#### **ORIGINAL RESEARCH ARTICLE**



# Host selection of Aedes aegypti and Culex quinquefasciatus females in a semi-desert environment in Northern Mexico

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#### Abstract

Transmission of arboviral diseases such as those caused by dengue, chikungunya and West Nile viruses involves hematophagous insects. Arbovirus transmission is a complex process in which host selection plays an important role that determines the contact rates between vulnerable hosts and infective vectors. This paper describes host selection of *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) and *Culex quinquefasciatus* in suburban neighborhood houses located in the semi-desert environment of northern Mexico. All bloodmeal analyses were completed in engorged females collected by means of BG-GAT (BIOGENTS ®) traps and blood identification was made through PCR analysis using cytochrome b as reference gene. The results show that *Ae. aegypti* is feeding primarily on birds from the Galliformes and Columbiformes orders. On the other hand, *Culex quinquefasciatus* exhibits the same behavior as *Ae. aegypti*, selecting Galliformes as the main blood source group but exhibits different behavior regarding humans, feeding preferentially on humans over non-human mammals. Regarding resource utilization, *Ae. aegypti* had a broader niche (0.79) than *Cx. quinquefasciatus* (0.46). Pianka's niche overlap index (0.79) showed that both species share many common hosts. Host selection for the mosquito species analyzed were found to be atypical from what is reported in the literature, which could have an impact on the vectorial capacity of the mosquitoes within the study area.

Keywords Arthropod vectors · Arbovirus · Niche overlap · Blood meals · Hematophagous behavior

### Introduction

The yellow fever mosquito, *Ae. aegypti*, is the main vector of Yellow fever, dengue, chikungunya, and Zika viruses while the southern house mosquito, *Cx. quinquefasciatus*, is a competent vector of West Nile, Saint Louis encephalitis, and Venezuelan equine encephalitis viruses. These pathogens are transmitted directly to humans or other species by the bites of infected mosquitoes during blood feeding and are the etiological agents of some of the most important infectious diseases around the world. For example, it has been estimated that dengue infected approximately 390 million people in 2013 (Bhatt et al. 2013) and, in the Americas, there were over 3 140 649 cases in 2019 (PAHO) while West Nile contagions have re-emerged several times since its introduction to North

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America in 1999 (Gray and Webb 2014). Understanding outbreaks of these diseases requires investigation of arboviral transmission as a complex process affected by several factors associated with viruses, vectors and hosts (Smith et al. 2014) such as mosquito infection rates, arboviral prevalence, and blood-feeding behavior (Gu et al. 2008; Labeaud et al. 2011). Blood-feeding behavior is an important characteristic in the vector capacity of mosquitos since it determines the cycle of arbovirus transmission between mosquitoes and vulnerable hosts (Kilpatrick et al. 2006). It is predicted that only approximately 10 000 species of insects possess hematophagous habits (McBride et al. 2014), from which female mosquitoes dominate disease transmission. Physiologically, female mosquitoes consume blood for reproductive purposes, while they ingest carbohydrates for energy reserves (Harrington et al. 2001). The origin of blood-feeding behavior is not clearly defined, but it might have originated when sapfeeding insects accidentally bit a vertebrate host or could be associated with chewing organisms that developed mechanisms to recognize chemical clues upon chewing on vertebrates (Takken and Verhulst 2013). The main strategy that

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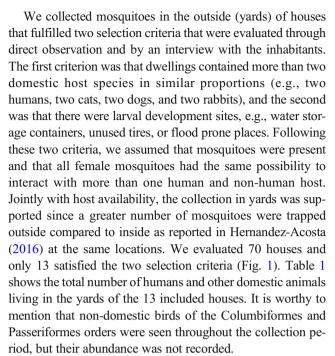
enables mosquitoes to find new hosts is through the detection of olfactory cues, such as carbon dioxide, lactic acid, and octenol, by receptor proteins present in sensory organs (Day 2005). Despite the presence of intricate mechanisms to identify possible hosts, the selection process is mediated by other factors such as host defense (Kelly 2001), host availability that is influenced by seasonality and microhabitat, previous encounters with a specific host species (Lyimo and Ferguson 2009) and viral infection (Vogels et al. 2017).

Host preference by mosquitoes is an intricate feature that varies among mosquito species and habitat based on the factors mentioned earlier. For example, *Anopheles minimus* is a mosquito capable of transmitting malaria parasites that has a strong affinity for human blood (Tisgratog et al. 2012), which increases its threat to public health. This feeding pattern is also reported for *Ae. aegypti* showing its significance as a threat to public health (Faraji et al. 2014; Sivan et al. 2015). *Culex quinquefasciatus* is a species known to have ornithophilic habits (Azmi et al. 2015; Faraji and Gaugler 2015); however, other species in this genus exhibit opportunistic feeding behavior (Thiemann et al. 2012).

Most studies focused on determining host preference for mosquitoes are performed in large areas to ensure the capture of numerous blood fed mosquitos, but these studies ignore the fact that many of those places may contain an unequal distribution of possible hosts. Nonetheless, investigating host preference of mosquitoes must be examined carefully because selecting sites with the presence of just one host species can misrepresent host usage regardless of the methods used (e.g., forage ratio) to correct for the abundance of host individuals per species (Hess et al. 1968). The aim of this study was to use molecular techniques to identify host selection for two mosquito species collected in locations with equally distributed hosts and compare their dietary resource use and overlap using ecological indices.

### **Materials and methods**

The study was conducted in a suburban area located northwest of Ciudad Juarez, Chihuahua, Mexico (31° 44′ 22″ N, 106 ° 29′ 13″ S, 1137 masl). Its weather is characterized as very dry and temperate, with an average temperature of 18 °C, high temperatures up to 40 °C during June, July, and September, an average annual precipitation of 262 mm and an average relative humidity of 38% (INEGI 2015). This zone has a total population of more than 16 000 people with previous studies showing the presence of arbovirus (mainly DENV, CHIKV and WNV) in human and mosquito populations, and this area is catalogued as high risk for arboviral transmission because of the socio-economic conditions of its inhabitants (Hernández-Acosta 2016).



We performed the collection of mosquitoes using one BG-GAT (BIOGENTS ®) trap per house situated outdoors in shaded spaces near as possible to sites where people usually rested and played with their pets, chicken coops, and others, so all host species (humans, dogs, cats, chickens, ducks, and other not domestic birds) inhabiting the dwelling could be encountered by mosquitoes and thus take a bloodmeal. Hay infusion and BG-Lure (BIOGENTS®) were used as attractants, and alpha-cypermethrin treated nets were used inside passive traps to knock down all trapped mosquitoes. The collection period included months with high mosquito density as reported in Hernandez-Acosta (2016), specifically from July to November of 2016 and from September to November of 2017. Every Monday, Wednesday and Friday of these two collection periods the traps were evaluated for mosquitoes and all captured individuals were transported to the laboratory in bags that were placed inside a cold container for further sorting and species identification according to Darsie and Ward (2005). To obtain the maximum sample size, collection of mosquitoes was stopped when no specimens could be obtained in any trap during three consecutive visits (this occurred in November where temperatures descend abruptly).

Blood-fed female mosquitoes were dissected to obtain the midgut and salivary glands, which were then placed separately in a microtube containing 100  $\mu L$  of TRI reagent® (MRC, Cincinnati, OH). RNA extraction was performed following the manufacturer's instructions. The midgut was homogenized mechanically using a sterile tissue grinder pestle, and 20  $\mu L$  of chloroform was added and mixed. Samples were then incubated for 10 min followed by a centrifugation at 12 000 x g for 15 min at 4 °C. After centrifugation, the aqueous phase was transferred to a new microtube, and 50  $\mu L$  of isopropanol was



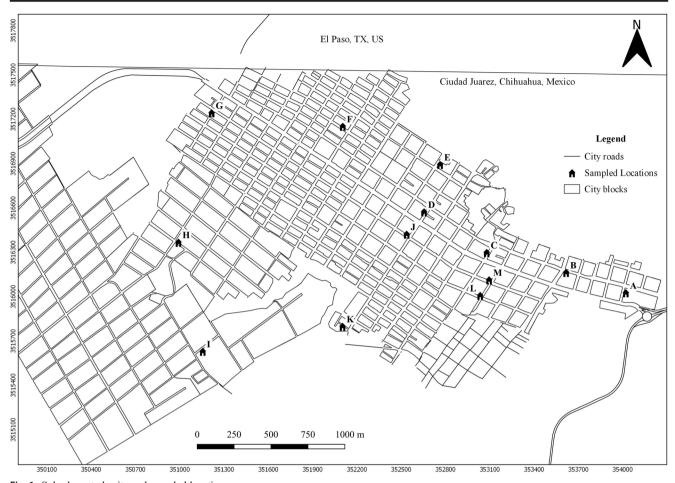


Fig. 1 Suburban study site and sampled locations

incorporated to precipitate RNA; this mix was incubated for 10 min at ambient temperature and centrifuged at 12 000 x g for 8 min at 4 °C. To clean the RNA pellet, 100  $\mu L$  of 75% ethanol was added, and this combination was mixed and centrifuged at 7 500 x g for 5 min at 4 °C. The RNA obtained was

resuspended in 20  $\mu$ L of sterile water and quantified using spectrophotometric analysis. All RNA samples were stored at  $-80~^{\circ}\text{C}$  for further use. An RNA extraction method was preferred over DNA extraction to analyze the origin of bloodmeals since all samples were also tested for RNA-arbovirus for

 Table 1
 Amount and type of host found in sampled locations

House	People	Dogs	Cats	Chickens	Ducks	Rabbits	Turkeys	Sheeps	Pigs
A	6	3	1	0	0	0	0	0	0
В	5	2	1	10	0	0	0	0	0
C	4	4	0	5	0	0	0	0	0
D	8	2	2	2	0	0	0	0	0
E	8	1	0	15	3	7	0	0	0
F	4	3	0	8	0	0	0	0	0
G	3	2	3	0	0	0	0	0	0
Н	6	1	0	30	0	2	0	2	0
I	4	13	1	5	0	0	2	0	24
J	2	3	1	0	0	0	0	0	0
K	9	8	0	4	1	0	6	0	0
L	4	0	1	6	0	1	0	0	0
M	5	2	0	6	0	0	0	0	0



forward studies. After RNA was extracted, cDNA was synthetized by Improm-II Reverse Transcription System ® using 1 µg of total RNA in combination with 0.5 µg of Oligo dT and with the necessary quantity of sterile water to reach a final volume of 8.5 µL. The mixture was incubated at 70 °C for 5 min and afterwards 1.5 mM MgCl<sub>2</sub>, 4 µL of reverse transcription buffer (5x), 0.5 mM of dNTP mixture, 20 U of RNAsin inhibitor and 1 µL of ImProm-II<sup>TM</sup> Reverse Transcriptase were incorporated. This reaction was incubated for 5 min at 25 °C, then for 60 min at 42 °C and finally for 15 min at 70 °C. The synthetized cDNA was quantified through spectrophotometric analysis. Host identification was made by PCR using midgut cDNA as a template and was based on the amplification of cytochrome b sequences. For avian or mammalian hosts identification we employed the primers presented in Table 2 and were obtained from De Cicero and Johnson (2001), Egizi et al. (2013), and Ngo and Kramer (2003) or were designed in the laboratory. These primers were used for identifying host at order level, however, we tried to identify ducks at a species level for their role in WNV transmission. All PCR reactions were composed of 1400 ng of cDNA, 0.8 mM of forward and reverse primers and 12.5 µL Master Mix 2x ®, brought to a final volume of 25 µL using sterile water. The PCR scheme to identify the origin of the bloodmeals is described as follows. All samples were subject to PCR using the primers specific to birds, mammal, and Galliformes. If a sample presented an amplification product using birds' primers, then it was taken as a bird positive sample. Later, only bird positive samples were further tested for Columbiformes, Passeriformes and duck (Anas platvrrhvnchos) to identify if the bird-derived blood matched those organisms. A similar approach was used for mammal identification. All mammal positive samples were then tested for human and non-human mammals separately to identify the specific host from which bloodmeal was obtained. All PCR reactions incorporated positive and negative (sterilized water as template) controls to assess the validity of the experiment. Positive controls included DNA extracted from host species found in the dwellings. These positive controls encompassed DNA from humans, dogs, cats, chickens, rabbits, Columbiformes (Zenaida asiatica and Columba livia), and Passeriformes (Passer domesticus and Quiscalus mexicanus).

The proportion of blood meals obtained from each host was determined and compared using a Chi-squared test in SPSS (version 23) to determine if proportions were different. To ensure that mosquitoes were collected in houses with equally available domestic hosts, the proportion of non-human per human individual was calculated. We also evaluated dietary specialization, estimating niche breadth and overlap through Levin's standardized niche breadth index and Pianka's overlapping index. A null model test was conducted using EcoSim version 1.1 for R to conclude whether the overlap value observed was greater than that expected by chance.



#### Results

A total of 948 Ae. aegypti (609 females) and 590 Cx. quinquefasciatus (173 females) mosquitoes were collected during both collection periods. Of these, 15 Ae. aegypti and 58 Cx. quinquefasciatus were partially or fully engorged and were included in the study. Blood fed Ae. aegypti were collected only in the outdoors of houses A, C, F, J, and M; the number and types of hosts registered in these houses is shown in Table 1. In Ae. aegypti positive houses we registered a combined total of 21 humans, 17 non-human mammals and 19 domestic birds that results in a proportion of 0.81 nonhuman mammals and 0.90 domestic birds for each human situated in the dwelling, demonstrating a similar proportion of hosts. The results of the molecular diagnosis analysis used to determinate the identification of mosquito's host selection is shown below. Figure 2 illustrates the expected amplification products as described methodologically by the amplification of cytochrome b as a reference gene. Host identification was successful for only 10 blood fed mosquitoes, representing a total of 17 partial or full ingests from Ae. aegypti. 40% (4/10) of these blood fed mosquitoes contained blood from one host type, 50% (5/10) from two hosts and 10% (1/10) from three hosts. Blood derived from avian hosts represented 52.9% (9/ 17) of total ingestions, of which 55.5% (5/9) were from Galliformes, 33.3% (3/9) were from Columbiformes and 11.1% (1/9) were from other bird groups. The remaining 47% (8/17) blood ingestions were acquired from mammal hosts, 37.5% (3/8) of which was taken from humans and 62.5% (5/8) from non-human hosts (Fig. 3).

Blood fed Cx, quinquefasciatus mosquitoes were collected in the outdoors of houses A, B, C, E, F, G, H, I, L, and M; the distribution and number of hosts found at these houses is presented in Table 1. In Cx. quinquefasciatus positive houses we registered a combined total of 49 humans, 74 non-human mammals, 87 Galliformes and 3 ducks which results a proportion of 1.51 non-human mammals, 1.78 Galliformes, and 0.06 ducks for every human present, indicating a similar proportion of host. Of the total Cx. quinquefasciatus mosquitoes analyzed, only 44 tested positive for the presence of any host representing a total of 68 ingests; 61.3% (27/44) of these mosquitoes fed upon one host, 22.7% (10/44) upon two hosts and 15.9% (7/44) upon three hosts. Of the total of ingestions 80.8% (55/68) were obtained from birds. The most frequent groups were Galliformes with 65.4% (36/55) and Columbiformes with 18.1% (10/55); other groups represented a total of 16.3% (9/55). The rest of the blood meals were obtained from mammals representing 19.1% (13/68) of the total. From these, humans scored for a total of 92.3% (12/ 13) while other mammals represented 7.6% (1/13) as showed in the Fig. 4. It is worthy to mention that neither Ae. aegypti nor Cx. quinquefasciatus tested positive for passeriformes or duck blood meals, and the blood from other groups of birds

Table 2 Specific primers used in Aedes and Culex blood intake by PCR

Primer name	Sequence (5'-3')		Product Size (bp)
Bird <sup>(2)</sup>	Fw GACTGTGACAAAATCCCNTTCCA	508	_
	Rv GGTCTTCATCTYHGGYTTACAAGAC		
Columbiformes <sup>(3)</sup>	Fw CTMACMGGMYTACTACTMGCCG Rv GGTTTGGCCAATGTAGGGGAC		333
Galliformes <sup>(3)</sup>	FW ATTTCGGCTCCCTATTAGCAG Rv GTCCGATGTGAAGGAAGATACAGATGAAGAAGAA	A	210
Passeriformes <sup>(4)</sup>	Fw TTCTCHGCHATCCCHTACATYGGHCAAACACT Rv CCRATRATRATGAATGGGTGTTCDACTGGYTGGCT	Γ	604
Anas platyrhynchos <sup>(4)</sup>	Fw CTCACTCTTATAGCAACTGCC Rv GAAGGTTAGGTGAGAATAGGGC		388
Mammal <sup>(4)</sup>	Fw CGAAGCTTGATATGAAAAACCATCGTTG Rv TGTAGTTRTCWGGGTCHCCTA		772
Non-Human Mammal <sup>(4)</sup>	Fw ATATTYTTTATYTGCCTVTWCMTACAHGT Rv GGRTRTCYTTGATTGTRTAGTADGGGTGRAA		423
Human <sup>(1)</sup>	Fw GGACGAGGACTATACTACGG Rv CTAGTTTGTTAGGGATGGAKCG		571
Human Nested <sup>(1)</sup>	Fw GGCTTACTTCTCTTCATTCTCTCCT Rv CTAGTTTGTTAGGGATGGAKCG		175

- (1) Primer sequence obtained from Ngo and Kramer (2003)
- (2) Primer sequence provided by Cicero and Johnson (2001)
- (3) Primer sequence obtained from Egizi et al. (2013)
- (4) Primer sequence designed by the authors

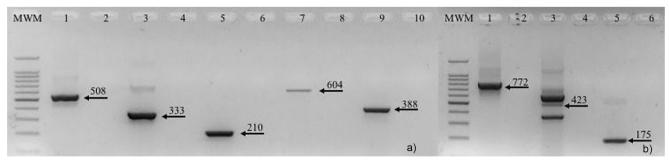
might not be included in the primers used. The Chi-square test revealed a statistically difference in proportions ( $\chi^2 = 5.708$ ; p = 0.017).

The Levin's standardized index of *Ae. aegypti* was 0.79, which indicates that this species exploits available resources in almost equal proportions. On the other hand, the calculated niche breadth index for *Cx. quinquefasciatus* was 0.46, showing a moderate use of hosts and a tendency to feed upon one species. Pianka's niche overlap index resulted in 0.79, showing that these species share common hosts and that when compared to a simulated value, there is insufficient information to conclude whether these two species compete in nature (P observed = null = 0.033).

### Discussion

Understanding the feeding habits of the principal vector species is of vital importance in the study of risk transmission of pathogens such as arboviruses because it determines the degree of interaction between vulnerable hosts and mosquitoes carrying a disease (Kilpatrick et al. 2006).

Numerous studies have demonstrated the anthropophilic behavior of *Ae. aegypti*, showing that despite the area, the percentage of blood meals obtained from human beings ranges between 70 and 99%. For example, in Thailand, around 97–99% of blood meals were from humans (Ponlawat and Harrington 2005); a pattern that was later corroborated by Sivan et al.



**Fig. 2** Electrophoretic analysis of host identification PCR products MWM is a molecular weight marker of 100 bp. Panel (a) lane 1 shows control Bird DNA; lane 3 Columbiformes; lane 5 Galliformes; lane 7 Passeriformes; and lane 9 Anseriforme. Lanes 2, 4, 6, 8, and 10 are

negative controls used in PCR protocol. Panel (b) lane 1 shows control Mammal DNA; lane 3 Non-human; and lane 5 Human. Lanes 2, 4, and 6 are negative controls used in PCR protocol. Numbers above arrows indicate the estimated weight in bp of amplification products



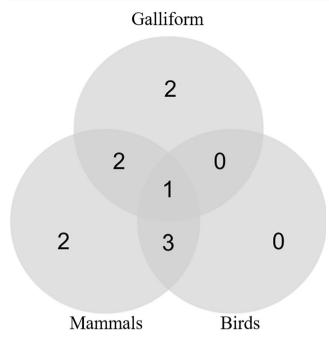


Fig. 3 Venn Diagram showing Aedes aegypti bloodmeals

(2015) where more than 80% of this mosquito specie fed on humans. In Puerto Rico, a similar pattern was found with 79% of female mosquitoes consuming human-derived blood (Scott et al. 2000). However, our results show an atypical behavior where *Ae. aegypti* tends to consume blood from birds. This is also reported for some populations although this divergent pattern can be derived of mixed forest and non-forest populations of *Ae. aegypti* (Mukwaya 1977). Results presented in this investigation might be different from what is reported in the literature because of the method used to situate traps in the collection sites,

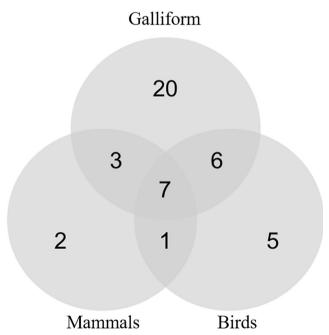


Fig. 4 Venn Diagram showing Culex quinquefasciatus bloodmeals



nevertheless, there is a clearly low number of blood fed mosquitoes and conclusions must be considered within this frame until replication of this collection protocol is done and the number of mosquitoes analyzed is raised. In most of the studies the individual sampled locations did not provide mosquitoes with a variety of available hosts at the same household, even though, there may be other hosts available in the surrounding sites. Thus, the anthropophagic behavior may be the result of mostly human host availability. To overcome this confounding factor, we collected blood-fed Ae. aegypti from spots where there was a similar probability of encountering a human, a bird or other mammal. Despite this, Ae. aegypti exhibited an ornithophilic oriented behavior. From an evolutionary point of view, several internal physiological characteristics must be present as evidence of host preference and of specialization for blood from a specific host, such as the cibarial armature (Lyimo and Ferguson 2009) or mechanisms used for host identification such as odor receptor proteins (McBride et al. 2014). Genes that code for odor receptor proteins have been found or predicted in genomes of several species such as An. gambiae, Ae. aegypti or Cx. quinquefasciatus, and its expression increases in those genera with greater distribution such as Aedes spp. and Culex spp. (Syed 2015). An expression analysis of odor receptors proteins specific to different host odorants may be a proxy of host location and discrimination but further studies must be implemented to confirm this hypothesis that could reinforce the findings showed in this work. Host behavior represents another component that must be deemed while studying blood feeding behavior of mosquitoes as some individuals can be more defensive than others (Kelly 2001). Humans employ defensive actions to avoid the nuisance of mosquitoes including the use of repellents and insecticides, chemical substances that may alter behavior of species like Ae. aegypti. In Mexico, the government recommends the use of repellents authorized by the United States Environmental Protection Agency like N,N-diethyl-metatoluamide (DEET), 2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester (Picaridin), and other plant derived chemicals for mosquito bite protection (Secretaria de Salud 2016). For DEET and Picaridin it is now demonstrated their capacity as repellents (agents that promote movement of insects away from these chemicals) and deterrents (substances that inhibit feeding or oviposition of insects in places where they normally would in the absence of the compounds) (Klun et al. 2006; Uc-Puc et al. 2016). As well as for repellents, pyrethroids insecticides have protective properties reducing the number of blood meals taken on humans wearing a fabric impregnated with this type of pesticides (Bowman et al. 2018). It is worth to mention that local stores sell commercial products containing repellent active ingredients like DEET. Moreover, pyrethroids are the most suggested pesticides to control mosquito populations and are applied according to Mexican national legislation (Diario Oficial de la Federacion 2014). Then, it is possible that the pattern of host selection found in Ae. aegypti is related to the use of repellents by the people located at the houses as well as the application of pyrethroids in vector control activities. However, we did not conduct a survey on repellent usage and only hypothetical scenarios can be proposed. Also, it must be noted that adulticides are applied to the environment (e.g. indoor residual spraying) and, because of the socioeconomic status of inhabitants, wearing pyrethroid-impregnated fabrics is almost impossible.

Culex quinquefasciatus is characterized by its ornithophilic behavior. For example, Molaei et al. (2006) reported that in the Northern United States, 93.1% of blood meals were obtained from birds. A similar pattern was observed in Yucatan, Mexico, where 82% of blood meals were obtained from the same group (García-Rejon et al. 2010) and in Queens, New York, where the blood meals obtained from the same group were observed as high 97.2% (Apperson et al. 2002). However, this vector presents a high plasticity in its blood-feeding habits as exhibited in studies where mammals were the primary source of blood (Azmi et al. 2015), although this was tested using an immunologic test which have some issues like cross-reactions.

It is important to note that host availability plays a significant role in defining the host selection pattern of this species. Our results show similarities to those of Garcia et al. (2010), with Galliformes as the dominant avian order, but differ from what was expected from mammal host selection; we found that mosquitoes consumed blood from humans over other mammals even though they were less abundant at the selected dwellings. This finding has strong implications for WNV transmission as mosquitoes can spread viruses into human populations if mosquitos select birds as well as humans for feeding (Weaver and Barret 2004). Human selection over other mammals can be the result of the presence of different species of the Cx. pipiens complex. It is now demonstrated that progeny of crossed Cx. pipiens pipiens and Cx. pipiens molestus, which have ornithophilic and anthropophilic behavior, respectively, display an intermediate host preference (Fritz et al. 2015); therefore, it is possible that a genetic component is affecting the observed behavior. Previous research at the study site found that most of the populations are present as hybrids known as Cx. pipiens quinquefasciatus and to a lesser degree Cx. quinquefasciatus (Loya Fierro 2017).

Most of the studies involving host preference focus only on determining the percentage of blood meals obtained from a "dominant" mosquito over a variety of hosts, neglecting the possible interactions between vectors present in the area and competition for dietary resources. Here, we describe that *Ae. aegypti* exploits more hosts (dietary resources) than *Cx. quinquefasciatus*, contrary to what we expected based on the anthropophilic behavior of *Ae. aegypti* reported previously (Ponlawat and Harrington 2005). In addition, as seen through the overlapping index, both species share hosts, but competition for dietary resources cannot be confirmed through the null model proposed. This relationship has been poorly studied. Of the

few studies, Chaves et al. (2010) analyzed the hematophagous pattern of mosquitoes within communities and found that at least one host is shared by vectors. Research done in the hemipterans *Triatoma infestans* and *Mepraia spinolai*, both *Trypanosome cruzi* vectors, established that the *M. spinolai* has a broader niche amplitude that *T. infestans* (0.29 and 0.15, respectively), and niche overlapping showed that these species exploit different dietary resources (0.229) (Canals et al. 2001). The results of this investigation may be related to host availability since *M. spinolai* is in contact with a greater number of likely hosts. Compared with our results, a greater availability of host species allows mosquitoes complex plasticity in their feeding habits and caused the observed results in niche breadth and overlap.

Here, we describe an atypical pattern of blood-feeding behavior for *Ae. aegypti*, where this mosquito specie feeds primarily on blood from birds whereas *Cx. quinquefasciatus* populations at the study site remain ornithophilic but show higher selection for humans when compared with other nonhuman mammals. However, it is important to remind that for all conclusions regarding blood-feeding behavior a low number of mosquitoes were collected and analyzed. Further studies using the same collection protocol in combination with other sampling techniques (e.g. backpack aspirators) should be performed to corroborate the results obtained in this research. Additionally, we demonstrated that these species share hosts and competition may occur, but this hypothesis has not yet been confirmed.

Author contributions The above authors acknowledge the activities realized in the present manuscript. Alan Esteban Juache made the experimental procedure and wrote the manuscript, Florinda Jiménez was the professor that planning the molecular diagnostic and all the laboratory procedures and Antonio de la Mora contributed in the design of fieldwork, and reviewing of the manuscript and Angélica Escárcega was the professor that made the statistical analysis and reviewing of the manuscript.

Data Availability Data transparency, we have all data and material available.

## Compliance with ethical standards

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

**Ethics approval** This work was approved by the bioethics committee of the Autonomous University of Ciudad Juarez CBE-ICB-071-10-15.

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