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Research paper

Biting Realities: Culicidae's Role in The Transmission of Bloodborne Pathogens

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Abstract

Mosquitoes (family Culicidae) are established vectors for many infectious agents, including arboviruses and protozoan parasites. The transmission of bloodborne viruses like the hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) by mosquitoes has been less studied than that of other agents. This study aimed to assess the potential association between mosquito exposure and seroprevalence of selected virological markers—HBsAg, HCV Ab, and HIV Ab—among individuals recently bitten by mosquitoes.

was not statistically significant when compared to the unexposed population, it does indicate that there were some underlying HBsAg and HCV infections in the mosquito-exposed population and no detectable infection in the unexposed. More epidemiological studies are warranted to improve understanding of the potential infectious nature of mosquito exposure. This occupational and social health approach of uncovering knowledge about disease among mosquito exposed population is warranted for researchers to improve knowledge and health, based on mutual agreement. For the study design, social and occupational approaches require blended methodologies, a rigorous approach, and the collaboration of public health researchers.

Raises initial questions about possible environmental or vector-related risks, although results were not statistically significant and cannot demonstrate causality. Findings suggest the need for larger, longitudinal studies including viral load quantification and molecular typing to assess whether mosquito exposure is associated with blood-borne viral infections in particular ecological circumstances. The current findings can be interpreted at face value as hypothesis-generating, and yet, contribute additional knowledge towards interdisciplinary concepts of unconventional transmission pathways for blood-borne pathogens - a field in need of more research.

Keywords: *Mosquito Bites; HBsAg; HCV Ab; HIV Ab; Bloodborne Pathogens; Seroprevalence; Transmission Risk; Vector-Borne Diseases; Statistical Analysis; Iraq.*

1. Introduction

Mosquitoes, members of the family Culicidae, are one of the most studied taxa of arthropods in the world primarily because of their capacity to transmit pathogens such as viruses (e.g., dengue, Zika, and yellow fever) or protozoa (e.g., Plasmodium spp., the cause of malaria) [1]. Vector-borne diseases pose a significant global public health burden, particularly in tropical and sub-tropical areas where they cause substantial morbidity and mortality and are also associated with major economic costs to health systems [2]. The ability of mosquitoes to be vectors is conferred by a blood feeding process known as hematophagy, as female mosquitoes consume blood meals to obtain proteins for developing eggs [3]. This process involves the ingestion of pathogens via blood feeding from infected hosts and then re-transmitting pathogens to new hosts the next time the mosquito feeds, characteristically followed by an overall cycle of transmission [4]. To control the transmission of diseases that cause substantial suffering and lessen morbidity and mortality, it is important to understand the key factors associated with pathogen transmission in mosquitoes. Mosquitoes in particular, Anopheles mosquitoes in Nigeria, have shown very adaptable changes in response to their environment, which may allow for an entire species-level change to spread [5]. The adaptability of mosquitoes further highlights the need for continual monitoring and research to identify the changing nature of mosquito-borne diseases, as well as to develop targeted interventions for disease control [6]. Additionally, when a region has several species of mosquitoes, management efforts can be significantly more complicated, since each species could have different behaviors, preferred habitats, and susceptibility to insecticides, thus increasing the need for a multifactorial approach for mosquito management.

This feeding behavior, by necessity, is the piercing of the host skin and consumption of blood, which leads to a natural and direct pathway for pathogens to cross into and out of the vertebrate host. Additionally, the mosquito environment varies tremendously, site by site and infection by infection, in terms of immune responses, physiology, and behavior. This will create very strong selection for phenotypic variation and rapid adaptation in the parasite population. Because of this, the vector-host contact rate should be considered a key component in the epidemiology of parasites or pathogens, because contact rate is directly related to the basic reproduction number, which is the indicator of transmissibility. Mosquitoes can transmit a plethora of pathogens, including bacteria and helminths in addition to viruses and protozoans [10]. Thus, the dynamics for mosquito-borne disease transmission are complex, arising from a combination of vector ecology, pathogen biology, host immunity, environmental factors, and human behaviors. Therefore, understanding these factors and interactions will be the determining factor for developing successful disease control and mitigation plans.

To grasp the complexities of pathogen transmission from mosquitoes, we will need to engage in a discussion of the factors driving vector competence, vector capacity, and human-vector interaction, along with the factors determining overall disease transmission risk and the potential for outbreaks. In addition, global changes that may increase density and expand the geographical range of mosquitoes are public health threats that could have global ramifications resulting in emergence or re-emergence of mosquito-borne diseases [12].

2. Literature review

2.1. Global prevalence and impact of mosquito-borne illnesses

Mosquito-borne disease is an important part of the worldwide burden of infectious diseases, with many of the cases occurring in low-income countries that do not have equitable access to healthcare and sanitation. One of the oldest forms of neglected tropical disease is Malaria (caused by parasites that use Anopheles mosquitoes as a vector). It accounts for a substantial proportion of global vector-borne disease, and the number of cases and deaths is staggering, with hundreds of thousands of deaths each year, mainly among young children in sub-Saharan Africa [13]. Dengue fever is another mosquito-borne illness, which is caused by a virus and transmitted by Aedes mosquitoes. Globalized dengue fever is emerging as a ubiquitous and expanding public health burden, threatening existing public health systems, as evidenced by global outbreaks among these mosquitoes in tropical and subtropical regions [14]. Furthermore, the last half century has resulted in the emergence of dengue fever throughout the world like never before, leading to increases in international burden attributed to urbanization, climate change, and increased global circulation of travel and tourism. The World Health Organization reported there were 228 million malaria infections and 405,000 deaths in the world in 2018 [15]. The same has occurred with dengue fever as a public health threat on a global scale. In addition, there were over 5 million dengue fever cases and 5,000 deaths in 2023, which is an eight-fold increase over 2019 [16]. The trend of increasing incidence and mortality underscores the need for effective mosquito-borne disease prevention and control efforts, at both a local and global level to reduce the incidence. It will take to show urgency and coordination to address the complexities of the impact of mosquito-borne illness.

In addition to malaria and dengue fever, other mosquito-borne diseases, including Zika virus, chikungunya, and West Nile virus, have been a major cause for public health concern in recent and recent years, causing rampant outbreaks and raising fears about long-term health effects. These diseases are caused by RNA viruses from the family Flaviviridae and are transmitted to humans by means of one or more mosquito vectors [17]. The rise and re-rise of these illnesses stresses the fluid nature of mosquito-borne disease transmission, and the inherent difficulties associated with controlling them, particularly in the context of globalization and environmental change [18]. The economic implications of mosquito-borne infectious diseases are considerable, both as direct healthcare costs and indirect from lost productivity and lost tourism revenue, and hampering economic development in regions afflicted by these diseases [19].

The interdependent nature of vector ecology, pathogen biology, human behavior, and environmental influences makes the prevention and control of mosquito-borne disease more complex, advancing the need for a holistic approach that links vector control, disease surveillance, health education, and community ownership. Approximately 75% of all emerging infectious diseases are zoonoses [20]. Not surprisingly, contributions from global warming, deforestation, and other environmental changes have all increased the emergence of zoonotic diseases [21]. Climate change affects many aspects of the transmission of vector-borne diseases [22]. Climate changes, which may include increased temperatures and altered precipitation, may extend the geographic distribution of mosquito populations, cause them to breed more frequently, and increase disease transmission [23].

Anthropogenic alterations to the Earth established new habitats for mosquitoes and increased contact between humans and mosquitoes. Urban development, agricultural expansion, and deforestation are some of the significant contributors to emerging and emerging zoonoses [24]. The principal reason for the establishment of new habitats is the alterations to the natural space previously occupied by the mosquitoes and the humans, which ultimately poses increasing contact and enables exposure to new pathogens. Travelers, including foreign a group of people who increase risk by traveling to areas they have never been, have also been exposed in more recent history to dengue viruses, the most reported arboviral infections in traveling/group of travelers [25]. For example, mass air travel and incidental recreational tourism, which provide the avenue for prospective hosts from different parts of the world, are fundamental to increasing the global incidence of arboviruses, particularly dengue [26],[27]. Resources and infrastructures for effective medical care, especially in impoverished countries, and appropriate and innovative mosquito management strategies are lacking in most regions of the world, contributing to the risk of arboviral infection [28].

2.2. Mosquito vectors and bloodborne pathogens

Mosquitoes in the family Culicidae are vectors for many bloodborne pathogens, including viruses, parasites, and helminths, causing diseases in humans and animals [29]. Mosquito genera that are of medical importance include Anopheles, Aedes, and Culex, each of which has different habitat preferences, host-feeding patterns, and vector competence for specific pathogens [30]. Anopheles mosquitoes are capable of vectoring Plasmodium (malaria), and saliva is injected during blood-feeding; the mosquito transmits the disease [31]. Aedes mosquitoes are responsible for transmitting multiple arboviruses, including dengue fever, Zika virus, chikungunya virus, and yellow fever virus, and these viruses can cause febrile illnesses, encephalitis, and hemorrhagic fevers in humans [32]. Culex mosquitoes transmit viruses such as West Nile virus, Japanese encephalitis virus, lymphatic filariasis, and more [33]. Transmission of these pathogens can affect humans and other vertebrates and invertebrates. Vector competence is defined as the ability of a mosquito to acquire, maintain, and subsequently transmit a pathogen to a susceptible host [30]. Vector competence is different among mosquito species, populations, and even individuals due to genetic, physiological, and environmental factors. We need to understand how different mosquito species have different vector competence for a variety of pathogens to anticipate disease risk and prioritize appropriate vector control [33].

The cycle of pathogen transmission by mosquito-borne vectors is influenced by complex interactions of the mosquito vector, the vertebrate host, and the pathogen itself and can be affected by factors such as environmental factors, host immunity, and pathogen virulence (McKenzie et al. 2012). Mosquitoes can pick up pathogens during a blood meal from an infected host to replicate and disseminate inside the mosquito before transmitting from its next blood feeding to a new host.

Factors that may affect the transmission of pathogens include multiple host status, pathogen replication time during and between the feeding cycles, the mosquito species' biting rate, longevity, and vector competence, and the host's immune status/contribution and behavior. Environmental factors such as temperature, humidity, and rainfall can affect breeding, development, and survival of mosquitoes and vector competence.

Due to temperature differences, A. stephensi population fluctuation is dependent [34]. Different categories of arboviruses take advantage of the mosquito bite to access vertebrate hosts. Female Aedes aegypti mosquitoes are more attracted to blood meals in indoor habitats, and peak biting occurs two to three hours after daybreak and three to four hours before nightfall [35]. Female Aedes aegypti mosquitoes have also been observed to blood feed on multiple hosts in each gonotrophic cycle, which has introduced adaptability to urban centers.

The Asian tiger mosquito, *Aedes albopictus*, is responsible for the transmission of more than 20 arboviruses including chikungunya, dengue, and Zika viruses [36]. *Ae. albopictus* is a native of Asia but has now become established across the globe due to international trade and transport, establishing itself in urban, suburban, and rural environments [37]. tsetse flies could spread from sub-Saharan Africa as they do in their normal distribution range[38]. A few disease vectors are affected by the distributional features of the landscape consisting of forests, agricultural land, and urban features [39].

The distribution of mosquitoes is contingent upon climate, vegetation, and human factors. Colonization of a new environment will depend on the presence of host availability and suitable habitat [38]. Spatial autocorrelation models provide a framework correct for spatially autocorrelated data by defining a neighborhood that may be used for prevention and control of disease [40]. Releasing Wolbachia-infected mosquitoes is a successful, sustainable tool for controlling dengue and other *Ae. aegypti* associated diseases even in highly urbanized, complex systems [41].

2.3. Arboviral diseases: dengue, zika, and chikungunya

Dengue fever, a condition caused by the dengue viruses (DENV 1-4), is the most common arboviral disease in the world, with estimates of 50 to 100 million dengue infections occurring annually [1]. Dengue is mainly transmitted by the mosquito *Aedes aegypti*, which breeds in artificial containers and has adapted to urban areas so well that it can be found in virtually every urban area that experiences tropical or subtropical weather conditions. In the last couple of decades, the incidence of dengue has increased rapidly due to changes associated with global urbanization, the rapid growth of urbanized populations, and changes in climate [2]. It is estimated that the dengue virus causes 100-400 million infections and between 40,000 and 70,000 deaths annually, with half of the world's population at risk for infection [3].

Zika virus emergence occurred during the 2015-2016 timeframe and was identified as a concern for global public health during widespread outbreaks in the Americas [4]. Like dengue, Zika virus is transmitted by mosquito vectors in the genus *Aedes*, commonly *Aedes aegypti*. Zika virus is also transmitted sexually between individuals and can also be transmitted vertically from a mother to a fetus [5][6]. Infection with Zika virus during pregnancy has been shown to result in congenital Zika syndrome, a collection of birth defects including microcephaly, abnormal brain development, and other neurological defects [7].

Chikungunya fever (CF), specifically defined by fever, rash, and often debilitating and persistent joint pain, is caused by chikungunya virus (CHIKV) and transmitted by *Aedes* species mosquitoes, primarily *A. aegypti* and *A. albopictus*. CF has grown rapidly in many countries in recent years, with outbreaks in Africa, Asia, and the Americas [46]. Reports of *Aedes* infestations and increasing chikungunya cases in the United States were reported in 2022 [47]. Reports are also documenting DENV-ZIKV co-infected cases in various countries [48]. Dengue virus (DENV) and Zika virus (ZIKV) are already present in Latin America, Africa, Asia, and the Pacific Islands and are both transmitted by *Aedes*, which has led to many endemic areas where ZIKV and DENV co-exist [49].

The co-circulation of and/or co-infection of several arboviruses in the same geographic area gives rise to difficulties in diagnosis, surveillance, and control [50]. The global expansion of many arboviral diseases worldwide can be facilitated by several problems: climate change, globalization and urbanization of human life, and international travel, all indicating the potential need for organizations to take a One Health approach to enabling vector management and diagnostic capacity [51]. In recent years, Africa has seen notable epidemics of urban arboviral diseases, including yellow fever and dengue fever [52]. It was shown that after a previous ZIKV infection, there was an increased frequency of more severe forms of disease resulting from a subsequent dengue serotype infection [53].

2.4. Strategies for mitigating mosquito-borne diseases

A successful method for managing mosquito-transmitted diseases entails a comprehensive strategy that involves collaboration by vector control, disease surveillance and monitoring, as well as health education. Vector control strategies include source reduction, larviciding, adulticiding, and personal protective measures such as the application of mosquito repellents and sleeping under mosquito bed nets.

Source reduction refers to eliminating or modifying mosquito breeding sites, eliminating standing water in containers, draining stagnant pools, as well as improving sanitation practices. Mosquito control practices have successfully reduced mosquito populations and the risk of disease transmission.

Larviciding practices involve chemical or biological insecticides to target and kill the immature mosquito stage in the aquatic habitats. Whereas adulticiding practices use insecticides to kill adult mosquitoes. Integrated vector management refers to vector control that employs a combination of the above strategies in consideration of the local context and available scientific evidence. The notion that approaches to vector control will constantly evolve using new strategies like Wolbachia-based control demonstrates the importance of reducing morbidity and mortality [54].

Indoor residual spraying (IRS), which consists of applying insecticides to the walls and ceilings of houses, has been shown to be effective at reducing malaria transmission in sub-Saharan Africa [55]. Insecticide-treated nets, insecticide-treated clothing, and spatial repellents are additional strategies to reduce human-vector contact with mosquitoes [56 - 58]. Epidemiological surveillance is important for tracking the incidence and distribution of mosquito-borne disease, for disease outbreak detection, and for assessing the effects of control measures [59]. Identifying and implementing mosquito vector control interventions represents a high priority need in areas with heavy disease burdens [55].

Public health education is vital for raising awareness of mosquito-borne disease and promoting personal protective measures, and to engage the community in vector control initiatives. Community engagement, education, and mobilization are key aspects of mosquito control programs and are beneficial in terms of community ownership, sustainability of intervention, and collaboration [60]. Long-lasting insecticidal nets is the most common intervention strategy used for the prevention of malaria in endemic countries around the world [61].

The development of new tools and technology for mosquito control remains an area of research that is evolving with exciting development across genetic control, Wolbachia-based control, and better formulations of insecticides. The malaria elimination community appears to be on the insecticide treadmill. With very few effective public health insecticides available, that means we should be assessing the insecticide-use practices [62]. New developments such as microbial larvicides have unique killing mechanisms and do not have the same resistance patterns as current pyrethroids, which provides a potential new supplement for vector control [63]. Targeted surveillance can help develop datasets that public health systems can employ to track and assess frameworks to understand the drivers of transmission [64]. Some possibilities include increasing IRS coverage, developing effective new insecticides, and fully employing multiple interventions [65]. Insecticide resistance will be a significant threat to the future of malaria prevention programs [65 - 67].

2.5. Addressing challenges in mosquito-borne disease control

Despite major advances in the control of diseases transmitted by mosquitoes, many challenges still exist that may include environmental change, urbanization, insecticide resistance and proliferation, and new or emerging infectious diseases. Increasing levels and intensities of resistance to pyrethroids in *Anopheles* vectors have limited malaria control progress in many places, and over the last decade, the prevalence of resistance to insecticides has increased [68]. Insecticide resistance occurs when the mosquito can survive exposure to an insecticide. The development and increase of insecticide resistance has the potential to disrupt existing insecticide-based interventions or use and create a need for new insecticide-based vector control strategies. Climate change may change the distribution, abundance, and capacity of a mosquito to transmit pathogens, sometimes resulting in diseases being transmitted to people in areas that fall outside historic geographic distribution [40]. Urbanization often results in a change of environmental conditions, which can favor mosquito breeding sites or create favorable environments for transmission, which increase mosquito-human contact. Emerging infectious diseases like the Zika virus and chikungunya virus create new challenges for vector-borne disease control as there is limited time to detect, classify, and implement an adequate response. The growing threat of resistance and the growing prevalence of mosquitoes observed around the globe, along with limited understanding of malaria epidemiology, still provide significant challenges for malaria elimination [61]. Addressing these issues requires active collaboration across multiple sectors, investment in research and development, and sustained political engagement. [69].

In addition, and despite the complex nature of parasite and vector life cycles, there is also an urgent requirement to create mathematical models to examine the effect of combined interventions. Mathematical models can provide essential information about the transmission dynamics associated with the disease, and information on indirect effects caused by different control interventions. For example, vector control strategies have developed new methods to control vector populations, and advanced diagnostic tools, multi-disciplinary commitment, and surveillance systems all require community participation to effectively deliver community-based interventions to eliminate malaria and prevent the emergence of other vector-associated diseases.

Continuing research programs should also reflect on finding new drug targets, providing new vaccine strategies, and studying the complex mechanisms of drug resistance [70]. In a complex world, it is now possible to invent strategies to circumvent resistance, reduce environmental damage, and increase activity [71]. Therefore, products used indoors or outdoors may be expanded in terms of notions of vector control. Research also has to address the problem of an outdoor mosquito vector and will continue to work towards developing an effective vaccination. However, there is still a need for timely and quality entomological surveillance to make evidence-based decisions, and in the context of vector control, [15], [72].

Malaria is a debilitating and life-threatening disease caused by infectious agents transmitted to humans via the bites of an infected vecter. continues to be a major worldwide health issue [73]. Following the most recent reports, the World Health Organization stated there were approximately 219 million cases of malaria in 87 countries, and indicated four regions as areas of high-risk: South-East Asia, Eastern Mediterranean, Western Pacific, and the Americas [74]. Most malaria cases and deaths occur in sub-Saharan Africa. Children under five years of age are particularly vulnerable [75]. Of the five species of *Plasmodium* that can cause disease in humans, *Plasmodium falciparum* has always been associated with the most notorious levels of mortality and morbidity due to infection [76]. It is also important that any infected persons are promptly treated to help reduce transmission risk [77].

Each year, nearly 200 million people die because of mosquito-borne illnesses, including malaria. The greatest number of deaths occurs in children under age five [78]. Malaria is preventable as well as curable with early and effective diagnosis [79]. Molecular methods, like the polymerase chain reaction, are much more sensitive and specific than traditional microscopy and can detect low-density infections and mixed-species infections. While detecting *Plasmodium* parasites by microscopy in blood smear is the gold standard for diagnosis of malaria due to low cost and simplicity, it is limited in that sensitivity varies, especially in the case of low parasite density.

Use of malaria RDTs has increased as they become more available, and easier access to malaria RDTs is associated with decreased child mortality [81]. WHO has recommended that, before treatment, for any suspected malaria case, microscopy or rapid diagnostic tests be used to confirm the diagnosis. Light microscopy is still the standard method along with rapid diagnostic tests [82]. However, asymptomatic and low-density Plasmodium infections confer decreased efficacy of traditional diagnostic approaches [83].

2.6. Viral hepatitis transmitted by mosquitoes

While typically associated with transmission through contaminated food, water, or blood products, emerging evidence suggests a potential role for mosquitoes in the transmission of viral hepatitis, particularly hepatitis A and hepatitis E. Hepatitis A virus and hepatitis E virus, primarily transmitted through the fecal-oral route, can cause significant liver inflammation and morbidity [84]. While mosquito-borne transmission of hepatitis viruses is not yet definitively established, several factors support the plausibility of this route. Mosquitoes, as indiscriminate blood feeders, can acquire viruses during blood meals from infected individuals and potentially transmit them to susceptible hosts.

The mechanical transmission, where viruses adhere to the mosquito's mouthparts or proboscis and are directly transferred during subsequent bites, is also possible. Furthermore, mosquitoes can contaminate surfaces and food sources through regurgitation or defecation, potentially introducing hepatitis viruses into the environment. The relatively high viremia levels observed in acute hepatitis A and E infections could increase the likelihood of mosquitoes acquiring the virus during blood meals. It is also important to consider the possibility of indirect transmission, where mosquitoes contribute to the spread of hepatitis viruses by contaminating water sources or serving as vectors for other pathogens that exacerbate liver inflammation.

The use of interventions aimed at reducing parasite load in mosquitoes can affect the incubation period or longevity of the mosquitoes, potentially increasing their efficiency in transmitting the infection [85]. The parasite undergoes several bottlenecks, potentially reducing the oocysts load in mosquitoes [86]. Mosquitoes acquire *Plasmodium* parasites during blood meals from infected humans. Inside the mosquito, the parasites undergo a complex series of developmental stages, ultimately producing sporozoites that migrate to the salivary

glands [87]. When the mosquito takes another blood meal, these sporozoites are injected into the new host, initiating a new cycle of infection [88]. After injection into the mammalian host, *Plasmodium* sporozoites migrate to the liver and invade hepatocytes [89].
After a period of asexual replication within liver cells, merozoites are released into the bloodstream, where they invade red blood cells [90]. Within the red blood cells, the parasites undergo further asexual replication, causing the characteristic symptoms of malaria. A subset of parasites differentiate into sexual forms called gametocytes, which are ingested by mosquitoes during a blood meal [91] [92]. These then turn into schizonts and can develop up to 30,000 merozoites, which rupture the hepatocytes [71] [88]. The parasites develop within the erythrocyte in distinct forms until erythrocyte lysis occurs, releasing merozoites for reinvasion [90]. The asexual multiplication of *Plasmodium* parasites within red blood cells is responsible for the clinical manifestations of malaria. The infected cell is ruptured in a process known as egress to release a new generation of parasites [93].

3. Material and methods

3.1. Study design

This study employed a cross-sectional observational design embedded within a broader literature-based review. It aimed to explore the plausibility of mosquito-mediated transmission of bloodborne pathogens, focusing on hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab), and human immunodeficiency virus antibody (HIV Ab). The observational data were used to support and contextualize the findings of an extensive literature review.

3.2. Study location

The observational component of the study was conducted in Babylon Governorate, specifically in Hilla City, Iraq, a region with endemic mosquito activity, particularly during the warmer months. The area exhibits typical urban and peri-urban mosquito breeding conditions such as stagnant water, open drains, and poor waste management practices.

3.3. Ethical approval

This study was approved by the Scientific and Ethical Committee of the Biology Department, College of Science, University of Babylon. Written informed consent was obtained from all participants after explaining the purpose of the study, the procedures involved, and the confidentiality of data.

3.4. Study population

The study involved 100 human participants, divided into two groups:

- Study Group: 80 individuals who reported recent mosquito bites (within the past 72 hours)
- Control Group: 20 individuals with no reported mosquito bites in the preceding month

Participants were selected based on the following inclusion and exclusion criteria:
Inclusion Criteria:

- Aged 18 to 55 years
- No history of blood transfusions, intravenous drug use, or recent surgery
- No known prior diagnosis of HBV, HCV, or HIV

Exclusion Criteria:

- Immunocompromised individuals
- Those with chronic liver or renal disease
- Those on antiviral therapy

3.5. Sample collection and processing

- Blood Collection: 5 mL of venous blood was drawn from each participant using sterile, single-use vacutainer systems and collected into gel-separator serum tubes.
- Centrifugation: Blood samples were centrifuged at 5000 rpm for 5 minutes to separate serum.
- Storage: Serum samples were stored at −20°C until virological analysis.

3.6. Laboratory analysis

3.6.1. Detection of viral markers

The serum samples were tested for:

- Hepatitis B surface antigen (HBsAg)
- Hepatitis C virus antibodies (HCV Ab)
- HIV-1 and HIV-2 antibodies (HIV Ab)

3.6.2. Testing system

- All tests were conducted using Electro-Chemiluminescence Immunoassay (ECLIA) technology
- Equipment: Roche Cobas e411 Immunoassay Analyzer
- Reagents and kits used were pre-calibrated and quality-controlled according to the manufacturer's standards (Roche Diagnostics, Germany)

3.7. Quality control

- Internal controls (positive and negative) were run with every batch
- Each sample was tested in triplicate to ensure reproducibility
- Cross-contamination was minimized by performing procedures in a biosafety level 2 (BSL-2) laboratory

3.8. Statistical analysis

- Data were entered into Microsoft Excel 2019 and analyzed using IBM SPSS Statistics version 25
- Descriptive statistics (percentages, means, standard deviations) were calculated
- Prevalence of each marker was calculated separately for study and control groups
- Chi-square (χ^2) test was used to determine significance between groups at a 95% confidence interval ($p < 0.05$ considered statistically significant)

3.9. Literature review methodology

The literature synthesis accompanying this study followed a narrative review approach to integrate epidemiological, environmental, entomological, and virological perspectives.

- Databases searched: PubMed, ScienceDirect, Scopus, WHO Global Health Observatory, and Google Scholar
- Keywords: “mosquito-borne transmission,” “hepatitis B,” “hepatitis C,” “vector competence,” “arboviruses,” “Culicidae,” “bloodborne pathogens,” “environmental factors.”
- Timeframe: Studies published between 2000 and 2025, with a focus on literature from the last 5 years
- A total of 160 sources were reviewed; over 100 peer-reviewed studies were cited in the final manuscript

4. Results

4.1. Participant characteristics and grouping

The study involved 100 participants, divided into:

- Study group (n = 80): recent exposure to mosquito bites
- Control group (n = 20): no mosquito exposure

Participants were screened for HBsAg, HCV Ab, and HIV Ab to explore any association between mosquito exposure and virological marker positivity.

4.2. Marker prevalence across groups

The overall prevalence rates are summarized in Table 1.

Table 1: Prevalence (%) of Virological Markers by Group

Group	HBsAg (%)	HCV Ab (%)
Study	1.25	2.5
Control	0.0	0.0

These results indicate low-level presence of HBsAg and HCV Ab exclusively in the study group, while no positive results were recorded in the control group. HIV Ab was uniformly negative.

4.3. Statistical analysis

To determine the significance of observed differences, Chi-square tests were conducted for each marker using the 2x2 contingency tables derived from group status and test outcomes.

Table 2: Statistical Analysis Results (Chi-Square Tests)

Marker	Chi-Square Value	p-value
HBsAg	0.000	1.0000
HCV Ab	0.146	0.7019
HIV Ab	0.000	1.0000

Although positivity was observed in the study group, the differences were not statistically significant due to small effect sizes and low event counts. This highlights the limitation of sample power in establishing causal inference.

4.4. Case-level observations

A review of individual-level data (refer to Minimal Positivity Dataset) showed:

- 1 participant tested positive for HBsAg
- 2 participants tested positive for HCV Ab
- 0 participants were positive for HIV Ab
- No individuals exhibited more than one positive marker

These rare, non-overlapping cases suggest isolated positivity events potentially due to background seroprevalence, subclinical infections, or historical exposures.

4.5. Visualization of virological marker distributions

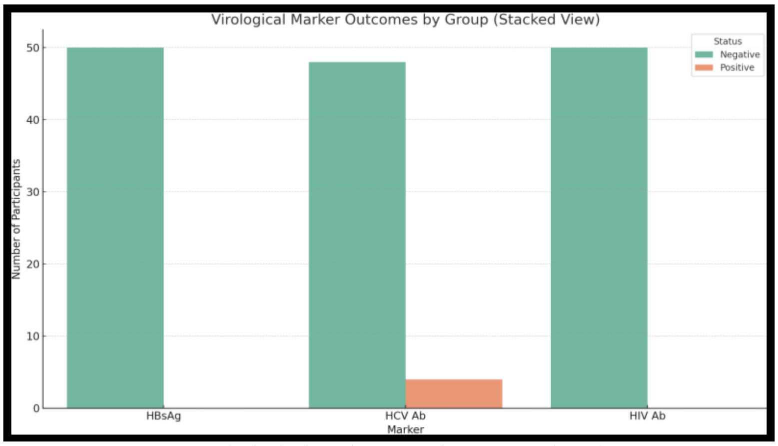

Fig. 1: Virological Marker Status by Group and Marker.

This stacked bar chart highlights the dominance of "Negative" results, with a small fraction of "Positive" cases appearing only in the study group for HBsAg and HCV Ab. The visual contrast between groups helps underscore the specificity of observed marker positivity to those recently exposed to mosquitoes.

4.6. Interpretation of findings

- The exclusive detection of virological markers in the study group, though statistically non-significant, may suggest an environmental or behavioral exposure difference.
- Absence of HIV Ab positivity and no co-infections limit the generalizability of findings.
- These results do not support a definitive link between mosquito bites and transmission of HBV or HCV but do raise hypothesis-generating questions for future research.

4.6.1. Conclusion of statistical evaluation

While seropositivity was detected in a small proportion of the mosquito-exposed group, the lack of statistical significance and the absence of any positives in the control group call for:

- Larger, well-powered studies
- Inclusion of viral load quantification
- Longitudinal design to track incident cases post-exposure

5. Discussion

The data from this study show no statistically significant correlation between the recent exposure to mosquito bites and the presence of virological markers for Hepatitis B and Hepatitis C. The lack of statistical significance suggests that any observed seropositivity in the study group may have been due to chance or other confounding factors.

The serological analysis (alongside ecological context) presents a clear example of how evidence should be integrated; the need for a variety of evidence when considering risk for virus transmission is needed [94]. The low frequency of HBsAg and HCV antibodies among the study participants may be due to several reasons including the low endemicity of the viruses in the study population, successful sample conditions and vaccination programs, or the lower sensitivity of the serological assays in which these tests were performed. In addition, it is possible that the sample size in this study was too small to reveal a statistically significant difference between study and control groups, especially considering that the low incidence of HBsAg and HCV antibodies could not be detected [95]. Thus, to investigate more thoroughly to better determine if mosquitoes contribute to the transmission of blood-borne viruses, larger numbers of participants and extended follow-up periods are potentially necessary [96]. Another limitation to the current study is the issue of recall bias; individuals do not accurately remember or report exposure to mosquito bites. Regardless, this limitation applies to both groups and was deemed acceptable [97].

The absence of detectable HIV antibodies in both study and control groups indicates the lack of plausible evidence for mosquito-borne HIV transmission, supporting the overall understanding of HIV transmission routes.

There are limitations to this investigation, particularly in contextualizing the results.

Because of the small sample size, our statistical power is compromised, which may have limited generalizability and resulted in a failure to identify a true association between mosquitoes and viral transmission [98]. More investigations are warranted, with greater sample size and sample diversity to validate or reject the present association [99].

The absence of statistically significant findings highlights a need for more robust study designs, larger sample sizes, and the inclusion of additional variables onto which potential etiological determinants can map, like individual behaviours, environmental determinants, and vector competence studies in future assessment of the risk of bloodborne viruses transmitted by mosquitoes [100]. Future studies should be aimed at species identification for their associated role in flu pandemic epidemiology (or viruses transmitted via personal behaviours) and for samples for potential viral load (mosquito and human), and characterizing environmental determinants that may alter the dynamics for underlying viral transmission. For example, the IgM response which is highly crucial for the diagnostic assessment of flavivirus infections can be inconclusive, particularly in areas where vaccination is prevalent due to cross-reactivity among flaviviruses in areas where the same flavivirus invades designated areas frequently [101]. This has implications for false-positivity feedback, making it particularly challenging to efficiently assess causative agents of clinical syndromes in undifferentiated presentations [6]. It is an important endeavour to use these considerations to determine serological assessments of data to determine the overall influence of mosquito-borne viral transmission.

Conclusion

This research investigated a possible relationship between recent mosquito exposure and virological indicators for hepatitis B (HBsAg), hepatitis C (HCV Ab), and human immunodeficiency virus (HIV Ab) in a population living in an endemic region, utilizing a mixture of observational data and confirmatory laboratory testing with electro-chemiluminescence immunoassay. The research wanted to determine whether mosquito bites may have contributed or were just coincidentally related to the seropositivity of bloodborne viral infection.

The findings revealed minimal but detectable seropositivity for HBsAg and HCV Ab among participants in the mosquito-exposed (study) group, whereas no such markers were identified in the control group. Notably, HIV Ab was absent in all individuals across both cohorts. The exclusive occurrence of HBsAg and HCV Ab in the study group is epidemiologically intriguing and merits further scrutiny. However, Chi-square statistical analysis did not reveal significant differences ($p > 0.05$) between the two groups for any of the three virological markers. This underscores a crucial limitation in the power of this study to draw causal or associative conclusions regarding mosquito-related transmission of these pathogens.

These results align with existing literature, which largely classifies mosquito-mediated transmission of hepatitis B, C, and HIV as negligible or unproven, particularly in the absence of mechanical contamination, high viremia in hosts, or direct inoculation pathways. The biological plausibility of such transmission remains controversial and is yet to be mechanistically validated in controlled experimental settings. Nevertheless, the zero-positivity in the control group, although not statistically significant, adds a descriptive layer of concern regarding environmental or behavioral exposures correlated with mosquito activity in hyperendemic areas.

From a public health perspective, the findings support the need to maintain high vigilance in mosquito control efforts, particularly in regions where both vector-borne and bloodborne diseases overlap. While this study does not substantiate vectorial competence for viral hepatitis or HIV among mosquitoes, it identifies a potential window of inquiry for environmental risk amplification. The intersection of mosquito ecology, poor sanitation, population density, and underlying viral burden may create complex transmission dynamics not fully captured by traditional paradigms of virology and entomology.

Future studies should be incorporated.

- Larger and more diverse sample populations
- Longitudinal follow-up to distinguish between historical and new infections
- Viral load quantification to assess infectivity thresholds
- Molecular genotyping to correlate source attribution of detected pathogens
- Entomological sampling to determine the presence of viral genomes within vector tissues

In conclusion, this study does not provide evidence for statistically significant transmission of bloodborne viruses via mosquitoes, but it raises important hypotheses about ecological interactions and co-exposure risks in endemic settings. The results should be interpreted as preliminary and hypothesis-generating, warranting deeper investigations through interdisciplinary approaches integrating virology, vector biology, epidemiology, and environmental health sciences. Such comprehensive frameworks will be essential in understanding and mitigating complex infectious disease dynamics in increasingly interconnected global populations.

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