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SHORT COMMUNICATION

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Fluralaner treatment of chickens kills the southern house mosquito, *Culex quinquefasciatus*

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Abstract

The control of zoonotic and vector-borne pathogens is challenging due to the limited availability of intervention tools. West Nile virus (WNV) is an example of a globally distributed zoonotic arbovirus that circulates between *Culex* species (Diptera: Culicidae) mosquitoes and avian hosts, with spillover transmission to humans, resulting in disease cases. Interventions delivering systemic insecticides to vertebrate hosts used by vector species, known as xenointoxication, are potential tools for managing vector populations by creating toxic bloodmeals. In this study, we evaluated the impact of two systemic pesticides (ivermectin; Ivomec® Pour-On and fluralaner; Bravecto®), and one anthelmintic (fenbendazole; Safe-Guard® Aquasol) on the mortality of *Cx. quinquefasciatus* Say (Diptera: Culicidae). We found no significant difference in the feeding rates of mosquitoes that fed on treated chickens compared with those fed on untreated chickens, suggesting that the treatment did not repel mosquitoes. The mortality of *Cx. quinquefasciatus* mosquitoes feeding on fluralaner-treated chickens was significantly higher ($p < 0.01$) than those fed on control chickens at 3 and 7 days post-treatment, but this effect was not observed in mosquitoes fed on chickens treated with fenbendazole or ivermectin. No differences in mortality were observed among the groups at 14, 26 or 56 days post-treatment. These data support fluralaner as a xenointoxication tool to control *Cx. quinquefasciatus* populations and decrease the risk of human exposure to their associated pathogens.

KEYWORDS
systemic pesticide, West Nile virus, Xenointoxication

INTRODUCTION

Zoonotic and vector-borne pathogens are emerging globally, and current control tools are insufficient. Given the many host animals involved, it is particularly difficult to manage the amplification of zoonotic agents among domestic or wild animals with spillover to humans. A pathosystem in this category is West Nile virus (WNV), one of the most widely distributed zoonotic, arthropod-borne viruses in the world. It is primarily transmitted by *Culex* spp. (Diptera: Culicidae) after blood feeding on viremic avian amplification hosts (McLean

Koyle Knape and Yuexun Tian contributed equally to this study.

et al., 2001). In the United States, the primary vector for WNV varies in different regions, with *Cx. tarsalis* Coquillett being the primary vector in western states, *Cx. quinquefasciatus* Say in southern states and *Cx. pipiens* Linnaeus in northern states (Ciota, 2017). Since the introduction of WNV to New York City in 1999, WNV has become widespread and the most common human mosquito-borne pathogen in the United States (Soto et al., 2022), with over 7 million human cases (Ronca et al., 2021). In the United States, approximately 1 out of 150 WNV-infected people develop severe neurological illness and functional sequelae. Among those with severe illness, the case-fatality ratio is approximately 10% (CDC, 2024), generating an estimated $56 million US dollars in annual medical expenses (Ronca et al., 2021).

Due to a lack of commercially available vaccines, controlling WNV is primarily achieved by population suppression of *Culex* spp. through larval source reduction, larvicides, adulticides and public education to prevent vector bites (Nasci et al., 2013). In addition to its high cost, using pesticide to control mosquitoes raises concerns about impacts on human health (Ross et al., 2013), effects on non-target invertebrates (Rasmussen et al., 2013) and pesticide resistance development. This highlights the need to develop novel approaches for pesticide applications to manage WNV. Host-targeted (systemic) pesticides, such as ectoparasiticides and endectocides (i.e. active ingredients that are lethal on ecto- and endoparasites), offer strategies for controlling human malaria (Foy et al., 2011), Chagas disease (Dias et al., 2005) and more recently, WNV (Nguyen et al., 2019). Ivermectin, a broad-spectrum endectocide, provided as treated seeds via artificial feeders to free-ranging passerine birds resulted in lethal bloodmeals that killed WNV vectors (Holcomb et al., 2022; Nguyen et al., 2019). However, ivermectin reaches maximum concentration immediately after treatment and may be undetectable within 24 h (Arisova, 2020). Similarly, Nguyen et al. (2019) found the levels of ivermectin in chicken serum dropped quickly, with observed mosquito mortality only until 3 days post-treatment. The need to repeatedly treat hosts with systemic pesticides could be accomplished using treated feed yet limits the feasibility of scale-up for population control of vectors. Accordingly, the use of alternative active ingredients to deliver toxic bloodmeals to mosquito vectors for longer durations is desirable for host-targeted vector control interventions. One alternative active ingredient is fluralaner, which was recently shown to produce toxic bloodmeals to *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) for up to 15 weeks post-treatment in dogs (Evans et al., 2023) and to triatomines for up to 14 days post-treatment in chickens (Durden et al., 2023). It has been proposed to be used to control vector-borne human diseases (Miglianico et al., 2018).

Culex vectors of WNV in the United States regularly utilise avian hosts for bloodmeals (Hannon et al., 2019). While ongoing studies are using host-targeted pesticide treatment of wild granivorous birds (Holcomb et al., 2023), the primary vector of WNV in the southern half of the United States, Mexico and Central America is *Cx. quinquefasciatus*, which was documented to feed on chickens 67% of the time in South Texas, with an estimated chicken density of 2299 chickens per km^2 (Olson et al., 2020), 45.3%–85.0% in Guatemala (Kading et al., 2013; Kent et al., 2010), and 6.1% in Reynosa, Mexico with an average of 37 chickens in each household (Estrada-Franco et al., 2020). Chickens are ubiquitous in the peridomestic environment in some regions, which offers a convenient target for creating toxic bloodmeals by systemic pesticides and are easier to treat than wild passerines. Additionally, sentinel chicken flocks are routinely used as a surveillance tool to detect WNV circulation by detecting seroconversion (Chaskopoulou et al., 2013). Therefore, xenointoxication with chickens is a potential tool for the wide-scale population suppression of *Culex* to reduce the risk of human and other animal exposure to WNV.

In this study, we evaluated the off-label, systemic treatment of chickens with three commercially available products to explore their insecticidal efficacy on *Cx. quinquefasciatus* when fed directly on treated chickens. We tested three active ingredients (fenbendazole, ivermectin and fluralaner). To our knowledge, this study is the first to document the utility of fluralaner systemic treatment of chickens for the control of mosquitoes.

MATERIALS AND METHODS

Mosquito colony

Culex quinquefasciatus Sebring strain (SEB) was used in this study (Sbrana et al., 2005). Mosquitoes were maintained in BugDorm (MegaView Science Co., Ltd., Taichung, Taiwan) on a natural day and night light cycle (10 h light, 14 h dark) with a constant 50% humidity at 27°C and adults were provided a 10% sucrose solution. Maintenance feeding of the colony was comprised of whole chicken blood treated with heparin (Sagent Pharmaceuticals, Schaumburg, IL) using Hemotek membrane feeders (Hemotek, Ltd., Blackburn, UK).

Chicken hosts

A flock of laying chickens (*Gallus gallus domesticus* (Linnaeus) (Galliformes: Phasianidae)) was obtained from a commercial hatchery (Lohmann LSL-Lite, Cuxhaven, Germany). These chickens were enrolled in this study at 28 weeks of age and were confirmed as healthy based on daily clinical health evaluations, egg-laying records and body weight records. Chickens were housed in an environmentally controlled layer house with a light/dark cycle of 16 h/8 h, which is suitable for egg production. Within the laying house, the chickens were housed with two per cage with a nipple waterer that serves two cages (four chickens) and fed on a standard commercial layer diet before experiments.

Chicken treatment

Chicken treatment was previously described in Durden et al. (2023). Briefly, individual chickens were treated with one of the three active ingredients: ivermectin (Ivomec®, Boehringer Ingelheim, Ingelheim am

Rhein, Germany), fenbendazole (Safe-Guard® AquaSol, Merck Animal Health USA, Rahway, NJ) and fluralaner (Bravecto®, Merck, Rahway, NJ, USA), or regular food/water as a control. The dose of each treatment was calculated based on the average hen weight (1.34 kg). Ivermectin and fenbendazole were delivered to chickens in a liquid formulation using a gravity flow nipple watering system, with the chemical mixed into their water and dosed at 0.4 mg/kg of body weight for ivermectin (Moreno et al., 2015) and 1 mg/kg for fenbendazole, as indicated on the product label. Fluralaner was delivered as a small oral chew in a dose of 0.5 mg/kg (Thomas et al., 2017) before the daily food was provided to ensure full consumption of the oral chew. Ivermectin and fenbendazole treatments were conducted daily for five consecutive days (Moreno et al., 2015), while fluralaner was given to the chickens twice, seven days apart (Thomas et al., 2017).

Mosquito feeding

Experiments were carried out using 7- to 12-day-old *Cx. quinquefasciatus* female adults, which were starved for 24 h before the experiment. During each trial, 42–99 mosquitoes were used to feed on a treated or control chicken at 3, 7, 14, 28 and 56 days post-treatment (DPT; after the last day of each treatment). Each chicken was used only once to avoid the confounding effects of acquired immunity to mosquito salivary antigens. Mosquitoes were briefly knocked down by placing their cages into a −20°C freezer for 5–10 min to sort females. The females were placed into a plastic container with a square opening (10 × 10 cm) on the side to which a mesh sleeve was glued on. The chicken's feet were inserted into the mosquito container through the mesh sleeve for mosquitoes to feed on for 45 min, while the chicken was re-strained using a stretchable self-adherent wrap (Healqu, Jersey City, New Jersey) on a padded stainless steel oven grate. After the 45-min feeding period, the mosquitoes were knocked down immediately by putting the container into a cooler (61 L × 38H × 61 W cm) half-filled with ice for 15 min. The immobilised mosquitoes were sorted with unfed mosquitoes removed and blood-fed mosquitoes transferred into a new container (5342 cm^3) with a 10% sucrose solution. The container was then placed into an incubator at 27°C and 50% humidity to monitor the survivorship of the blood-fed mosquitoes, which was checked daily for 10 days. The trial was repeated three times, resulting in a total of 60 chickens ([three treatment + one control] × five time points × three replicates) used in this study.

Statistics

All statistical analyses were conducted using R studio software (Version 4.2.2R Foundation for Statistical Computing, Vienna, Austria). Feeding success was calculated by dividing the number of blood-fed mosquitoes by the total number of mosquitoes in the container. The effects of treatment and DPT were analysed using analysis of variance (ANOVA) followed by Tukey's post hoc test (R package: stats (Team, 2020)). Mosquito survivorship was analysed using the Kaplan–Meier survival curve (R package: survival (Therneau, 2020)) followed by a paired log-rank test (R package: survminer (Kassambara et al., 2021)) to compare the survival curves with different treatments.

RESULTS

Feeding success

There was no significant difference in *Cx. quinquefasciatus* feeding success among the treatments within each DPT or among the DPT within each treatment (Figure 1). However, a significant difference was observed under different DPT (14 vs. 56, p-value = 0.013), while no significant difference was detected between the treatments and the interaction of DPT and treatment.

Survivorship

Significant differences in *Cx. quinquefasciatus* survivorship were observed only at 3, 7 and 56 DPT, but not at 14 and 28 DPT (Figure 2). At 3 DPT, the mosquito survivorship with fluralaner was significantly lower compared with the control (p-value <0.001). Mosquitoes fed on ivermectin (p-value = 0.001) and fenbendazole-treated chickens (p-value <0.001) had higher survivorship than the mosquitoes fed on control chickens. The same result was observed for fluralaner at 7 DPT (p-value = 0.004), but ivermectin- (p-value = 0.476) and fenbendazole-treated chickens (p-value = 0.477) were not significantly different at this time point. At 56 DPT, mosquitoes with fenbendazole had a significantly higher survivorship than mosquitoes in the control group (p-value = 0.014), but no significant difference was observed for ivermectin (p-value = 0.866) and fluralaner (p-value = 0.866).

DISCUSSION

In this study, we evaluated the mortality of *Cx. quinquefasciatus* after feeding on chickens that were treated with two systematic pesticides: fluralaner and ivermectin, and one anthelmintic, fenbendazole, for up to 56 days post-treatment. Only fluralaner-treated chickens resulted in significantly higher mortality in mosquitoes compared with the control group at 3 and 7 DPT, but not 14 or longer DPT. These results were consistent with Durden et al. (2023), showing that fluralaner, but not ivermectin or fenbendazole, was detectable in chicken plasma at 3, 7 and 14 DPT.

Evans et al. (2023) and Duncan et al. (2023) documented a longer effective period, up to 15 and 12 weeks, respectively, against *Ae. aegypti* from fluralaner (Bravecto)-treated dogs. Fluralaner was detected from dog plasma at all time points in Evans et al. (2023). The differences between the effective periods may be due to the doses administered to chickens and dogs. Bravecto®, as a commercial

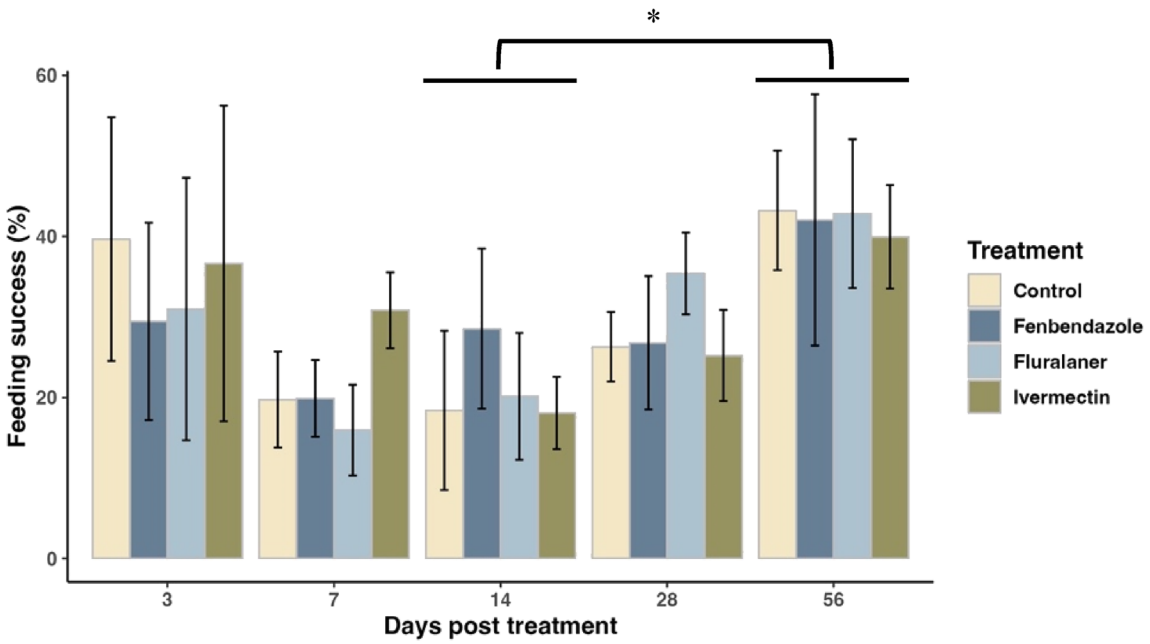

FIGURE 1 The mean (±SE) of *Culex quinquefasciatus* feeding success on chickens with different systemic treatments at different time points post-treatment.

product for dogs to treat fleas and ticks, contains the optimal doses for dog treatment, but not for chickens. Previous studies have identified the lethal dose concentrations (LD_{50}) of fluralaner to *Ae. aegypti* and *Cx. quinquefasciatus* to be 24.04 and 49.82 ng/mL respectively, based on artificial inoculations of heparinized chicken blood (Shah et al., 2024). In Durden et al. (2023), this LC_{50} for *Cx. quinquefasciatus* was only achieved in chicken plasma for up to 7 DPT, which supports our observed mosquito survival results. Fluralaner has been previously shown to be safe in chickens up to five times the dose used in our study (Prohaczik et al., 2017), so exploring the optimal dose of fluralaner for chicken treatment to achieve a longer effective period against mosquitoes is necessary. In addition, fluralaner resistance has only been reported in house flies with cytochrome P450 mediated detoxification as a major resistance mechanism (Burgess et al., 2020; Norris et al., 2023). In Europe, fluralaner has been commercialised as Exzolt™ (Merck Animal Health USA, Rahway, NJ) to treat poultry mites in a liquid formulation with 0-day egg withdrawal and 14-day meat withdrawal according to the product manual. However, it has not yet been approved in the United States and there are no other commercial fluralaner products available for poultry.

Ivermectin has been used as an endectocide to treat nematodes and ectoparasites on domestic animals (Foy et al., 2011) with high lethal and sub-lethal effects on mosquitoes, including *Anopheles* spp. (Derua et al., 2016; Foley et al., 2000), *Aedes* spp. (Deus et al., 2012) and *Culex* spp. (Deus et al., 2012; Nguyen et al., 2019). However, its short half-life limits the effects of arthropod vector control. Nguyen et al. (2019) reported that ivermectin concentration in chicken serum rapidly decreased after consumption and was only detectable up to two days, which was positively correlated with the mortality of *Cx. tarsalis* that was fed on those treated with chickens. Our results are consistent with the observations of Nguyen et al. (2019), as the earliest mosquito feeding was conducted on 3 DPT and survivorship of mosquitoes fed on ivermectin-fed chickens was not lower than those fed on control birds. This result indicates the need for repeated chicken treatments with ivermectin to achieve long-term management of *Cx. quinquefasciatus*, which increases control costs.

Fenbendazole is an antiparasitic used as the active ingredient in the commercial product Safe-Guard® AquaSol, which is labelled for parasite treatment for chickens. Derouen et al. (2009) evaluated the effects of Safe-Guard® (fenbendazole, cattle formulation, Intervet Inc., Millsboro, DE) on gastrointestinal nematodes in calves, which were treated at a dose of 5 mg/kg. The treatment of fenbendazole significantly reduced nematode eggs in faeces with a reduction rate of 96–100%, suggesting that fenbendazole was effective in controlling nematode infestation in calves (Derouen et al., 2009). However, few studies have evaluated the effects of fenbendazole on arthropod feeding. In this study, fenbendazole had no significant effect on increasing mosquito mortality, indicating either a lack of insecticidal effects or that the minimum dose was not reached in chicken blood to kill mosquitoes, which is consistent in *Triatoma gerstaeckeri* (Stal) (Hemiptera: Heteroptera) (Durden et al., 2023). However, on 56 days post-treatment, mosquitoes fed on fenbendazole-treated chickens had a significantly lower mortality than the control group, which may be due to the difference between the individual chickens or the groups of mosquitoes. Further research is needed across dose ranges to confirm the effects of fenbendazole on mosquitoes, triatomines and other ectoparasites.

Overall, less than 50% of the mosquitoes fed on treated or control chickens. This low feeding percentage is consistent with prior studies demonstrating low feeding rates in laboratory environments

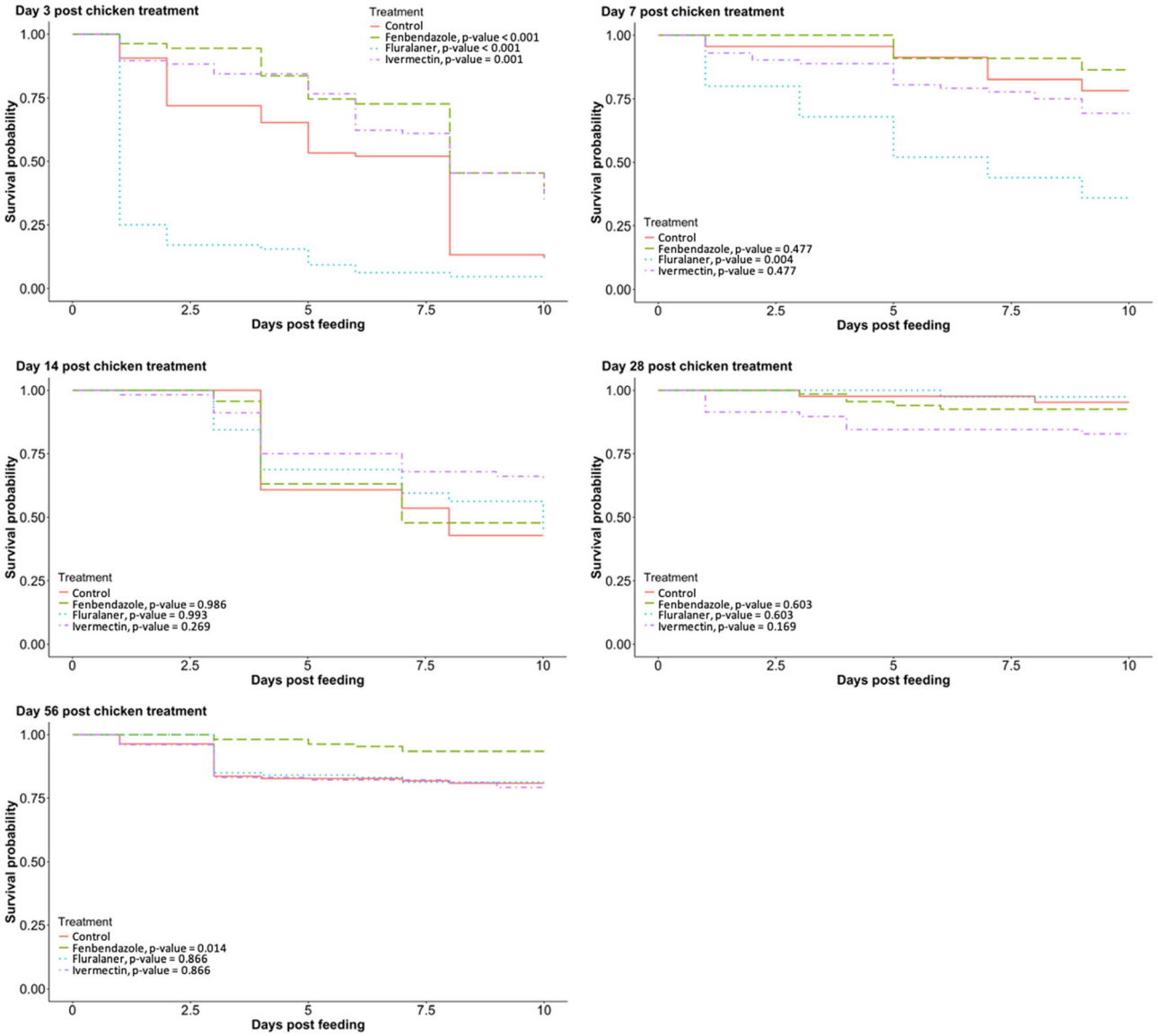

FIGURE 2 Kaplan–Meier survival curves of *Culex quinquefasciatus* fed on chickens as control or chickens treated with fenbendazole, ivermectin or fluralaner at 3, 7, 14, 28 and 56 days post-treatment.

by *Culex* sp., while other colonised mosquito species, such as *Ae. aegypti*, are more willing to take bloodmeals (Lyski et al., 2011; Meuti et al., 2023). Our low feeding rates could also be attributed to other factors. For example, although the chickens were re-strained, their movements during feeding may have still prevented bloodmeal acquisition. Mosquitoes may also be damaged or stressed while transferring in vehicles from the insectary to the experimental feeding room 2 km away. Despite the low feeding rate, no significant differences were observed within each time point or each treatment, suggesting no repellent effects of the products evaluated in this study on mosquitoes. Members of the *Cx. pipiens* complex, which includes *Cx. quinquefasciatus*, are ornithophilic (Farajollahi et al., 2011) and frequently feed on chickens when available, suggesting that this low feeding rate observed in this current study would not reflect the utility of this control approach in nature.

The results reported in our study and previous studies (Alcantara et al., 2023; Duncan et al., 2023; Durden et al., 2023; Evans et al., 2023; Gurtler et al., 2022) suggest fluralaner is a promising candidate as a host-targeted pesticide due to high efficacy against blood-feeding arthropods, lack of repellency and non-toxicity in a wide range of domestic animals. This approach of xenointoxication could allow for area-wide treatment of chickens in peridomestic environments, which could result in population suppression of medically relevant bird-biting mosquitoes such as *Cx. quinquefasciatus*.

Culex quinquefasciatus is nearly globally distributed and considered to be an important vector of multiple pathogens including WNV (Ciota, 2017), St. Louis encephalitis virus (Diaz et al., 2013) and filarial nematodes such as Wuchereria bancrofti (Cobbold) (Rhabditida: Onchocericidae) (Calheiros et al., 1998). Xenointoxication is an alternative mosquito control tool that could achieve population

suppression of a variety of blood-feeding arthropods of public health importance and future studies should evaluate the impact of xenointoxication on disease transmission in nature.

AUTHOR CONTRIBUTIONS

Koyle Knape: Methodology; data curation; writing – original draft; writing – review and editing. **Yuexun Tian:** Formal analysis; writing – original draft; writing – review and editing; visualization. **Cassandra Durden:** Methodology; data curation; writing – review and editing. **Dayvion R. Adams:** Conceptualization; writing – review and editing. **Macie Garza:** Data curation; writing – review and editing. **John B. Carey:** Methodology; writing – review and editing. **Sarah A. Hamer:** Conceptualization; writing – review and editing. **Gabriel L. Hamer:** Conceptualization; methodology; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing interests to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Texas Data Repository at https://doi.org/10.18738/T8/W7T98S.

ETHICS STATEMENT AND CONSENT TO PARTICIPATE

Protocols for the use of chickens were reviewed and approved by the Texas A&M University Institutional Animal Care and Use Committee (Animal Use Protocol IACUC 2021–0109) on 05/11/2021.

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REFERENCES

Alcantara, J.A., de Araujo, F.S.A., da Costa, P.A., Alencar, R.M., de Albuquerque Caldas, B.Y., Godoy, R.S.M. et al. (2023) Effect of fluralaner on the biology, survival, and reproductive fitness of the neotropical malaria vector *Anopheles aquasalis*. *Malaria Journal*, 22(1), 337. Available from: https://doi.org/10.1186/s12936-023-04767-0

Arisova, G.B. (2020) Efficacy of ivermectin-based drugs against ectoparasites in broiler chickens. *World's Veterinary Journal*, 10(2), 160–164.

Burgess, E.R., Geden, C.J., Lohmeyer, K.H., King, B.H., Machtinger, E.T. & Scott, J.G. (2020) Toxicity of fluralaner, a companion animal insecticide, relative to industry-leading agricultural insecticides against resistant and susceptible strains of filth flies. *Scientific Reports*, 10(1), 11166. Available from: https://doi.org/10.1038/s41598-020-68121-z

Calheiros, C.M., Fontes, G., Williams, P. & Rocha, E.M. (1998) Experimental infection of *Culex* (*Culex*) *quinquefasciatus* and *Aedes* (*Stegomyia*) *aegypti* with *Wuchereria bancrofti*. *Memórias Do Instituto Oswaldo Cruz*, 93(6), 855–860. Available from: https://doi.org/10.1590/s0074-02761998000600029

CDC. (2024) Clinical Signs and Symptoms of West Nile Virus Disease.

Chaskopoulou, A., Dovas, C.I., Chaintoutis, S.C., Kashefi, J., Koehler, P. & Papanastassopoulou, M. (2013) Detection and early warning of West Nile virus circulation in Central Macedonia, Greece, using sentinel chickens and mosquitoes. *Vector Borne and Zoonotic Diseases*, 13(10), 723–732. Available from: https://doi.org/10.1089/vbz.2012.1176

Ciota, A.T. (2017) West Nile virus and its vectors. Curr Opin. *Insect Sci.*, 22, 28–36. Available from: https://doi.org/10.1016/j.cois.2017.05.002

Derouen, S.M., Miller, J.E., Foil, L.D. & Gentry, G.T. (2009) Control of horn flies (*Haematobia irritans*) and gastrointestinal parasites and its relation with cow-calf performance. *Veterinary Parasitology*, 162(3–4), 320–326. Available from: https://doi.org/10.1016/j.vetpar.2009.03.021

Derua, Y.A., Malongo, B.B. & Simonsen, P.E. (2016) Effect of ivermectin on the larvae of *Anopheles gambiae* and *Culex quinquefasciatus*. *Parasites & Vectors*, 9, 131. Available from: https://doi.org/10.1186/s13071-016-1417-5

Deus, K.M., Saavedra-Rodriguez, K., Butters, M.P., Black, W.C. & Foy, B.D. (2012) The effect of ivermectin in seven strains of *Aedes aegypti* (Diptera: Culicidae) including a genetically diverse laboratory strain and three permethrin resistant strains. *Journal of Medical Entomology*, 49(2), 356–363. Available from: https://doi.org/10.1603/me11164

Dias, J.C., Schofield, C.J., Machado, E.M. & Fernandes, A.J. (2005) Ticks, ivermectin, and experimental Chagas disease. *Memórias Do Instituto Oswaldo Cruz*, 100(8), 829–832. Available from: https://doi.org/10.1590/s0074-02762005000800002

Diaz, L.A., Flores, F.S., Beranek, M., Rivarola, M.E., Almiron, W.R. & Contigiani, M.S. (2013) Transmission of endemic St Louis encephalitis virus strains by local *Culex quinquefasciatus* populations in Cordoba, Argentina. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 107(5), 332–334. Available from: https://doi.org/10.1093/trstmh/trt023

Duncan, K., Barrett, A.W., Little, S.E., Sundstrom, K.D. & Guerino, F. (2023) Fluralaner (Bravecto((R))) treatment kills *Aedes aegypti* after feeding on *Dirofilaria immitis*-infected dogs. *Parasites & Vectors*, 16(1), 208. Available from: https://doi.org/10.1186/s13071-023-05819-9

Durden, C., Tian, Y., Knape, K., Klemashevich, C., Norman, K.N., Carey, J.B. et al. (2023) Fluralaner systemic treatment of chickens results in mortality in *Triatoma gerstaeckeri*, vector of the agent of Chagas disease. *Parasites & Vectors*, 16(1), 1–10.

Estrada-Franco, J.G., Fernandez-Santos, N.A., Adebiyi, A.A., Lopez-Lopez, M.J., Aguilar-Duran, J.A., Hernandez-Triana, L.M. et al. (2020) Vertebrate-Aedes aegypti and *Culex quinquefasciatus* (Diptera)-arbovirus transmission networks: non-human feeding revealed by meta-barcoding and next-generation sequencing. *PLoS Neglected Tropical Diseases*, 14(12), e0008867. Available from: https://doi.org/10.1371/journal.pntd.0008867

Evans, C.C., Normile, D., Gamble, S., Guerino, F., Dzimianski, M.T. & Moorhead, A.R. (2023) Treatment of dogs with Bravecto((R)) (fluralaner) reduces mosquito survival and fecundity. *Parasites & Vectors*,

16(1), 147. Available from: https://doi.org/10.1186/s13071-023-05682-8

Farajollahi, A., Fonseca, D.M., Kramer, L.D. & Kilpatrick, A.M. (2011) "bird biting" mosquitoes and human disease: a review of the role of complex mosquitoes in epidemiology. *Infection, Genetics and Evolution*, 11(7), 1577–1585. Available from: https://doi.org/10.1016/j.meegid.2011.08.013

Foley, D.H., Bryan, J.H. & Lawrence, G.W. (2000) The potential of ivermectin to control the malaria vector *Anopheles farauti*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 94(6), 625–628. Available from: https://doi.org/10.1016/s0035-9203(00)90211-6

Foy, B.D., Kobylinski, K.C., da Silva, I.M., Rasgon, J.L. & Sylla, M. (2011) Endectocides for malaria control. *Trends in Parasitology*, 27(10), 423–428. Available from: https://doi.org/10.1016/j.pt.2011.05.007

Gurtler, R.E., Laino, M.A., Alvedro, A., Enriquez, G.F., Macchiaverna, N.P., Gaspe, M.S. et al. (2022) Treatment of dogs with fluralaner reduced pyrethroid-resistant *Triatoma infestans* abundance, *Trypanosoma cruzi* infection and human-triatomine contact in the Argentine Chaco. *Parasites & Vectors*, 15(1), 257. Available from: https://doi.org/10.1186/s13071-022-05343-2

Hannon, E.R., Jackson, K.C., Biggerstaff, B.J., Raman, V. & Komar, N. (2019) Bloodmeal host selection of *Culex quinquefasciatus* (Diptera: Culicidae) in Las Vegas, Nevada, United States. *Journal of Medical Entomology*, 56(3), 603–608. Available from: https://doi.org/10.1093/jme/tjy237

Holcomb, K.M., Nguyen, C., Foy, B.D., Ahn, M., Cramer, K., Lonstrup, E.T. et al. (2022) Effects of ivermectin treatment of backyard chickens on mosquito dynamics and West Nile virus transmission. *PLoS Neglected Tropical Diseases*, 16(3), e0010260. Available from: https://doi.org/10.1371/journal.pntd.0010260

Holcomb, K.M., Nguyen, C., Komar, N., Foy, B.D., Panella, N.A., Baskett, M.L. et al. (2023) Predicted reduction in transmission from deployment of ivermectin-treated birdfeeders for local control of West Nile virus. *Epidemics*, 44, 100697. Available from: https://doi.org/10.1016/j.epidem.2023.100697

Kading, R.C., Reiche, A.S., Morales-Betoulle, M.E. & Komar, N. (2013) Host selection of potential West Nile virus vectors in Puerto Barrios, Guatemala, 2007. *The American Journal of Tropical Medicine and Hygiene*, 88(1), 108–115. Available from: https://doi.org/10.4269/ajtmh.2012.12-0223

Kassambara, A., Kosinski, M., Biecek, P. & Fabian, S. (2021) survminer: Drawing Survival Curves using 'ggplot2'. R package version 0.4 9 (2021).

Kent, R.J., Reiche, A.S., Morales-Betoulle, M.E. & Komar, N. (2010) Comparison of engorged *Culex quinquefasciatus* collection and blood-feeding pattern among four mosquito collection methods in Puerto Barrios, Guatemala, 2007. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 26(3), 332–336. Available from: https://doi.org/10.2987/09-5953.1

Lyski, Z.L., Saredy, J.J., Ciano, K.A., Stem, J. & Bowers, D.F. (2011) Blood feeding position increases success of recalcitrant mosquitoes. *Vector Borne and Zoonotic Diseases*, 11(8), 1165–1171. Available from: https://doi.org/10.1089/vbz.2010.0164

McLean, R.G., Ubico, S.R., Docherty, D.E., Hansen, W.R., Sileo, L. & McNamara, T.S. (2001) West Nile virus transmission and ecology in birds. *Annals of the new York Academy of Sciences*, 951, 54–57. Available from: https://doi.org/10.1111/j.1749-6632.2001.tb02684.x

Meuti, M.E., Siperstein, A. & Wolkoff, M. (2023) Points to consider when establishing and rearing *Culex* mosquitoes in the laboratory. *Cold Spring Harbor Protocols*, 2023(8), pdb. top107823.

Miglianico, M., Eldering, M., Slater, H., Ferguson, N., Ambrose, P., Lees, R.S. et al. (2018) Repurposing isoxazoline veterinary drugs for control of vector-borne human diseases. *Proceedings of the National Academy of Sciences of the United States of America*, 115(29), E6920–E6926. Available from: https://doi.org/10.1073/pnas.1801338115

Moreno, L., Dominguez, P., Farias, C., Canton, L., Virkel, G., Mate, L. et al. (2015) Ivermectin pharmacokinetics, metabolism, and tissue/egg residue profiles in laying hens. *Journal of Agricultural and Food Chemistry*, 63(47), 10327–10332. Available from: https://doi.org/10.1021/acs.jafc.5b04632

Nasci, R.S., Fischer, M., Lindsey, N., Lanciotti, R.S., Savage, H.M., Komar, N. et al. (2013) *West Nile virus in the United States: guidelines for surveillance, prevention, and control*, 4th revision edition. Fort Collins, Colorado, USA: Centers for Disease Control and Prevention. https://stacks.cdc.gov/view/cdc/22064

Nguyen, C., Gray, M., Burton, T.A., Foy, S.L., Foster, J.R., Gendernalik, A.L. et al. (2019) Evaluation of a novel West Nile virus transmission control strategy that targets *Culex tarsalis* with endectocide-containing blood meals. *PLoS Neglected Tropical Diseases*, 13(3), ARTN e0007210. Available from: https://doi.org/10.1371/journal.pntd.0007210

Norris, R.H., Baker, O.S., Burgess, E.R., Tarone, A., Gerry, A., Trout Fryxell, R.T. et al. (2023) Selection for, and characterization of, fluralaner resistance in the house fly, *Musca domestica*. *Pesticide Biochemistry and Physiology*, 191, 105355. Available from: https://doi.org/10.1016/j.pestbp.2023.105355

Olson, M.F., Ndeffo-Mbah, M.L., Juarez, J.G., Garcia-Luna, S., Martin, E., Borucki, M.K. et al. (2020) High rate of non-human feeding by *Aedes aegypti* reduces zika virus transmission in South Texas. *Viruses*, 12(4):1–20. Available from: https://doi.org/10.3390/v12040453

Prohaczik, A., Menge, M., Huyghe, B., Flochlay-Sigognault, A. & Traon, G.L. (2017) Safety of fluralaner oral solution, a novel systemic antiparasitic treatment for chickens, in laying hens after oral administration via drinking water. *Parasites & Vectors*, 10(1), 363. Available from: https://doi.org/10.1186/s13071-017-2291-5

Rasmussen, J.J., Wiberg-Larsen, P., Kristensen, E.A., Cedergreen, N. & Friberg, N. (2013) Pyrethroid effects on freshwater invertebrates: a meta-analysis of pulse exposures. *Environmental Pollution*, 182, 479–485. Available from: https://doi.org/10.1016/j.envpol.2013.08.012

Ronca, S.E., Ruff, J.C. & Murray, K.O. (2021) A 20-year historical review of West Nile virus since its initial emergence in North America: has West Nile virus become a neglected tropical disease? *PLoS Neglected Tropical Diseases*, 15(5), e0009190. Available from: https://doi.org/10.1371/journal.pntd.0009190

Ross, S.M., McManus, I.C., Harrison, V. & Mason, O. (2013) Neurobehavioral problems following low-level exposure to organophosphate pesticides: a systematic and meta-analytic review. *Critical Reviews in Toxicology*, 43(1), 21–44. Available from: https://doi.org/10.3109/10408444.2012.738645

Sbrana, E., Tonry, J.H., Xiao, S.Y., da Rosa, A.P., Higgs, S. & Tesh, R.B. (2005) Oral transmission of West Nile virus in a hamster model. *The American Journal of Tropical Medicine and Hygiene*, 72(3), 325–329.

Shah, H.K., Srinivasan, V., Venkatesan, S., Balakrishnan, V., Candasamy, S., Mathew, N. et al. (2024) Evaluation of the mosquitocidal efficacy of fluralaner, a potential candidate for drug based vector control. *Scientific Reports*, 14(1), 5628. Available from: https://doi.org/10.1038/s41598-024-56053-x

Soto, R.A., Hughes, M.L., Staples, J.E. & Lindsey, N.P. (2022) West Nile virus and other domestic nationally notifiable arboviral diseases - United States, 2020. *MMWR. Morbidity and Mortality Weekly Report*, 71(18), 628–632. Available from: https://doi.org/10.15585/mmwr.mm7118a3

Team RC. (2020) *RA language and environment for statistical computing*. R Foundation for Statistical. Computing.

Therneau, T. (2020) A Package for Survival Analysis in R. R package version 3.2-7. 2020. https://CRAN.R-project.org/package=survival

Thomas, E., Chiquet, M., Sander, B., Zschiesche, E. & Flochlay, A.S. (2017) Field efficacy and safety of fluralaner solution for administration in drinking water for the treatment of poultry red mite (*Dermanyssus gallinae*) infestations in commercial flocks in Europe. *Parasites & Vectors*, 10(1), 457. Available from: https://doi.org/10.1186/s13071-017-2390-3

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