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ORIGINAL ARTICLE

Survival effects of antibiotic exposure during the larval and adult stages in the West Nile virus vector *Culex pipiens*

Marta Garrigós[1], Mario Garrido[1], Manuel Morales-Yuste[1], Josué Martínez-de la Puente[1,2] and Jesús Veiga[1]

[1] *Faculty of Pharmacy, Department of Parasitology, University of Granada, Granada, Spain and* [2] *CIBER de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain*

Abstract The ability of mosquitoes to transmit a pathogen is affected, among other factors, by their survival rate, which is partly modulated by their microbiota. Mosquito microbiota is acquired during the larval phase and modified during their development and adult feeding behavior, being highly dependent on environmental factors. Pharmaceutical residues including antibiotics are widespread pollutants potentially being present in mosquito breeding waters likely affecting their microbiota. Here, we used *Culex pipiens* mosquitoes to assess the impact of antibiotic exposure during the larval and adult stages on the survival rate of adult mosquitoes. Wild-collected larvae were randomly assigned to two treatments: larvae maintained in water supplemented with antibiotics and control larvae. Emerged adults were subsequently assigned to each of two treatments, fed with sugar solution with antibiotics and fed only with sugar solution (controls). Larval exposure to antibiotics significantly increased the survival rate of adult females that received a control diet. In addition, the effect of adult exposure to antibiotics on the survival rate of both male and female mosquitoes depended on the number of days that larvae fed *ad libitum* in the laboratory before emergence. In particular, shorter larval *ad libitum* feeding periods reduced the survival rate of antibiotic-treated adult mosquitoes compared with those that emerged after a longer larval feeding period. These differences were not found in control adult mosquitoes. Our results extend the current understanding of the impact of antibiotic exposure of mosquitoes on a key component of vectorial capacity, that is the vector survival rate.

Key words antibiotics; avian malaria; microbiota; mosquitoes; vector competence; vector survival

Introduction

Vector-borne pathogens are currently a major concern due to their impact on public health (WHO, 2020), livestock production (Ndiva Mongoh *et al.*, 2008; Pendell *et al.*, 2016), and wildlife (Folly *et al.*, 2020). Among them, mosquito-borne pathogens play a central role in causing important diseases such as malaria, dengue, West Nile fever and yellow fever (WHO, 2020). The epidemiological relevance of mosquitoes in the transmission of different pathogens may be inferred by the vectorial capacity. This term, defined by an equation proposed by Garret-Jones (1964), estimates the ability of a vector population to effectively transmit a pathogen to a host based on parameters including the host-biting rate and the expectation of infective life. The vectorial capacity of mosquitoes is therefore modulated by both abiotic and biotic factors that alter these parameters, such as

Correspondence: Marta Garrigós and Josué Martínez-de la Puente, Faculty of Pharmacy, Department of Parasitology, University of Granada, Campus Universitario de Cartuja sn, 18071 Granada, Spain. Tel: +34958243857. Email: garrigosm@ugr.es and jmp@ugr.es

temperature, humidity and larval nutrition (Lefèvre *et al.*, 2013; Carvajal-Lago *et al.*, 2021), among others. Additionally, recent studies identified the mosquito microbiota, which is the microbial community that lives in contact with the mosquito epithelia, as a major factor affecting different components of the vectorial capacity, through their effects on the mosquito lifespan and pathogen development (Caragata *et al.*, 2013; Martínez-de la Puente *et al.*, 2018; Cansado-Utrilla *et al.*, 2021). Mosquito larvae partially obtain the microbiota from their breeding sites and, therefore, it is highly dependent on the environment (Coon *et al.*, 2014). After emergence, adult mosquitoes maintain part of the larval microbial community (Lindh *et al.*, 2008) but also acquire new symbionts from their diet (Muturi *et al.*, 2019; Sarma *et al.*, 2022).

Antibiotics and other pharmaceutical residues are widespread pollutants in freshwater worldwide (Wilkinson *et al.*, 2022), with mosquitoes and other aquatic organisms being commonly exposed to them (Endersby-Harshman *et al.*, 2019). In addition, adult mosquitoes feeding on animals treated with antibiotics, including humans, may be also exposed to these pollutants (Gendrin *et al.*, 2015). Antibiotic exposure may largely affect the composition of the mosquito microbiota and, consequently, affect their lifespan and their competence for the transmission of vector-borne pathogens (e.g., Dong *et al.*, 2009; Gendrin *et al.*, 2015; Martínez-de la Puente *et al.*, 2021). In addition, antibiotic treatments may also affect mosquito fecundity (Ha *et al.*, 2021), blood digestion and egg production (Gaio *et al.*, 2011), and the larval development period (Chouaia *et al.*, 2012). Despite the importance of antibiotics in the epidemiology of mosquito-borne pathogens, studies so far have mainly focused on female mosquitoes belonging to the genera *Anopheles* (Dos Santos *et al.*, 2022) and *Aedes* (Gómez-Govea *et al.*, 2022), with very limited information available for other major mosquito vector groups. This is especially the case of the carry-over effects of antibiotic exposure during the larval and adult stages on adult survival that, to our knowledge, have not been investigated in detail.

Here we assessed the synergistic impact of antibiotic exposure during the larval and adult stages on the survival rate of both male and female adult mosquitoes. We used wild-collected *Culex pipiens* mosquitoes due to: (i) the abundance of this species in human habitats in the temperate northern hemisphere (Haba & McBride, 2022) and (ii) their role as major vectors of pathogens such as West Nile virus and avian malaria parasites (Santiago-Alarcón *et al.*, 2012; Engler *et al.*, 2013; Gutiérrez-López *et al.*, 2020). We predict that exposing *Cx. pipiens* mosquitoes to antibiotics solely during the adult stage will enhance survival rates of mosquitoes, as suggested by prior research (Gendrin *et al.*, 2015; Martínez-de la Puente *et al.*, 2021; Santos *et al.*, 2022). However, we hypothesize that exposure to antibiotics during both larval and adult stages will negatively affect adult survival rates, as this treatment may lead to a more profound alteration of the microbiome, potentially removing crucial bacterial taxa. In our analyses, we control for the potential effect of the development time of mosquitoes feeding *ad libitum* as larva, because this variable may determine the nutrition and size of adults with potential consequences on the survival rate of mosquitoes (Carvajal-Lago *et al.*, 2021).

Materials and methods

Mosquito collection and rearing conditions

We collected *Cx. pipiens* larvae in September 2022 from two sampling points in a rural area in Granada, southern Spain. The larvae were then placed in separate sterilized plastic trays containing 1.5 L of dechlorinated water according to treatment (see below), collection date and sampling point. Each tray was kept in a distinct incubation tent and fed *ad libitum* with 40 mg of shredded fish food (JBL Propond All Seasons S®) per liter of dechlorinated water. Food was provided to larvae every day. Emerged adults were isolated in insectaries according to emergence date, sampling point, sex and treatment as larvae. From emergence until assignment to the different experimental groups, mosquitoes were provided *ad libitum* with sterilized, dechlorinated 10% sugar solution. Larvae and adult mosquitoes were maintained in a climatic chamber under controlled conditions at a mean temperature of 26.7 °C (Range: 25.2−27.7 °C), with a mean relative humidity of 58% (range: 52%−67%) and a 16 : 8 light : dark photoperiod cycle.

Experimental design and antibiotic treatments

We used a paired two-way factorial design to investigate how antibiotic exposure during the larval and/or adult stages affected the survival rate of female and male adult mosquitoes. Larvae from each sampling point and collection date were separated into two experimental groups, each placed in separate trays and incubation tents: (i) larvae in dechlorinated water (larvae control group), and (ii) larvae in dechlorinated water with antibiotics (larvae antibiotic-treated group). For the antibiotic-treated group, we added a single dose of antibiotics to the dechlorinated water at the beginning of the experiment to a final concentration of 0.6 μg of gentamicin sulfate

Table 1 Number of replicates and total number of mosquitoes (in brackets) included in this study according to the larval and adult treatments.

Treatment	Females	Males
Control larvae—control adults	17 (384)	14 (289)
Control larvae—antibiotic-treated adults	17 (381)	15 (311)
Antibiotic-treated larvae—control adults	15 (323)	14 (339)
Antibiotic-treated larvae—antibiotic-treated adults	15 (334)	15 (362)

(Sigma-Aldrich, Stockholm, Sweden), and 1.2 units/1.2 μg of penicillin-streptomycin (Gibco^{TM}, Grand Island, NY, USA) per liter of water solution. These concentrations are in line with those reported in treated wastewater (Mutuku *et al.*, 2022).

Adults that emerged during the first day after capture were discarded in order to include in the experiment only mosquitoes that were exposed to antibiotics for at least 24 h. The rest of mosquitoes were extracted from each tent every 1−3 d, anesthetized with ether, sexed and identified to the species level following Schaffner *et al.* (2001). *Culex pipiens* mosquitoes of each sex and experimental origin (control and antibiotic-treated larvae) were divided into two experimental groups: (i) adults fed with sterilized 10% sugar solution (adult control group), and (ii) adults fed with sterilized 10% sugar solution and antibiotics (adult antibiotic-treated group). The adult antibiotic treatment included 15 μg gentamicin sulfate and 10 units/10 μg of penicillin-streptomycin per milliliter of water solution (Dong *et al.*, 2009; Martínez-de la Puente *et al.*, 2021). The sugar solution with or without antibiotics was replaced every day to avoid product degradation. To avoid density being a confounding factor, we used a paired experimental design, assigning the same number of individuals ($\pm$1; average = 22.32, SD = 5.43) to each replicate of control and antibiotic treatments for males and females emerged at the same date (Table 1). We monitored the daily mosquito survival rate of adult mosquitoes for 68 d postemergence.

Statistical analysis

To test the effect of antibiotic treatment on mosquito survival rate, we generated survival curves using Kaplan–Meier estimates, and employed a Cox proportional-hazards model to fit the data (estimated as the probability of a mosquito of surviving 24 h). We included three independent variables in the model: treatment of larvae (control or antibiotic-treated larvae), treatment of adults (control and antibiotic-treated adults), and the number of days that the larvae remain in the trays feeding *ad libitum* until their emergence. We performed a backward stepwise model selection beginning with the model including each independent variable and all the interactions among them. Then, we simplified the models by removing the least significant variable or interaction at each step until the coefficient of all variables and interactions were significant ($P \leq 0.05$). We used Bonferroni correction for multiple-comparisons among the different levels of the interactions included in the final model. Separate models were constructed for female and male mosquitoes. Analyses were conducted in R (R Core Team, 2022), using the survival (v3.3-1; Therneau, 2022) and survminer (v0.4.9; Kassambara *et al.*, 2021) packages.

Results

We monitored the daily survival rate of 2723 adult mosquitoes corresponding to 122 replicates (14−17 insectaries per sex and larvae-adult treatment; Table 1). Results of the models for both females and males are summarized in Table 2.

For female mosquitoes, the final model included the interactions between larval and adult treatments and between adult treatment and larval feeding period (Table 2). *Post hoc* analyses revealed that the only significant differences were found in control adult females with respect to the antibiotic treatment received during the larval stage. In particular, control females treated with antibiotics during the larval stage showed a higher survival than those treated as controls (Fig. 1; $Z = 3.07$, $P = 0.01$). Non-significant differences between the treatments during the larval stage were found in female mosquitoes treated with antibiotics as adults (Fig. 1; $Z = 0.25$, $P = 1$), or between the different adult treatments for larvae treated as controls (Fig. 1; $Z = 1.14$, $P = 1$), and for those treated with antibiotics (Fig. 1; $Z = -1.68$, $P = 0.55$). Regarding the interaction between adult treatment and larval *ad libitum* feeding period, a shorter larval *ad libitum* feeding period reduced the survival rate of mosquitoes when they were exposed to antibiotics as adults, while adult females fed with the control diet showed a similar mortality rate regardless of the larval *ad libitum* feeding period (Fig. 2; Table 2).

For male mosquitoes, the final model included the interaction between adult treatment and the larval *ad libitum* feeding period (Table 2, $Z = -3.95$, $P < 0.0001$) showing a similar pattern to that found in females. Shorter larval feeding periods rapidly reduced the survival rate of adults when they were subsequently exposed to antibiotics, but as larval feeding period increased, the

Table 2 Summary of the adjusted Cox models for female and male mosquitoes showing the coefficient (Coef.) and its standard variation (Coef. Std. Dev.), the hazard ratio (HR), *Z* statistic (*Z*), and adjusted *P* value (*P*) corresponding to each variable or their interactions included in the final model: larval treatment (LT), adult treatment (AT), and larval *ad libitum* feeding period (LFP). For the larval and adult treatments, the reference level was control and, therefore, the coefficient was computed for the antibiotic-treated level (A).

Variable	Coef.	Coef. Std. Dev.	HR	*Z*	*P*
Female mosquitoes					
LT (A)	−0.271	0.088	0.763	−3.070	**0.002****
AT (A)	1.404	0.245	4.073	5.738	**< 0.001*****
LFP	−0.049	0.020	0.953	−2.375	**0.018***
LT (A) × AT (A)	0.249	0.124	1.283	2.014	**0.044***
AT (A) × LFP	−0.197	0.032	0.821	−6.191	**< 0.001*****
Male mosquitoes					
AT (A)	0.840	0.225	2.317	3.738	**< 0.001*****
LFP	−0.041	0.019	0.960	−2.180	**0.029***
AT (A) × LFP	−0.115	0.029	0.891	−3.947	**< 0.001*****

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

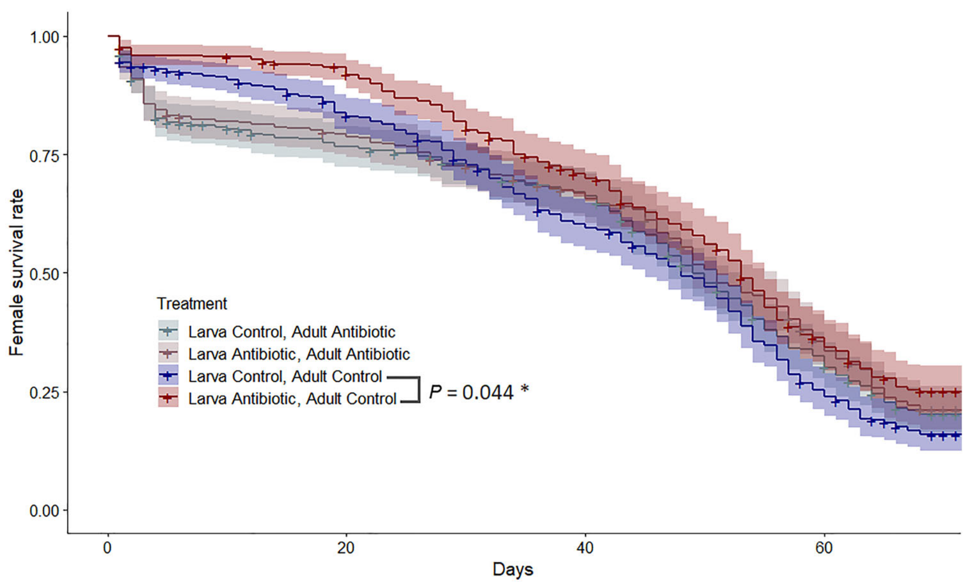

Fig. 1 Kaplan–Meier curve showing the survival rate of *Culex pipiens* female mosquitoes according to their treatments during the larval and adult stages. Blueish curves correspond to control larvae and reddish curves to antibiotic-treated larvae; darker curves correspond to control adults and lighter curves to antibiotic-treated adults. Significant differences were found only between control adults that were treated and those that were not treated (control) with antibiotics at the larval stage.

negative effect of antibiotics on adult survival dissipated (Fig. 2; Table 2).

Discussion

The habitat conditions during the larval stage, such as food availability and the presence of pollutants, can affect different mosquito traits including survival rate and vector competence (Lefèvre *et al.*, 2013; Carvajal-Lago *et al.*, 2021; Neff & Dharmarajan, 2021). However, despite the widespread distribution of antibiotics in nature, particularly in freshwater (Maghsodian *et al.*, 2022), little is known about their effects on mosquito life history traits. Here, we exposed larvae and adults of *Cx. pipiens* to antibiotics including penicillin, gentamycin and streptomycin. We found significant effects on the survival

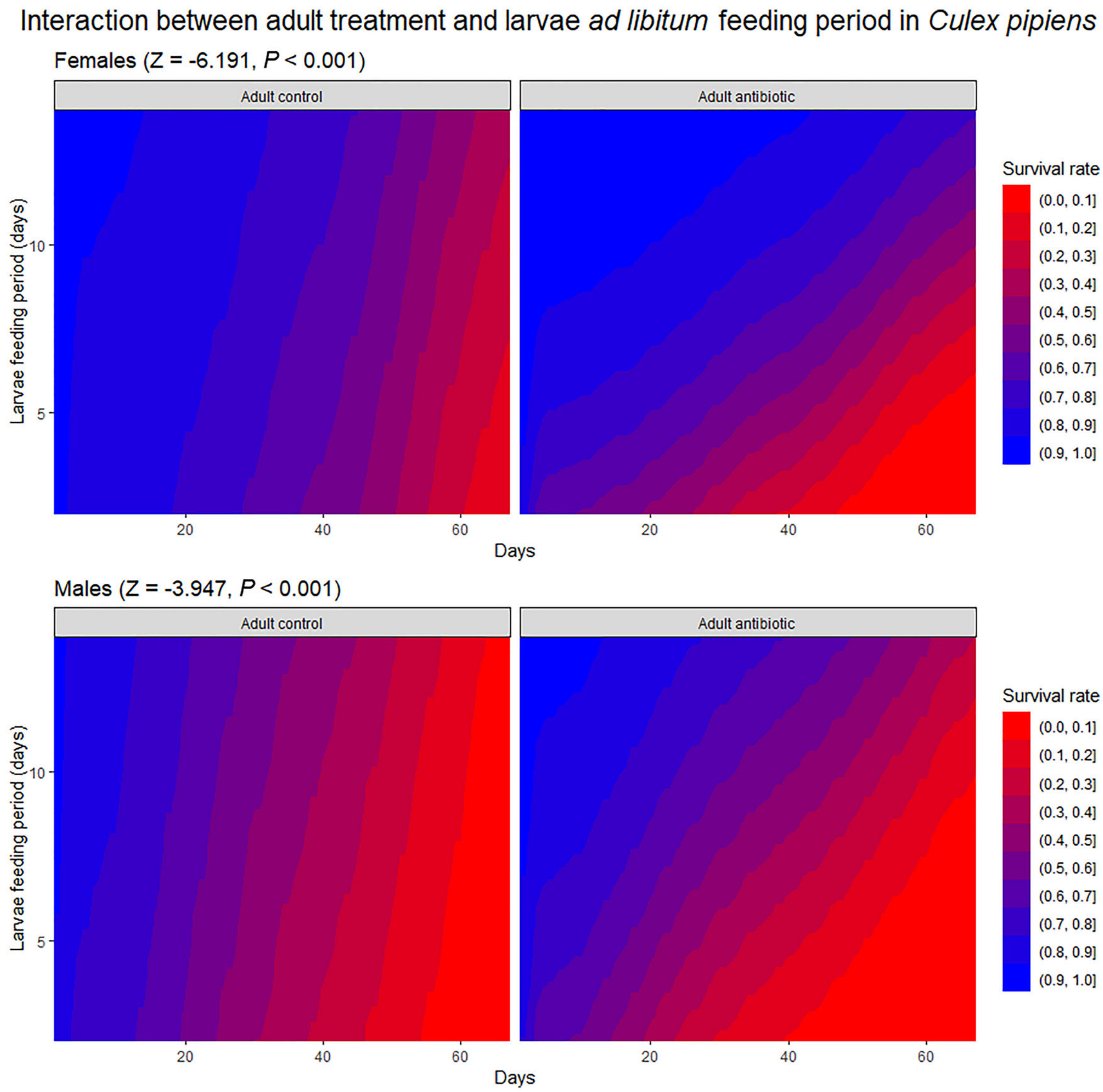

Fig. 2 Survival rate of *Culex pipiens* mosquitoes through the experiment duration (*x*-axis) according to the duration of the larval *ad libitum* feeding period in days (*y*-axis). Figures correspond to each adult treatment group (control and antibiotic-treated mosquitoes) for females (upper) and males (lower). The interaction between the adult treatment and the larvae *ad libitum* feeding period was significant for both males and females.

rates of adult males and females. Our findings indicate that larvae exposed to antibiotics showed increased survival rates in control adult female mosquitoes, and the duration of *ad libitum* feeding period during the larval stage determined the impact of antibiotic treatment during the adult stage on the survival rate of both female and male mosquitoes.

Disruption of larvae microbiota composition by antibiotics may result in a modification of adult microbiota (Qing *et al.*, 2020; Tikhe & Dimopoulos, 2022), which is known to modulate mosquito traits such as survival rate or reproduction (Caragata *et al.*, 2013). However, few studies have assessed the effect of larval microbiota disruption in adult survival, showing contradictory results. Using *Aedes aegypti* females, Dickson *et al.* (2017) did not find any significant difference in the adult lifespan among different groups of mosquitoes emerged from monoaxenic larvae with different bacterial symbionts. Giraud *et al.* (2022) found that several larval monoaxenic treatments resulted in differences in the adult lifespan. On the other hand, Carlson *et al.* (2020) exposed larvae to two different bacteria species and found no differences in the survival rate of adult mosquitoes. Here, we used an antibiotic cocktail to modify the microbiota composition of *Cx. pipiens* larvae and observed an increase in the survival rate of adult females, but only when they received a control diet. Taken together, these results suggest that, in mosquitoes, the effects of the larval environment on adult fitness, here measured as survival rate, may be due in part to differences in the microbial community present in their breeding habitat, although the underlying mechanisms are still unknown. One potential reason explaining this pattern could be that antibiotics may remove or reduce the presence of bacteria that are detrimental to mosquito survival, including pathogenic bacteria (Ramirez *et al.*, 2014; Contreras *et al.*, 2019). Additionally, mosquito microbiota triggers mosquito immune responses (Gabrieli *et al.*, 2021), which can induce important costs for mosquitoes (Ahmed *et al.*, 2002), affecting their survival. Thus, a simplified microbiota community due to antibiotic exposure during the larval period is expected to result in lower immune responses and, consequently, reduced survival cost for mosquitoes.

Larval *ad libitum* feeding period modulates the negative effect of antibiotics on adult survival rate, showing a similar pattern in both female and male mosquitoes. Microorganism-based detritus that larvae consume in the field usually contain lower amounts of macronutrients than laboratory diets (Merritt *et al.*, 1992; Souza *et al.*, 2019), and field-collected adult mosquitoes contain fewer nutritional reserves than those reared under laboratory conditions (Day & Van Handel, 1986). In addition, the associated microbiota plays a key role in mosquito nutrition being a direct source of macronutrients (Steyn *et al.*, 2016), providing essential nutrients like vitamins (Danchin & Braham, 2017; Guégan *et al.*, 2020; Wang *et al.*, 2021), and participating in functions such as fructose assimilation (Guégan *et al.*, 2020), and metabolism of sugar, protein, and nitrogen (Samaddar *et al.*, 2011; Chabanol *et al.*, 2020; Guégan *et al.*, 2020). Chabanol *et al.* (2020) exposed *Anopheles coluzzii* mosquitoes to the same antibiotic cocktail that we used here, which affected the tricarboxylic acid (TCA) cycle with subsequent loss of amino acids and other nitrogen-containing metabolites. In this context, the pattern found here with a lower survival rate of mosquitoes exposed to antibiotics as adults and fed during fewer days *ad libitum* as larvae suggests that microbiota disruption of adults may have higher deleterious effects in individuals with lower nutritional reserves. These results may have important implications for pathogen transmission as the effects of antibiotic exposure of adult mosquitoes (e.g., feeding on blood of antibiotic-treated individuals) may have different effects on mosquito survival depending on the development time and diet during the larval period, a factor that has not been considered in previous studies (e.g., Martínez-de la Puente *et al.*, 2021). Unfortunately, information on the exact instar of the larvae included in our experiment is lacking, and therefore we cannot separate the effect of the development time from that of the larval diet. Further studies are necessary to (1) control for the larval instar when studying the interaction between the larval diet and antibiotic treatment in adult mosquitoes and (2) identify the role of antibiotic treatments of humans and livestock on the survival rate of mosquitoes and their impacts on parasite epidemiology.

The conclusions of our study are limited by the fact that we do not know the identity and richness of microorganisms affected by the treatment. However, the antibiotics used were previously employed to disrupt the microbiota of mosquitoes, showing significant effects on survival and pathogen transmission (Dong *et al.*, 2009; Martínez-de la Puente *et al.*, 2021; Santos *et al.*, 2022). In addition, using field-collected larvae, we observed significant effects resulting from exposure to antibiotics at concentrations commonly found in environmental freshwater (Mutuku *et al.*, 2022). Our findings underscore the importance of considering the impact of pharmaceutical pollutants on vectorial capacity, affecting the survival rate of mosquito vectors of pathogens causing, among others, West Nile fever and avian malaria. A mosquito with a longer lifespan might have more opportunities to bite different hosts and, therefore, more chances of becoming infected and transmitting pathogens (Cansado-Utrilla *et al.*, 2021). Further research must be done to identify the mechanisms underlying these results and the consequences of antibiotic exposure on the epidemiology of mosquito-borne pathogens.

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Disclosure

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

References

Ahmed, A.M., Baggott, S.L., Maingon, R. and Hurd, H. (2002) The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. *Oikos*, 97, 371–377.

Cansado-Utrilla, C., Zhao, S.Y., McCall, P.J., Coon, K.L. and Hughes, G.L. (2021) The microbiome and mosquito vectorial capacity: rich potential for discovery and translation. *Microbiome*, 9, 111.

Caragata, E.P., Rancès, E., Hedges, L.M., Gofton, A.W., Johnson, K.N., O'Neill, S.L. *et al.* (2013) Dietary cholesterol modulates pathogen blocking by *Wolbachia*. *PLoS Pathogens*, 9, e1003459.

Carlson, J.S., Short, S.M., Angleró-Rodríguez, Y.I. and Dimopoulos, G. (2020) Larval exposure to bacteria modulates arbovirus infection and immune gene expression in adult *Aedes aegypti*. *Developmental and Comparative Immunology*, 104, 103540.

Carvajal-Lago, L., Ruiz-López, M.J., Figuerola, J. and Martínez-de la Puente, J. (2021) Implications of diet on mosquito life history traits and pathogen transmission. *Environmental Research*, 195, 110893.

Chabanol, E., Behrends, V., Prévot, G., Christophides, G.K. and Gendrin, M. (2020) Antibiotic treatment in *Anopheles coluzzii* affects carbon and nitrogen metabolism. *Pathogens*, 9, 679.

Chouaia, B., Rossi, P., Epis, S., Mosca, M., Ricci, I., Damiani, C. *et al.* (2012) Delayed larval development in *Anopheles* mosquitoes deprived of *Asaia* bacterial symbionts. *BMC Microbiology*, 12, S2.

Contreras, E., Masuyer, G., Qureshi, N., Chawla, S., Dhillon, H.S., Lee, H.L. *et al.* (2019) A neurotoxin that specifically targets *Anopheles* mosquitoes. *Nature Communications*, 10, 2869.

Coon, K.L., Vogel, K.J., Brown, M.R. and Strand, M.R. (2014) Mosquitoes rely on their gut microbiota for development. *Molecular Ecology*, 23, 2727–2739.

Danchin, A. and Braham, S. (2017) Coenzyme B12 synthesis as a baseline to study metabolite contribution of animal microbiota. *Microbial Biotechnology*, 10, 688–701.

Dickson, L.B., Jiolle, D., Minard, G., Moltini-Conclois, I., Volant, S., Ghozlane, A. *et al.* (2017) Carryover effects of larval exposure to different environmental bacteria drive adult trait variation in a mosquito vector. *Science Advances*, 3, e1700585.

Day, J.F. and Van Handel, E. (1986) Differences between the nutritional reserves of laboratory-maintained and field-collected adult mosquitoes. *Journal of the American Mosquito Control Association*, 2, 154–157.

Dong, Y., Manfredini, F. and Dimopoulos, G. (2009) Implication of the mosquito midgut microbiota in the defense against malaria parasites. *PLoS Pathogens*, 5, e1000423.

Endersby-Harshman, N.M., Axford, J.K. and Hoffmann, A.A. (2019) Environmental concentrations of antibiotics may diminish *Wolbachia* infections in *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, 56, 1078–1086.

Engler, O., Savini, G., Papa, A., Figuerola, J., Groschup, M., Kampen, H. *et al.* (2013) European surveillance for west Nile virus in mosquito populations. *International Journal of Environmental Research and Public Health*, 10, 4869–4895.

Folly, A.J., Dorey-Robinson, D., Hernández-Triana, L.M., Phipps, L.P. and Johnson, N. (2020) Emerging threats to animals in the United Kingdom by arthropod-borne diseases. *Frontiers in Veterinary Science*, 7, 20.

Gabrieli, P., Caccia, S., Varotto-Boccazzi, I., Arnoldi, I., Barbieri, G., Comandatore, F. *et al.* (2021) Mosquito trilogy: microbiota, immunity and pathogens, and their implications for the control of disease transmission. *Frontiers in Microbiology*, 12, 630438.

Gaio, A.O., Gusmão, D.S., Santos, A.V., Berbert-Molina, M.A., Pimenta, P.F.P. and Lemos, F.J.A. (2011) Contribution of midgut bacteria to blood digestion and egg production in *Aedes aegypti* (Diptera: Culicidae) (L.). *Parasites & Vectors*, 4, 105.

Garrett-Jones, C. (1964) Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. *Nature*, 204, 1173–1175.

Gendrin, M., Rodgers, F.H., Yerbanga, R.S., Ouédraogo, J.B., Basáñez, M.G., Cohuet, A. *et al.* (2015) Antibiotics in ingested human blood affect the mosquito microbiota and capacity to transmit malaria. *Nature Communications*, 6, 5921.

Giraud, É., Varet, H., Legendre, R., Sismeiro, O., Aubry, F., Dabo, S. *et al.* (2022) Mosquito-bacteria interactions during larval development trigger metabolic changes with carryover effects on adult fitness. *Molecular Ecology*, 31, 1444–1460.

Gómez-Govea, M.A., Ramírez-Ahuja, M.L., Contreras-Perera, Y., Jiménez-Camacho, A.J., Ruiz-Ayma, G., Villanueva-Segura, O.K. *et al.* (2022) Suppression of midgut microbiota impact pyrethroid susceptibility in *Aedes aegypti*. *Frontiers in Microbiology*, 13, 761459.

Guégan, M., Tran Van, V., Martin, E., Minard, G., Tran, F.H., Fel, B. *et al.* (2020) Who is eating fructose within the *Aedes albopictus* gut microbiota? *Environmental Microbiology*, 22, 1193–1206.

Gutiérrez-López, R., Martínez-de la Puente, J., Gangoso, L., Soriguer, R. and Figuerola, J. (2020) *Plasmodium* transmission differs between mosquito species and parasite lineages. *Parasitology*, 147, 441–447.

Ha, Y.R., Jeong, S.J., Jang, C.W., Chang, K.S., Kim, H.W., Cho, S.H. *et al.* (2021) The effects of antibiotics on the reproductive physiology targeting ovaries in the Asian tiger mosquito, *Aedes albopictus*. *Entomological Research*, 51, 65–73.

Haba, Y. and McBride, L. (2022) Origin and status of *Culex pipiens* mosquito ecotypes. *Current Biology*, 32, R237–R246.

Kassambara, A., Kosinski, M. and Biecek, P. (2021) Survminer: drawing survival curves using 'ggplot2'. R package version 0.4.9. https://CRAN.R-project.org/package=survminer

Lefèvre, T., Vantaux, A., Dabiré, K.R., Mouline, K. and Cohuet, A. (2013) Non-genetic determinants of mosquito competence for malaria parasites. *PLoS Pathogens*, 9, e1003365.

Lindh, J.M., Borg-Karlson, A.K. and Faye, I. (2008) Transstadial and horizontal transfer of bacteria within a colony of *Anopheles gambiae* (Diptera: Culicidae) and oviposition response to bacteria-containing water. *Acta Tropica*, 107, 242–250.

Maghsodian, Z., Sanati, A.M., Mashifana, T., Sillanpää, M., Feng, S., Nhat, T. *et al.* (2022) Occurrence and distribution of antibiotics in the water, sediment, and biota of freshwater and marine environments: a review. *Antibiotics (Basel, Switzerland)*, 11, 1461.

Martínez-de la Puente, J., Gutiérrez-López, R. and Figuerola, J. (2018) Do avian malaria parasites reduce vector longevity? *Current Opinion in Insect Science*, 28, 113–117.

Martínez-de la Puente, J., Gutiérrez-López, R., Díez-Fernández, A., Soriguer, R.C., Moreno-Indias, I. and Figuerola, J. (2021) Effects of mosquito microbiota on the survival cost and development success of avian *Plasmodium*. *Frontiers in Microbiology*, 11, 562220.

Merritt, R.W., Dadd, R.H. and Walker, E.D. (1992) Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual Review of Entomology*, 37, 349–376.

Mutuku, C., Gazdag, Z. and Melegh, S. (2022) Occurrence of antibiotics and bacterial resistance genes in wastewater: resistance mechanisms and antimicrobial resistance control approaches. *World Journal of Microbiology and Biotechnology*, 38, 152.

Muturi, E.J., Dunlap, C., Ramirez, J.L., Rooney, A.P. and Kim, C.H. (2019) Host blood-meal source has a strong impact on gut microbiota of *Aedes aegypti*. *FEMS Microbiology Ecology*, 95, fiy213.

Ndiva Mongoh, M., Hearne, R., Dyer, N.W. and Khaitsa, M.L. (2008) The economic impact of West Nile virus infection in horses in the North Dakota equine industry in 2002. *Tropical Animal Health and Production*, 40, 69–76.

Neff, E. and Dharmarajan, G. (2021) The direct and indirect effects of copper on vector-borne disease dynamics. *Environmental Pollution*, 269, 116213.

Pendell, D.L., Lusk, J.L., Marsh, T.L., Coble, K.H. and Szmania, S.C. (2016) Economic assessment of zoonotic diseases: an illustrative study of Rift Valley Fever in the United States. *Transboundary and Emerging Diseases*, 63, 203–214.

Qing, W., Zhijing, X., Guangfu, Y., Fengxia, M., Qiyong, L., Zhong, Z. *et al.* (2020) Variation in the microbiota across different developmental stages of *Aedes albopictus* is affected by ampicillin exposure. *MicrobiologyOpen*, 9, 1162–1174.

R Core Team. (2022) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ramirez, J.L., Short, S.M., Bahia, A.C., Saraiva, R.G., Dong, Y., Kang, S. *et al.* (2014) *Chromobacterium* Csp_P reduces malaria and dengue infection in vector mosquitoes and has entomopathogenic and in vitro anti-pathogen activities. *PLoS Pathogens*, 10, e1004398.

Samaddar, N., Paul, A., Chakravorty, S., Chakraborty, W., Mukherjee, J., Chowdhuri, D. *et al.* (2011) Nitrogen fixation in *Asaia* sp. (family Acetobacteraceae). *Current Microbiology*, 63, 226–231.

Santiago-Alarcon, D., Palinauskas, V. and Schaefer, H.M. (2012) Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. *Biological Reviews of the Cambridge Philosophical Society*, 87, 928–964.

Santos, N.A.C., Magi, F.N., Andrade, A.O., Bastos, A.S., Pereira, S.D.S., Medeiros, J.F. *et al.* (2022) Assessment of antibiotic treatment on *Anopheles darlingi* survival and susceptibility to *Plasmodium vivax*. *Frontiers in Microbiology*, 13, 971083.

Sarma, D.K., Kumar, M., Dhurve, J., Pal, N., Sharma, P., James, M.M. *et al.* (2022) Influence of host blood meal source on gut microbiota of wild caught *Aedes aegypti*, a dominant arboviral disease vector. *Microorganisms*, 10, 332.

Schaffner, E., Angel, G., Geoffroy, B., Hervy, J.P., Rhaiem, A. and Brunhes, J. (2001) *The Mosquitoes of Europe: An Identification and Training Programme*. Paris (FRA); Montpellier: IRD; EID, 1 CD ROM. (Didactiques).

Souza, R.S., Virginio, F., Riback, T.I.S., Suesdek, L., Barufi, J.B. and Genta, F.A. (2019) Microorganism-based larval diets affect mosquito development, size and nutritional reserves in the yellow fever mosquito *Aedes aegypti* (Diptera: Culicidae). *Frontiers in Physiology*, 10, 152.

Steyn, A., Roets, F. and Botha, A. (2016) Yeasts associated with *Culex pipiens* and *Culex theileri* mosque larvae and the effect of selected yeast strains on the ontogeny of *Culex pipiens*. *Microbial Ecology*, 71, 747–760.

Therneau, T. (2022) A package for survival analysis in R. R package version 3.3-1. https://CRAN.R-project.org/package=survival

Tikhe, C.V. and Dimopoulos, G. (2022) Phage therapy for mosquito larval control: a proof-of-principle study. *Mbio*, 13, e0301722.

Wang, Y., Eum, J.H., Harrison, R.E., Valzania, L., Yang, X., Johnson, J.A. *et al.* (2021) Riboflavin instability is a key factor underlying the requirement of a gut microbiota for mosquito development. *Proceedings of the National Academy of Sciences USA*, 118, e2101080118.

WHO, World Health Organization. (2020) Vector-borne diseases. https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases

Wilkinson, J.L., Boxall, A.B.A., Kolpin, D.W., Leung, K.M.Y., Lai, R.W.S., Galbán-Malagón, C. *et al.* (2022) Pharmaceutical pollution of the world's rivers. *Proceedings of the National Academy of Sciences USA*, 119, e2113947119.

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