Journal of Medical Entomology, 57(2), 2020, 542–550
doi: 10.1093/jme/tjz209
Advance Access Publication Date: 22 November 2019
Research

Infected Mosquitoes Have Altered Behavior to Repellents: A Systematic Review and Meta-analysis

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Subject Editor: Douglas Norris

Received 28 June 2019; Editorial decision 18 October 2019

Abstract

Here we conducted a systematic review and meta-analysis to reach a consensus on whether infected and uninfected mosquitoes respond differently to repellents. After screening 2,316 published studies, theses, and conference abstracts, we identified 18 studies that tested whether infection status modulated the effectiveness of repellents. Thirteen of these studies had outcomes available for meta-analysis, and overall, seven repellents were tested (typically DEET with 62% of outcomes), six mosquito species had repellence behaviors measured (typically *Aedes aegypti* (L.) (Diptera: Culicidae) mosquitoes with 71% of outcomes), and a broad diversity of infections were tested including Sindbis virus (Togaviridae: Alphavirus) (33% of outcomes), Dengue (Flaviviridae: Flavivirus) (31%), malaria (*Plasmodium berghei* Vincke & Lips (Haemosporida: Plasmodiidae) or *P. falciparum* Welch (Haemosporida: Plasmodiidae); 25%), Zika (Flaviviridae: Flavivirus) (7%), and microsporidia (4%). Pooling all outcomes with meta-analysis, we found that repellents were less effective against infected mosquitoes—marking an average 62% reduction in protective efficacy relative to uninfected mosquitoes (pooled odds ratio = 0.38, 95% confidence interval = 0.22–0.66; $k = 96$). Older infected mosquitoes were also more likely to show altered responses and loss of sensitivity to repellents, emphasizing the challenge of distinguishing between age or incubation period effects. *Plasmodium*- or Dengue-infected mosquitoes also did not show altered responses to repellents; however, Dengue–mosquito systems used inoculation practices that can introduce variability in repellency responses. Given our findings that repellents offer less protection against infected mosquitoes and that these vectors are the most dangerous in terms of disease transmission, then trials on repellent effectiveness should incorporate infected mosquitoes to improve predictability in blocking vector–human contact.

Key Words: feeding behavior, malaria, mosquito-borne disease, mosquito repellent and attractant

Repellents help reduce mosquito bites and therefore play a central role in preventing the transmission of vector-borne diseases such as malaria, dengue fever, and Zika. Given that these parasites and viruses can be transmitted with a single bite, repellents must offer near-total protection against parasite-infected mosquitoes. Yet repellent protection is inconsistent among infected and uninfected mosquitoes (Qualls et al. 2014). Some studies find no relationship between infection status and feeding behavior (e.g., Robert et al. 1991, Costantini et al. 2004), whereas others find that repellents are less effective against infected mosquitoes (e.g., Barnard et al. 2007, Leal et al. 2017). Although parasites and viruses are expected to alter mosquito feeding behavior (reviewed in Moore 1993, Hurd 2003, Poulin 2010; but also see Koella and Packer 1996, Anderson et al. 1999), experiments testing for repellency alterations are fraught with experimental inconsistencies that can introduce heterogeneity in feeding behaviors. These include variability in inoculation practices (Qualls et al. 2011), testing age (i.e., days post-inoculation; Leal et al. 2017), and the behavioral response measured (e.g., spatial or contact repellency responses; Qualls et al. 2012a). Furthermore, not all repellents are alike, and their effectiveness can be highly

dependent on the mosquito and parasite species tested (Onyango and Moore 2014).

Here, we conduct a systematic review to find all of the available evidence on repellent effects among infected and uninfected mosquitoes. We then use meta-analysis to 1) reach a consensus on the mixed findings of parasite-altered behaviors to repellents and 2) test sources of heterogeneity that can explain mixed outcomes (e.g., inoculation method, mosquito age). We hope that by collating and synthesizing what has been done, we can identify research gaps and address concerns about current practices that affect the scope and quality of studies testing parasite-altering effects of repellents on mosquitoes.

Methods

We searched for published articles and gray literature (e.g., unpublished studies, theses, conference abstracts) that fit these study criteria: was an experiment (e.g., field, laboratory), that tested a repellent (e.g., synthetic, essential oil, insecticide with repellent activities), on uninfected (e.g., parasite-free, sham-infected) and infected mosquitoes that were either experimentally inoculated with parasites/viruses (e.g., injection, feeding, larval exposure) or had infection status determined after repellency tests (e.g., wild mosquitoes collected during field trials and tested afterward). Using *Web of Science* (WOS; 28 August 18 with University of South Florida [USF] Tampa Library institutional subscription), we found 1,835 publications with this keyword search: ((repel* OR deet OR protection*) AND (*infect* OR alter* OR response*) AND (mosquito* OR anopheles OR aedes OR culex OR mansonia)). We then screened only the titles and abstracts of these candidates using a dual-screening design, where 18 pairs of screeners (total 36 individuals) independently evaluated approximately 101 publications each. The screening team was composed of senior undergraduates taking a Medical Entomology course at USF; all were trained for screening studies via in-class screening exercises and by reading the review by Qualls et al. (2014). This study-selection effort was completed using PDF forms generated by the METAGEAR package for R (v. 0.5; Lajeunesse 2016), and to facilitate text screening, titles/abstracts had 'mosquito', 'repel', and 'infect' highlighted. This screening resulted in 377 of 1,835 studies with either dual or mixed agreement for inclusion. The interscreener reliability for inclusion/exclusion was strong (pooled inter-rater agreement: Byrt's kappa = 0.754 ± 0.193 SD; n = 18 observer pairs with coding categories 'keep' or 'discard'; Byrt et al. 1993). These 377 studies were then randomized and dual screened again on 6 September 2018, resulting in 140 of 377 with either dual or mixed agreement for inclusion (pooled Byrt's kappa = 0.421 ± 0.413 SD; n = 18 pairs screening approx. 21 studies each). Finally, via consensus vote among 26 screeners on 6 September 2018 (all students attending class on that date), it was agreed that 14 of 140 fit our inclusion criteria; the consensus vote was necessary to finalize inclusion of studies screened with mixed agreement. In total, through this screening design, titles/abstracts were vetted for inclusion five times.

Thirteen screeners also searched the Open Access Theses and Dissertations database (OATD; Dowling 2013) on 19 September 2018 with keywords (feeding AND behavior AND mosquito AND infect*) to find 23 candidates, and (mosquito AND repel* AND infect*) to find 19 candidates. After deduplication, these 36 theses were screened, and via consensus vote among 26 reviewers, two fit our inclusion criteria (i.e., Qualls 2012, Yang 2017). Finally, a separate group of thirteen screeners on 19 September 2018 performed a *Google* search, but limited screening to the first 50 of 13,900 hits based on keywords: ('infection status' AND mosquito AND repel). A consensus screening of these 50 found a publication (Van Roey et al. 2014) and conference abstract (Mulatier et al. 2018a) that met our inclusion criteria. The 13 screeners for OATD and the other 13 for the *Google* search were students that attended class on that day (total 26).

Full-text screening of these 18 candidate documents (14 WOS + 2 OATD + 2 Google) excluded a literature review (Qualls et al. 2014), a thesis that had all chapters already included as published studies (Qualls 2012), a laboratory trial that did not test repellency on uninfected mosquitoes (McCall et al. 2017), and field trials that either did not report the repellent used (Bockarie and Dagoro 2006) or had zero to near-zero prevalence of parasites among field-collected mosquitoes (e.g., Van Roey et al. 2014). Finally, two additional unpublished studies were discovered in the discussion sections of Sugiharto et al. (2016) and Yang (2017).

A reviewer of our study requested an update to our literature search, and on 30 August 2019, M.J. Lajeunesse repeated the WOS search with the same keywords but limiting search results to 2018 and 2019, resulting in 322 candidate studies. Screening studies with the abstract_screener() tool from the METAGEAR package for R with the same keywords highlighted as in the previous screening, three studies were identified: two new studies by Mulatier et al. (2019) and Thiévent et al. (2019), and a third study that was already included from our previous search (Thiévent et al. 2018).

In total, full-text screening found that 13 of 18 relevant studies (11 of 16 from the first search and 2 of 2 from the updated search) had numerical outcomes available for meta-analysis (see Table 1). Study outcomes were quantified as log odds ratio (logOR) effect sizes, which uses reported counts (N) of responses (R) and no responses (NR) to repellents between infected mosquitoes (I; treatment group) and uninfected mosquitoes (U; control group), and is defined as $\text{logOR} = \ln([N_\text{I}^\text{R} N_\text{U}^\text{NR}]/[N_\text{I}^\text{NR} N_\text{U}^\text{R}])$. The variance (var) of logOR is used to weight effect sizes in meta-analysis, such that outcomes with high sampling error are given less weight when pooling outcomes (Hedges and Olkin 1986), and was estimated $\text{asvar}\,(\text{logOR}) = 1/N_\text{I}^\text{R} + 1/N_\text{U}^\text{NR} + 1/N_\text{I}^\text{NR} + 1/N_\text{U}^\text{R}$ (Bland and Altman 2000). Negative logOR indicate that infected mosquitoes were less sensitive to repellents than uninfected mosquitoes, and positive logOR that infected mosquitoes were more sensitive. In some studies, outcomes with infected mosquitoes were compared with multiple control groups (e.g., uninfected and sham-infected mosquitoes), or multiple repellents were compared with a single control group (e.g., repellent-free or alcohol only). This shared information introduces covariances among pairs of effect sizes and violates the nonindependence assumption of meta-analysis models; therefore following Lajeunesse (2011) and Bagos (2012), pairs of effect sizes that share common information had covariances modeled as $\text{cov}\,(\text{logOR},\ \text{logOR}) = 1/N_\text{I}^\text{R} + 1/N_\text{U}^\text{NR}$. Some studies reported mean outcomes ($\bar{X}$) rather than counts, and in these cases, we first estimated effect sizes using Hedges' d (Hedges 1982), which is the standardized mean difference between responses to repellents among infected and uninfected mosquitoes, or $d = (1 - 3/[4N_T - 9])(\bar{X}_\text{I} - \bar{X}_\text{U})/s*$, where N is the sample size with $N_T = N_\text{I} + N_\text{U}$, and $s* = ([(N_\text{I} - 1)\text{SD}_\text{I}^2 + (N_\text{U} - 1)\text{SD}_\text{U}^2]/[N_T - 2])^{1/2}$ with SD. The variance of d is $\text{var}(d) = 1/N_\text{I} + 1/N_U + d^2/2N_T$. In cases when $\bar{X}$ and SD (or confidence intervals, sampling errors) were only reported in figures, these were manually extracted from images using the METAGEAR package for R. Finally, following Lajeunesse (2013a), d were then converted to logOR using $\text{logOR} = d\pi/\sqrt{3}$ and $\text{var}(\text{logOR}) = \text{var}(d)\pi^2/3$.

Table 1. Studies found with a systematic review that tested repellents on infected and uninfected mosquitoes

Study	Mosquito (strain)	Parasite (strain)	Repellent (% active ingredient)	Trial design	Mosquito age (d)	Contrast design
Studies included in meta-analysis						
Mulatier et al. (2019)	*Anopheles gambiae* (pyrethroid resistant, KdrKis)	*Plasmodium falciparum* (Ivory Coast)	DEET (500 mg/m^2)	L	3–5 PE (6–8 and 12–14 PI)	I–U
Thiévent et al. (2019)	*Anopheles gambiae* (KISUMU)	*Plasmodium berghei* (ANKA)	Permethrin (0.75%)	L	3–4 PE (11 and 21 PI)	I–U
Thiévent et al. (2018)	*Anopheles arabiensis* (Tanzania)	*Plasmodium falciparum* (Tanzania)	Permethrin (270 mg/m^2)	L	NA PE (14 PI)	I–U
	Anopheles gambiae (KISUMU)	*Plasmodium berghei* (ANKA)	Permethrin (270 mg/m^2)	L	3–4 PE (21 PI)	I–U
Leal et al. (2017)	*Aedes aegypti* (Brazil)	Zika virus (Brazil)	DEET (1%, 5%**)	L	NA PE (10 PI)	I–U
	Aedes aegypti (Brazil)	Zika virus (Brazil)	Picaridin (1%, 5%**)	L	NA PE (10 PI)	I–U
Sugiharto et al. (2016)	*Aedes aegypti* (Liverpool)	Dengue virus (D02-005)	DEET (0.14%, 2.5%)	L	3–5 PE (1, 4, 7, 10, 14, and 17 PI)	II–SI, II–U
Qualls et al. (2012a)	*Aedes aegypti* (Orlando)	Sindbis virus (SVHR)	DEET (15%)	L	7 PE (10 PI)	I–U
	Aedes aegypti (Orlando)	Sindbis virus (SVHR)	Picaridin (15%)	L	7 PE (10 PI)	I–U
	Aedes aegypti (Orlando)	Sindbis virus (SVHR)	Lemon eucalyptus (37%)	L	7 PE (10 PI)	I–U
	Aedes aegypti (Orlando)	Sindbis virus (SVHR)	2-Undecanone (7.75%)	L	7 PE (10 PI)	I–U
Qualls et al. (2012b)	*Aedes aegypti* (Orlando)	Sindbis virus (SVHR)	DEET (30%)	L	7 PE (7/14 PI)	I–U
Qualls et al. (2011)	*Aedes aegypti* (Orlando)	Sindbis virus (SVHR)	DEET (3%)	L	7 PE (3, 5, 7, 10, and 18 PI)	I–U
Frances et al. (2011)	*Aedes aegypti* (Bangkok)	Dengue virus (D82-041)	DEET (5%)	L	5–7 PE (14 PI)	II–SI
	Aedes aegypti (Bangkok)	Dengue virus (New Guinea)	DEET (5%)	L	5–7 PE (14 PI)	II–SI
	Aedes aegypti (Bangkok)	Dengue virus (CH53489)	DEET (5%)	L	5–7 PE (14 PI)	II–SI
	Aedes aegypti (Bangkok)	Dengue virus (816689)	DEET (5%)	L	5–7 PE (14 PI)	II–SI
	Aedes albopictus (Krabi, Thailand)	Dengue virus (D82-041)	DEET (5%)	L	5–7 PE (14 PI)	II–SI
	Aedes albopictus (Krabi, Thailand)	Dengue virus (New Guinea)	DEET (5%)	L	5–7 PE (14 PI)	II–SI
	Aedes albopictus (Krabi, Thailand)	Dengue virus (CH53489)	DEET (5%)	L	5–7 PE (14 PI)	II–SI
	Aedes albopictus (Krabi, Thailand)	Dengue virus (816689)	DEET (5%)	L	5–7 PE (14 PI)	II–SI
Barnard et al. (2007)	*Aedes aegypti* (Orlando)	*Edhazardia aedis* (Thailand)	DEET (15%)	L	7 and 14 PE (NA PI)	LI–U
Costantini et al. (2004)	*Anopheles gambiae* s.l. (Burkina Faso)	*Plasmodium falciparum* (NA)	DEET (2–13%)	F	NA PE (NA PI)	NI–NU
	Anopheles gambiae s.l. (Burkina Faso)	*Plasmodium falciparum* (NA)	Picaridin (2–13%)	F	NA PE (NA PI)	NI–NU
	Anopheles gambiae s.l. (Burkina Faso)	*Plasmodium falciparum* (NA)	IR3535 (2–13%)	F	NA PE (NA PI)	NI–NU
Copeland et al. (1995)	*Anopheles funestus* (Kenya)	*Plasmodium falciparum* (NA)	DEET (5%)	F	NA PE (NA PI)	NI–NU
	Anopheles funestus (Kenya)	*Plasmodium falciparum* (NA)	AI3-37220 (5%)	F	NA PE (NA PI)	NI–NU

Table 1. *Continued*

Study	Mosquito (strain)	Parasite (strain)	Repellent (% active ingredient)	Trial design	Mosquito age (d)	Contrast design
Robert et al. (1991)	*Anopheles stephensi* (India)	*Plasmodium falciparum* (NF54)	DEET (NA)	L	NA PE (NA PI)	I–U
	Anopheles stephensi (India)	*Plasmodium berghei* (NK65)	DEET (NA)	L	NA PE (NA PI)	I–U
	Anopheles stephensi (India)	*Plasmodium falciparum* (NF54)	Permethrin (NA)	L	NA PE (NA PI)	I–U
	Anopheles stephensi (India)	*Plasmodium berghei* (NK65)	Permethrin (NA)	L	NA PE (NA PI)	I–U
Gray literature (outcomes unavailable for meta-analysis)					Outcome description	Notes
Mulatier et al. (2018a)	*Anopheles gambiae* s.s. (pyrethroid resistant)	*Plasmodium falciparum* (NA)	DEET (NA)	L	No effect	Unpublished study reported as conference abstract, possibly published as Mulatier et al. (2019)
McCall et al. (2017)	*Aedes aegypti* (Liverpool)	*Dirofilaria immitis*	Permethrin + dinotefuran + pyriproxyfen	L	Untestable effect	Uninfected mosquitoes were not tested
Van Roey et al. (2014)	20 *Anopheles* spp. (Cambodia)	*Plasmodium falciparum* (NA) and *Plasmodium vivax* (NA)	DEET (20%) and picaridin (10%, 20%)	F	Untestable effect	None of the mosquitoes collected were infected
K. Chan and S.L. Paulson (unpublished data)	*Aedes triseriatus* (NA)	La Crosse virus (NA)	Multiple tested but not reported	NA	Effect	Discussed in Yang (2017)
M. J. Turell (unpublished data)	*Culex tarsalis* (NA)	Rift Valley fever virus (NA)	DEET (NA)	NA	Effect	Discussed in Sugiharto et al. (2016)
	Aedes taeniorhynchus (NA)	Rift Valley fever virus (NA)	DEET (NA)	NA	Effect	

Trial designs include laboratory (L) or field studies (F; i.e., landing-catch experiments), mosquito age in days post-emergence (PE) and post-inoculation (PI), and finally treatment–control contrast design: inoculated via natural means (I; e.g., feeding), inoculated via injection (II), sham-injection (SI), unmanipulated (U), natural (wild) infections (N), and mosquito larva exposed (LI). Unreported information presented as NA.

[a]Chamber experiment equivalent to 30% active ingredient in arm-in-cage experiment.

Publication bias was assessed using Egger's test (Egger et al. 1997) based on inverse variances (i.e., meta-analysis weights) of logOR and while assuming a fixed-effect model for meta-analysis. Mixed-effect meta-analyses were used to pool and compare k number of effect sizes using the *rma.mv*() function of the METAFOR package for R (Viechtbauer 2010). All meta-analyses included a between-study random-effects component (τ^2; as all random-effects meta-analyses; see Koricheva et al. 2013), and an additional random-effect component (γ^2) that modeled the overrepresentation of multiple effect sizes from single studies. These random effects were estimated using maximum likelihood. The nonindependence of common controls among pairs of effect sizes was integrated by including the covariances (cov) of effect size pairs as off-diagonals in the $k \times k$ variance–covariance matrix used in meta-analysis to model sampling error (see Lajeunesse 2011). Pooled effect sizes were significantly nonzero when 95% confidence intervals (CI) did not overlap with zero. Differences among groups of effect sizes (added as fixed-effect categorical moderators in meta-analysis) were evaluated with Q^B-tests (akin to omnibus ANOVA; Hedges and Olkin 1986), and pairwise differences among groups were evaluated with z-test contrasts.

Results

Our search strategy found 18 published and unpublished experiments comparing behavioral responses to repellents between infected and uninfected mosquitoes (Table 1), adding 13 to the 5 previously summarized by Qualls et al. (2014). Among these studies, 13 had numerical outcomes available for calculating 96 effect sizes (i.e., difference in repellency sensitivity among infected and uninfected mosquitoes) across six mosquito species, six parasites, seven repellents, and a diversity of age classes and experimental designs (Table 1). An assessment of publication bias indicated that negative outcomes (e.g., loss of repellency effects on infected mosquitoes) with small sample sizes were more common than those reporting greater repellency among uninfected mosquitoes (Egger's test: $z = -12.18$, $P < 0.0001$, $k = 96$; see funnel plot inlay in Fig. 1). However, we must caution that there were too few studies for this test to convincingly assess whether publication bias is a valid concern with this literature (i.e., it failed two of the four eligibility criteria for test appropriateness; see Ioannidis and Trikalinos 2007, Lajeunesse 2013b).

In aggregate, our meta-analysis found that infected mosquitoes were less sensitive to repellents than uninfected mosquitoes (Fig. 1), even after excluding outcomes from one over-represented research group (e.g., Qualls et al. 2011, 2012a,b; subset grand mean logOR = −0.47, LCI = −0.82, UCI = −0.13; $k = 64$). The effects of infection status on repellency were tested with six mosquito species (Fig. 2), and although only *Aedes aegypti* mosquitoes had altered effects to repellents, excluding this species revealed that, in aggregate across the remaining five species, the loss of repellency effects due

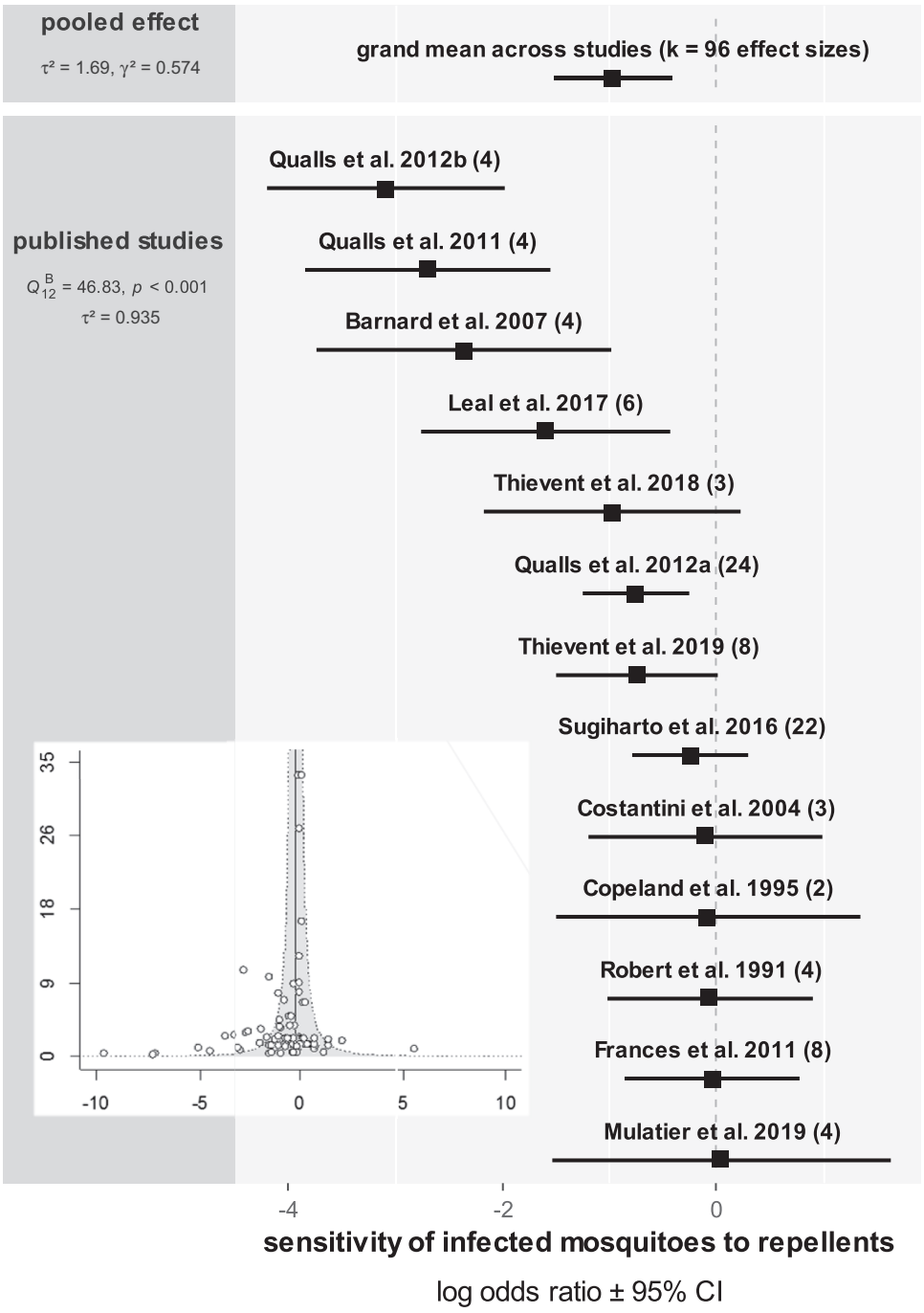

Fig. 1. Meta-analysis of pooled repellency effects among infected and uninfected mosquitoes, and parsed effects among published studies (see Table 1). Negative log odds ratios indicate that infected mosquitoes were less sensitive to repellents then uninfected controls. Pooled effects were estimated with a meta-analysis that modeled random-effects estimates for between-study variance (τ^2) and multiple effects per study (γ^2). The number of effect sizes pooled (k) is presented in the rightmost parentheses of each group subheadings. When confidence intervals (CI) do not overlap with zero (dashed line), pooled effects are significantly nonzero. Finally, Q^B tests with subscripts indicating degrees of freedom are omnibus tests for differences among groups of pooled effect sizes. Inlayed is the funnel plot of repellency effects of log odds ratios on x-axis (k = 96; log odds ratios on x-axis) and their inverse variance on the y-axis (i.e., meta-analysis fixed-effect weights). In the absence of heterogeneity and bias, 95% of effect sizes are expected to lie within the shaded funnel area (Ioannidis and Trikalinos 2007).

to infections remained (subset grand mean excluding *A. aegypti*: logOR = −0.26, LCI = −0.51, UCI = −0.01; k = 28). Likewise, although only experiments testing DEET had enough outcomes to detect a loss of repellency sensitivity (Fig. 2), loss of repellency effect was still detectable when aggregating effects across non-DEET repellents (subset grand mean excluding DEET: logOR = −0.61, LCI = −0.97, UCI = −0.24; k = 37). Finally, there was significant variability in repellency effects among infection types, where reductions in repellent sensitivity were found in mosquitoes infected by microsporidia (*Edhazardia aedis*), Zika virus, and Sindbis virus, but not with *Plasmodium* (malaria) and Dengue virus infections (Fig. 2).

The inoculation method used to infect mosquitoes and the type of control group used to compare these mosquitoes were both important sources of variability in repellency (Fig. 3). Outcomes based on

inoculation methods that did not injure mosquitoes were more likely to detect a loss in repellency effects on mosquitoes, whereas studies that either experimentally injected parasites or studies that measured parasitism levels after experiments did not. There was also variability among different inoculation practices; in particular, there was a significant difference among naturally infected injections versus thoracic injections. However, it is important to note that all thoracic injection studies were studies focusing on Dengue inoculations.

We did not find significant variability across six behavioral feeding responses (Fig. 3), including behaviors that are activated

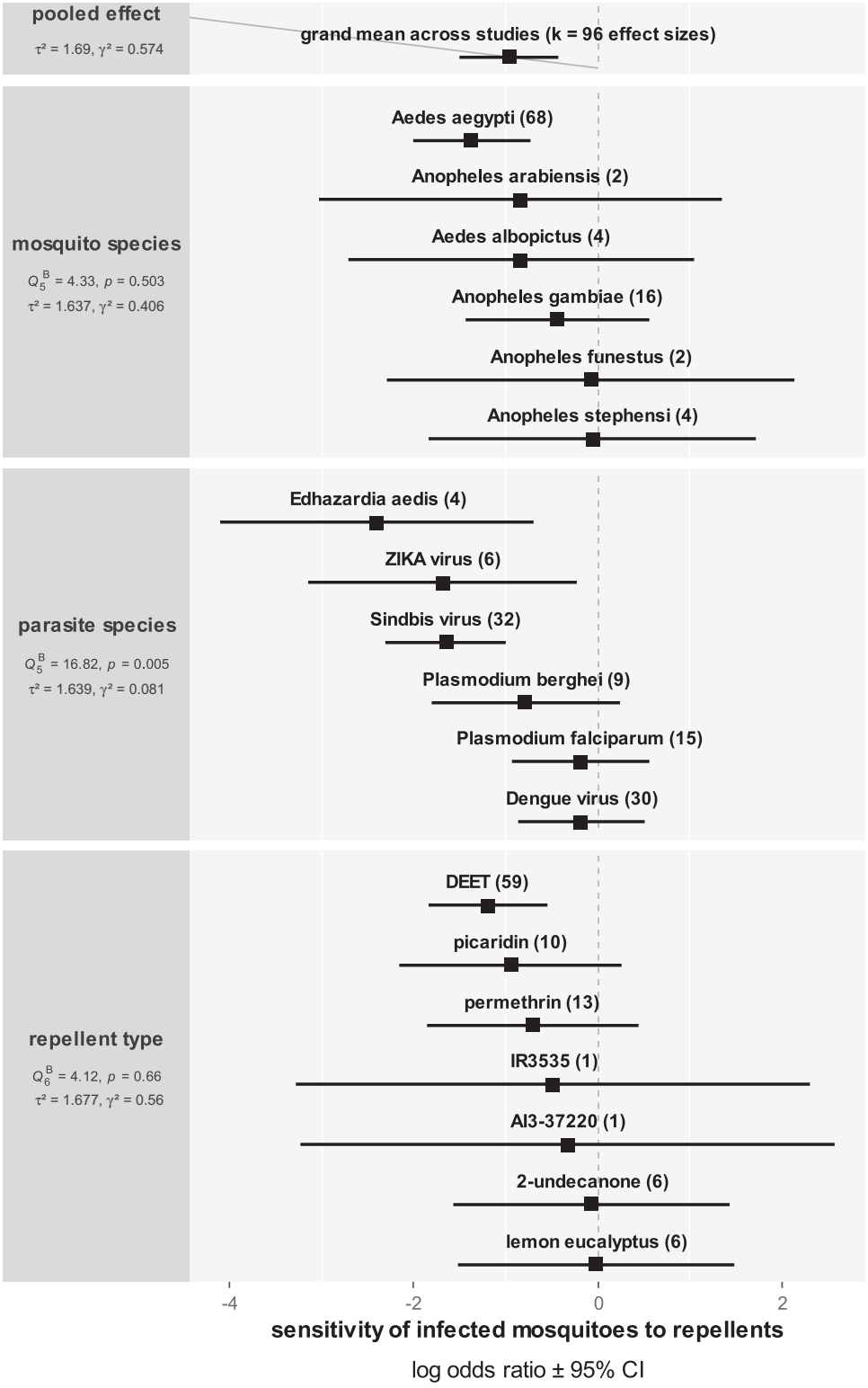

Fig. 2. Pooled repellency effects parsed by mosquito species, infection type (parasite species), and repellent tested (see Table 1). Interpretation of effects same as Fig. 1.

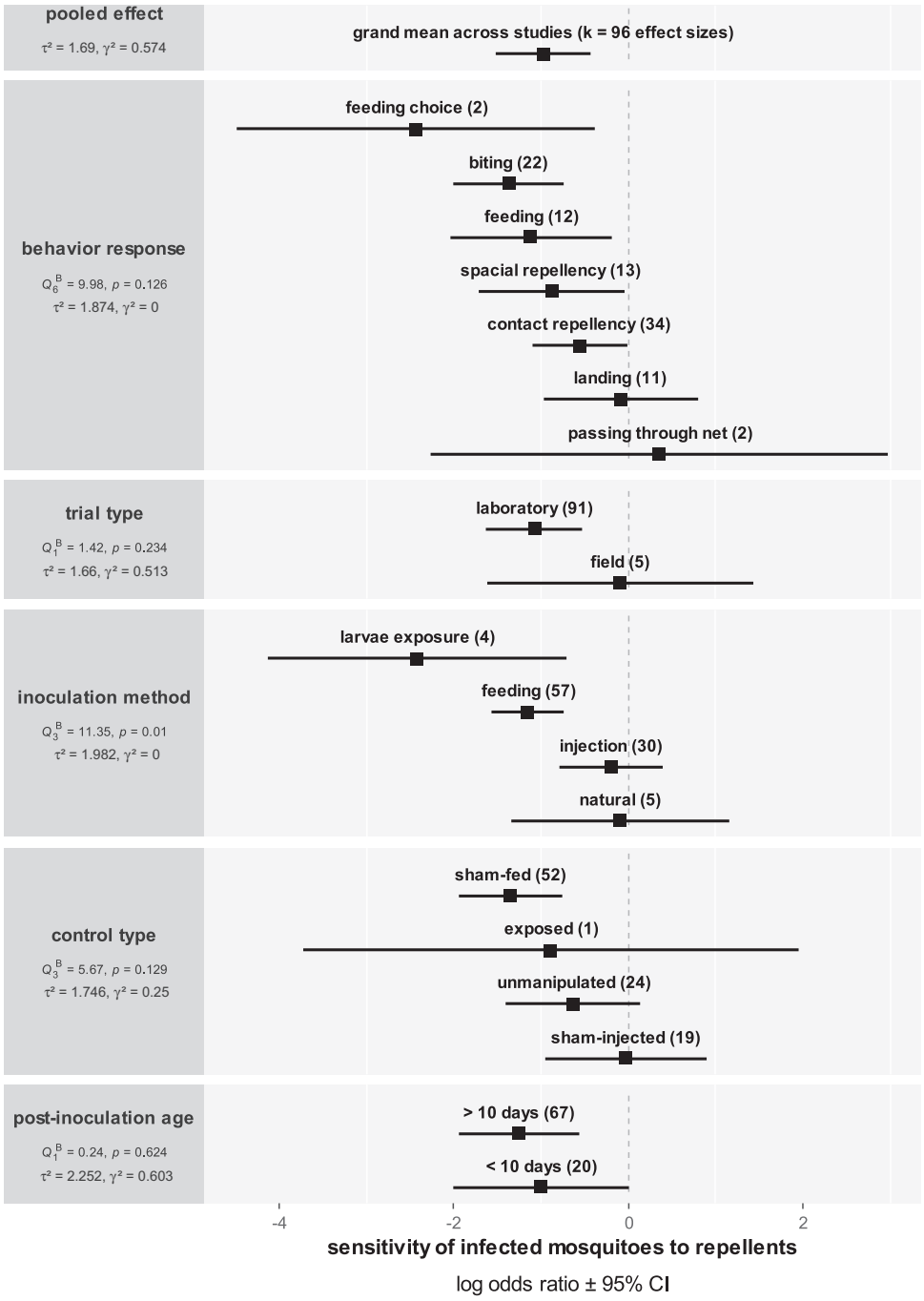

Fig. 3. Pooled repellency effects parsed by experimental practices (see Table 1). These include the mosquito feeding behaviors measured as a response to repellents, such as feeding choice, biting, feeding, spatial repellency, contact repellency, and landing behaviors. Experiments conducted in the field or laboratory (trial type), and how mosquitoes were experimentally infected with parasites; larvae exposure, feeding, injection, natural (wild mosquitoes that had infection status evaluated after they were sampled during repellency tests). What type of control group was used to compare with infected mosquitoes; these include unmanipulated individuals or procedural that were sham-fed, exposed, or sham-injected. Finally, the age of mosquitoes tested after inoculation of parasites. Interpretation of pooled effects same as Fig. 1.

spatially or on contact; however, responses based on biting and feeding behaviors were the most common and therefore had the most power, in aggregate, to detect altered responses to infection. There were also no differences between laboratory or field experiments; however, there were too few field studies to adequately make this comparison. Finally, there was less evidence for parasite effects on repellency with young mosquitoes (Fig. 3).

Discussion

We found that the personal protection offered by repellents decreased when mosquitoes had infections. We also found that we could not fully generalize this negative effect to all experimental designs—given that conflicting outcomes to repellency responses were associated with parasite-specific experimental practices (e.g., inoculation protocol; see Fig. 2). Because our synthesis cannot address

the causal explanations for why parasites or viruses alter repellent efficacy, we primarily focus on knowledge gaps and how our findings can be used to improve future tests of impacts of infection status and repellent efficacy.

Our systematic review found that studies testing parasitism effects on mosquito repellency were dominated by laboratory experiments testing DEET on *A. aegypti* mosquitoes infected with viruses (Figure 2). Our systematic review also identified several key knowledge gaps: there were no repellency tests using several culicine vectors (e.g., *Culex*, *Mansonia*, *Haemagogus*), roundworms (e.g., filarial worms), flaviviruses (e.g., West Nile, yellow fever), alphaviruses (e.g., Chikungunya), or phleboviruses (e.g., Rift Valley Fever). Furthermore, popular extract-based repellents have not been tested (e.g., citronella), and given that DEET may repel through multiple dimensions of disengagement (i.e., via both noncontact and contact effects; Syed and Leal 2008), future studies can use different repellent types to better pinpoint which behavioral responses are most altered by parasitism (see Fig. 2). Our gray literature search found studies that could fill some of these gaps (see Table 1); however, numerical outcomes of these studies were not available and could not be included in our meta-analysis.

We also found evidence that older infected mosquitoes may be less sensitive to repellents than younger infected mosquitoes (Fig. 3)—indicating potential interactive effects between mosquito age and infection-status. Few studies explore the effects of mosquito age on repellency, but when they do, they offer conflicting reports (see Xue et al. 1995, Barnard 1998). A more recent study by Mulatier et al. (2018b) using multiple mosquito species found that older (uninfected) adults tended to be more sensitive to DEET than younger (uninfected) ones. Our findings suggest that infection status can counterbalance these positive age-dependent effects on repellency, which is in agreement with the broad diversity of altered positive effects that parasitism can have on host-seeking and host-feeding behaviors (see Moore 1993). Future studies should seek to distinguish between these two moderating effects to better understand the mechanisms of loss of repellency, and whether age and infection status consistently act interactively to influence repellency efficacy or whether there are conditions when they can act independently or additively.

Studies testing repellency and infection status were also confounded by invasive inoculation practices. We found that studies using intrathoracic injection techniques to experimentally infect mosquitoes were less likely to find altered responses to repellents than studies exposing mosquitoes to parasites via feeding (Fig. 3). In particular, this negative effect is especially discernable when procedural (sham-injected) controls are used as the baseline of comparison to infected mosquitoes. Our findings reaffirm precious criticisms of the potential confounding effects of these invasive inoculation practices (see Sugiharto et al. 2016), which by-pass natural routes of infection (Qualls et al. 2014), and themselves via injury can alter the physiology and behavior of mosquitoes.

Finally, repellency guidelines developed using nuisance (uninfected) mosquitoes may not adequately deliver the protection needed to prevent disease transmission. The current EPA (2010) protocol targets 5- to 10-d-old post-emergence adults, whereas WHO (2009) recommends trials using 5- to 7-d old adults. However, our synthesis confirms and endorses previous calls to develop recommendations that also target old (>10 d), parous, infected mosquitoes (see Copeland et al. 1995, Frances et al. 2011, Qualls et al. 2011, Sugiharto et al. 2016, Leal et al. 2017). Clearly, repelling nulliparous mosquitoes seeking their primary bloodmeal helps to prevent disease transmission because it limits opportunities for mosquitoes to become vectors. However, there are many conditions where first hosts are not protected (or cannot be protected, as in systems with sylvatic phases in transmission cycles; e.g., yellow fever). In these cases, effective protection of disease-transmitting mosquitoes may require higher concentrations of repellents that can compensate for the altered behaviors of infected mosquitoes. There are currently too few studies available to develop guidelines for these conditions—in particular field studies, which had very low representation in our synthesis (see Table 1), but are key to validate the repeatability of laboratory outcomes under natural conditions (Thiévent et al. 2018). However, developing predictable repellent-based protection is possible if the following open questions are addressed. First, how do mosquito age and infection status interact to affect repellent efficiency? In a comparative context among a diversity of mosquito–parasite systems, is this interaction consistent? Given the logistic challenges with testing repellency with infected mosquitoes (e.g., maintaining laboratory cultures of parasites or pathogens, mosquito inoculation methods, ethical use of human volunteers as attractants), can old, parous but uninfected mosquitoes be used as practical surrogates for developing better protection against mosquito vectors?

Acknowledgments

This study was a class project by the Fall 2018 Medical & Applied Entomology course at the University of South Florida taught by M.J.L.

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