results were also contrasted with the study of Konate et al. (1999) who documented a prevalence of mixed meals in Dielmo. Notably, the vast majority (93.55%) of identified blood meals originated from animals, with only a minor fraction (6.45%) sourced from humans. Interestingly, cows emerged as the clear preferred host across all studied locations and mosquito species. Furthermore, findings of this study align with previous research by Massebo et al. (2015), Finney et al. (2021), and Gueye et al. (2023), which are collectively highlighting the predominant zoophagic behavior of *Anopheles* mosquitoes in these regions. Conversely, a study in Iran by Yaghoobi-Ershadi et al. (2001) reported that a significant portion of *An. sacharovi* females was found as feeding on humans (ranging from 7.52% to 38% depending on location) using the ELISA method. Furthermore, some individuals had mixed blood meals, suggesting they entered dwellings after acquiring their initial blood meal outdoors (Saili et al., 2023). This observed zoophagic behavior in *An. sacharovi* contradictory to the previously documented highly anthropophilic nature of *An. gambiae* and *An. funestus* (Konate et al., 1994), presenting a challenge for malaria control efforts. Asale et al. (2017) attributed this shift in feeding preference to the widespread implementation of control measures, potentially forcing mosquitoes to seek alternative readily available hosts like livestock. Despite the abundance of chickens and birds in the study area, no blood meals from these avian species were detected in *An. sacharovi*, which aligns with previous observations in the region (Konate et al., 1994).

The presence of domestic animals has been associated with a decrease in malaria transmission rates due to zoophilic deviation (Bruce-Chwatt and De Zulueta, 1980). Cows were found to be the most common vertebrate hosts. Host similarities were detected in the host-feeding patterns (Bedir et al., 2022). While chickens and birds found to be the most commonly encountered potential domestic hosts in both studied villages, no blood meals from these animals were identified in the analysis of *An. sacharovi*. This finding suggests that, despite their high density, these birds were not preferred by *An. sacharovi*. This selectivity likely stems from the accessibility and abundance of cow hosts, which are commonly kept near human dwellings (Konate et al., 1994). Conversely, cow blood meals were significantly more prevalent, likely due to the higher abundance of cattle in the villages. Notably, cattle were kept close to or even inside residences, making them readily accessible to the *Anopheles* mosquitoes compared to other potential hosts. Boreham and Garrett-Jones (1973) performed a study in the Greek village; the results of their precipitation test study with *An. sacharovi* revealed three different biotopes (sheep/goat, horse and pig). Studies investigating the anthropophilic index (AI) of *An. sacharovi* revealed geographic variability in its host-feeding behavior. Early reports from Iran (Zahar, 1974) documented AI values ranging from 4.2% to 30.6%, but the information on blood meal source identification is lacking. Edrissian et al. (1985) observed a higher human blood feeding rate (26.5%) in Iranian dwellings compared to animal shelters (9.4%). Similar variations are evident across other regions. Bruce-Chwatt et al. (1966) reported human blood meal proportions of 5.6% and 30.5% in Greece and Syria, respectively. Hadjinicolaou and Betzios (1973) further supported this trend in Greece, with human blood feeding reactions found in 38.5% of mosquitoes collected from human dwellings compared to only 1.1% from animal shelters. Demirhan and Kasap (1995) observed an HPI (host preference index) of less than one for humans in Türkiye, suggesting a preference for alternative hosts when available. Tavşanoğlu and Çağlar (2008) also reported no significant difference in host preferences within Turkish villages, with precipitin tests indicating a high animal blood index (cattle/sheep) from houses, likely due to the proximity of human settlements and livestock enclosures.

Research on *Culex* mosquito feeding preferences across America, Europe, and Portugal (Muñoz et al., 2011; Guta et al., 2021) reveals significant variations in host selection within *Cx. pipiens* populations. Blood feeding behavior ranges from primarily targeting birds to predominantly feeding on humans. In this study, the majority of the *Cx. pipiens* (84%) and *Cx. tritaeniorhynchus* (98.3%) preferred cow blood in their abdomen and no blood meals from sheep, horses, or dogs were detected in *Cx. pipiens*. In contrast, studies by Guta et al. (2021) in Ethiopia identified a higher proportion of human blood meals (33.2%) compared to cow meals (15.2%) in *Culex*. Also, in various studies conducted in America and Europe, the avian host spectrum of *Cx. pipiens* determined as 64–97%, and avian host preferences found to be important compared to mammalian hosts (Figuerola et al., 2007; Vázquez et al., 2010; Gómez-Díaz and Figuerola, 2010). Conversely Zimmerman et al. (1985) reported that *Culex pipiens* and *Cx. antennatus* fed exclusively on mammals (human, cow/buffalo, sheep/goat/hose/donkey, dog, cat, and rat, totally 98.7%). In Portugal, Gomes et al. (2013) demonstrated that *Cx. pipiens molestus* and *Cx. pipiens pipiens* forms primarily fed on Passeriformes birds (90%). Similar trends were observed in other regions. Muñoz et al. (2011) collected 65 *Cx. pipiens* from various regions of Spain. They identified 43 of the *Cx. pipiens* samples as positive for host blood; with mt-COI sequence analysis, the host species distribution was determined in the mammalian group as dog, cat, and human; in the poultry group, they determined them as pigeons, chickens, sparrows, doves, and blackbirds. In Türkiye, while studying the host preference of *Cx. pipiens* complex, Korkmaz et al. (2016) showed that positive specimens were determined to be positive for only mammalian, avian, and both avian and mammalian blood, respectively. Avian host preference in blood meal of the specimens belonging to *Cx. pipiens* was found to be significant. Also, Bedir et al. (2022) collected different mosquito species from Aras Valley; they used the PCR-based reverse line blotting method and their results showed that these mosquito species fed on eight mammal species, i.e. humans, cows, sheep, horses, dogs, cats, goats, and porcupines, as well as avian species.

Likewise, *Cx. tritaeniorhynchus* displayed a stronger preference for human blood meals, albeit with regional variations documented in Senegal (Gordon et al., 1991). Tuno et al. (2017) conducted controlled laboratory experiments using *Cx. tritaeniorhynchus* and observed a preference for cow blood meals (65.2–66.1%) over pigs (42.4–56.6%). Interestingly, 3067 blood-engorged *Cx. tritaeniorhynchus* were collected from Kerala in southern India, fed mainly (56.6%) on cattle. Pig feeding accounted for 6.3% of the total. Mosquito samples also revealed serologic mixed origin, mixed blood meals were mostly (96.7%) from cattle and goats (Arunachalam et al., 2005). The high proportion of multiple feeding of exophilic vectors such as *Cx. tritaeniorhynchus* on dampening (dead-end) hosts such as cattle and goats may impede the transmission of JE virus to humans by diverting host-seeking mosquitoes away from potential hosts such as pigs and birds.

Also, Pennington and Phelps (1968) were performed a study in Okinawa, Ryukyu Islands; 20,522 *Cx.*

tritaeniorhynchus blood meals were identified. Their results showed that *Cx. tritaeniorhynchus* generally fed on larger domestic animals such as cows and pigs. Over 80% of the mosquitoes tested had fed on cows and pigs. Ramesh et al. (2015) used the ELISA method and revealed that *Cx. tritaeniorhynchus* primarily fed on cows (94% and 81% in two regions, respectively) with a negligible preference for goats (0.5% and 0.75%). Additionally, their study did not detect the feeding preference of *Cx. tritaeniorhynchus* on humans, dogs, or pigs.

This study demonstrated the exophagic, zoophagic and opportunistic behaviors of important *Anopheles* and *Culex* mosquitoes from Türkiye. Determining the blood sources of such vectors is essential when designing new vector control strategies. Further investigation is necessary to understand the specific feeding patterns of *Anopheles* and *Culex* mosquitoes in the region, considering their potential for human blood feeding. Also, more studies should investigate the feeding host profile of outdoor resting populations and how the presence of certain animals may influence disease epidemiology.

Conflict of interest
The authors declare that they have no conflict of interest.

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