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Host-Feeding Patterns of Mosquito Species in Aras Valley, Turkey

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ABSTRACT

Identifying hosts of blood-feeding mosquito species is important to elucidate the role of vector species in disease transmission cycles. Herein, the aim was to identify the vertebrate hosts of mosquito species using the polymerase chain reaction-based reverse line blotting method. The mosquito species were *Anopheles maculipennis* sensu lato Meigen, 1818; *Aedes caspius* Pallas, 1771; *Aedes vexans* Meigen, 1830; *Culex theileri* Theobald, 1903; *Anopheles hyrcanus* Pallas 1771; *Culex pipiens* sensu lato Linnaeus, 1758; and *Culiseta annulata* Schrank, 1776, which were collected from the Aras Valley between July-August 2012 and June-September 2013. The analysis of mosquito blood-meal samples revealed several mammalian and avian host species. And also varying degrees of opportunistic mosquito feeding behavior in both birds and mammals were noted. These mosquito species fed on eight mammal species, i.e., humans, cows, sheep, horses, dogs, cats, goats, and porcupines, as well as avian species. Studies on the host-feeding patterns of blood-feeding vector arthropods may provide information about the vector potential of these species. However, the host-feeding patterns of the vector species are only one part of the vector capacity. To detect the exact potential of vector species, further studies are needed on vector competence for different pathogens.

Key words: Aras Valley, host-feeding patterns, mosquito species, reverse line blotting, vector biology.

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INTRODUCTION

The host-feeding patterns define the hosts that mosquitoes feed on in their natural habitats and can be influenced by numerous intrinsic and extrinsic factors such as host availability, host defences and mosquito host preference (Lyimo & Ferguson, 2019). The host-feeding patterns of mosquitoes are important for humans in the epidemiology of mosquito-borne pathogens. Female mosquitoes require blood to develop eggs; therefore, they obtain blood meal from a variety of hosts, such as humans and/or animals. The degree of contact with different vertebrate hosts may result in the adaptation and transmission of mosquito-borne parasites or pathogens to susceptible vertebrate species (Kilpatrick et al, 2013). Species exhibiting strong and genetic-based host selection behavior are the most important vectors of infectious diseases, and it has been predicted that this behavioral trait may have evolved parallel to host–parasite coevolution (Takken & Verhulst, 2013). The host-feeding patterns of mosquitoes are directed by various genetic and ecological factors, e.g., environmental conditions and host characteristics that affect host-seeking and selection patterns (Clements, 1992; Takken &Verhulst, 2013).

Determining the variety of vertebrate hosts as blood-meal sources for mosquitoes is essential to understand the dynamics of mosquito-borne diseases and to describe the role of various mosquito species in being a nuisance for humans and animals (Mukabana, Takken, & Knols, 2002).

In Turkey, various native mosquito species have been identified and demonstrated as potential pathogen and exotic vectors, including *Aedes albopictus* (Skuse, 1895) and *Aedes aegypti* (Linnaeus, 1762) (Akıner, Demirci, Babuadze, Robert, & Schaffner 2016). Virus screening of *Ae*. *albopictus* samples collected from several locations in the Black Sea Region of Turkey from 2016-2017 revealed the presence of West Nile virus (WNV), *Aedes flavivirus*, and the cell fusing agent virus. Merida-like virus Turkey, a recently described novel rhabdovirus, was also co-detected in a *Culex pipiens* sensu lato Linnaeus, 1758 pool that was positive for WNV (Akıner et al, 2019).

WNV is an extremely important virus that maintains its cycle between mosquitoes, birds, and mammals, such as humans and horses, as dead-end hosts because they do not develop sufficient quantities of the virus in their blood to infect mosquitoes that may feed on them, and horse-to-horse, horse-to-person, and person-to-person virus transmission does not occur.

Although several wild and domestic avian species are susceptible to WNV infection, birds often serve as natural reservoir hosts for WNV infections (Mihailović et al, 2020). In Europe, *Cx*. *pipiens* s.l. and *Aedes caspius* are the most important vectors associated with WNV, whereas *Culex theileri* Theobald, 1903 and *Anopheles maculipennis* sensu lato Meigen, 1818 are suggested to play a potential role in virus circulation (Calistri et al, 2010; Engler et al, 2013). WNV is common in Turkey, and *Cx*. *pipiens* s.l. and *Ae*. *caspius* Pallas, 1771 are potential vector species for WNV, similar to that in Europe (Ergünay et al, 2017; Öncü et al, 2018; Akıner et al, 2019). Furthermore, WNV-neutralizing antibodies were detected at a rate of 9.9% in ducks in Kars Province, which is located

at the border of our study region (Ergünay et al, 2014). The Aras Valley is located on one of the most important migratory bird routes in the world and provides nesting and breeding grounds for several bird species that migrate to the Middle East and Africa annually from Russia and the Caucasus (Şekercioğlu, 2008). The movement of infected migratory birds may play a role in the introduction and spread of arboviruses, such as WNV, on the European mainland (Calistri et al, 2010).

Previously, the presence of *Dirofilaria immitis* (Leidy, 1856) and *Dirofilaria repens* Railliet & Henry, 1911 (Nematoda: Filarioidea) DNA was shown in *An. maculipennis* s.l., *Ae. caspius*, *Ae. vexans*, *Cx. theileri*, *Cx. pipiens* s.l., and *Anopheles hyrcanus* Pallas 1771 in the Aras Valley (Demirci, Bedir, Taskin Tasci, & Vatansever, 2021). *D. immitis* and *D. repens* are present worldwide and cause cardiopulmonary and subcutaneous dirofilariasis in canines, felines, and other mammalian carnivores (Simon et al, 2012). Several mosquito species from different genera, such as *Anopheles*, *Aedes*, and *Culex*, have been identified as potential vectors for *Dirofilaria* spp. around the world (Simon et al, 2012).

The information presently available on the host-feeding patterns of mosquito species in Turkey is based on only two studies. One of them reported the host species preferences of blood-feeding mosquitoes belonging to *Cx. pipiens* s.l. collected from the Central Anatolia Kayseri province in Turkey (Korkmaz et al, 2017), and the other reported the host species preferences of blood-feeding mosquitoes belonging to *Cx. pipiens* s.l., Favre, 1903 and *Culex tritaeniorhynchus* Giles, 1901, from the Mediterranean and Aegean Regions in Turkey (Bursalı, 2020).

The present study was performed to determine the host-feeding patterns of mosquito species in the Aras Valley. The blood-meal hosts of seven mosquito species have been reported through blood-meal identification using reverse line blotting (RLB). This technique has allowed the successful identification of blood hosts of the tick *Ixodes ricinus* (Acari: Ixodidae) (Humair et al, 2007). RLB facilitates the simultaneous amplification of different species using polymerase chain reaction (PCR) and then distinguishes them using species-specific oligonucleotides. Therefore, RLB involves a hybridization step following PCR amplification, and it shows higher sensitivity than PCR alone. The use of chemiluminescent reagents instead of radioactive substances in imaging does not lead to harmful effects in people who perform this technique, thereby making the technique more user-friendly. In addition, the oligonucleotide-containing blot can be reused at least 20 times, thus providing an economic advantage for the users (Georges et al, 2001).

MATERIALS AND METHODS

Study region

Samples were collected from six villages in the Aras Valley, where the Iğdır Plain is located: Mürşitali, Küllük, Zülfikarköy, Pirli, Yukarıçamurlu, and Yukarıçıyrıklı (Fig. 1 and Table 2). The municipality authorities were informed about mosquito trapping, and sampling was performed on private lands after permission was obtained from the owner.

The Aras Valley, located in Northeast Anatolia, Turkey, is an important ecological corridor considered the entrance route for desert fauna into Anatolia (Demirsoy, 2002). The Aras Valley has high population densities of mosquito species due to the intense irrigation of agricultural lands and suitable climate conditions. Furthermore, sustainable agriculture and livestock activities host many cattle, sheep, goats, and poultry. Moreover, from a biological viewpoint, this region is an important basin located on the migratory route of several birds migrating from Russia and the Caucasus to the Middle East and Africa every autumn and returning in the spring (Şekercioğlu et al, 2011).

Fig. 1. Map showing the location of the six villages in the Aras Valley.

Mosquito collection, identification, and storage

Mosquitoes were collected in 2012 (July-August) and 2013 (June-September) using New Jersey light traps (JW Hock Company, Gainesville, FL) and mouth aspirators. During the sampling period approximately 3 traps (i.e., close to the barns or in the barns, close to the houses and open areas neighboring croplands where people work) were operated for each village for one night per month. In addition, indoor resting mosquitoes were sampled with mouth aspirators by two experienced collectors from the walls and ceilings in the same sampling sites where light traps catches were conducted. Morphological identification of the Culicidae was done mainly using the electronic identification key of Schaffner et al (2001). The samples were stored at -80°C until DNA extraction.

DNA extraction

Mosquitoes were scanned under a stereo microscope for abdomens that contained blood. The abdomens of blood fed female mosquitoes were excised and removed from the head/body sections using sterile forceps and placed in sterile 1.5-ml microtubes. Blood samples of vertebrates obtained from the Faculty of Veterinary Medicine, Kafkas University, with the approval of the Ethics Board, Kafkas University, Kars, Turkey, were

used as positive control samples. Genomic DNA was isolated from whole blood using the Purelink® Genomic DNA Purification Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

Species-specific probes

Species-specific 5′-amino-linked oligonucleotide probes were designed by Abbasi et al, (2009) according to mammalian and avian vertebrate cytochrome b (CytB) sequences obtained from the direct sequencing of PCR products (Table 1).

Table 1. Species-specific oligonucleotide probes designed to identify different vertebrate blood types based on their hybridization with the CytB PCR product.

Species	Oligonucleotide sequence
Human (*Homo sapiens*)	5'-amino-ATG CAC TAC TCA CCA GAC GC
Cow (*Bos taurus*)	5'-amino-ATT ATG GGT CTT ACA CTT T
Sheep (*Ovis aries*)	5'-amino-TCC TAT TTG CGA CAA TAG CTTCCT
Goat (*Capra hircus*)	5'-amino-ATA CAT ATC GGA CGA GGT CTA
Dog species	5'-amino-CAG ATT CTA ACA GGT TTA
Horse/Donkey *(Equus asinus)*	5'-amino-CTA CTT TTC ACA GTT TAG CTA CA
Domestic cat (*Felis domesticus*)	5'-amino-CAT TGG AAT CAT ACT ATT
Porcupine (*Hystrix indica*)	5'-amino-ACA CTG CCT ACA CAA CTA CA
Brown rat (*Rattus rattus*)	5'-amino-CAG TCA CCC ACA TCT GC
Rock hyrax (*Procavia capensis*)	5'-amino-CCT ATT CTT CGT ATG TCT TTA
General avian probe 1 (Avian)	5'-amino-TAC ACA GCA GAC AC
General avian probe 2 (Avian)	5'-amino-GCC TCA TTC TTC AT
Domestic chicken (*Gallus gallus*)	5'-amino-CAT CCG GAA TCT CCA C
Rock pigeon (*Columbia livia*)	5'-amino-ACT ACT CGC CGC ACA TTA
House sparraow (*Passer domesticus*)	5'-amino-GAG ACG TCC AAT TCG GAT
Rock partridge (*Alectoris graeca*)	5'-amino-TCA CCG GCC TCC TCC TA
Turkey (*Meleagris gallopavo*)	5'-amino-CTT CTG TGG CCA ACA CAT

Polymerase chain reaction and reverse line blotting

Universal primers designed for the PCR amplification of 344 base pairs of the conserved region of CytB were used for amplification: Cyto1: 5′-CCA TCA AAC ATC TCA GCA TGA AA-3′ and Cyto2: 5′-biotin-CCC CTC AGA ATG ATA TTT GTC CTC-3′. The 50-µL PCR reaction included the following components: 5 U/µl Taq DNA polymerase (0.4 µl), 10 mM dNTPs (1 µl), 25 mM $MgCl_2$ (1.5 µl), each primer (1 µl), genomic DNA (2 µl), and dH_2O (38.1 µl). The thermal cycling conditions were as follows: initial denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, primer extension at 72°C for 1 min; and final extension at 72°C for 8 min. The obtained PCR products were used as probes in RLB hybridization reactions.

The Biodyne C membrane (Pall Biosupport, Germany) was activated using 10 ml of 16% 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDAC; Carl Roth GmbH, Karlsruhe, Germany) for 9 min at room temperature. The activated membrane was washed with distilled water for 30 s and placed in the Miniblotter. The liquid remaining on the membrane (EDAC and distilled water) was aspirated. Each probe channel (150 µl), diluted to 50-1200 pmol/150 ml in 500 mM NaHCO3 (pH 8.4), was added to the Miniblotter channels, excluding the first and last; 4% ink diluted with 2X SSPE and 0.5% SDS was added into the first and last channels. After the loading process, the membrane was incubated for 5 min at room temperature and then aspirated. The membrane was washed with 100 ml 2X SSPE and 0.1% SDS at 60°C for 5 min. The oligonucleotide-immobilized membrane was rotated 90° in the opposite direction of the probes and placed in the Miniblotter, and the liquid was aspirated. Thereafter, 20 µl of PCR product was diluted with 150 µl 2X SSPE and 0.1% SDS. The diluted PCR products were denatured at 100°C for 10 min in a PCR thermal cycler. The denatured PCR products were immediately cooled on ice for 5 min to prevent reattachment of the DNA strands. Then, the PCR products were added to the Miniblotter channels and allowed to hybridize for 30 min at 75°C and for 60 min at 53°C in an incubator. The membrane was washed twice with 100 ml 2X SSPE and 0.5% SDS at 62°C in 10-min intervals, followed by incubation with 15 ml 2X SSPE and 0.5% SDS with 3 µl peroxidase-labeled streptavidin dilution at 42°C for 1 h in the hybridizer. Thereafter, the membrane was washed twice with 100 ml 2X SSPE and 0.5% SDS at 62°C in 10-min intervals and then washed twice with 100 ml 2X SSPE at 5-min intervals. Subsequently, the membrane was placed in 10 ml ECL detection liquid (Roche Diagnostics, Mannheim, Germany) for 5 min at room temperature. Finally, an X-ray image was obtained by placing the membrane between two acetate papers in a film cassette placed under Hyperfilm for 30 min. The formation of black spots upon the union of oligonucleotide probes to PCR products after the development of the film was considered positive. To control the sensitivity and specificity of the RLB, control RLB assays were performed with CytB products amplified from positive controls. Of the mammalian species-specific CytB products, only goat CytB PCR products showed faded cross-hybridization with sheep probes. While all other avian products hybridized with their own probes, only the partridge-specific probe showed some cross-reactivity with chicken DNA. However, while the chicken samples amplified with both Avian 1 and Avian 2 probes, the partridge samples amplified with only Avian 2 probes. Additionally, to avoid the consequences of human DNA contamination during collection, identification, extraction, etc., human signals were considered positive when they were strong.

To remove the PCR products from the membrane, it was washed twice in 100 ml 1% SDS at 80°C for 30 min. Then, it was rinsed in 240 ml 20 mM EDTA at room temperature for 15 min. Finally, the membrane was sealed in a plastic bag with 10 ml 20 mM EDTA and refrigerated at 4°C for future re-use.

RESULTS

In total, 26,654 mosquito species were sampled during this study and of those, 11,403 were mosquitoes were visually determined to contain blood (Completely engorged, semi engorged and nearly digested blood fed females). Only completely engorged and semi engorged females were chosen for this study (916 mosquitoes). Of those, 655 (71.50%) representing seven species belonging to four genera were positive for CytB PCR (Table 2). The mosquitoes that were negative for PCR may have had dried blood in which DNA could not be extracted or < 0.1 pg DNA was present in the ingested blood (Abbasi et al, 2008). A gel image of CytB PCR-targeting DNA, extracted from blood-fed mosquitoes, is shown in Fig. 2. The results of mammalian and avian-derived blood meals are shown in Tables 4 and 5, respectively. The mosquitoes were found to feed on eight mammalian (i.e., humans, cows, sheep, horses, dogs, cats, goats, and porcupines) and several avian species. Opportunistic feeding behavior on both mammalian and avian hosts was detected at varying degrees, except for *Cs*. *annulata* and *An*. *hyrcanus* (Table 3). These two species fed only on mammalian species, whereas the other five species (*An*. *maculipennis* s.l., *Ae. vexans*, *Ae*. *caspius*, *Cx*. *pipiens* s.l., and *Cx*. *theileri*) fed on both mammalian and avian hosts.

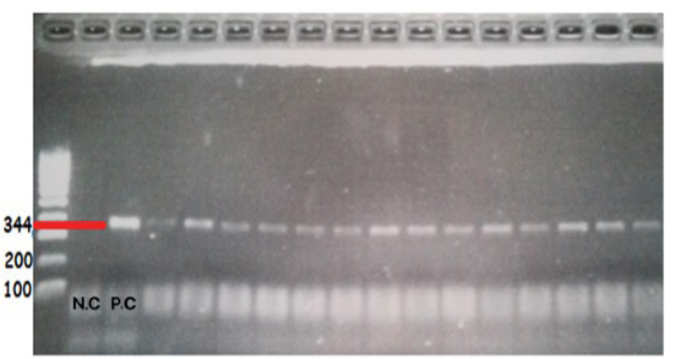

Fig. 2. PCR products of vertebrate cytochrome b sequences (344 bp) in the presence of mosquito species genomic CytB DNA- P.C-Positive control, N.C-Negative control.

The blood-meal samples belonging to mammalian and avian hosts predominantly included blood meal from humans (83.81%), followed by cow (53.28%), sheep (5.19%), partridge (4.88%), pigeon (4.12%), cat (1.06%) unidentified avian (0.61%), sparrow (0.45%), horse (0.30%), goat (0.15%), dog (0.15%), and porcupine (0.15%; Tables 4 and 5). Single and mixed blood-meal sources are shown in Table 6.

The pattern for single hosts was as follows: 219 (33.43%) of human blood, 97 (14.80%) of cow blood, 5 (0.76%) of sheep blood, 1 (0.15%) of horse blood, and 1 (0.15%) of pigeon blood. Mixed blood meal from two, three, and four hosts were also identified as follows: 234 (35.72%) of humans/cows, 24 (3.66%) of humans/sheep and humans/partridges, 6 (0.91%) of humans/pigeons, 4 (0.61%) of humans/avian, 1 (0.15%) of humans/goats, pigeons/partridges, and chickens/pigeons, 1 (0.15) of humans/cows/pigeons, 4 (0.61%) of humans/cows/sheep and humans/cats/pigeons, 2 (0.30%)

of humans/cows/cats, humans/cows/chickens, and humans/sheep/pigeons, 1 (0.15) of humans/cows/horses, humans/cows/dogs, humans/partridges/pigeons, humans/chickens/pigeons, humans/cows/partridges, and humans/cows/avian, 4 (0.61%) of humans/sheep/pigeons/partridges, and 1 (0.15%) of humans/cows/partridges/pigeons, humans/cows/cats/pigeons, and humans/pigeons/sparrows/porcupines (Table 6).

The seasonal proportion of human and cow-derived blood meal is presented in Fig. 3. The proportion of human-derived blood was significantly different between July and August, whereas the proportion of cow-derived blood was significantly different among all months except June and August ($p < 0.05$). The proportion of cow-derived blood decreased in July and August compared with that in June and September.

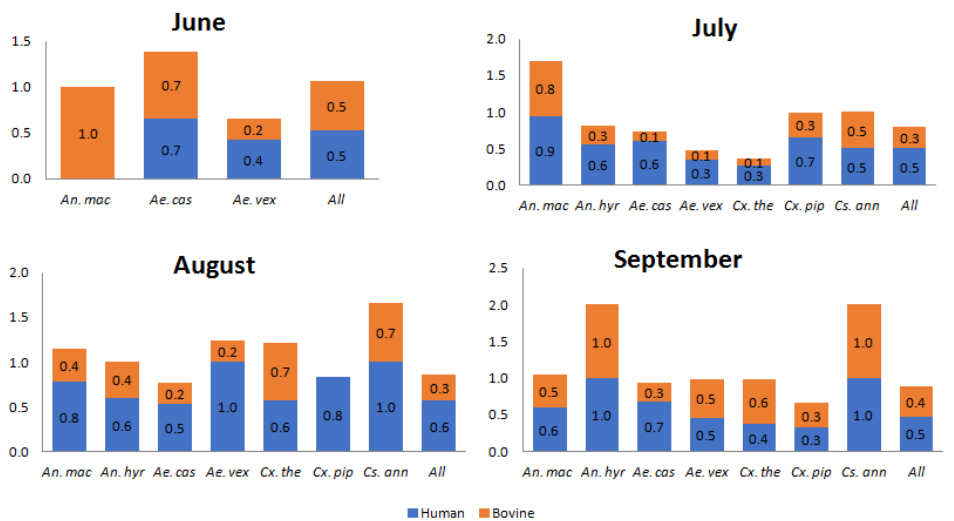

Fig. 3. The proportion of human and cattle-derived blood meals by months. *An. mac: An. maculipennis s.l., An. hyr: An. hyrcanus, Ae. cas: Ae. caspius, Ae. vex: Ae. vexans, Cx. the: Cx. theileri, Cx. pip: Cx. pipiens s.l., Cs. ann: Cs. annulata*, All: All species.

Table 2. CytB positive blooded mosquito species collected from six villages in the Aras Valley.

Month/Year	Sampling villages	Coordinates	*An. maculipennis* s.l.	*An. hyrcanus*	*Ae. caspius*	*Ae. vexans*	*Cx. theileri*	*Cx. pipiens* s.l.	*Cs. annulata*
July/2012	Yukarı Çıyrıklı	40.12773° 43.60654°	2	2					
	Pirli	40.06559° 43.78573°	71			1			
	Küllük	40.02663° 44.13784°				1			
August/2012	Pirli		23	3					
	Küllük		10						
June/2013	Pirli		1		4	9			
	Zülfikarköy	39.99232° 44.14654°			25	38			
	Yukarı Çıyrıklı			1					
July/2013	Yukarı Çıyrıklı			3	3				1
	Pirli			1					
	Mürşitali	42.636222° 44.306610°	20	2	26	21	21	5	
	Yukarı Çamurlu	39.94974° 44.39266°	3	2	35		28	1	2
August/2013	Küllük		7						
	Zülfikarköy			2	8	19	2	1	2
	Y. Çamurlu		2	1	30	11	32	3	
	Yukarı Çıyrıklı		38		2				
	Pirli					10	11		
September/2013	Küllük		3						
	Zülfikarköy					11	8	3	2
	Mürşitali		27	1	22	9	4		
	Yukarı Çamurlu				19				
Total			207	18	174	130	106	13	7

Table 3. Number of the blood-meal sources by host class identified (mammals/avians) from 7 species of mosquitoes collected in the Aras Valley.

Mosquito species	Total number of mosquitoes	Blood detected from only mammals	Blood detected from only avians	Blood detected from both mammals and avians
An. maculipennis s.l.	207	174	0	33
An. hyrcanus	18	18	0	0
Ae. caspius	174	149	1	24
Ae. vexans	130	129	0	1
Cx. theileri	106	103	1	2
Cx. pipiens s.l.	13	10	1	2
Cs. annulata	7	7	0	0
Total	655	590	3	62

Table 4. Types of mammalian-derived (included all single and mixed feedings) blood-meal sources identified from mosquitoes collected in the Aras Valley.

Mosquito species	Blood detected from humans	Blood detected from cows	Blood detected from sheeps	Blood detected from horses	Blood detected from goats	Blood detected from dogs	Blood detected from cats	Blood detected from porcupines
An. maculipennis s.l.	185	135	26	0	1	0	5	0
An. hyrcanus	14	4	0	2	0	0	0	0
Ae. caspius	146	72	5	0	0	0	0	1
Ae. vexans	115	57	1	0	0	1	2	0
Cx. theileri	72	73	2	0	0	0	0	0
Cx. pipiens s.l.	11	3	0	0	0	0	0	0
Cs. annulata	6	5	0	1	0	0	0	0
Total	549	349	34	3	1	1	7	1

Table 5. Number of avian-derived (included all single and mixed feedings) blood meals identified from mosquitoes collected in the Aras Valley.

Mosquito species	Blood detected from pigeons	Blood detected from sparrows	Blood detected from chickens	Blood detected from partridges	Other avian species
An. maculipennis s.l.	25	0	2	12	0
Ae. caspius	1	2	0	18	4
Ae. vexans	0	0	0	1	0
Cx. theileri	1	0	2	1	0
Cx. pipiens s.l.	3	0	0	0	0
Total	27	2	4	32	4

Table 6. Single and mixed blood meal sources identified from mosquitoes collected in Aras Valley.

Blood-meal sources	Species							
	***An. maculipennis* s.l. (%)**	***An. hyrcanus* (%)**	***Ae. caspius* (%)**	***Ae. vexans* (%)**	***Cx. theileri* (%)**	***Cx. pipiens* s.l (%)**	***Cs. annulata* (%)**	**Total (%)**
Humans	28 (4.27)	9 (1.37)	72 (11.0)	71 (10.8)	30 (4.58)	7 (1.07)	2 (0.30)	219 (33.4)
Cows	19 (2.90)	3 (0.46)	27 (4.12)	15 (2.29)	31 (4.73)	1 (0.15)	1 (0.15)	97 (14.8)
Sheeps	2 (0.30)	0	1 (0.15)	0	2 (0.30)	0	0	5 (0.76)
Horses	0	1 (0.15)	0	0	0	0	0	1 (0.15)
Pigeons	0	0	0	0	0	1 (0.15)	0	1 (0.15)
Humans/cows	101 (15.4)	4 (0.61)	45 (6.87)	39 (5.95)	40 (6.10)	2 (0.30)	3 (0.46)	234 (35.7)
Humans/sheeps	19 (2.90)	0	4 (0.61)	1 (0.15)	0	0	0	24 (3.66)
Humans/goats	1 (0.15)	0	0	0	0	0	0	1 (0.15)
Humans/partridges	6 (0.91)	0	17 (2.59)	1 (0.35)	0	0	0	24 (3.66)
Humans/pigeons	4 (0.61)	0		0	0	2 (0.30)	0	6 (0.91)
Humans/sparrows	0	0	2 (0.30)	0	0	0	0	2 (0.30)
Humans/avians	0	0	4 (0.61)	0	0	0	0	4 (0.61)
Chickens/pigeons	0	0	0	0	1 (0.15)	0	0	1 (0.15)
Pigeons/partridges	0	0	1 (0.15)	0	0	0	0	1 (0.15)
Humans/cows/ sheeps	4 (0.61)	0	0	0	0	0	0	4 (0.61)
Humans/cows/horses	0	1 (0.15)	0	0	0	0	1 (0.15)	2 (0.30)
Humans/cows/dogs	0	0	0	1 (0.15)	0	0	0	1 (0.15)
Humans/cows/cats	0	0	0	2 (0.30)	0	0	0	2 (0.30)
Humans/cows/ pigeons	7 (1.07)	0	0	0	0	0	0	7(1.06)
Humans/cows/ chickens	1 (0.15)	0	0	0	1 (0.15)	0	0	2 (0.30)
Humans/cows/ partridges	1 (0.15)	0	0	0	0	0	0	1 (0.15)
Humans/cows/avians	0	0	0	0	1 (0.15)	0	0	1 (0.15)
Humans/sheeps/ pigeons	2 (0.30)	0	0	0	0	0	0	2 (0.30)
Humans/cats/ pigeons	4 (0.61)	0	0	0	0	0	0	4 (0.61)
Humans/chickens/ pigeons	1 (0.15)	0	0	0	0	0	0	1 (0.15)
Humans/partridges/ pigeons	1 (0.15)	0	0	0	0	0	0	1 (0.15)
Humans/cows/cats/ pigeons	1 (0.15)	0	0	0	0	0	0	1 (0.15)
Humans/cows/ partridges/pigeons	1 (0.15)	0	0	0	0	0	0	1 (0.15)
Humans/sheeps/ pigeons/partridges	4 (0.61)	0	0	0	0	0	0	4 (0.61)
Humans/pigeons/ sparrows/porcupines	0	0	1 (0.15)	0	0	0	0	1 (0.15)
Total	207	18	174	130	106	13	7	655

CONCLUSIONS AND DISCUSSION

Feeding pattern studies based on blood meal analysis can be biased in several ways such as sampling site location (host absence/presence and indoor/outdoor) and trapping methods, leading to a predominant host that does not necessarily reflect innate mosquito preference for that host (Fikrig & Harrington, 2021). In the present study, seven mosquito species were collected from six sampling sites located in the Aras Valley in the Northeastern Anatolia Region of Turkey, which fed on eight mammalian (humans, cows, sheep, horses, dogs, cats, goats, and porcupines) and several avian species. Humans were found to be the most common vertebrate hosts. Host similarities were detected in the host-feeding patterns, i.e., all the mosquito species in the area shared mostly humans and cows host species, as previously highlighted in similar studies from Iran (Shahhosseini et al, 2018), Germany (Börstler et al, 2016), and Switzerland (Schönenberger et al, 2016). Human-derived blood meals accounted for > 83% of all feedings, and human and cattle hosts comprised nearly three-fourths of all analyzed blood samples, similar to a study in Iran (Shahhosseini et al, 2018). A relatively high abundance of selected hosts present in the available host communities or a strong overlap in the host preference may be probable explanations for the detected host similarities between the mosquito species (Shahhosseini et al, 2018).

Moreover, the proportion of cattle-derived blood-feeding decreased in July and August compared to that in June and September. Although host selection is a genetic-based characteristic in response to certain host characteristics, host availability, and diversity in the environment where mosquito species exist considerably affect host orientation (Clements, 1992). Furthermore, mosquitoes have higher feeding success on inactive hosts (Day & Edman, 1984). Our study region has microclimate features based on its location and is influenced by the Mediterranean climate. Agriculture and animal husbandry are the main sources of income in the region, and residents work in orchards and fields throughout the day, particularly in the summer. In addition, barns are located close to the houses, and cattle are the main livestock reared in the region. Furthermore, cattle herding is moved to plateau lands between July and August, especially considering that the cattle population notably decreases during these months. The results of the present study showed that the proportion of blood-feeding mosquitoes on cattle hosts significantly decreased in July and August. Many factors, such as trap placement, availability of vertebrate host species, collection methods, and weather, can influence the mosquito species composition in the collection area, as well as the represented blood-meal hosts (Thiemann & Reisen, 2012). During our sampling process, only New Jersey light traps were used, and they were placed close to the barns or in the barns, as well as in open areas neighboring croplands where people work. In addition, indoor samplings were performed in houses and animal barns. Shahhosseini et al, (2018) detected certain differences between the host-feeding patterns of the Biogents Sentinel traps (BG trap; Biogents, Regensburg, Germany) Heavy Duty Encephalitis Vector Survey traps (EVS trap; BioQuipProducts, Rancho Dominguez, CA, USA) and handheld aspirators. Thus, more detailed studies with different collection methods and trap placements may have different results in the area. The density and diversity of vertebrate species were not

surveyed at the collection sites; thus, the significance of the relative representation of a species or lack thereof in the blood-meal host identification cannot be suggested. Rather than suggesting seasonal variation in host utilization, the results may indicate that mosquito species tend toward accessible hosts, and the sampled species tend to be present near hosts that are easily accessible, densely populated, and more tolerant to bite pressure, with minimum energy and maximum efficiency.

All mosquito species in the present study fed on humans, and/or other mammals, birds, and domestic mammals. These results may indicate the potential transmission risk of zoonotic pathogens, such as filarial nematodes (Azari-Hamidian et al, 2009), WNV (Nikolay, 2015), Sindbis virus (Brugman et al, 2017), and Usutu virus (Calzolari et al, 2013), which are transmitted between different mammals and birds, and Rift Valley fever virus (McDaniel et al, 2014), which causes outbreaks in cattle and humans. Our previous study conducted in the same area (the Aras Valley) showed that *An*. *maculipennis* s.l., *Ae*. *caspius*, *Ae*. *vexans*, *Cx*. *theileri, Cx*. *pipiens s.l. are* potential vectors of *D*. *immitis* and *D*. *repens* with DNA in head-thorax pools; *An*. *hyrcanus* is also a likely vector, but Dirofilaria DNA was only found in abdomen pools for the study area (Demirci et al, 2021). And also as we mentioned before WNV is common in Turkey, and *Cx*. *pipiens* s.l. and *Ae.caspius* are potential vector species for WNV (Ergünay et al, 2017; Öncü et al, 2018; Akıner et al, 2019). In Europe, *Cx*. *pipiens* s.l. and *Ae*. *caspius* are the most important vectors associated with WNV too, whereas *Cx. theileri* and *An. maculipennis* s.l. are suggested to play a potential role in virus circulation (Calistri et al, 2010; Engler et al, 2013). Furthermore, WNV-neutralizing antibodies were detected at a rate of 9.9% in ducks in Kars Province, which is located at the border of our study region (Ergunay et al, 2014). The four most common mosquito species in this study (*An*. *maculipennis* s.l., *Ae. caspius*, *Ae*. *vexans* and *Cx*. *theileri*) and also *Cx*. *pipiens* all fed on humans, non humans mammals and birds. Therefore these species may be classified as potential vectors between mammals and/or birds for the area. Field data on the host-feeding patterns of blood-feeding vector arthropods may provide information regarding the vector potential of these species. However, the host-feeding patterns of the vector species are only a part of the vector capacity. To detect the actual potential of a species as a vector, further studies are needed on vector competence for different pathogens.

The most abundant species, *An*. *maculipennis* s.l., commonly described to feed on mammals and birds (Brugman et al, 2017), showed opportunistic feeding behavior on a wide range of host species in the present study, which is in line with the literature (Danabalan et al, 2014, Schönenberger et al, 2016, Heym et al, 2019). The mixed feeding behavior of *Anopheles* mosquitoes could serve as a strategy to increase reproduction (McDaniel et al, 2014). Members of the *An*. *maculipennis* complex in the study were not identified at the species level. The sibling species in this complex may significantly differ in feeding preferences and therefore in vector capacity (Takken and Verhulst, 2013). The *An*. *maculipennis* complex has eleven members and the most important malaria vectors in the Palearctic region. In particular, *An*. *(Anopheles)* atroparvus van Thiel, 1927, *An*. *labranchiae* Falleroni, 1926 and *An*. *sacharovi* Favre, *1903* are considered the most important malaria vectors (BruceChwatt & de Zulueta 1980). Of these members,

four species were recorded in Turkey: *An. maculipennis* s.s. Meigen, 1818 (Diptera, Culicidae), *An. messeae* Falleroni, 1926 (Diptera, Culicidae), *An. melanoon* Hackett, 1934 (Diptera, Culicidae), and *An. sacharovi* Favre, 1903 (Diptera, Culicidae) (Kasap and Kasap, 1983; Parrish, 1959; Postiglione et al, 1973; Ramsdale et al, 2001; Şimşek et al, 2011). Furthermore, *An. hyrcanus* is one of the secondary vectors of malaria (WHO, 2018). Studies on the host preference of *An. hyrcanus* are limited; a study conducted in Bangladesh determined that the species fed on only mammalian hosts (Bashar et al, 2012). The Aras Valley, in addition to the Marmara region (north-western region of Turkey), the Mediterranean region (southern coast of Turkey) and low-lying parts of Southeast Turkey, is a suitable place in Turkey for the transmission of *Plasmodium falciparum* infection, as the temperature is convenient (Özbilgin et al, 2011). Thus, the presence of potential malaria vectors and their human host preference behavior indicate that the study area may be at risk for malaria and the importance of future studies to detect the presence of *Plasmodium* in the study area.

The species *Cx. pipiens* s.l. comprises two morphologically identical biotypes, *pipiens* and *molestus*, that can form hybrids. The biotypes of *Cx. pipiens* s.l. species were not identified in the present study. Biotype *pipiens* preferred birds, whereas biotype *molestus* preferred mammals, including humans (Byrne and Nichols, 1999; Dohm and Turell, 2001; Sanburg & Larsen, 1973). Several studies (Brugman et al, 2017, 2018; Shahhosseini et al, 2018) have shown that *Cx. pipiens* s.l. has a strong feeding preference for avian blood, and various laboratory (Fritz et al, 2015) and field studies (Apperson et al, 2004; Börstler et al, 2016) have shown the high mammalian orientation of this species, which is consistent with our results. A study conducted in Turkey found that this species fed on both avian and mammalian blood, and the preference for human blood was higher than that for other mammalian species, which is similar to our findings (Korkmaz et al, 2017). Another study showed that *Cx. pipens* s.l. fed only on birds and horses and had a strong feeding preference for avian blood (Bursalı, 2020). As mentioned previously, many factors, such as trap placement, vertebrate host species' availability, collection methods, and weather, may cause different results for the same species.

Host-feeding patterns for opportunistic mosquitoes are typically determined by the most abundant or readily available host species. Additionally, it reflects the fact that the host-feeding patterns of a single species can greatly vary in different regions, thereby affecting the role of species as a bridge vector in different regions. The global host range of *Ae. vexans* is primarily mammals and rarely birds (Fall et al, 2012; Molaei et al, 2006). In the present study, *Ae. vexans* was found to feed on mammalian hosts, and only one individual fed on avian blood. In Senegal, this species was shown to feed on mammals, but mostly on blood derived from horses (Biteye et al, 2019). Conversely, *Ae. caspius* is a highly anthropophilic species and is known to aggressively bite humans throughout the day (Napp et al, 2018; Soliman et al, 2016).

A study conducted in Kenya (Tchouassi et al, 2016) found that *Cx. theileri* feeds solely on mammals, whereas studies conducted in Spain and Iran found that they feed on both mammalian and avian species, with a wide host range, similar to our results (Martínez-De La Puente et al, 2012; Shahhosseini et al, 2018). In the present study,

Cs. *annulata* and *An*. *hyrcanus*, unlike other species, fed only on mammalian blood. Although this result was similar to that reported by previous studies conducted in the United Kingdom (Service, 1971) and Portugal (Osório et al, 2012) for *Cs*. *annulata*, another study conducted in the United Kingdom, showed that this species also fed on avian blood (Brugman et al, 2017).

Although a wide range of studies have been conducted on mosquitoes in Turkey, the analysis of host-feeding patterns of mosquitoes is a highly unattended area of research. This is the most detailed study of the host-feeding patterns of mosquitoes in Turkey to date. The results indicate a strong overlap of host taxa with the highest frequencies for humans and cows. The overlap of host species' feeding patterns among mosquito species indicates that host selection is highly variable and may be correlated with the presence/abundance of the hosts. Vector competence is described the capacity of a mosquito to obtain the pathogen and support its transmission. Thus, these results suggest that most species in the area can potentially transmit pathogens between mammals and between birds and mammals. Therefore, for more concrete conclusions about the host-feeding patterns of mosquito species, it is necessary to give attention to the abundance and availability of host species in the field. A better understanding of the host-feeding patterns of mosquitoes will advance our comprehension of mosquito-borne disease epidemiology and enable the development of effective disease control strategies, such as by targeting host-specific vector species more accurately.

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