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Evidence-based vector control? Improving the quality of vector control trials

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Vector-borne diseases (VBDs) such as malaria, dengue, and leishmaniasis cause a high level of morbidity and mortality. Although vector control tools can play a major role in controlling and eliminating these diseases, in many cases the evidence base for assessing the efficacy of vector control interventions is limited or not available. Studies assessing the efficacy of vector control interventions are often poorly conducted, which limits the return on investment of research funding. Here we outline the principal design features of Phase III vector control field studies, highlight major failings and strengths of published studies, and provide guidance on improving the design and conduct of vector control studies. We hope that this critical assessment will increase the impetus for more carefully considered and rigorous design of vector control studies.

Evidence-based policy making on vector control

VBDs such as malaria, dengue, and leishmaniasis are responsible for considerable morbidity and mortality and fall disproportionately on the poorest communities in the developing world [1–4]. One of the key methods by which VBDs can be controlled and eliminated is through vector control [5–10]; for example, long-lasting insecticidal nets (LLINs) for malaria or indoor residual spraying (IRS) for Chagas disease.

Development of vector control interventions follows a multistage process [11] (Figure 1). First, a draft target product profile should be generated. This document guides the development process by outlining the features and performance targets of the intended vector control tool. The next step is demonstrating the proof of concept by

conducting Phase I studies (laboratory assays to determine the mode of action) and Phase II (semi-field and small-scale field) studies, which generally have entomological end points. Large-scale Phase III field studies (efficacy studies) (see Glossary) are then conducted, which measure the efficacy of the vector control tool against epidemiological outcomes when implemented under optimal conditions.

Based on the results of Phase III trials, the World Health Organization (WHO) will make recommendations for pilot implementation. These Phase IV studies will assess the effectiveness of the vector control tool when it is delivered and used operationally (i.e., under 'real-world' conditions), as well as collecting information on feasibility, distribution mechanisms, acceptability, economics, and safety. Information gathered from the Phase III and IV studies will enable the WHO to draw up policy recommendations and, in parallel, member states will develop country-level policy.

Evidence-based policy making on vector control tools is now regarded as essential and is adopted by the WHO [12,13] (Box 1). The quality of evidence on vector control interventions from epidemiological trials or systematic reviews needs to be rated before recommendations and policy can be formulated. Since 2008, the WHO has adopted the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) methodology for evaluating evidence for policy and guideline recommendations [14,15]. According to the GRADE methodology, an initial rating is given based on the study design. Randomised controlled trials (RCTs) are rated as high-quality evidence and non-RCTs as low quality. Studies are then up- or downgraded based on several factors. RCTs can be downgraded depending on risk of bias, inconsistency, indirectness, imprecision, or publication bias. Non-RCTs can be upgraded based on the effect size observed, dose response, or plausible residual confounding. The final score generated can range from high (i.e., further research is very unlikely to change our confidence in the estimate of

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Glossary (adapted from [24,88–90], http://www.cochrane-handbook.org)

Allocation concealment: refers to keeping the investigator unaware of to which group (i.e., treatment or control) an individual or cluster is assigned. Selection bias can be introduced if the investigator or participant can foresee the assignment (e.g., use of alternation or rotation, assignment envelopes not sealed, not opaque, or not sequentially numbered).

Attrition bias: refers to systematic differences between those individuals or communities that withdraw from the study or those that are lost to follow up versus those that continue in the study.

Blinding: a procedure used in trials in which participants/investigators/outcome assessors do not know to which group the individual or cluster has been assigned. Single blind refers to either the participant or investigator/outcome assessor being blinded, while double blind refers to both the participant and the investigator/outcome assessor being blinded.

Case-control study: a study in which a group of people with the disease of interest (cases) and a group of people without the disease (controls), but representing the population from which the cases originated, are identified. The prevalence of the exposure of interest (e.g., use of protective intervention) is compared between these two groups.

Cluster randomisation: a study in which clusters are randomly assigned to either control or intervention groups. Clusters can be geographical areas (e.g., sectors of a large city), communities (e.g., villages), administrative units (e.g., district, region), institutions (e.g., schools), health facilities, or households.

Cohort study: a study in which two groups of disease-free people are identified – exposed (using a protective intervention) and unexposed (not using a protective intervention). The groups are then followed over a period of time for the outcome of interest (usually disease or infection). In this study type, the people are not allocated to the intervention of interest.

Confounding bias: according to Porta, 'confounding occurs when all or part of the apparent association between the exposure and the outcome is in fact accounted for by other variables that affect the outcome and are not themselves affected by exposure' [90]. A variable that is on the causal pathway between the exposure and the outcome is not a confounder. Confounding bias refers to 'bias of the estimated effect of an exposure on an outcome due to the presence of common causes of the exposure and the outcome' according to Porta [90]. This is a common type of bias in observational studies and nonrandomised trials. For example, in an observational study of the association between house screening and malaria incidence, the relationship is likely to be confounded by socioeconomic status since people in superior houses that use screening are likely to be of higher socioeconomic status, who may, for example, have greater access to other protective measures against malaria such as LLINs.
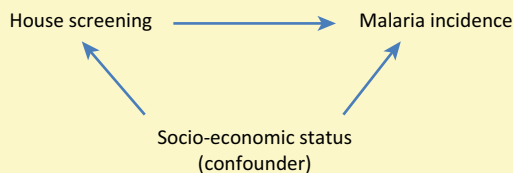
Control group: a group of study participants that receive no intervention, a placebo, or the standard of care depending on the study design and thereby serve as a comparison group when the intervention results are evaluated.

Controlled before-and-after study (CBA): also known as a pre–post study. A study in which observations are made before and after implementation of an intervention in both the intervention group and a control group that does not receive the intervention.

Courtesy bias: a tendency for study participants to give favourable answers out of courtesy to the investigator (e.g., incorrect reporting of high compliance with an intervention).

Crossover study: a study in which individuals/clusters receive the intervention or control for a period of time before switching to receive control or intervention. There is usually a washout period in-between to avoid carry-over effects.

Cross-sectional study: in an analytical cross-sectional study, information is collected at one point in time on the prevalence of the outcome of interest (e.g., disease, infection) and the exposure (e.g., use of a protective intervention).

Detection bias: refers to systematic differences between groups in how outcomes are determined. For example, clinicians assessing patients may be more or less likely to diagnose a particular disease if they know that a person received a protective intervention in the study. Detection bias can be reduced by ensuring that investigators and outcome assessors are not aware of which intervention participants have received.

Effectiveness study: these studies estimate the effect of an intervention under pragmatic or 'real-life' conditions (e.g., intervention delivery under routine conditions so that the relevance of the findings for policy and practice is maximised).

Effect size: the magnitude of difference between treatment and control groups (e.g., risk or rate ratio, percentage reduction in prevalence).

Efficacy trial: these studies estimate the effect of an intervention under highly controlled conditions (e.g., maximal coverage of the target population and adherence to the intervention).

Experimental study: a study design in which we allocate exposure to study subjects and observe the outcome.

Interrupted time series (ITS): a study in which the outcome is measured on several occasions both before and following introduction of an intervention (the 'interruption'). This allows us to see whether an intervention has had an impact greater than any underlying trend in the data. This study design may or may not include a parallel control group.

Observational study: a study design in which we observe the effect of the exposure on the study subjects but no role is played in assigning the exposure to the participants.

Performance bias: according to Porta, refers to 'systematic differences in the care provided to members of the different study groups other than the intervention under investigation' [90]. For example, if participants know they are in the control group of a trial of repellents, they may be more likely to use other forms of vector control, such as protective clothing. Alternatively, health-care providers may care for patients differently if they are aware of which study group they are in. Performance bias can be reduced by blinding to ensure that participants, health-care providers, and researchers are unaware of which intervention participants have received, although this is not always possible.

Randomisation: individuals or clusters are allocated to intervention and control using a random method. Randomisation comprises two interrelated steps, sequence generation and allocation concealment (not to be confused with blinding).

Randomised controlled trial (RCT): individuals or clusters (cluster-randomised controlled trial) are randomly allocated to receive either intervention or control. Intervention and control groups are then followed up for the outcome of interest.

Recall bias: refers to systematic differences between groups in the recall of information regarding exposures. It is a particular problem in case-control studies where surveys are used to gather information on past exposures.

Selection bias: refers to 'bias in the estimated association or effect of an exposure on an outcome that arises from the procedures used to select individuals into the study or the analysis', according to Porta [90]. Often, selection bias refers to systematic differences between the characteristics of the study population and those of other populations and thus there is a lack of generalisability. Nonrandomised studies are particularly susceptible to selection bias, although randomised studies can suffer from selection bias if randomisation procedures are not followed correctly. Selection bias can also be introduced into observational studies. For example, in case-control studies selection bias is introduced if cases are selected that are not representative of all cases within the population or controls are selected that are not representative of the population that produced the cases.

Sequence generation: a method of generating an allocation sequence. The method can be nonrandom (e.g., odd or even date of birth, investigator preference) or random (e.g., random number generator, drawing lots, coin tossing).

Step–wedge design: studies in which the intervention is rolled out to clusters in a staged fashion. At the end of the study, all clusters will have received the intervention. The order in which clusters receive the intervention is usually determined at random.

Stratification/stratified randomisation: a technique used to ensure that equal numbers of individuals or clusters with a characteristic thought to affect response to the vector control intervention (e.g., baseline incidence) will be allocated to each study arm. Multiple clusters are grouped to form strata based on a characteristic (e.g., low versus high incidence of disease) and clusters are randomly allocated within the strata such that equal numbers are assigned to intervention and control. Within each strata more than one cluster is assigned to an arm.

Systematic review: according to Porta, a systematic review is 'a review of the scientific evidence which applies strategies that limit bias in the assembly, critical appraisal, and synthesis of all relevant studies on the specific topic' [90]. The Cochrane Collaboration produces 'gold-standard' systematic reviews that are conducted in a highly rigorous fashion.

Time series: a study in which the outcome is measured on several occasions following the introduction of an intervention. This study design generally has a parallel control group, but may not be randomised.

effect) to very low (i.e., very uncertain about the estimate of effect).

While vector control interventions are the backbone of many disease control programmes, the evidence supporting their use remains weak. Based on our experience systematically reviewing the literature [16–20], we have identified repeated problems with vector control studies. To advance evidence-based policy making, the quality of evidence on vector control interventions – specifically the
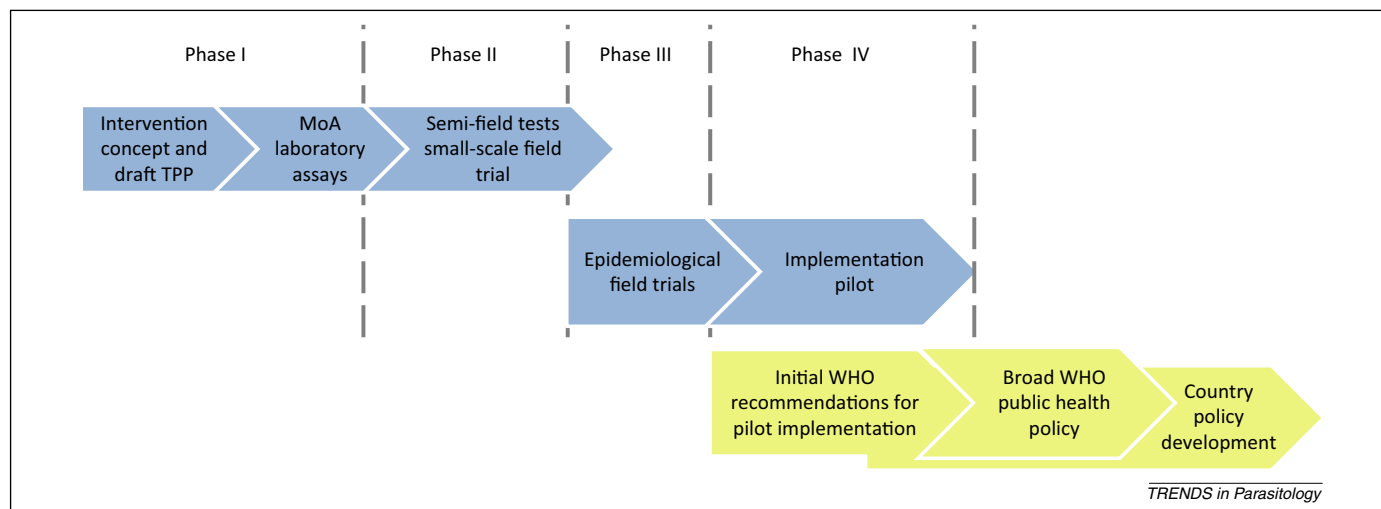
Figure 1. Stages in development of a new vector control product. The first step in the development of a new vector control product is to define the target product profile (TPP), including target efficacy characteristics, safety, and cost. Laboratory assays are then conducted to establish the mode of action (MoA) of the product followed by Phase II studies (semi-field and small-scale field trials) to determine the efficacy of the product against entomological outcomes. Phase III field trials to assess the efficacy of the intervention against epidemiological outcomes are then conducted and, based on the results of these trials, the World Health Organization (WHO; http://www.who.int/neglected_diseases/vector_ecology/VCAG_resources/en/) will make recommendations for pilot implementation. Phase IV pilot implementation studies assess the effectiveness of the vector control tool when it is used under 'real-world' conditions and collect information on feasibility, distribution mechanisms, acceptability, cost, cost-effectiveness, and safety. On the basis of Phase III and Phase IV studies, the WHO develops broad WHO public health policy on which many member states base country-level policy. Adapted from [11].

design, conduct, analysis, and reporting of vector control studies – needs to be improved. The problem of waste in research has recently been highlighted in a series in *The Lancet* that calls for better design, conduct, analysis, and reporting of studies [21,22]. Here we respond to *The Lancet*'s demand to reduce waste in research by highlighting

the essence of good study design for evaluating the efficacy of vector control interventions. Given the importance of study design and risk of bias to the GRADE assessment of quality of evidence, we first provide a primer on study designs and bias to illustrate the hierarchy of experimental designs for estimating intervention efficacy. Second, we review common failings of vector control efficacy studies in terms of their design and conduct and suggest how these studies can be improved.

General considerations on study designs for vector control studies

The methodological quality of study designs varies such that some are better than others in being able to answer the question 'Does the intervention work?' or 'Does this intervention work better than that intervention?' [23]. In Figure 2 we provide a hierarchy of study designs for evaluating the efficacy of vector control interventions – ranking studies as level 1, 2a, or 2b according to their methodological quality – and list nonrecommended studies. We accept that different study types may be better for answering other questions, such as the acceptability of the intervention [23].

RCTs are generally considered the 'gold-standard' study design for evaluating the efficacy of a protective intervention since they have a low risk of selection bias [24] (http://www.cochrane-handbook.org), which is arguably the most important type of bias in experimental studies. Such is the importance of randomisation that we consider RCTs as level 1 evidence. If the number of randomisation units is sufficiently large, randomisation will ensure that, in a two-armed study, any factors that may affect an outcome are similar in the two arms [24]. Even if one randomises, it is good practice to check that the baseline characteristics of the groups are similar to verify whether the randomisation was successful [25]. If there is no random allocation of intervention and control communities, potential bias can
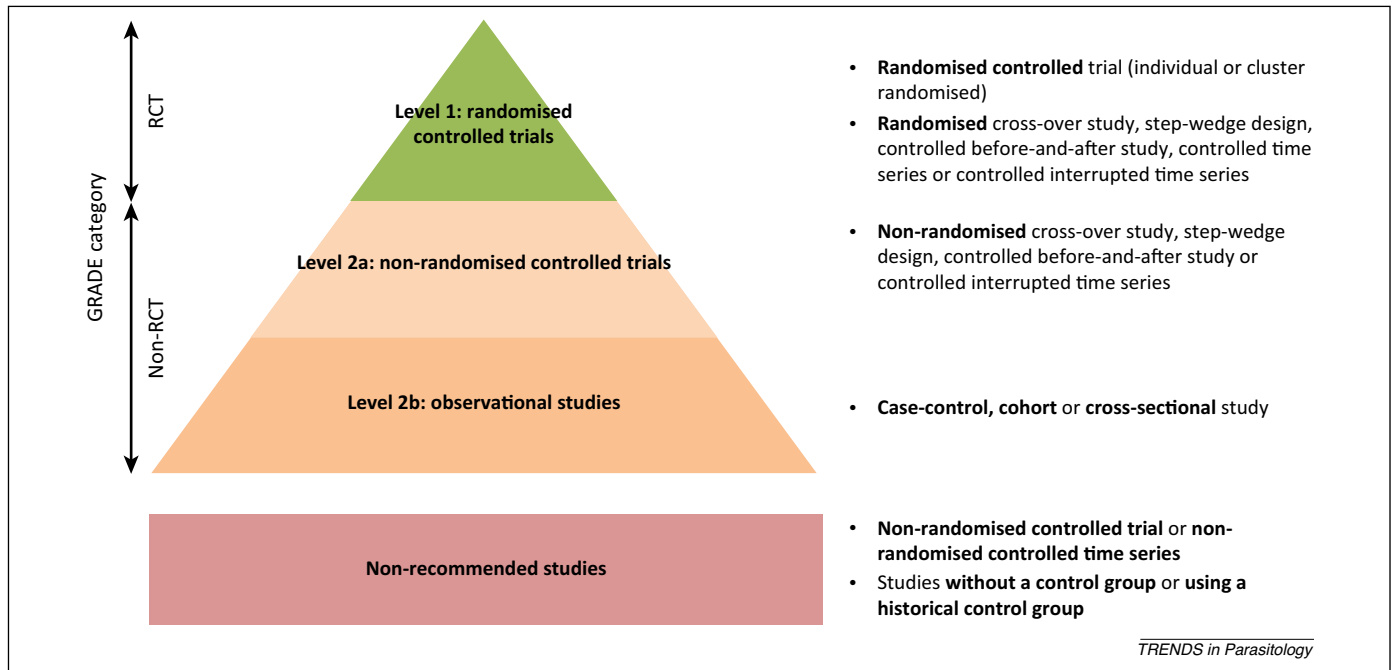
Figure 2. Hierarchy of study designs for assessing the efficacy of vector control interventions. Study designs for assessing the efficacy of vector control interventions can be ranked according to their methodological quality. Randomised controlled trials (RCTs) (level 1) are the 'gold-standard' study design for evaluating the efficacy of vector control interventions. Randomisation reduces the risk of selection bias by ensuring that control and intervention groups are similar to each other. Level 1 studies include cluster or individually randomised controlled trials as well as randomised crossover, randomised step–wedge, randomised controlled before-and-after, randomised controlled time series, and randomised controlled interrupted time series studies. Nonrandomised trials (including nonrandomised crossover, nonrandomised step–wedge, nonrandomised controlled before-and-after, and nonrandomised controlled interrupted time series studies) are at a higher risk of bias and so are ranked lower (level 2a). Observational studies, such as case-control, cohort, and cross-sectional studies (level 2b), provide weaker evidence on the efficacy of protective interventions than experimental designs since they can be subject to bias due to confounding factors and flaws in measuring exposures and outcomes. Nonrandomised controlled trials, nonrandomised controlled time series designs, and studies without a control group or using a noncontemporaneous control group are not recommended. Adapted from an Australian Government National Health and Medical Research Council 2009 document on additional levels of evidence and grades for recommendations for developers of guidelines (http://www.nhmrc.gov.au/guidelines-publications/information-guideline-developers/resources-guideline-developers) and the Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence (http://www.cebm.net/index.aspx?o=5653). GRADE (Grading of Recommendations Assessment, Development, and Evaluation) levels defined as in [14].

be reduced by adjusting for pre-intervention differences in the two groups using multivariate analysis (e.g., [26]). There is, however, no guarantee that this will fully control for confounders that may be unknown or unmeasured.

In vector control studies, the intervention is often allocated to a group of individuals known as a cluster (e.g., district, village, household) rather than at the individual level. There are several reasons why cluster allocation is common [24]. First, many vector control tools are, by their nature, applied to groups of people or communities. For example, spatial repellent may be allocated to a household or an environmental sanitation intervention against dengue may be allocated at the community level. Second, cluster allocation can help reduce contamination between study arms that might occur if individuals within the same community received different interventions; for example, sharing of insect repellent with family members within the same household or village. Last, cluster allocation means that we are able to assess the community-level effect of the intervention. For example, mass killing of mosquitoes coming into contact with LLINs can reduce transmission so that indirect protection is provided to individuals not using LLINs.

There are numerous other study design types, including controlled before-and-after (CBA) studies, controlled time series, controlled interrupted time series (ITS), crossover studies, and step–wedge designs (Figure 3), that may be more suitable for evaluating the efficacy of some vector

control tools. For example, time series or ITS are probably more appropriate for studies of human African trypanosomiasis in which vectors are highly mobile and control efforts need to be implemented over large areas [27]. Step–wedge studies involve rolling out the intervention to clusters in a staged fashion. This design is often used where logistical, practical, or financial constraints make the staged roll out of the intervention desirable. We classify randomised CBA, randomised time series, randomised ITS, and randomised step–wedge studies as level 1 and nonrandomised CBA, nonrandomised ITS, and nonrandomised step–wedge studies as level 2a. We do not recommend the use of nonrandomised controlled trials or nonrandomised time series designs since selection bias is likely to be high and there are no pre-intervention data to assess the comparability of groups.

Observational studies such as case-control, cohort, or cross-sectional studies (Figure 4) have been used to generate evidence of the efficacy of vector control interventions. However, these designs provide weaker evidence than experimental (randomised) designs since they can be subject to bias (e.g., recall bias, detection bias, confounding). For this reason we have ranked these studies as level 2b.

We also do not recommend the use of studies without a control group or those using a noncontemporaneous control group. This is because longitudinal changes, such as rainfall, may impact epidemiological outcomes and can exaggerate or mask an intervention effect.
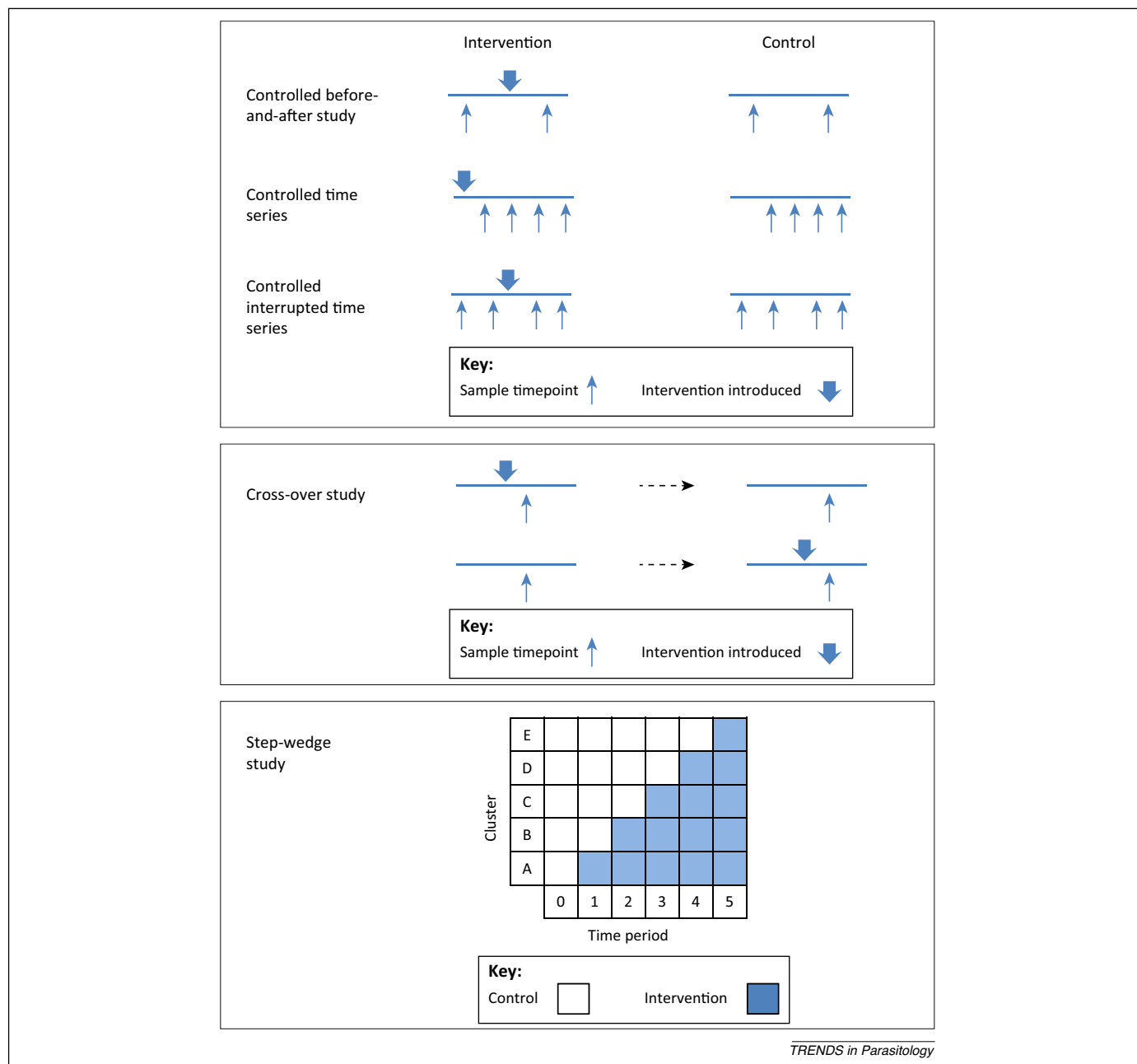
Figure 3. Schematic illustrating design of controlled before-and-after, controlled time series, controlled interrupted time series, crossover, and step–wedge studies. Controlled before-and-after studies involve collecting data on outcome measures before and after implementation of the intervention in the intervention group and at the same time points in the control group. In controlled time series studies, data on outcome measures are collected at several time points once the intervention has been implemented in the intervention group and at the same time points in the control group. Controlled interrupted time series studies involve collecting data on outcome measures at several time points before and after implementation of the intervention in the intervention group and at the same time points in the control group. In crossover studies, two groups are allocated (usually randomly) to control or intervention and outcome measures are assessed once the intervention has been implemented. Following a suitable washout period, the intervention and control are switched around and outcome measures are assessed again. In a step–wedge study, the intervention is rolled out randomly to clusters in a staged fashion so that by the end of the study all clusters will have received the intervention. Adapted from [32].

Common failings of vector control studies and recommendations

Here we describe common problems with the design of vector control studies illustrated with examples and make recommendations for improvements.

Implementation and adherence to the intervention

In efficacy trials, vector control interventions should ideally be implemented in an optimal manner with attention to quality control, high coverage, and user compliance.

Unless these parameters are measured, it is impossible to know whether an observed lack of effect is due to low quality, coverage, and/or compliance or lack of efficacy of the vector control method.

Quality control checks should be put in place to ensure that vector control interventions such as IRS are implemented optimally (e.g., correct application of insecticides, coverage of all assigned structures). This can be achieved through accurate record keeping, random spot checks, and supervision [28,29].
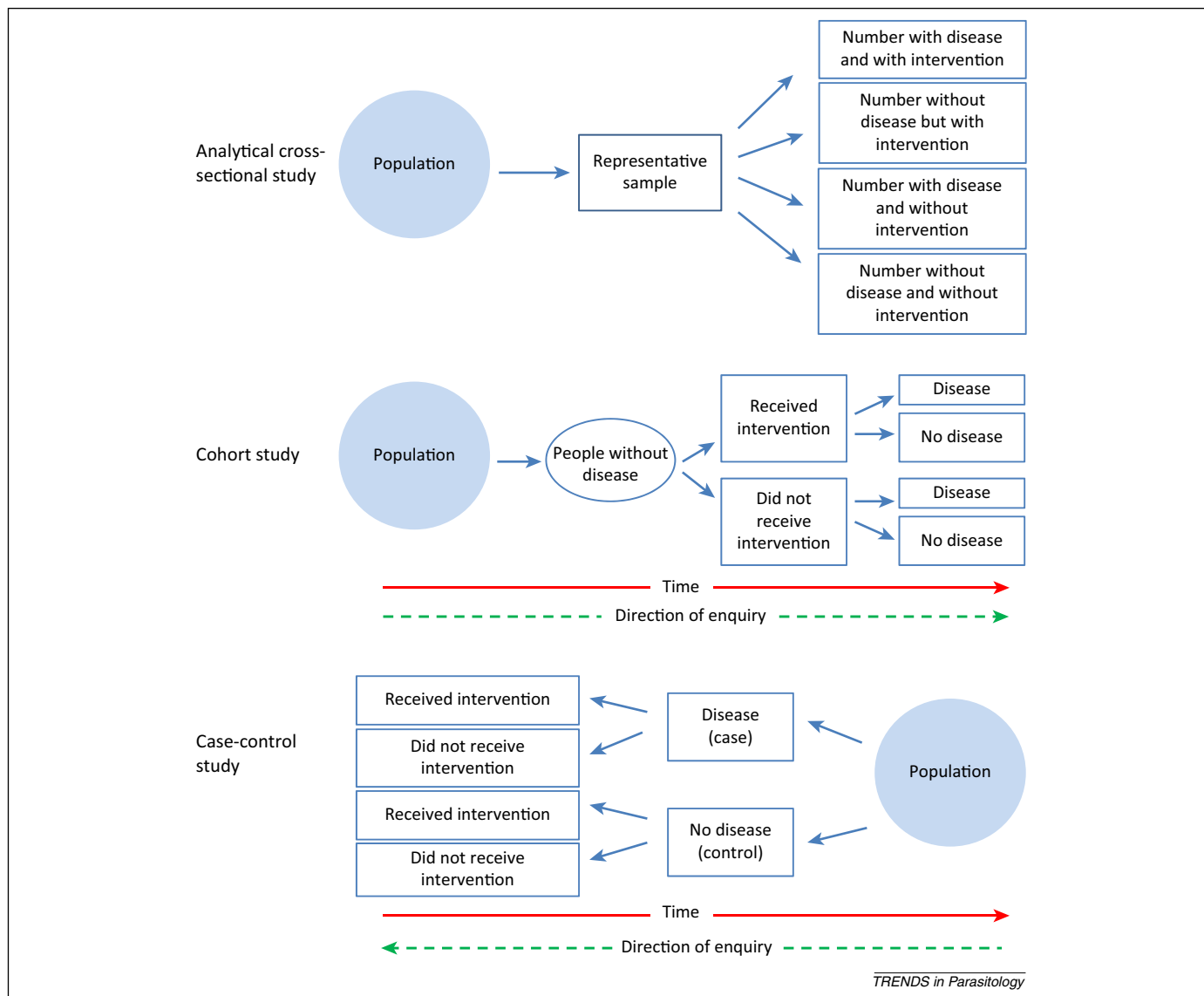
Figure 4. Schematic illustrating design of observational studies for vector control interventions. In an analytical cross-sectional study, a cross-sectional survey is taken from a representative sample of a population. The survey gathers information on outcomes (e.g., disease/infection) and exposure to the intervention from individuals at the same time so the sample can be split into four groups: those with the disease who were exposed to the intervention; those without the disease who were exposed to the intervention; those with the disease who were not exposed to the intervention; and those without the disease who were not exposed to the intervention. In a cohort study, a sample of the population is chosen that is free of disease. Individuals without the disease are split into two groups (those exposed to the intervention and those not exposed to the intervention) and are followed up over time to determine how many develop the disease or infection. In a case-control study, individuals are selected on the basis of their disease or infection status. A group of individuals with the disease or infection (cases) and a group without the disease/infection (controls) are selected. The prevalence of exposure to the intervention is then compared between the cases and controls.

Adherence to the intervention being tested is very important. Efficacy studies usually employ specific techniques (e.g., behaviour change communication) to encourage optimal uptake and use of the intervention where user compliance is required (e.g., [30]). Adherence to the intervention should be measured while taking into account that there is also the potential for introduction of bias here (e.g., courtesy bias). In some cases, innovative methods need to be identified to assess compliance. For example, a RCT of topical repellents against malaria measured compliance through self-reporting of use, the proportion of lotion used estimated from returned bottles, and 'sniff checks' whereby trial staff visited villages at dusk and smelled the arms of participants to check whether lotion had been applied [31].

Choice and measurement of outcome measures
Epidemiological outcomes are necessary to demonstrate the efficacy of the intervention in protecting human populations and to ensure the relevance of these studies to public health. To date, however, many Phase III studies often focus exclusively on entomological outcomes, which are generally useful only for demonstrating proof of concept or as a secondary outcome in support of an epidemiological primary outcome. For example, a Cochrane systematic review on larvivorous fish for malaria control did not identify any studies with epidemiological outcomes [32]. The best epidemiological measure is the incidence of clinical disease or disease-specific mortality, but for some diseases, such as dengue, seroincidence (seroconversion in sequential blood draws) and the prevalence of infection in

single blood draws, including age-specific antibody prevalence, can be good substitutes [30,33]. Studies should use WHO-recommended case definitions with parasitological diagnosis, or serological or molecular verification [34–38] to allow comparison of data between studies. Outcome measures such as self-reported malaria as used by Kroeger *et al*. in a study of repellent soap [39] are unreliable.

Detection bias can be reduced by blinding outcome assessors to the identity of study arms and by the use of objective and well-standardised epidemiological and entomological outcomes. The latter should particularly be used in nonblinded studies.

Entomological data should be collected in a standardised fashion across study arms and sites and over time. Ideally these sampling tools should be automated (e.g., CDC light trap, sticky trap, other trap or target) and not depend on the ability of the fieldworker to collect specimens (e.g., human landing catches, aspiration of resting adults, larval surveys). Several other techniques can help avoid introduction of bias in the measurement of entomological outcomes, including separating the field teams that are implementing and monitoring the intervention (e.g., [40]).

Entomological end points are not always good predictors of epidemiological outcomes. For example, a RCT of LLINs for visceral leishmaniasis reported a reduction in sandfly density in homes but did not show any effect on infection in study participants [41,42]. The authors postulated that transmission was also occurring outside the home and so, although there was a reduction in indoor sandfly density, this did not reduce disease burden. Where possible, it is preferable to use entomological outcomes that relate to disease transmission, such as entomological inoculation rate, rather than measures that do not, such as vector density.

Traditional indicators of immature *Aedes* abundance, such as house index (percentage of houses with larvae and/or pupae), are a poor indication of adult production [43] (http://apps.who.int/iris/handle/10665/68575). Pupal demographic surveys (pupae per person/area index) or measurement of adult vector density are likely to be more appropriate for assessing transmission risk and directing control operations [44,45] (https://extranet.who.int/iris/restricted/handle/10665/69354, http://apps.who.int/iris/handle/10665/68575). However, both measures are far more labour intensive than larval surveys and so may not be feasible for routine monitoring of vector populations [35]. Because, unlike infections caused by protozoa and nematodes, dengue virus infection results in sterilising immunity, pupal and adult surveys are not consistently informative about dengue risk without an understanding of the underlying susceptibility of the human population to dengue virus [46–48].

Avoiding performance bias
Blinding of trial participants, health-care providers, and researchers to the intervention received by participants can reduce performance bias. However, blinding of vector control studies is often impossible. For example, it was not possible to blind study participants in a RCT assessing the efficacy of house screening versus no house screening

against malaria [49]. The study found that children living in screened homes were less likely to use bed nets than children residing in homes that were unscreened, which may reflect a belief among householders that screening was a substitute for bed nets. However, the effect of performance bias in this study was minimised because bed net use was carefully recorded and its effect could be adjusted for in the statistical analysis. Alternatively, an originally blinded study may become unblinded during the study. For example, some participants in a RCT of topical repellents became aware that the placebo lotion they were allocated was not providing protection against mosquito bites, which led to the withdrawal of all households in one village [31]. This kind of participant response can lead to introduction of attrition bias.

Selection of sites for entomological monitoring
Sampling sites for entomological surveys are often chosen purposely based on where high vector densities are likely; for example, sites close to suspected larval habitats or houses with unplastered walls or wood construction for *Triatoma* surveys [50–52]. However, this does not measure average community exposure to infection and there is potential for the introduction of sampling bias if sites are not selected in a consistent way across intervention and control arms. We therefore recommend that sampling sites for entomological surveys be selected randomly. It is also possible to separate the sampling frame into strata and sample from each stratum independently, if there is likely to be substantial variation within subpopulations. For example, Joshi *et al*. stratified dwellings into two groups (houses occupied by humans alone and houses occupied by humans and animals) before using simple random sampling to select dwellings in which to measure sandfly density [53].

Contamination or spillover effects
Contamination or spillover effects between different study arms due to the movement of vectors [54,55] or humans between clusters can make interpretation of study findings difficult. Spillover that has a conservative effect (i.e., it biases results towards the null) can occur through one of two routes. First, community-level effects of the intervention can reduce the transmission intensity in neighbouring control clusters, as occurred in a study of insecticide-treated water-jar covers and window curtains against dengue in Mexico and Venezuela [56]. Second, movement of people between intervention and control clusters (and vice versa) is also able to dilute the intervention effect because a person's risk of infection is proportional to the amount of time he or she spends in versus out of the treatment area. If the protective effect of an intervention or the sample size of the study is sufficiently large, a positive result can still be demonstrated in a superiority trial, albeit with reduced intervention effect. However, a negative finding of 'no difference' in such a trial is harder to interpret and a critical question arises. Is the lack of effect due to spillover or due to the absence of efficacy of the new intervention?

A more serious problem arises if the spillover effect is anticonservative, because it exaggerates the difference in outcomes between the intervention and control arms of the

study. For example, topical repellents or house structural changes that have no killing effect on mosquitoes may divert vectors to nonusers in the control arm of the study, putting them at higher risk of infection than they would otherwise have been [57,58].

Hayes and Moulton [24] outline several methods for reducing contamination, including ensuring clusters are well separated, using a buffer zone so there is no common boundary between intervention and control clusters, as shown in a larval source management study conducted in Tanzania [59], or a 'fried-egg' design where the intervention and control are administered throughout the cluster but only the central portion is used for outcome measurement [60]. When designing these types of studies it is, therefore, important to have an estimate of how far the vector is likely to fly in seeking a blood meal or a breeding site. Georeferences of cases that constitute the outcome measure should be recorded to show whether there were edge effects due to contamination. This technique has been used to estimate the size of area-wide effects in studies of LLINs for malaria control [61]. Unintended consequences of topical repellents can be avoided by randomising only a relatively low proportion of individuals or households in a village to receive the intervention [31,62,63]. Tackling the problem of human movement in dengue studies is more difficult because *Aedes aegypti* feeds during the day when people are engaged in their daily activities. Potential strategies to avoid this would be to use larger cluster areas or monitor epidemiological outcomes in a sentinel cohort that is less mobile (e.g., young children) [64]. Even if these steps are taken it is a good idea to collect travel histories from study participants, particularly if the intervention is located in a household. In this way, participants can be excluded from the per-protocol study analysis if they have travelled for significant periods of time and, therefore, spent a relatively brief time being exposed to the intervention (e.g., [65]).

Contamination can also be a problem in crossover trials if the washout period is insufficient. While crossover trials may be suitable where the washout period is short (e.g., larvicide with a short half-life [66]), they should be used with caution where interventions are persistent (e.g., DDT, habitat manipulation).

Need for sample size calculations

Sample size calculations are performed before conducting a study to quantify the power that the study has to show an effect of the intervention and thereby answer the study question (Box 2). The effect of a small sample size is on the standard error of the outcome measure; that is, it will lead to large confidence intervals around the estimated effect and hence poor precision. The sample size needs to be large enough to ensure that the probability of a type II error is reasonably small, generally 10% (= 90% power) or 20% (= 80% power). Sample size calculations should be performed for all study outcomes, whether epidemiological or entomological. We identified several studies that did not report conducting sample size calculations for epidemiological and/or entomological (e.g., [67–71]) outcomes, including several studies that failed to show an effect of the intervention [72,73], indicating that the lack of an effect

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Box 2. Power and sample size calculations [91–93]

When conducting a study there are two hypotheses that need to be considered: the null hypothesis (there is no difference between the two interventions) and the alternative hypothesis (there is a difference between the two interventions or, more commonly for superiority trials, the novel intervention is more protective than standard practice). When testing a hypothesis there are two types of error possible:

- Type I error, or α. We reject the null hypothesis incorrectly (i.e., there is no effect but we report that there is).
- Type II error, or β. We incorrectly do not reject the null hypothesis (i.e., there is an effect but we fail to detect it).

Several factors need to be considered when calculating sample sizes.

- The prevalence or incidence of the outcome in the control group.
- The expected effect size of the new intervention. It is important to be clear about what is the smallest size of effect we deem to be relevant from a public health or clinical perspective; for example, a study assessing the effect of house screening against exposure to malaria vectors established at the beginning of the trial that full screening or screened ceilings would be recommended if they reduced house entry by malaria mosquitoes by at least 50% [49].
- Significance level (*P* value). This represents the probability of a type I error; generally 0.05 is used, which means that we have a 5% probability of a type I error.
- Power. The power of a study is the probability of not committing a type II error, or $1 - \beta$ (e.g., if we have a 20% probability of a type II error, the power is 80%).

Many vector control trials use a clustered design. For cluster-randomised trials, two additional factors need to be taken into account:

- Average cluster size.
- The coefficient of variation, *k*, which measures the level of between-cluster variation of the outcome.

This is important because outcomes measured in individuals or sampling sites within the same cluster are likely to be correlated. A large value of *k* implies substantial between-cluster variation in the outcome, which makes it harder to show an intervention effect unless the sample size is increased.

It is recommended to consult an experienced statistician to assist with sample size calculations, particularly for cluster-randomised trials.

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may simply be due to the study being underpowered. Parameters required for sample size calculations such as the prevalence or incidence of the outcome in the control group or the coefficient of variation may not be readily available [30], although the former can be estimated from a survey conducted before the study's start if it is not known.

Vector control trials generally use a cluster design. Since outcomes measured in individuals or sampling sites within the same cluster are likely to be more similar than those between clusters, the sample size calculation needs to take this into account and a larger sample size is required than when a nonclustered design is used (Box 2). Hayes and Moulton recommend the use of six clusters per arm as an absolute minimum and it is generally better for cluster-randomised trials to have a higher number of smaller clusters than fewer large clusters [24]. We identified a large number of published vector control trials that used two villages [74,75] or two areas [76,77], one in which the intervention was introduced and the other acting as a control. This is a poor design because the use of only two clusters means that the intervention effect is completely confounded by study site and effectively constitutes a sample size of one [78,79].

Table 1. Minimum recommended follow-up periods by study type[a]

Study design	Preintervention	Postintervention
Randomised controlled trial	Desirable to check baseline characteristics of study population. At least one transmission season for entomological data if sampling sites are nonrandomly selected	At least one transmission season (two seasons is desirable)
Controlled before-and-after study	At least one transmission season, especially if entomological sampling sites are nonrandomly selected	At least one transmission season
Randomised controlled time series	Not applicable	Two or more transmission seasons
Interrupted time series	Two or more transmission seasons	Two or more transmission seasons
Crossover study	At least one transmission season before crossover (and washout) and one transmission season after	

[a]Transmission season may be shorter than a 1-year period or a whole year if transmission is perennial.

Deciding on the duration of the follow-up period

Insufficient periods of follow up plague many vector control trials. For example, a RCT of topical repellents against malaria in Ethiopia conducted two malaria prevalence follow-up surveys 1 month and 2 months after the baseline survey [80]. This study is unlikely to give a true picture of the efficacy of the repellent since compliance with the repellent would probably remain high during this short time period but decline over a longer time period. It is also worth noting that *Plasmodium falciparum* infections last on average 1 year [81,82], although they can persist for up to a decade or longer [83], and it takes several years for this indicator to re-equilibrate fully following a reduction of transmission [84,85].

For entomological outcomes, follow-up periods need to be sufficiently long and repeat measurements need to be taken to gain a picture of transmission in the area (e.g., [86,87]). This is because there is likely to be large variation in vector density between sampling sites and across different sampling periods (night to night, week to week, or over a transmission season) due to environmental factors such as rainfall. Designs in which entomological sampling is conducted once during the follow-up period are less likely to give reliable results due to inherent variability in vector populations even if the number of sampling units is high. Longer periods of follow up with repeat measurements can be used to assess whether the effect of an intervention is waning (e.g., IRS with a short-lasting insecticide) and to determine how often the intervention needs to be replaced or reapplied.

We recommend that minimum pre- and postintervention follow-up periods be used for epidemiological and entomological data collection, the duration of which differs depending on the study design chosen and the context of pathogen transmission (Table 1).

Concluding remarks

We have identified common problems with vector control studies and provide suggestions on how these can be improved. We also illustrate that some study designs are methodologically stronger than others. While hierarchies based on study design are somewhat controversial (http://www.alliance4usefulevidence.org/publication/what-counts-as-good-evidence-february-2013/), we believe they remain useful in addressing the evidence for what

interventions work, particularly when combined with a broader evaluation of the quality of the evidence as offered by GRADE [14,15]. More specifically, the GRADE rating of evidence takes into account numerous factors in addition to study design [14,15]. This means, for example, that a poorly conducted RCT with a high risk of bias does not necessarily constitute better evidence than a sound observational study with a large effect size.

We suggest that there are several reasons why many vector control studies have historically been designed and conducted in a less-than-optimal fashion. First, a lack of resources may have limited the extent to which entomologists could conduct large-scale, well-designed studies. This may help explain the large number of two-village comparison studies and studies without epidemiological outcomes. The impact of shortfalls in resources is exacerbated by issues associated with implementing environmental interventions on a large scale and the urgent need for VBD control. Second, medical entomologists have traditionally not been taught epidemiology or have not worked in an integrated fashion with epidemiologists. It is necessary to upgrade this aspect of the skill set of medical entomologists, to include epidemiology in medical entomology course curricula, and for epidemiologists to partner with entomologists in conducting intervention assessments.

New vector control tools are urgently needed to reduce the burden of VBDs. In highlighting key problems with the design and conduct of vector control tools and suggesting remedies we hope that this manuscript will provide an impetus for upgrading the evidence base on vector control interventions. The present lack of rigorous, evidence-based vector-borne intervention assessments is an obstacle to innovation in disease reduction. It also wastes a considerable amount of money, time, and energy. Improving the quality of future vector control trials will not only save valuable resources but will also expedite the process of achieving recommendation from the WHO for the roll out of effective new interventions.

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