Title: What is known about modified insects for disease prevention?: a systematic review

Short title: Modified insects for disease prevention

Authors

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ABSTRACT

The modification and release of insects to suppress or replace natural insect vectors constitutes a promising tool for vector control and disease prevention, facing the unprecedented global emergence of vector-borne diseases. Little is known regarding these innovative modification strategies and available evidence is not standardized turning it difficult to reflect on their actual efficacy and eventual effects.

This work conducted a systematic review, gathering and analyzing research articles from PubMed and Biblioteca Virtual em Saúde databases whose results directly report efficacy and effects of the use of modified insects for disease prevention until 2016. Within more than 1500 publications that were screened a total of 349 were analyzed.

A total of 12/3.4% reported field-based evidence, and 41/11.7% covered modification strategies’ efficacy after insects’ release, their epidemiology impact or its long-term efficacy. Examples of successful results were the replacement of natural field populations by wolbachia-infected mosquitoes in 5 weeks, and the elimination of a population in laboratory cages after transgenic mosquitoes release over 10–20 weeks. Variability in the effective results were described (90/25.7%) questioning its reproducibility in different settings. We also found 38/10.9% publications reporting reversal outcomes, such as an increase of vector population after release.

Ecological effects such as horizontal transfer events to non-target species (54/15.5%), and wolbachia-induced worsening pathogenesis on mammal filarial diseases (10/2.9%) were also reported.

Present work revealed promising outcomes of both suppressing and replacing approaches. However, it also revealed a need of field-based evidence mainly regarding epidemiologic and long-term impact of insect modification strategies. It pointed out some eventual irreversible and important effects that must not be ignored when considering open-field releases, and that may constitute constraints to generate the missing field evidence. Moreover, the level of variability of existing evidence suggests the need of local/specific evidence in each setting of an eventual release.
Innovative strategies are needed to arrest the unprecedented increase of vector-borne disease incidence, distribution and severity. Several modification techniques are being tried all over the world. However this is still an emergent topic with scarce available information and of complex understanding.

Present work is the unique structured review regarding the use of modified insects for vector-borne disease prevention, bringing neutral and robust evidence that will contribute with critical insights regarding these approaches.

Here we explored more than 1200 publications and analyzed 349 publications on this subject, describing the actual efficacy and reported effects of several modification strategies. More than 30 categories were reported such as, the type of modification, the year of the publication, the species were results were tested, the type of study, and also type of Efficacy Outcome (from modification to long-term) and/or the type of Effects Outcome (from physiologic to ecologic effects). Analysis revealed promising outcomes regarding vector-control and disease prevention. However insects' modification strategies still lack field-based evidence mainly regarding epidemiological and long-term efficacy. Eventual reversal outcomes on disease transmission, or irreversible biological effects (including horizontal transfer to non-target species or worsening pathogenesis in particular diseases in mammals), were also described. These effects need to be explored, dispelled or resolved before field trials occur in human residential areas. Some of these questions could only have a robust answer if these strategies would be implemented, needing to take the risk to observe reversal outcomes and/or irreversible effects. These findings reflect the big dilemma that is under the use of modified insects to prevent vector-borne diseases. Findings could support health authorities in decision-making and regulatory committees during advisory processes, by evaluating the pros/cons of each modifying technique for a particular setting. Moreover they could also summarize what is crucial to inform to communities if planning open field releases in residential areas.
INTRODUCTION

Vector-borne diseases have a wide impact on human health being an mandatory topic on global health agendas (1)(2). Even with the significant reduction of the global burden of malaria since the beginning of the century, in 2016, this infectious disease was still responsible for 445 000 deaths (3). Due to human population growth, globalization, and climate change, arboviral diseases outbreaks have been increasing in frequency, expansion, diversity and severity (4). Only dengue’s incidence grew more than 30-fold in the last 50 years (5). Although arboviruses dispersal is partially conditioned by the environmental constraints that limit the distribution of its main vectors, outbreaks of diseases such as, yellow fever, chikungunya and Zika have been reported all over the world (6-10). The severity of Zika fetal malformations during 2015/2016 epidemics turn it a public health emergency of international concern according to World Health Organization (11). The lack of effective approved vaccines for some of these infections and the increase of insecticide resistance in its most competent vectors, impose an urgent need for innovative effective strategies to minimize these diseases (12)(13).

The release of modified insects is considered a promising approach for prevention and control of vector-borne diseases. Innumerous techniques and insects’ modification strategies had been laboratorial tested, all of them fitting one of the two broad approaches: (i) modification and release of sterile insects aiming the reduction/eradication of natural vector populations (suppression approach / vector control approach) or (ii) modification and release of insects refractory to pathogen transmission aiming the replacement of natural vector populations (replacement approach / transmission prevention approach). Open releases of modified insects have been occurring all over the world in an attempt to cope to the unprecedented vector-borne diseases burden (14-21). However, none of these modifying technologies has yet been approved by the WHO’s Vector Control Advisory Group(22).

Few studies reported the effectiveness of insects modification strategies, and even less their eventual effects exploring them only barely and theoretically(23-25). Important reviews on this topic were recently published, but corresponding to the perspective of the author regarding the subject or a summary of the authors’ selection of publications (23)(26-29). This work presents a unique structured review on the use of modified insects to control and prevent vector-borne diseases, gathering, exploring, and classifying evidence available up to 2016 regarding efficacy and effects of the use of modified insects for disease prevention.
METHODS

Present work is enrolled in a bigger project whose aim is the description of the strengths/weaknesses/opportunities/threats of modified insects for disease prevention. During analysis and reviewers’ consensus, it was realized that all evidence found constituting strengths/weaknesses/opportunities/threats of the insects’ modification for disease prevention, were fitting in two main themes: the efficacy and the effects of the insects’ modifications. Hence, results were extracted and are presented according to this classification.

Search strategy

To identify relevant documents focusing on strengths, weaknesses, opportunities and threats of modifying insects to prevent diseases, two electronic databases (PubMed and Biblioteca Virtual em Saúde, BVS) were searched using combinations of MeSH terms and free text words such as: “organisms, genetically modified” (MeSH), “wolbachia”, “lethal”, “sterile insect”, “vector-borne”, “replacement” and “suppression”. To help increase sensitivity and specificity, combinations of different search strings were used for each electronic database. Results from all searches were downloaded into Mendeley program (Elsevier); duplicates were withdrawn automatically using Mendeley and verified manually, followed by the inclusion process implementation.

Study selection

Publications were included in the study when all of the following inclusion criteria were met:

1. Research articles i.e. publications structured as Introduction, Material and Methods, and Results/Discussion, or similar.
2. Available as Free Full-Text at NOVA Discovery platform
3. Written in English, French, Portuguese or Spanish.
4. Published until the date of the search (1st March 2016)
5. Publications covering modified insects or the modifications itself. It was considered ‘modification’ any process, species or condition, described in the literature as able to be used to modify insects (rather genetic or other type of modification), even if not explicit in the collected paper.
6. Publications whose results explicitly report strengths, weaknesses, opportunities and threats of the modifications concerned with regard to health impact and/or biological impact
7. Publications whose studies were performed in Insect or Mammal species, rather in vitro, in vivo, ex vivo or in archetypal modeled species.
A two-stage inclusion process was applied. All references were initially screened by title and abstract and included in the study if they met the selection criteria. In the second stage, the full text reading of each publication was undertaken. To establish consensus in criteria application, part of the publications (5% of the 1st screening, and 50% of the 2nd screening) were screened by two reviewers (inter-reviewer check). Disagreements were resolved by discussion. In the end of the 2nd screening and after criteria had been discussed, the full-text screening was repeated by one reviewer to ensure homogeneity of the criteria during the process (temporal check). All documents considered relevant went to the next phase of extracting data and analysis.

Data synthesis and analysis

Data was extracted from the included publications into a digital data-extraction form. Two investigators performed data extraction and analysis of 50% of the included publications (inter-reviewer check). All extracted data was structured into two major themes, efficacy and effects of the modification strategies, and each of them divided into several topics and sub-topics (see more detailed information in Results section). These hierarchical categories were defined by the two reviewers through a consensual process. Disagreements were resolved by discussion. When consensus was attained, categorization and analysis of all included articles were re-checked in order to ensure a homogeneous analysis (temporal check). According to the evidence reported, publications were classified into as many categories as possible, in order to reduce the likelihood of missing key points in the data. It was also extracted information regarding the year, type of study (laboratory, semi-field, field and computational modelling), species involved in the experiments, and modification strategy (Wolbachia, anti-pathogen Transgenesis, lethal Transgenesis, etc.). As to Wolbachia-based studies, publications were classified according to the endosymbiont origin: natural occurrence, artificially introduced or removed from natural or artificially infected hosts. The classification regarding the type of study was used as a proxy of the publication robustness, considering semi-field and field studies the most robust ones, and computational modelling and laboratorial the less robust. Apart from qualitative analysis, descriptive statistics analysis was performed. The softwares Excel (Microsoft Office, Windows 10) and NVivo 10 (QSR International Pty Ldt, Doncaster, Victoria, Australia) were used during the analysis. Results are presented by theme, modification strategy and species, publications are also referred according to their chronological order on the manuscript' sections, tables and supplementary information. This literature review followed the proceeding of a PRISMA methodology (S1 Checklist).
RESULTS

Databases searches resulted in a total of 1567 publications (Fig 1). Following the removal of duplicates, 1205 references were selected. After the two-stage selection process 377 articles were included in the study, and 349 publications were analyzed. References from analyzed publications were ordered from 1 up to 349 and cited in italic for differentiation from manuscript's references (see full list of analyzed publications and summary of analysis in S1 Appendix).

Fig 1 - PRISMA Flowchart reporting the number of publications in each stage of the review

From the 349 publications selected for analysis, 340/97.1% were published after the year of 2000. The majority constituted laboratory studies, i.e. performed in a controlled experimental environment (310/88.6%) and referred to Wolbachia or other symbiont-based modification strategy (307/88.0%). Out of those 307, 2/0.7% publications referred to other symbiont (Rickettsia and Sodalis) (1),(2). Several organisms’ species integrated in the experiments of the analyzed publications: five genera of insects vectors (Aedes, Anopheles, Culex, Mansonia, Phlebotomos and Glossina) 54 genera of non-vector insects, and four mammals genera (see all data regarding quantitative analysis in S1 Figure).

In what concerns the content of the publications, two major themes emerged from the analyzed research articles: (i) efficacy of the modification strategies, and; (ii) effects induced by the modifications. Both themes (efficacy and effects) were divided into several topics (see Fig 2).

Fig 2 - Schematic representation of the themes, topics and type of outcomes described in the Results’ section

Efficacy outcomes were also divided into effective outcomes - reporting the success of the modification strategy - and ineffective outcomes - reporting the failure of the modification strategy. Ineffective outcomes includes outcomes that: (i) achieved no results, (ii) described indirect results that call into question the efficacy of the modification strategy and/or (iii) reported reversal results, i.e. that lead to the reverse of the aim of the modification strategy.

There were more publications contributing to efficacy (237/67.7%) than publications with results regarding effects (156/44.6%) (Fig 3). Regarding themes and topics’ analysis, each publication may have contributed to more than one category.
Fig 3 - Distribution of the publications whose results contributed to each of the major themes
(n=349)

In what concerns publications covering efficacy outcomes, there were more publications reporting ineffective outcomes (164/69.2%) than publications reporting effective ones (150/63.3%). Out of the latter, only 41/27.3% constitute main effective outcomes (regarding its release, epidemiologic and long-term efficacy), and out of the former, 38/23.2% constitute reversal outcomes (Fig 4).

Fig 4 - Distribution of the publications whose results contributed to each type of outcomes in Efficacy (n=237)

Next sections summarize the analysis by themes and topics, and by modification strategies as follows: (i) Wolbachia and other symbiont-based modification strategies and (ii) Transgenesis and other non-symbiont-based modification strategies. Some modifications are the combination of the two above types of modification strategies. Examples of those are: (i) Paratrangenesis, i.e. the introduction of a transgene in a symbiont bacteria infecting the insect (rather than introducing the transgene in the genome of the insect itself) (2); (ii) the simultaneous introduction of a Wolbachia and a transgene in the same organism (3) (both included in ‘Wolbachia and other symbiont-based’ section); and (iii) Transgenesis using Wolbachia as gene drive (4-9) (included in ‘Transgenesis and other’ section).

Efficacy

Wolbachia and other symbiont-based modification strategies (effective and ineffective outcomes)

A total of 195/82.3% research articles, out of the 237 with efficacy outcomes, presented results regarding the efficacy of Wolbachia and other symbiont-based insect modifications (111/56.9% reported effective outcomes, 145/74.4% reported ineffective outcomes, nTotal=195). One publication reported the efficacy of other symbiont-based insect modification strategy (a Sodalis modified by paratrangenesis) (2), and two publications referred to computational studies using archetypal modeled endosymbionts (10),(11). Efficacy results covered all the topics (Modification, Technique, Fitness, Release, Epidemiology, Long-term and Variability) as presented in Fig 5 and described in the following paragraphs.
According to analyzed publications, different Wolbachia strains were microinjected into several insect species, leading to a successful germline infection of the insect (stable transinfection). Stable transinfections were reported in the following insect species: Aedes aegypti (12-31), Aedes albopictus (32-36), Aedes polyniensis (37,38), Anopheles gambiae (39), Anopheles stephensi (40),(39),(41), and Culex tarsalis (42) (vector mosquitoes), and Bemisia tabaci (whitefly) (43), Ceratitis capitate (medfly) (44), Drosophila melanogaster (fruit fly) (45),(46), Drosophila simulans (fruit fly) (47-50),(45),(51),(46),(52), Ephestia kuehniella (butterfly) (53),(54), Laodelphax striatellus (planthopper) (55) (non-vector insects). Apart from transinfections, analyzed articles also described other types of Wolbachia successful introduction such as: transient somatic infection (56-58), infections in cell lines (59-65), ex vivo organ culture (66), outcrossing (67),(68) and introgression (69-71),(45),(72),(19),(67),(73-77). One article reported the loss of wMel on an Aedes albopictus cell line 12 passages post infection (61).

In what concerns the traits that ensure the Wolbachia effectiveness, several publications reported high cytoplasmic incompatibility (CI), i.e. the survival of offspring from infected females (>90%) and/or perfect (100%) maternal transmission, i.e. the transmission of Wolbachia to the insect host’s offspring, on vectors species mainly in Aedine species (12),(17),(19),(68),(27),(30),(74),(78-80),(75),(37), but also in Anopheles stephensi (40),(39), and Culex pipiens quinquefasciatus (81),(82). Wolbachia-induced pathogen protection was first reported in Drosophilinae species, protecting from infection of several RNA virus (83). Later, pathogen protection induced by artificial introduction of Wolbachia was also demonstrated in several vector species: not only in those naturally not infected by Wolbachia, such as, Aedes aegypti (14),(16),(19),(20),(28), and Anopheles stephensi (40),(84); but also in those that naturally host it, such as, Aedes albopictus (35),(85),(86), Aedes polyniensis (37),(38), Anopheles gambiae (57),(58), and Culex quinquefasciatus (87). In Aedine vector species, transinfected Wolbachia blocked the development of several human pathogens: yellow fever virus (YF) (20), chikungunya virus (CHIKV) (16),(20),(88),(86) three serotypes of dengue virus (DENV1, DENV2 and DENV3) (16),(19),(28),(64),(35),(85),(38), and the filarial nematode Brugia Pahangi (37). Wolbachia-infected Aedes aegypti was also protected against plasmodium gallinaceum (16). In Anopheline species, transinfected Wolbachia induced protection against Plasmodium falciparum, the most
virulent plasmodium species (57),(40), modestly suppressed *Plasmodium berghei* oocyst levels (58), and at some temperatures also protected from *Plasmodium yoelii* infection (84). In *Culex pipiens quinquefasciatus*, transinfected *Wolbachia* diminished the West Nile virus (WNV) transmission (87). Six publications also reported that transinfected *Wolbachia* could enhance infection of some pathogens in vector species such as, WNV in *Culex tarsalis* (42) and in vitro (59), *Plasmodium yoelii* at some temperatures in *Anopheles stephensi* (84), *Plasmodium gallinaceum in Aedes fluvialitilis* (80), *Plasmodium relictum in Culex quinquefasciatus* (89), and DENV2 in *Aedes aegypti* (90). These constitute reversal outcomes, i.e. the reverse to what was intended with an effective *Wolbachia*-based modification.

- **Fitness**
  None or low fitness costs, caused by the introduction of *Wolbachia* in insects species, allow the modified insect to be reproductively competitive against their natural counterparts, and therefore to easily invade natural populations after its release. Analyzed publications described no or low fitness costs after *Wolbachia* introduction in several vector insect species: *Aedes aegypti* (67),(22),(24),(73),(30), *Aedes albopictus* (91),(78),(79),(86), *Aedes fluvialitilis* (80), *Aedes polyniensis* (75-77), and *Culex pipens quinquefasciatus* (81),(82). Some articles reported a fitness cost that act as part of the control strategy, i.e. constituting part of the modification efficacy. These were the report of *Wolbachia*-induced life shortening of vectors (that reduce or eliminate the time vectors can transmit the pathogen) (16-19),(68),(34),(57) or decreased viability of desiccated eggs (preventing the next generation of mosquitoes from hatching after the dry season) (18),(68). Despite referring to a fitness cost, since they constitute *Wolbachia* traits that ensure its effectiveness, are herein considered effective outcomes of the Technique topic. In some cases *Wolbachia* induced fitness costs on mating competitiveness (41), fecundity (22),(27), (80),(34),(41), fertility (22), larvae competitiveness (92), life span (34), or development time (68),(25) of the modified insect. Moreover, some publications reported *Wolbachia*-induced fitness benefits in vector insects (13),(90),(93),(74),(92),(41),(94),(95),(82). Once fitness benefits lead to an increase of the insect vectorial capacity and consequently to an increase on disease transmission, they constitute reversal outcomes.

- **Release**
  A successful release of modified insects describe either: (i) their effective invasion and establishment in the field (replacing natural population); or (ii) incompatible mattings between modified and natural insects (suppressing the population). The release of the non-vector *Ceratitis capitata* (fruit fly) males, transinfected and inducing complete CI, led to the complete
suppression of a laboratory cage population of natural specimens (44). Insects with introduced Wolbachia successfully replaced natural specimens in laboratory cages (12),(44), in semi-field cages (19),(40), and in the field, (67),(27). Similar results were suggested by computational modelling studies (96),(30). Other articles reported invasion but only under certain meteorological (68) or entomological conditions (26), or if some technical ordeals could be overcome (97). However, release of Wolbachia-insects also led to no/low invasion rates (98),(99). Several studies suggested the need to release prohibitively large number of insects (100-102). To overcome that, two solutions were reported: releases in a ratio of 95% male mosquitoes (requiring a mass rear capacity) (11) or the introduction of insecticide resistance genes along with Wolbachia in the host insect, combined with a pre-release intervention to reduce (adult) insect vector numbers (29), (3). The unintended increase of the insect population after the release of the modified insects (reversal outcomes) was estimated by computational modelling studies (103), some of them based on field data of wMelPop-aegypti (100) and of superinfected Aedes albopictus (93).

- Epidemiology

No publications described the impact of Wolbachia-based modified insects on human disease incidence. However, several computational modelling studies estimated a successful epidemiological impact after the release of Wolbachia-insects (96),(11), specifically using wMel-aegypti combination which seems to eliminate DENV transmission in low or moderate transmission settings (104),(105).

- Long-term

Released wMel-aegypti populations persisted in near fixation and maintained the Wolbachia-induced DENV protection, two years after the release (27),(28). Laboratory and/or computational modelling studies also revealed that Wolbachia (natural or introduced) may persist over time in its symbiotic host (106),(107),(10),(108). Despite that, the long-term efficacy of Wolbachia-based modification strategy was questioned due to the report of: (i) a change in CI rates or in Wolbachia density with age, time or over generations (109-111),(69),(112),(47),(113-115); (ii) a change in other effective traits (45),(60); (iii) loss of Wolbachia infection (106),(116-122); and (iv) its natural replacement by other Wolbachia strain (123-125). Moreover, long-term efficacy was also questioned by the risk of immigration/re-invasion of other insect populations after the release and fixation of the modified insect population (21),(27). How and how much this phenomena will affect efficacy is not known (104),(21).
Variability

Finally, also affecting Wolbachia efficacy is its variability. It was reported that a considerable degree of variability may evolve in short evolutionary periods (126). Several articles described Wolbachia evolution (127),(128), including its transition from facultative parasite to a nutritional mutualist (129) or obligatory symbiont (130). Wolbachia density inside an insect-host changed according to a multitude of factors, such as, host genetic background (112),(131),(61),(132),(133),(62),(134), presence of resistance genes (135), host gender (109),(136),(111),(134), development stage (111), nutrition (57),(41),(137), immunity status (111), presence of pathogens (23),(83), or host microbiome (39),(138-140). It also varied according to Wolbachia strain (141),(142), even when coexisting in the same host (140), insect larvae density (143), and environmental conditions (84),(144),(145),(143),(111) (such as humidity and temperature). However, Wolbachia density in Ae. aegypti did not alter after repeated human blood feeding (31), neither insecticide susceptibility of Ae. aegypti changed after Wolbachia infection (36). Somewhat surprising Aedes albopictus cell lines infected with wStr or wAlbB showed resistance to streptomycin (63) Wolbachia main effective outcomes in insect vectors are presented in Table 1 and its reversal outcomes in insect vectors is described in Table 2, see the complete data (also (146-153) and (154-201)) in S1 Table.

Table 1- Main effective outcomes of Wolbachia-based and other symbiont-based insect modification (vector insect species).
Publications reporting effective outcomes in release, epidemiology and long-term topics (the ones closer to the strategy aim, that is transmission blockage or vector suppressing). Ineffective outcomes of the mentioned publications are also presented (in italic).

Table 2- Reversal outcomes of Wolbachia-based and other symbiont-based insect modification (vector insect species cell-lines not included)
Publications reporting reversal outcomes (reverse to the strategy aim, that is transmission blockage or vector suppressing). Effective outcomes of the mentioned publications are also presented (in italic).
| Species | Wolb strain | Release | Epidemiology | Long-term | Type of Study | Reference |
|---------|-------------|---------|--------------|-----------|---------------|-----------|
| *Aedes aegypti* | wAlbB | reaching infection fixation within seven generations | wolb-mosquitoes would be successfully maintained in wild populations | | Lab | Xi et al., 2005a |
| | wMel/wMelPopCLA | near fixation in 30 days-wMel (much quicker than wMelPopCLA) | | | Lab and semi field | Walker et al., 2011 |
| | wMel | near-fixation in 5 weeks / 90% infected mosquitoes at 5 weeks after releases | | | Field and Model | Hoffmann et al., 2011 |
| | wMelPop-CLA | invasion is possible under humid conditions (*under dry conditions invasion will be difficult*) | can eliminate dengue transmission in low or moderate transmission settings | wolb-mosquitoes once established these are not vulnerable to invasion | Lab and Model | Yeap et al., 2011 |
| | n.a. | (achieve fixation in a comparable time but with half mosquitoes) | the approach can be used to bolster wolb frequency if reinvasion by uninfected mosquitoes occur. | | Model | Hoffmann and Turelli, 2013 |
| | wMel | Residential blocks with relatively low numbers were more easily invaded | >2 years after release (traits were reevaluated) | | Field and Model | Hoffmann et al., 2014a |
| | wMel | near fixation in both locations, *but a persistent low frequency of uninfected mosquitoes* | >2 years after release protection persist | | Lab and field | Frentiu et al., 2014 |
| | n.a. | | 66-75% reduction in DENV transmission (*it may be insufficient in high transmission settings*) | | Model | Ferguson et al., 2015 |
| | wMel | can spread effectively in different urban environments | | | Lab, field and Model | Dutra et al., 2015 |
| *An. stephensi* | wAlbB | invasion of laboratory mosquito populations | | | Lab and semi field | Bian et al., 2013a |
| *Culex pipiens* | | If technical ordeals can be overcome, wolb can invade vector populations | | | Lab and Model | Rasgon and Scott, 2003 |
Glossina mors. mors modified Sodalis (Paratransgenesis) Fixation of the modified tse tse flies potential to eradicate trypanosome infections in humans, animal reservoir

* Lab stands for laboratorial and Model stands for computational modelling

Model Medlock et al., 2013
| Species        | Wolb strain          | (Modification)                  | Technique (Pathogen protection) | Fitness                                                                 | Release                                                                 | Type of Study                  | Reference                        |
|---------------|----------------------|---------------------------------|---------------------------------|--------------------------------------------------------------------------|-------------------------------------------------------------------------|--------------------------------|----------------------------------|
| Aedes aegypti | wAlbA and wAlbB      | Stable transinfection           | higher fecundity females        |                                                                          | net increase in mosquito numbers may occur                              | Lab and Model                  | Ruang-areerate and Kittayapong, 2006, Jeffery et al., 2009 |
|               | wMelPop              | not applicable                   |                                 |                                                                          |                                                                         | Lab and Model                  |                                  |
|               | wAlbB                | Stable transinfection (inhibit DENV2 infection) | increased longevity             |                                                                          |                                                                         | Lab                             | Bian et al., 2010                |
| Aedes albopictus | wAlbA & wAlbB        |                                 |                                 |                                                                          |                                                                         | Lab                             | Dobson et al., 2002             |
|               | wAlbA and wAlbB      | natural, introgressed           | longer lived, higher egg hatch in compatible crosses, and more fecund     |                                                                          |                                                                         | Lab                             | Dobson et al., 2004             |
|               |                      |                                 | under low competitive pressures, females experience higher survivorship   |                                                                          |                                                                         | Lab                             | Gavotte et al., 2010            |
| Aedes fluviatilis | wFlu (natural)      |                                 | enhances oocyst infection of *plasmodium gallicaceum*                     |                                                                          |                                                                         | Lab                             | Baton et al., 2013              |
| An. gambiae    | wAlbB                | somatic infection               | increases *plasmodium berghei* oocysts                                   |                                                                          |                                                                         | Lab                             | Hughes et al., 2012             |
| An. stephensi  | wAlbB                | Somatic transinfection          | *plasmodium yoelii* —(increased oocysts at 24ºC)                         |                                                                          |                                                                         | Lab                             | Murdock et al., 2014            |
| Culex pipiens | wPip                 | protection from *Plasmodium*-induced mortality                   |                                                                              |                                                                          |                                                                         | Lab                             | Zélé et al., 2012               |
|               | wPip (ARwp line)     | increasing *plasmodium relictum* transmission stages               |                                                                              |                                                                          |                                                                         | Lab                             | Zélé et al., 2014               |
| Culex quinquefaciatus | wPip (natural)    |                                 | live longer, lay eggs earlier and higher hatching rates                   |                                                                          |                                                                         | Lab                             | Almeida et al., 2011            |
| Culex tarsalis | wPip                 |                                 | enhanced WNV infection (not transmission)                                |                                                                          |                                                                         | Lab                             | Dodson et al., 2014             |
| NA | (natural) | eventual undesirable increase in the density of adult population | Model Dobson et al., 2002 |

* Lab stands for laboratorial, Model stands for computational modelling, and NA stands for not applicable
Transgenesis and other non-symbiont-based modification strategies (effective and ineffective outcomes)

A total of 37 research articles (15.6% out of the 237 with efficacy outcomes) have results regarding the efficacy of transgenesis or other non-transgenic and non-symbiont-based insect modification (37/100% reported effective outcomes, 15/40.5% reported ineffective outcomes).

Two types of transgenesis were found in analyzed publications: (i) anti-pathogen transgenesis, i.e. transgenesis with an anti-pathogen effector gene (30 publications) (202-205), (9), (206), (7), (207), (208), (5), (209-215), (6), (4), (216), (8), (217-225); and (ii) results on lethal transgenesis, i.e. transgenesis with a lethality inducing gene, covering the release of insects with a dominant (RIDL) (three publications) (226-228), and with a female-killing transgene (three publications) (229), (224), (230). Two non-transgenic non-symbiont-based insect modifications were also reported: (i) a radiation-based sterilization insect technique (SIT) (one publication) (231), and (ii) a RNAi-mediated sterilization (one publication) (232).
Not all modifications covered all efficacy topics (Modification, Technique, Fitness, Release, and Variability). No studies reported results regarding epidemiology efficacy using these types of modifications. Results are described in the following paragraphs and quantified in Fig 6.

Fig 6 - Distribution of the publications whose results contribute to each efficacy topic distinguishing effective/ineffective outcomes (n=37)

- Modification (anti-pathogen transgenesis)
Several studies described successful gene vectors mainly transposable elements, such us, Hermes and piggyBac (230), (202), (205), (211), (213), (216), (218), (221), (223), (228), but also transcription activator-like effector nuclease (TALEN) (225). Moreover, several distinct promoters were described generating successful sex, tissue or stage-specific expression of effector genes (211), (212), (204), (213), (203), (217), (219), (215). Successful insertion of an anti-pathogen effector gene was reported in Aedes aegypti (203), (208), (211), (212), (216), (220), (217), (224), Anopheles gambiae (204), (213), (218), (222), (205), (207), (210), (221), Anopheles stephensi (205), (207), (210), (214), (221), Culex pipiens (7), Glossina morsitans morsitans (4), and in some non-vector insects (202), (9), (6), (215), (8), (219), (223), (225).

- Technique (anti-pathogen transgenesis)
A subsequent blockage or reduction of pathogen transmission was reported in Aedes aegypti (208), (211), (212), in Anopheles gambiae (204), (213), (222), Anopheles stephensi (207), (214), (221), and in the silkworm Bombyx mori (225). However, in one publication the Plasmodium falciparum protection induced by an anti-malarial gene was inconsistent (213).
• Fitness (anti-pathogen transgenesis)

In some cases, the anti-pathogen transgene led to no or low fitness cost in the modified insects (208),(222), thus allowing the modified insect to be reproductively competitive against their natural counterparts. However, anti-pathogen gene insertion also caused fitness benefits in *Anopheles gambiae* (206), and *Anopheles stephensi* that fed *Plasmodium*-infected blood (207),(221). Since these outcomes increase vectors abundances and vectorial capacity, they constitute reversal outcomes.

• Release (anti-pathogen transgenesis)

Several publications, all of them computational modelling or laboratorial studies, estimated successful release of insects modified with an anti-pathogen transgene (7),(207),(4),(220),(221),(224). Some out oh those described the efficacy of different gene drives (which bias the inheritance of a particular gene to quickly and irreversibly spread it through a population) such as, Multi-locus assortment (209), Medea and Killer-Rescue (220), and *Wolbachia* (7),(4),(9),(5). Two publications presented comparative studies describing advantages and disadvantages of several gene drives (6),(8). Numerous computational modellings studies suggested a hard compromise between invasiveness and confinement (a high migration rate required to become established in neighboring populations, and low frequency persistence in neighboring populations for moderate migration rates) (209),(220),(7),(4),(9),(5),(6),(8). *Wolbachia* was referred as an efficient gene drive in some studies (9),(7),(5),(4) but according to Marshal and Hay, 2012 (8), was not reliable for confinement properties. Semele, Merea and two-locus engineered underdominance were the most promising in confinement properties and required lower introduction frequencies (compared to *Wolbachia*, Medea, single-allele underdominance, single-locus engineered underdominance and killer-rescue) (8). Multi Locus Assessment, despite being less effective as gene drive, allows the test of ecological components before releases with more invasive gene drives (209).

• Modification and Technique (lethal transgenesis)

Successful insertion of a lethal transgene was reported in *Aedes aegypti* (226), *Drosophila melanogaster* (230) and *Ceratitis capitata* (228) and subsequent lethality was laboratorial confirmed in *Aedes aegypti* (226), and *Drosophila melanogaster* (230).

• Fitness (lethal transgenesis)

One publication reported no fitness costs caused by the modification on the modified insect and confirmed that the lethal transgene did not affect insecticide susceptibility (228).
- **Release (lethal transgenesis)**

  Computational modelling studies reported that elimination of vector insects might be an unrealistic objective. However, substantial suppression can nonetheless be achieved in certain conditions, such as an uniform spatial pattern and multiple lethal elements (227), or a certain release ratio and population size (229),(224). Elimination of a natural population after the release of insects with a dominant lethal was though reported in semi field studies (228),(226).

- **Long-term (lethal transgenesis)**

  One computational modelling study suggested lethal transgenesis long-term efficacy to be compromised by invasion of wild type insects (224).

From all publications in this section, only one publication reported a field study, describing the ability to mate and copulate of a radiated insect, modified by a sterilizing technique (SIT) (231).

Also only three publications reported reversal outcomes, related to fitness benefits (as above mentioned). Main effective outcomes of transgenic and other non-symbiont-based modified insect vectors are presented in Table 3 (the complete data is presented in S2 Table).

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**Table 3- Main effective outcomes of transgenesis and other non-symbiont-based insect modification (vector insect species)**

Publications reporting effective outcomes on the topics: release, epidemiology and long-term (the ones closer to the strategy’s aim, that is transmission blockage or vector suppressing). Ineffective outcomes of the mentioned publications are also presented (in italic).
| Species               | Modification Strategy | Release                                                                 | Long-term                                                                 | Type of Study | Reference                  |
|----------------------|-----------------------|-------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------|---------------------------|
| Aedes aegypti        | anti-pathogen Transgenesis (MLA*) | the risk of an accidental premature release into nature is minimized and can be used as a back-up transgene dispersal mechanism while not as efficient as active drive mechanisms | Model | Rasgon, 2009 |
| Lethal Transgenesis (RIDL) | males introduced weekly eliminated the populations within 10–20 weeks.       | Lab | Wise de Valdez et al., 2011 |
| Lethal Transgenesis (Female killing) | substantial suppression can be achieved if releases are deployed in a uniform spatial pattern using strains combining multiple lethal elements | Model | Legros et al., 2012 |
| Lethal Transgenesis (Female killing) | Release ratio and population size can impact mean extinction time. Eradication may not always be obtainable in an operationally realistic time frame | Model | Robert et al., 2013 |
| An. stephensi        | anti-pathogen Transgenesis | generally to release adults of both sexes in multiple releases over time. (the less efficient male-only release impose less public concern) | Model | Legros et al., 2013 |
| Culex pipiens sl     | anti-pathogen Transgenesis | can substantially decrease vector competence of a natural population, even at release ratios well below those required for population reduction. Are considerably more robust to immigration. | Model | Okamoto et al., 2014 |
| G. morsitans          | anti-pathogen Transgenesis | (in lab cages) gradually replaced non-transgenic mosquitoes when fed on Plasmodium-infected blood but not when fed on non-infected blood | Lab and Model | Marrelli et al., 2007 |
| An. stephensi        | anti-pathogen Transgenesis | (in lab cages) transgenic mosquitoes invade when maintained on Plasmodium-infected blood. | Lab and Model | Smith, 2013 |
| Culex pipiens sl     | anti-pathogen Transgenesis | the number of transgenic mosquitoes that must be eventually released may be low and the gene of interest could spread in a relatively short period of time | Lab and Model | Rasgon et al., 2006 |
| G. morsitans          | anti-pathogen Transgenesis | experimental CI results were incorporated into a mathematical model, confirmed that Wolbachia can be used successfully as a gene drive | Lab and Model | Alam et al., 2011 |

*MLA – Multi Locus Assessment ; Lab stands for laboratorial and Model stands for computational modelling
Five articles (2.1% out of the 237 with efficacy outcomes) contributed to the efficacy of insect modification as a vector control approach, regardless of the modification strategy used (see S8 Table). They reported results as diverse as: effective releases, in what concerns numbers of insects, and sex ratio, (233), the impact of laboratory rearing (234), or descriptions of gene flow of eventual release sites (235-237). Interestingly, in all analyzed publications regarding gene flow, no isolation was found between Islands and mainland, neither in Society Islands of French Polynesia (236), nor Lake Victoria in Western Kenya (235), nor in Bijagós archipelago in Guiné-Bissau (237).

EFFECTS

Apart from the intended effective traits, modifications also induced other effects into the modified insects, as reported in 155/44.4% publications (nTotal=349). Modifications induced effects at several levels: (i) at the specimen level, i.e. physiological effects such as, reproduction, immune response or microbiome of the modified insect; (ii) at the insect species level, concerning its evolution and/or behavior; and (iii) at the ecosystem level, affecting any other organism of the modified insect ecosystem (Fig 2 and Fig 7).

Fig 7- Number of publications reporting each Wolbachia-induced effect per taxonomic group

Majority of publications contributing to this theme covered effects specifically induced by Wolbachia. There were two exceptions only, all of them reporting populational effects: one publication describing effects on evolution, induced by other symbiont (Rickettsia) (1), and one publication describing loss of assortative mating induced by laboratory rearing (234).

Physiological effects (at the insect specimen level)

A total of 63/18.1% publications (nTotal=349) reported that insect modification strategies may induce physiological effects on the target insect. Most frequent physiological effects found in analyzed publications were Wolbachia-induced effects on reproduction. The following reproductive modifications were reported: (i) male-killing (the death of male embryos during early embryonic development, with advantage for the surviving infected female siblings), stated in four non-vector species (Ephestia Kueheniella – butterfly, Hypolimnas bolina- butterfly, Ostrinia scapulalis moth, and Tribolium madens - beetle) (53),(54),(155),(238-240); (ii) feminization (the conversion of genetic males into functional females), described in two non-vector species (Eurema hecabe, and Zyginidia pullula) (241-243),(172); and (iii) parthenogenesis (the exclusive participation of females on reproduction, and production of female offspring), reported in several
species of parasitoid wasps (173),(244),(245). Although cytoplasmic incompatibility (CI) can be considered a Wolbachia reproductive effect, since it is a required trait for Wolbachia use as control strategy, it was herein considered an efficacy trait rather than a reproductive effect (see CI results on Technique topic). Moreover, effects on reproduction also included sex ratio alterations (246),(157),(247),(248), exceptional sex mosaics (168), requirement of Wolbachia for oogenesis (130),(131), or changes in expression of genes associated with reproduction (249),(172). Wolbachia also induced effects on the immunity of the modified insect mainly through the up-regulation of effector genes. Report of Wolbachia-mediated induction of immune system was described in Aedes aegypti (14),(90),(250),(187),(251),(23), Aedes albopictus (252),(253),(35), Aedes polyniensis (37), Anopheles gambiae (254),(253),(57), Anopheles stephensi (40), and Drosophila melanogaster (250),(249). Wolbachia also induced reduction of the immune response by decreasing the ability to encapsulate parasitoid eggs in Drosophila simulans (161) or decreasing the ability to produce lead peroxides (255). Other physiological effects were also reported, such as, alteration in the insect’s microbiome, (164),(138),(150),(39),(256), gene expression (genes, microRNA, sRNA, or epigenetic effects), (172),(257),(249),(258-260), or in its nutrition and metabolic mechanism (261),(170),(71),(262-265),(129),(249),(266),(267),(137),(195), see all information regarding physiological effects (also (268),(269),(182),(270),(189),(271)) in S4 Table.

Populational effects (at insect population level)

According to 40/11.5% analyzed articles (nTotal=349), Wolbachia also affected its host population in several ways such as, altering its mitochondrial DNA (mtDNA) pattern, interfering in speciation process, on its behavior ecology or others. Changes in mtDNA patterns were reported in several non-vector insects (272-283), and in the vector mosquito Culex pipiens (7). Phylogenetic analysis of Culex pipiens complex populations from three continents indicated a Wolbachia-induced drastic reduction of mitochondrial variability, thus profoundly interfering in its population structure (7). Several articles suggested that Wolbachia induced speciation, altering genomic diversity (284), leading to pre-mating behavioral isolation (285),(286) or to reproductive divergence (due to variable phenotypic effects) (10). Wolbachia-induced behavioral isolation is more likely in diploid and haploid than in haplodiploids hosts (287), and can be more evident in hybrid zones (288). Examples of behavioral changes, all of them reported in non-vector insects or suggested by computational modelling studies, are the sex-role inversion on reproductive ritual (246), the increase of sexual promiscuity (159),(289), and the irreversible loss of sexual reproduction (290),(173),(291).
Symbiont-induced speciation was also reported in *Neochrysocharis formosa* infected with *Ricketia* (1). All data regarding populational effects (also (292),(293),(160),(170),(247),(294),(172),(295),(144),(296-299)) is presented in S5 Table.

**Ecological effects (at insect ecosystem level)**

Finally, 64/18.3% publications reported that *Wolbachia* is also able to induce alterations in other organism rather than its host, interfering thus, with host ecosystem. The majority of the publications covering this type of effect described the report or the estimation of horizontal transfer events (i.e. transfer between neighboring contemporary species) of genetic material, such as a gene or a symbiont. Horizontal transfers (HT) can occur through bacteriophages, parasitoids, hemolymph, etc. Reported HT events comprise innumerous type of transfers such as between symbionts (300),(292),(301), (302-304),(137), from *Wolbachia* to insect vector (305-309), from *Wolbachia* to nematode species (305), from insect to insect (155),(116),(310-312),(118),(313),(294),(107),(314),(248),(315-320),(296),(321-324),(201), and from insect to non-insect species (325),(326), insect to bacteria (327). All data regarding HT (also (328),(329),(119),(167),(305),(330),(122),(331),(302),(245),(325),(332),(333),(307),(334-339)) is described in S6 Table.

Apart from HT, *Wolbachia* can reach other organisms also inducing ecological effects. All publications reporting non-HT ecological effects consisted in studies in mammals. Those had contact with *Wolbachia* mainly via filarial infection. A *Wolbachia*-infected filarial nematode induced or exacerbates the filarial diseases pathogenesis such as, human subcutaneous dirofilariasis (340), onchocerciasis (river blindness) (341),(342), and lymphatic filariasis (343),(344) (Table 4).

Table 4 – Other ecological effects: publications reporting *Wolbachia*-induced effects on mammals and respective main results
| Wolbachia Origin | Results | Analysed Mammal cells | Related disease | 1st author, year |
|------------------|---------|-----------------------|-----------------|-----------------|
| Cats (cows)      | Presence of Wolbachia confirmed in aorta sections from different Onchocerca Armillata-infected animals | tissue sections of infected animals | Onchocerciasis (river blindness) | Neary et al., 2011 |
| Cats            | Dirofilaria immitis infection leads to an immune response against Wolbachia proteins. | Sera cats and owners’ cats | Heartworm disease (cats/dogs) | Bazzocchi et al., 2000 |
| Cats            | Wolbachia-induced a greater acute inflammatory response worsening the broncho-reactivity | infected and non-infected breathing patterns | Heartworm-associated respiratory disease (cats/dogs) | García-Guasch et al., 2013 |
| Humans          | anti-Wolbachia surface protein antibody responses are associated with the presence of chronic filarial morbidity | human serum samples | Lymphatic filariasis | Punkosdy et al., 2003 |
| Humans          | specific immune response to Wolbachia in patients; Wolbachia may indeed participate in granuloma formation | human skin nodules | Human subcutaneous dirofilariasis | Grandi et al., 2008 |
| Humans          | Wolbachia surface protein may also contribute to the suppression of immune responses seen in filarial patients | human patients blood | Lymphatic filariasis | Shiny et al., 2008 |
| Humans          | Wolbachia is a major contributing factor in the development of chronic pathology in humans | blood neutrophils from adult healthy volunteers | Onchocerciasis (river blindness) | Tamarozzi et al., 2014 |
| Humans          | twice detection in 5 days of Wolbachia genes in human patient with an unknown infection. Later detection of a non-Hodgkin’s lymphoma | blood of a patient with apparent viral infection symptoms | non-Hodgkin’s lymphoma | Chen et al., 2014 |
| Mice            | Wolbachia-mediated neutrophil activation is an important mechanism to visual impairment and eventual blindness in ocular onchocerciasis | mouse cornea and peritoneal cavity neutrophils | Onchocerciasis (river blindness) | Gillette-Ferguson et al., 2004 |
| Cell lines      | Wolbachia lipopolysaccharides (LPS) may be one of the major mediators of inflammatory pathogenesis in filarial nematode disease | murine macrophage and mosquito cultures | Lymphatic filariasis | Taylor et al., 2014 |
LIST OF SUPPLEMENTARY INFORMATION

- S1 Checklist – PRISMA checklist
- S1 Appendix – List of analyzed publications and summary of analysis
- S2 Appendix - Research expressions, review assumptions and list of analyzed categories
- S1 Fig - Quantitative description of the included publications according to: type of insect modification strategy (A), type of study (B), study's species (C), year of publication (D)
- S1 Table - Results regarding effective and ineffective outcomes of wolbachia and other symbiont-based insect modification strategy: a) on Insects vectors; b) on Non-vector Insects (from pp.24)
- S2 Table - Results regarding effective and ineffective outcomes of Transgenesis and other non-symbiont-based insect modification strategy: a) on Insects vectors; b) on Non-vector Insects (from pp.7)
- S3 Table - Results regarding effective and ineffective outcomes regardless of the insect modification strategy
- S4 Table - Results regarding modification-induced physiological effects: a) on insect reproduction; b) on insect immune response (pp.3); c) other modification-induced physiological effects (pp.5)
- S5 Table - Results regarding modification-induced populational effects (evolution and behavior)
- S6 Table - Results reporting horizontal transfer events in modified insects
To our knowledge, the present work constitutes a unique review on modified insects for vector-borne disease prevention, for several reasons. Rather than being the perspective of an author or a summary of the authors’ selection of publications, this review followed a structured methodological procedure. Furthermore, authors from present review are not involved in scientific projects to modify insects having, thus, no conflict of interest in the outcomes of this reflection. Moreover, present review enclosed several types of modifications in any vector or non-vector insect species, covering an uncommon comprehensiveness, and thus, offering an exceptional opportunity to observe trends and to outline the big picture of the modified insects. In that sense, this review constitutes a baseline of knowledge that not only can be fed with forthcoming publications to follow up trends, but also point out to questions that may need to be further explored. Additionally, this review developed a framework of themes, topics and outcomes, that organizes the extensive information available. Also, it goes beyond modifications’ efficacy, most commonly covered in current reviews (26)(23), exploring research articles also related to modifications’ effects and even the variability of the efficacy outcomes in different settings. Finally, it is to our knowledge the sole review on the subject gathering publications on mammals and exploring the eventual effects of insects’ modifications on this important taxonomic group where humans are included. According to the epidemiology definition, efficacy determines whether an intervention produces the expected result under ideal circumstances, while effectiveness measures the degree of beneficial effect under actual settings(38). This works explored efficacy (modification, technique and fitness) and effectiveness related topics (release, epidemiology, long-term and variability). However, to simplify classification, ‘efficacy’ was used as a broad term that included all above-mentioned topics. Efficacy of the modifications was the most covered theme, being analyzed in 237 out of the 349 publications included in the review. Nevertheless, the amount of publications regarding modifications’ efficacy does not reflect the extent neither the robustness of the evidence available regarding it. Out of those 237, only 41 publications reported main effective outcomes: successful releases of modified insects, its positive epidemiological impact and/or its long-term efficacy (the remaining cover ineffective or primary effective outcomes). Out of the three main efficacy topics, the epidemiological impact was the less covered, reported in only five publications, none of them based on the most robust field studies, but all based on computational modelling studies instead. Main effective outcomes were obtained for several insect modifications, namely, Wolbachia-based strategies (replacement and suppression approaches), RIDL and female killing lethal transgenesis (both suppression approaches), computational modeled transgenesis regardless of the transgene (replacement and/or suppression approaches),
and paratransgenesis (replacement approach). Seven publications (out of the 41) reported main
effective outcomes based on semi-field or field studies, (corresponding to outcomes obtained
with Wolbachia or RIDL strategies). Even though not many, these publications achieved critical
outcomes: (i) field-released wMel-aegypti mosquitoes not only reached near fixation (despite a
persistent low frequency of uninfected mosquitoes), but also maintained their effective traits
such as CI, fixation and pathogen protection for at least two years after the release (27)(28) (from
S1 Appendix) (ii) weekly introduction of Aedes albopictus males with a dominant lethal (RIDL) led
to the eradication of a laboratory cages population in 10-20 weeks (228) (from S1 Appendix).

Present review also described reversal outcomes obtained after the release of transgenic insects
or insects modified with Wolbachia (reported in 39 publications). Out of those, only one
publication correspond to a field study, but in this case the release of modified insects may lead
to an increase in the vector insect population particularly if occurring when its natural abundance
is at its maximum (100) (from S1 Appendix).

Furthermore, analyzed publications also reported biological effects (at physiological, populational
and ecological level) of Wolbachia-based insect modifications. Effects on mammals should be
particularly and carefully explored. Monitoring of an eventual increase of lymphatic filariasis
severity, non-Hodgkin’s lymphoma incidence, or unrecognized infections may be advised in areas
already subjected to Wolbachia-insects releases.

Even though there were not found publications reporting eventual effects of transgenesis and
other non-symbiont modifications, some of their effects were already discussed in the literature.

In what concerns suppressing strategies, such as RIDL (lethal transgenesis), female killing (lethal
transgenesis), SIT or RNAi-mediated sterilization, the elimination of a species leads to profound
changes in its ecosystem, such as eventually putting some non-target species in risk or giving
opportunity to not-targeting species to expand (35)(23). Moreover, some authors have been
arguing that biodiversity loss may even be associated to emergence of vector-borne diseases (36)
(37). Regarding replacement approaches, such as anti-pathogen transgenesis, the effects of the
transgene in the ecosystem are unknown. When associated with Wolbachia as gene drive, there
are emerging questions regarding the lateral transfer of the inserted transgene to Wolbachia
itself or via Wolbachia to other organisms.

Since the inclusion criteria were restricted to studies on insects and mammals, results regarding
ecological effects may be limited to these taxonomic groups. Horizontal transfer events of
Wolbachia genetic material to non-insect non-mammal species were even though reported
(305),(325),(326) (from S1 Appendix). It is not surprising that publications covering Wolbachia
were the most founded in this review. Since Wolbachia is a natural bacterium, Wolbachia-based
strategies are much more accessible for study than other patented strategies such as, RIDL or
transgenesis. Moreover, besides those studies specifically oriented for a Wolbachia-based
strategy, other studies regarding Wolbachia were included (mainly regarding natural Wolbachia) whenever their results contributed to the efficacy or the effects of Wolbachia as a vector control strategy. Furthermore, Wolbachia is a unique term while for other strategies several diverse terms may be used (such as, the name of the particular transgene), and may occur that not all were covered within the research expressions.

In what concerns the year of the publications it is clear how recent this topic is, being almost the totality been published in the last 20 years. This can, at least partially, explain why in several topics we found a gap of knowledge such as the long-term efficacy or the epidemiological impact of the modifications.

In conclusion, insects modifications strategies appear as a promising innovative alternative to overcome an unprecedented increase of vector-borne, mainly arboviral, diseases. Nevertheless, these modification tools still lack evidence on field-based efficacy mainly regarding epidemiological and long-term impact. Field releases in endemic areas could provide that kind of missing evidence. However, relevant questions remain without a solid understanding. Eventual reversal outcomes on disease transmission, or irreversible biological effects (including effects on mammals) need to be explored, dispelled or resolved. This leads for demand of studies before testing their definite effectiveness in the field. However, some of these questions could only have a robust answer if these strategies would be implemented, needing to take the risk to observe reversal outcomes and/or irreversible effects, in order to confirm the efficacy of these strategies. This reflect the current dilemma that is under the use of modified insects to prevent vector-borne diseases. The level of variability of existing evidence suggests the need to generate local/specific evidence in each setting of an eventual release. Importantly, available preventive strategies should not remain on hold while modified insects do not offer an effective and safe solution. A comprehensive cost-effectiveness analysis could be an important tool when deciding to proceed or not with these innovative strategies, and/or to improve other available strategies. Therefore, an adequate decision could be made for each particular setting, evaluating the pros and cons of these approaches and of each modifying technique.

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Efficacy
regarding the modification aim

- Modification
  results associated with the modification of an insect

- Technique
  description of the modification-aimed traits

- Fitness
  impact of the modification on insect fitness

- Release
  impact of the release of modified insects, replacing or suppressing natural populations

Efficacy
main effective outcomes

- Epidemiology
  impact of the modification on human disease cases

- Longterm
  long-term persistence of the effective traits (applicable in replacement approaches)

- Variability
  variability of the results in different biological settings and conditions

- Effective outcomes

- Ineffective outcomes

Effects
not directly related with the modification aim

- Physiological effects
  effects on the modified insect specimen
  - Reproduction
  - Immune response
  - Other physiological

- Population effects
  effects on the modified insect species
  - Evolution
  - Behaviour

- Ecological effects
  on the modified insect ecosystem
  - Horizontal transfer
  - Other ecological

Fig 2
Fig 3, 4, 5 and 6
|                          | Reproduction | Immune response | Other physiological | Evolution | Behaviour | Horizontal transfer | Other ecological |
|--------------------------|--------------|-----------------|---------------------|-----------|-----------|--------------------|-----------------|
| **Aedes spp** (insect vectors) | (0)          | (11)            | (11)                | (0)       | (0)       | (4)                | (0)             |
| **Other insect vectors** | (0)          | (4)             | (3)                 | (2)       | (1)       | (5)                | (0)             |
| **Non-vector insects**   | (21)         | (5)             | (12)                | (22)      | (8)       | (44)               | (0)             |
| **Mammals**              | (0)          | (0)             | (0)                 | (0)       | (0)       | (0)                | (10)            |
| **Not applicable**       | (0)          | (0)             | (0)                 | (5)       | (2)       | (1)                | (0)             |
| **Total**                | (21)         | (20)            | (27)                | (29)      | (11)      | (54)               | (10)            |