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Quantitative Risk Assessment of a Potential Release of *Aedes aegypti* Carrying *Wolbachia* Strains for Dengue Control in Burkina Faso

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Abstract

One of the most promising vector control strategies for controlling dengue fever is the stable introduction of the obligate intracellular *Wolbachia* bacteria strain into *Aedes aegypti* mosquitoes. This method reduces the mosquito's ability to transmit dengue through reproductive strategies associated with *Wolbachia* infection, such as parthenogenesis, male elimination or feminization, sex ratio distortions and cytoplasmic incompatibility. Expert knowledge and a risk assessment framework were used to identify the risks associated with the release of *Wolbachia*-bearing mosquitoes. Then, consultations with individual participants were organized and a Bayesian network (BN) was developed to capture the relationship between the hazards as well as the likelihood of these adverse events occurring. Finally, risk was calculated from the probability and consequence estimates obtained from our Burkina Faso participants, using a questionnaire based on the identified risks. Our "Cause More Harm" study yielded 46.15% negligible risk, 44.23% very low risk and 9.62% low risk. The "socio-cultural change" parameter had the greatest influence, with the perception that the dissemination project would be poorly received by the local population. This parameter alone accounted for 80% of the most significant risks. This explains the fact that hazard RA 49 "negative messages in social media" is ranked as the highest individual risk (although the risk is low) with a calculated risk of 0.261. The risk assessment was designed to integrate the interdependent complexity of hazards likely to affect the dissemination of technology in the environment. It represents an important implementation phase in the success of this innovative research, introducing a new technology to combat the transmission of dengue fever.

Keywords

Assessment, Risks, Dengue, *Wolbachia*, *Aedes aegypti*, Dissemination, Burkina Faso

1. Introduction

Vector-borne diseases account for approximately 17% of the global burden of non-communicable diseases, causing more than 700,000 deaths annually, particularly in the African Region of the World Health Organization (WHO) [1]. Among these, dengue is a rapidly expanding mosquito-borne viral disease primarily transmitted by *Aedes aegypti* [2]. While dengue cases are widely reported worldwide, surveillance in Africa remains limited, and outbreaks often go undocumented. However, past outbreaks in Mozambique (1984-1985), Senegal (2006), Côte d'Ivoire (2008), Cape Verde (2009) and Burkina Faso (2016-2017, 2023) highlight the growing threat of dengue in the region [2]. Currently, vector control is the only effective means to reduce dengue transmission, but traditional interventions such as insecticide spraying and larval source reduction face challenges, including insecticide resistance and inconsistent implementation. To address these limitations, innovative approaches like *Wolbachia*-based mosquito control are being developed [3]. *Wolbachia* is an intracellular endosymbiotic bacterium that naturally infects approximately 66% of insect species, including *Aedes albopictus*, but it is not naturally found in *Aedes aegypti* [4]-[6]. When introduced into *Aedes aegypti*, *Wolbachia* spreads through maternal transmission and disrupts mosquito reproduction via mechanisms such as cytoplasmic incompatibility, sex ratio distortions, and parthenogenesis [4] [7] [8]. Importantly, *Wolbachia* blocks dengue virus replication within mosquitoes, reducing their ability to transmit the disease [9]. Decades of research have led to the successful establishment of *Wolbachia* strains in *Aedes aegypti* populations, and field trials are now planned in Africa, including Burkina Faso, to evaluate their effectiveness in controlling dengue. As these releases would be the first of their kind in the region, ethical and biosafety committees have emphasized the need for a comprehensive risk assessment to ensure that *Wolbachia*-infected *Aedes aegypti* do not pose unintended ecological or public health risks. This study aims to systematically assess these risks by identifying potential hazards, establishing a risk assessment framework, and quantifying the likelihood and severity of adverse outcomes. Given the limited empirical data on *Wolbachia* releases in Africa, this research will provide critical insights to inform decision-making and guide the implementation of *Wolbachia*-based dengue control strategies [10].

2. Methodology

2.1. Hazard Identification and Conceptual Model Development

The first step in any risk analysis is to identify all hazards associated with an event.

In the context of a release of *Aedes aegypti* infected with *Wolbachia* in Yogyakarta, Indonesia, a risk assessment was conducted using the "Cause More Harm" (CMH) criterion. The central evaluation team consisted of four experts in ecology, medical entomology, biological evolution, and medicine. Additionally, independent experts from universities, research institutes, non-governmental organizations, and governmental agencies from various disciplines were invited to participate in the risk assessment discussions. The expert panel included a virologist, two microbiologists/epidemiologists, four entomologists (medical and agricultural), a biodiversity expert, a parasitologist, an internist, an infectious disease specialist, an immunologist, a pediatrician, a psychologist, a public health expert, an economist, and a social scientist. The team employed a methodology developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia to assess risks associated with the release of *Aedes aegypti* infected with *Wolbachia*. Meetings and workshops were organized to gather expert opinions and evidence for identifying potential hazards to humans and the environment.

A Bayesian Belief Network (BBN) was subsequently used to visualize and develop the risk analysis framework, combining expert evaluations with conditional probabilities to determine the final risk value. According to Bayes' theorem, future events can be predicted based on all past events. The BBN was developed by structuring hazards into nodes and defining their probabilistic relationships. Experts were grouped according to the four elements identified as CMH criteria: ecological impacts, reduced mosquito management effectiveness, deterioration of public health standards, and negative socio-economic and cultural impacts. Each group discussed all potential hazards associated with each CMH component in the context of the release of *Aedes aegypti* infected with *Wolbachia* over the next 30 years. To construct the BBN, expert opinions were elicited through structured workshops where participants provided estimates of probabilities and potential consequences based on prior studies, field data, and their expertise. Nodes in the BBN were linked based on causal relationships identified through expert consensus, literature reviews, and logical dependencies. Sensitivity analysis was conducted by varying input probabilities to assess the model's robustness and identify key risk drivers.

The expert consultations for hazard identification and mapping followed several steps: event identification, determination of possible event states, development of a hazard list, and consensus on all hazards and their definitions. The hazard identification and mapping process based on expert input resulted in 56 hazards (nodes). These hazards were categorized into four CMH sub-components: negative impact on mosquito ecology (19 hazards), reduced mosquito management effectiveness (12 hazards), public health standards (14 hazards), and socio-economic and cultural impacts (13 hazards). While 56 hazards were identified, two hazards (increased biting rate and transmission of non-dengue pathogens) were shared between two groups (mosquito management effectiveness and public health standards). Thus, the total number of hazards increased to 58.

2.2. Study Organization

2.2.1. Study Type and Period

This study focused on assessing risks associated with the potential release of *Aedes aegypti* carrying the *Wolbachia* bacterium. It is a descriptive study conducted over six months, from September 2022 to February 2023.

2.2.2. Study Population and Sampling

The target population consisted of local participants, specifically graduate researchers and researchers at IRSS/DRO working on *Ae. aegypti* infections by *Wolbachia* in the fields of entomology, molecular biology, parasitology, and bacteriology. Due to the specificity of the study characteristics, the study population was restricted. Sampling was exhaustive, as the entire population was included. A total of 25 individuals participated in the study.

2.2.3. Study Variables

Five variables were assessed, three of which were qualitative and binary (two possible responses: yes or no):

The presence of a biosafety management system,

The participation of a biosafety team in research activities,

Respondent participation in biosafety training.

The remaining two were quantitative variables:

Respondent knowledge of biosafety,

Risks associated with the release of *Ae. aegypti* infected with *Wolbachia.*

The variable "Risks associated with the release of *Ae. aegypti* infected with *Wolbachia*" comprised four sub-variables:

Ecological impact of the release, Effectiveness of mosquito management, Public health standards, Economic and socio-cultural changes.

2.2.4. Data Collection

To evaluate the risks of releasing *Ae. aegypti* infected with *Wolbachia* in dengue control in Burkina Faso, a questionnaire was developed based on the 58 identified risks. The questionnaire included 58 risks and four questions about respondents' biosafety knowledge and the IRSS/DRO biosafety management system. Its purpose was to evaluate the CMH criterion to ensure that the release activity would be a solution rather than an additional problem in dengue control. Target participants were contacted for interviews after being briefed on the study, with appointments scheduled individually. The interviews lasted approximately one hour and followed these steps:

Presentation of the questionnaire framework (Supplementary file 1), Detailed presentation of the questionnaire and administration method, Consent signing and questionnaire administration.

The questionnaire was divided into three main sections: Administrative and demographic information, Evaluation of respondent biosafety knowledge, Evaluation of risks associated with *Ae. aegypti* release. The third section comprised four CMH components, each containing a set of risks: Ecological impact: 18 risks, Mosquito management effectiveness: 10 risks, Public health standards: 12 risks, Economic and socio-cultural impacts: 12 risks (Supplementary file 2).

The questionnaire was administered using ODK Collect on a smartphone. For each stated risk, respondents indicated whether it could result in a CMH effect. If yes, they assigned a score for both the probability of occurrence and the impact's severity on a scale of 0.1 to 1. If not, the survey moved to the next risk. Data was analyzed using R and Excel software.

2.3. Risk Calculation

Experts defined the hazards resulting from the release of *Ae. aegypti* infected with *Wolbachia* and the likelihood of these hazards based on existing information. The consequences of hazards were determined through discussions and consensus based on expert evaluations. Overall risk was then calculated using a simple equation:

$$\text{Risk} = \text{Probability} \times \text{Consequence}$$

The risk estimation scale proposed by Murray *et al.*, 2016 [11], was used as a reference to determine the likelihood and probable consequences. The estimation scales and intervals, along with their definitions, were established during a group discussion as part of a participatory process, following [12] (**Table 1**).

Table 1. Probability and consequence estimation scale for risk calculation.

Definition	Negligible	Very Low	Low	Moderate	High	Very High
Probability	0 - 0.01	0.02 - 0.10	0.11 - 0.40	0.41 - 0.74	0.75 - 0.89	0.90 - 1

3. Results

3.1. Evaluation of the System and Knowledge of the Respondent on Biosecurity

The presence of a biosafety management system is a crucial element for risk assessment and mitigation. **Table 2** shows the 25 respondents, 80% acknowledged the existence of such a system in their department, while 20% reported its absence. The lack of universal coverage suggests potential gaps in biosafety enforcement, which could impact containment and risk mitigation efforts.

Table 2. Expression of the presence or absence of a biosecurity management system.

Responses	Yes	No	Total
Number	20	05	25
Percentage	80%	20%	100%

Despite 80% of respondents affirming the presence of a biosafety management system, only 32% reported active engagement with a biosafety team (**Table 3**). This discrepancy highlights that while a system may exist, its effective implementation and oversight may be lacking. Strengthening biosecurity oversight mechanisms and improving participation could enhance risk mitigation strategies.

Table 3. Expression of participation of a biosecurity team in activities.

Responses	Yes	No	Total
Number	08	17	25
Percentage	32%	68%	100%

Training is a key component in ensuring adherence to biosafety protocols. Table 4 indicates that 88% of respondents have received biosafety training at least once. However, the 12% who have not undergone training represent a potential vulnerability. Regular refresher courses should be encouraged to maintain high compliance levels and reinforce good biosafety practices.

Table 4. Proportion of respondents who received training on biosafety.

Responses	Yes	No	Total
Number	22	03	25
Percentage	88%	12%	100%

Most respondents rated their knowledge of biosafety as acceptable (76%) or high (20%). Only one individual (4%) reported a low level of knowledge, and none rated themselves as having non-existent knowledge (Table 5). While this indicates a generally well-informed group, further training to elevate knowledge from acceptable to high or very high would be beneficial.

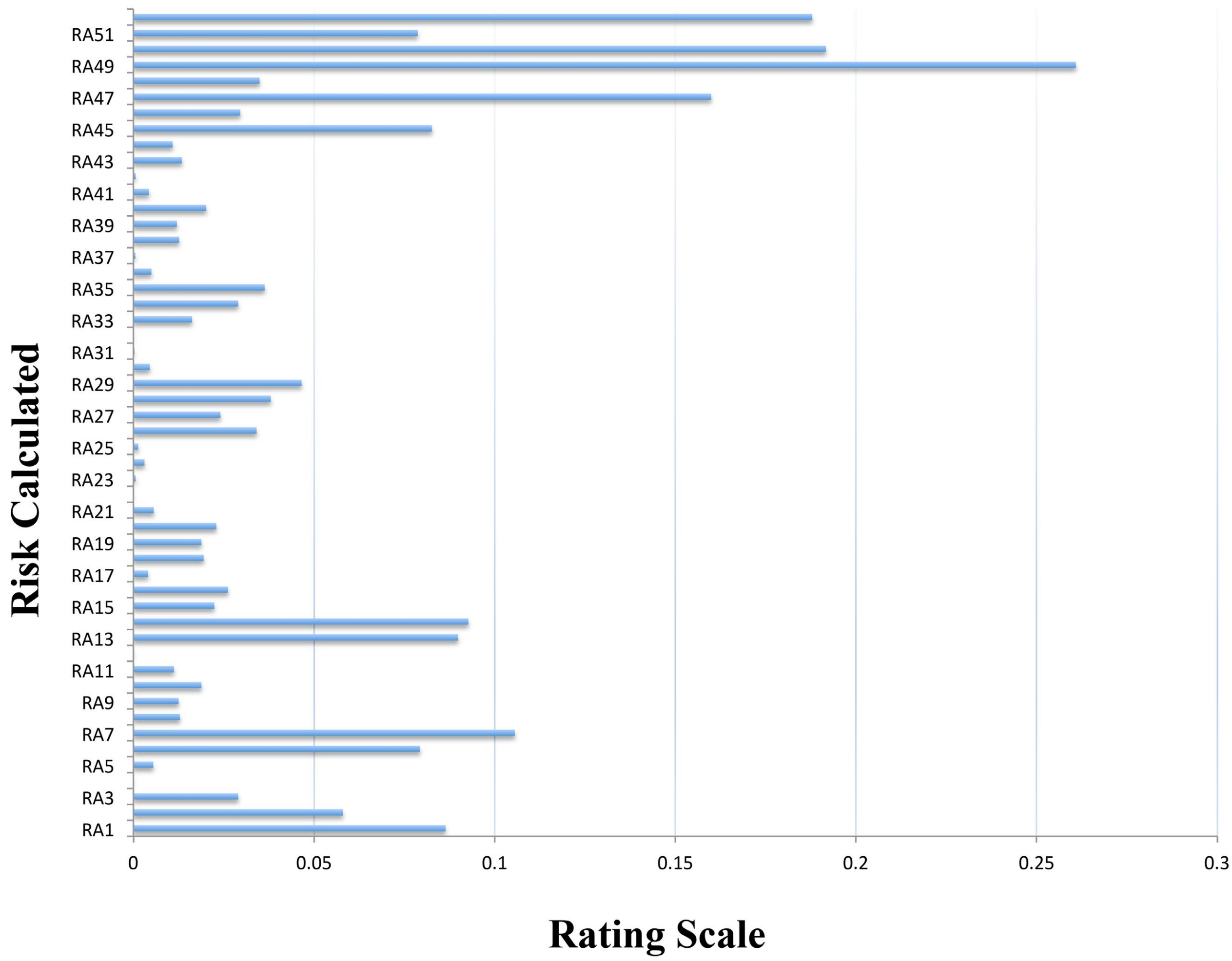
Table 5. Assessment of respondents' knowledge of biosafety.

Grade	1 (Non-Existent)	2 (Low)	3 (Acceptable)	4 (High)	5 (Very High)	Total
Number	00	01	19	5	00	25
Percentage	00%	4%	76%	20%	00%	100%

3.2. Assessment of the Risks Inherent in the Dissemination of *Ae. aegypti* Infected by *Wolbachia*

Figure 1 presents the distribution of calculated risks based on probability estimation. The highest calculated risks are linked to "economic and socio-cultural effects," with risk assessments (RA) of 47, 49, 50, and 52.

The risks were calculated by the simple operation risk = Probability × Consequence. The risk assessment in Table 6 reveals that all calculated risks fall within the "Negligible" to "Low" range, with no risks categorized as "Moderate," "High," or "Very High." The majority (90.38%) is in the "Negligible" or "Very Low" categories, indicating a limited likelihood of severe adverse events. However, the presence of risks classified as "Low" (9.62%) suggests that while the probability of significant harm is minimal, it is not entirely absent.



Legend: RA = Risk Assessment.

Figure 1. Assessment of calculated risks according to the probability estimation scale after obtaining the probabilities of occurrence of events and their potential consequences.

Table 6. Classification of calculated risks according to the probability estimation scale.

Definition	Negligible	Very Low	Low	Moderate	High	Very High	Total
Interval	0 - 0.01	0.02 - 0.10	0.11 - 0.40	0.41 - 0.74	0.75 - 0.89	0.90 - 1	
Number of Risks	24	23	05	00	00	00	52
Percentage	46.15%	44.23%	9.62%	00	00	00	100%

The risk assessment reveals that all calculated risks fall within the "Negligible" to "Low" range, with no risks categorized as "Moderate," "High," or "Very High." The majority (90.38%) is in the "Negligible" or "Very Low" categories, indicating a limited likelihood of severe adverse events. However, the presence of risks classified as "Low" (9.62%) suggests that while the probability of significant harm is minimal, it is not entirely absent.

4. Discussion

Our study focuses on assessing the risks inherent in releasing *Ae. aegypti* mosquitoes infected with *Wolbachia* as part of the fight against dengue. We identified and evaluated risks based on participant feedback and a Bayesian Belief Network (BBN). To achieve this, we designed a questionnaire informed by identified risks, which allowed us to collect participant opinions and conduct a superficial evaluation of the biosafety management systems of their respective departments.

Notably, 20% of study participants reported the absence of a biosafety management system in their departments. Additionally, 68% of respondents conducted their research activities without support from a biosafety team, and 12% had never participated in biosafety training. This highlights the presence of an existing biosafety management system but with significant structural deficiencies, as five out of 20 participants were unaware of its existence. Given the activities conducted at IRSS/DRO, a biosafety officer or team should accompany all experimental manipulations, and all personnel should undergo mandatory biosafety training tailored to their specific roles.

Using the questionnaire, participants assigned probability and consequence scores to hazards categorized as having "CMH" (economic, social, and cultural) effects. These scores were used to calculate the risk associated with each hazard. All calculated risks (100%) ranged between 0 and 0.261 (below 0.40), indicating that the identified hazards were either negligible, very low, or low. Specifically, 46.15% of the risks were negligible, 44.23% were very low, and 9.62% were low. These results might initially suggest a positive conclusion; however, the Bayesian Belief Network (BBN) reveals certain limitations.

The BBN is a tree-like structure that illustrates the relationships between risks, highlighting their interactions. It comprises a Directed Acyclic Graph (DAG) and a Conditional Probability Table (CPT). In the BBN, hazards (or risks) are represented as nodes, which interact in a way that the occurrence of one node can trigger or exacerbate another's consequences. The DAG consists of nodes and links that describe relationships between variables. Here, the nodes represent identified risks, and each is connected to others by links, arcs, or edges to show conditional dependencies. A link between a parent node and a child node indicates a functional or statistical correlation. Each child node (connected to one or more parent nodes) contains a CPT that specifies the conditional probability of the node being in a particular state, given the state configurations of its parent nodes. Conditional probability reflects the likelihood of an event occurring if another event occurs, and it was used to calculate the probability of each node. The absence of a link between two nodes means no CPT can be defined.

For instance, the "Economic and Sociocultural Change" component of "CMH," which contains four of the five highest-calculated risks, has four parent nodes (healthcare, tourism, reduced income, increased expenses) leading to the child node "economic change." It also has six other parent nodes (scapegoating, migration, unfavorable media, social conflicts, legal actions, social fear) leading to the child node "social behavior change." These two child nodes then converge on the aforementioned subcomponent. Given these interdependencies, it is crucial to consider all risks and implement precautionary measures across all subcomponents to mitigate the identified "CMH" effects.

Beyond the limitations of the BBN, additional uncertainties must be considered. During interviews, participants revealed that the classification of "CMH" risks was predominantly based on theoretical knowledge, such as reading articles and making deductions. For example, the endosymbiotic nature of the *Wolbachia* bacterium led them to assert that the genetic or behavioral modification of *Ae. aegypti* would have no "CMH" effects. Furthermore, the lack of a well-structured biosafety management system and insufficient biosafety training among personnel are uncertainties that must be addressed for an objective and effective risk assessment. The lack of empirical data on *Ae. aegypti* infected with *Wolbachia* limits the ability to conduct a more in-depth risk analysis.

Nonetheless, as observed in Yogyakarta, Indonesia [13], and Australia [11], the results are promising, with risks ranging from negligible to very low. Moreover, 80% of the highest risks are social rather than biological. The "sociocultural change" parameter had the most significant influence, driven by the perception that the population might poorly receive the dissemination project. This parameter alone accounted for 80% of the most significant risks. For instance, the hazard RA 49, "negative messages on social media," was classified as the highest individual risk (albeit low), with a probability of 0.548, a consequence of 0.476, and a calculated risk of 0.261. These hazards can be mitigated with tailored communication strategies and the involvement of social science professionals.

In contrast, in Yogyakarta, the parameter "mosquito management effectiveness" had the greatest impact, stemming from the perception that dengue had been eradicated, which could lead to reduce mosquito control efforts in households [13]. These differences highlight the importance of considering the specific ecological and socio-cultural contexts when implementing *Wolbachia*-based interventions. Burkina Faso differs significantly from Indonesia and Australia in terms of mosquito ecology, disease burden, public perception, and regulatory frameworks. For example, Burkina Faso has a high malaria burden, and mosquito control strategies often focus on malaria vectors rather than dengue-transmitting mosquitoes. Additionally, socio-cultural attitudes toward genetic or biological interventions in vector control may vary, influencing community acceptance. Unlike Indonesia, where community engagement played a crucial role in the project's success, Burkina Faso may require different outreach approaches tailored to local beliefs and concerns. Understanding these distinctions can help refine risk mitigation strategies, ensuring that potential socio-economic and cultural concerns are adequately addressed.

5. Conclusion

Ultimately, the importance of risk assessment cannot be overstated. It reassures stakeholders about the safety of the study for both humans and the environment. Additionally, it protects research teams and institutions from potential legal proceedings. At the conclusion of our assessment, the results are encouraging, showing that the dissemination of *Ae. aegypti* mosquitoes infected with *Wolbachia* poses little to no danger. The parameter "sociocultural change" had the most significant influence, accounting for 80% of the most impactful risks. This explains why hazard RA 49, "negative messages on social media," was identified as the highest individual risk, with a calculated risk of 0.261. However, in general, the identified hazards were either negligible, low, or very low. The calculated risks, as presented, are manageable, provided that an appropriate management plan is implemented. Furthermore, the Bayesian network allows for the identification of parent or source nodes, enabling proactive risk prevention at its root. That said, the limitations of the method, particularly the Bayesian Belief Network (BBN), must be considered. It is also important to acknowledge that the risks identified were based on studies conducted in Yogyakarta, Indonesia, and not under the specific conditions of Burkina Faso. Comparing Burkina Faso's ecological and socio-cultural landscape with prior *Wolbachia* release sites provides valuable insights into tailoring mitigation strategies to local needs, ensuring both the effectiveness and acceptability of the intervention.

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Author Contributions

Conceptualization, E.M.D.B. and A.D.; Methodology, A.S., M.B.B. and E.M.D.B.; Data collection, A.S. and E.M.D.B.; data curation, A.S., E.M.D.B. and R.W.S; writing-original draft preparation, E.M.D.B., Funding acquisition, E.M.D.B. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

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