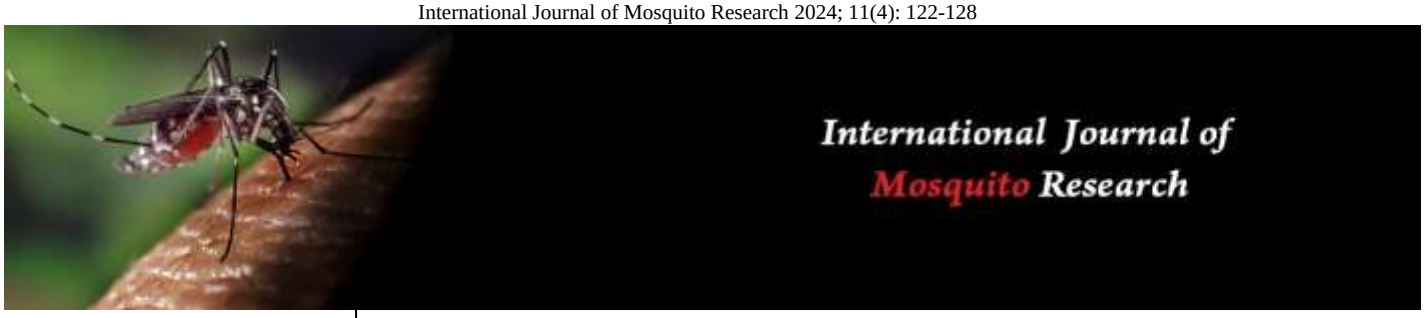

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2024; 11(4): 122-128

www.dipterajournal.com
Received: 08-06-2024
Accepted: 12-07-2024

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Quantifying mosquito populations for informed control of *Aedes aegypti* breeding in Sudanese homes in North Kordofan, Sudan

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DOI: https://doi.org/10.22271/23487941.2024.v11.i4b.795

Abstract
A cluster survey approach was conducted to identify indoor breeding sites of the primary vector, *Aedes aegypti* within El-Obeid City to contribute to a more comprehensive understanding of dengue fever epidemiology in North Kordofan and strengthen prevention and control strategies.
Methodology: Eighty clusters, each comprising ten households, were chosen via systematic random sampling. Households were examined identify indoor *Aedes aegypti* breeding sites within homes and to estimate entomological indices. The performance of the Integrated Vector Control (IVC) Program in North Kordofan State during the outbreak was conducted using a scoring system coined by IVC experts.
Results: The study identified *Aedes aegypti* mosquitoes as the culprit behind a dengue outbreak in El-Obeid City. These mosquitoes breed in man-made containers, especially uncovered clay water pots (zeers). High mosquito indices suggest a significant population. The survey identified several potential mosquito breeding risks that could have contributed to the outbreak's severity. Water shortages may have led residents to store more water around their homes, creating ideal breeding grounds. Additionally, stagnant water from leaks in the public water system situated close to houses likely provided further habitat for mosquito larvae. There is lack of public awareness regarding the daytime biting habits of the mosquito that transmits dengue fever; many interventions focus solely on night-time mosquito activity.
Conclusion: Aedes mosquito is responsible for a major dengue outbreak in North Kordofan (NK), Sudan, Targeting clay pots, the primary breeding ground for these mosquitoes, is likely the most effective control strategy. There is need to adapt dengue control measures to seasonal variations. Community involvement is key factor for successful prevention. Raising public awareness about the daytime threat posed by *Aedes aegypti* mosquitoes is crucial. Additionally, community participation in larviciding, clean-up initiatives, and improved waste management practices are essential for eliminating mosquito breeding sites. The effectiveness of the existing North Kordofan Integrated Vector Control Program requires further evaluation.

Keywords: *Aedes aegypti*, dengue fever, El-Obeid city, North Kordofan, Indoor breeding sites, cluster survey

Introduction
Dengue fever, a mosquito-borne viral illness transmitted by *Aedes aegypti* mosquitoes, poses a growing threat worldwide due to parallel increase in urbanization, water storage at household level, and travel that can introduce them to new areas. As cities expand, populations become denser, creating ideal breeding grounds for mosquitoes [1]. Dengue fever is endemic in Sudan, historically confined to the coastal and subcoastal regions, particularly Port Sudan city. However, recent heavy rains and flooding have led to outbreaks across the country, affecting populations in eastern, northern, southern, and central Sudan. Infection rates have ranged from 7% to 25% [2]. Recurring dengue fever outbreaks occurred in eastern and western regions bordering countries with established dengue transmission [3-8]. In 2019, a large outbreak struck El-Obeid City, North Kordofan, for the first time, following similar outbreaks in several

Sudanese states [5]. Entomological surveillance identified *Aedes aegypt*i, the primary urban vector of dengue virus, as the predominant mosquito species in the affected areas [9]. This mosquito thrives in artificial containers around human dwellings, such as discarded tires, flower pots, and water storage containers; hot and humid climates favor *Aedes aegypt*i breeding success [11]. North Kordofan experiences hot summers with some humidity, potentially creating suitable conditions for indoor breeding of this mosquito.

Entomological indices like House Index (HI), Container Index (CI), and Breteau Index (BI) serve as invaluable tools for scientists and public health officials. These indices offer a quantitative window into mosquito life cycles, population density, and potential outbreak risk. By analyzing these indices, researchers can gain crucial insights into mosquito ecology and inform targeted control measures. This data-driven approach allows for a more effective and efficient allocation of resources towards combating mosquito-borne diseases [10]. However, existing research on *Aedes aegypt*i in Sudan has focused primarily on outdoor breeding sites and adult mosquito populations, neglecting the potential for indoor breeding. This lack of specific data on *Aedes aegypt*i breeding ecology, particularly within homes in North Kordofan, hinders the development of effective control strategies for this critical vector.

This study utilizes entomological surveys within El-Obeid City homes during the 2019 dengue outbreak to identify *Aedes aegypt*i breeding sites, the primary dengue vector. The data will inform targeted vector control and guide resident education campaigns on *Aedes aegypt*i habits and preventative measures, empowering them to participate in mosquito population control and reduce future outbreak risk.

Material and Methods

Setting

El-Obeid City, the capital of North Kordofan State in Sudan and the epicenter of a recent dengue fever outbreak, serves as a vital transportation hub and regional commercial center for western Sudan. This semi-arid city, with a population exceeding 936,000, faces a chronic challenge: water scarcity that compels residents to store water in various containers,; mainly, clay pots (zeers), barrels, ground and roof water reservoirs. Containers that retained water for long periods of time make good or suitable breeding habitats for mosquitoes such as the different water holding artificial containers; thus, inadvertently creating ideal breeding grounds for Aedes mosquitoes [10]. The State Ministry of Health initiated limited vector control measures (source reduction and space spraying) two weeks after the first confirmed case. Public concern spurred community-led interventions.

Study Design and Sampling Strategy

As part of a larger investigation, this study employed a descriptive cross-sectional survey design to comprehensively assess the scale and spread of *Aedes eygpti* within El-Obeid City. A systematic random sampling technique was used to ensure a representative sample of households across the entire city. The city map was divided into a 27x27 grid system, resulting in 729 individual grid squares. Using random selection, 80 of these squares were chosen (Figure 1). Each selected square represented a 400-meter square area. This specific size and number of squares balanced capturing a representative sample with maintaining a manageable workload for the survey teams. The first house within each selected square was chosen randomly. To ensure ten households were included from each square, subsequent households were selected by identifying the nearest house to the previously chosen one. This approach ensured spatial spread within each sampled area. More details were reported elsewhere [12].

Survey Teams and Data Collection Strategies

Survey teams comprised three members: an entomologist, a surveyor, and (at least) one additional member to ensure gender balance. Each member had a specific role in data collection. All teams participated in a comprehensive role-playing exercise to ensure effective communication and data collection in the field. They were trained to respect residents' decisions regarding participation and to avoid arguments that could hinder data collection. For further details on team training, please refer to a separate publication. Each team received a comprehensive toolkit for efficient navigation and data collection. This toolkit included a pixel map pinpointing the specific grid square assigned to the team, a road map guiding navigation to the designated grid square location, and a Google map illustrating the distribution of houses within the assigned grid square.

Teams utilized a semi-structured questionnaire designed to capture a wide range of data relevant to dengue fever transmission and potential risk factors. The questionnaire covered sociodemographic characteristics (age, gender, education level, etc.), for suspected cases the date of symptom onset and disease outcome, housing conditions (water storage practices and waste disposal methods), water sources used by the household, travel history within the past two weeks, mosquito control efforts employed by the household, and healthcare seeking behavior in the event of illness.

In addition to questionnaires, trained teams performed visual inspections within each household. This included observing water storage containers and documenting practices, searching for potential mosquito breeding sites, and inquiring about mosquito bite prevention methods used by residents.

A phlebotomist, also part of the survey team, collected 5 ml blood samples from two individuals per household: one experiencing dengue-like symptoms and one asymptomatic individual. A total of 1,285 blood samples were collected (645 symptomatic, 640 asymptomatic). The fieldwork was conducted over three consecutive days (November 26-29, 2022). The survey team met with state health authorities to discuss and address challenges faced during outbreak management.

*Aedes aegypt*i Breeding Site Inspection:

Survey teams conducted thorough inspections within participating households to identify potential breeding sites for *Aedes aegypt*i mosquitoes. The focus was on artificial containers capable of holding stagnant water, such as water storage containers, buckets, bowls, discarded tires, flower pots and any other items that could accumulate water. Trained inspectors visually identified *Aedes aegypt*i eggs (Black, typically laid along the waterline of containers), larvae (Small, motile creatures residing in the water), and pupae (Comma-shaped with a breathing tube on their heads) based on their appearance. Flashlights aided inspection of dark and hidden areas. Dipped nets collected water samples for

examination under a microscope to confirm presence of larvae and pupae. Clay pots (zeers) used for storing drinking water were also examined due to their potential to attract egg-laying mosquitoes. Residents were interviewed about their water storage practices and any mosquito problems they experienced. Identified breeding sites and mosquito presence were documented, often mapped, to inform targeted vector control strategies.

Entomological Investigation

Four key **Entomological Indices** were used to monitor *Aedes aegypt*i breeding activity.

Container Index (CI): This widely used index expresses the percentage of inspected containers positive for immature stages (eggs, larvae, pupae) of *Aedes aegypt*i. It is calculated as: (Number of positive containers / Total number of containers inspected) x 100.

Breteau Index (BI): This index focuses specifically on the presence of *Aedes aegypt*i larvae and pupae. It represents the number of positive containers per 100 houses inspected and is calculated as: (Number of positive containers / Number of houses inspected) x 100.

House Index (HI): This index indicates the percentage of houses with at least one container positive for immature stages of *Aedes aegypt*i. It is calculated as: (Number of houses with positive containers / Total number of houses inspected) x 100.

Pupal Index (PI): Reflecting the potential for adult mosquito emergence, this index is calculated as the number of pupae collected per 100 houses inspected: (Number of pupae collected / Number of houses inspected) x 100.

An entomological investigation was conducted to assess the presence of *Aedes aegypt*i larvae and pupae in households and premises of dengue fever (DF) patients and their families. Houses were randomly selected from a complete list of confirmed DF cases. The World Health Organization (WHO) guidelines were used to interpret entomological indices and assess the risk of dengue virus transmission. High risk was indicated by: House Index (HI) > 35%: Percentage of houses with mosquito larvae or pupae; Breteau Index (BI) > 50: Number of containers per 100 houses positive for larvae or pupae; and Container Index (CI) > 20%: Percentage of containers positive for larvae or pupae. Low risk was indicated by: HI < 4%, BI < 5%, or CI < 3%.

Assessment of Integrated Vector Control (IVC) Response During the Dengue Outbreak

To assess the effectiveness of North Kordofan State's Integrated Vector Control (IVC) program during the dengue outbreak, researchers developed a comprehensive tool. This tool, consisting of 90 questions grouped under 14 Key Performance Indicators (KPIs), was based on World Health Organization (WHO) publications and peer-reviewed medical journals. It aimed to objectively assess progress and impact in response to the outbreak, focusing on ten key intervention pillars. The ultimate goal was to improve performance, quality, accountability, and reporting of IVC activities.

Using the Delphi Technique, researchers sought independent assessments from four IVC experts at the state and Federal Ministry of Health. A scoring system was developed, assigning weights to each KPI based on its relative importance. The total score for all indicators within a single pillar was 100. To ensure anonymity and confidentiality, the scoring process was meticulously designed. Scores were entered and analyzed in an Excel sheet, calculating the weighted mean for each observation. Based on this analysis, a scoring system was established: below 20%: major weakness, 21-40%: needs improvement, and above 40%: Strength

A cluster survey encompassing 791 houses across 80 clusters identified a significant number of dengue cases (950) from early August to late December 2022. This extended timeframe suggests ongoing transmission throughout the survey period.

Data Analysis

Data analysis primarily utilized two software programs:

Microsoft Excel (Version 10.0): Facilitated data entry, basic analysis, and generation of descriptive statistics (frequencies, means, standard deviations). Excel was also used to create epidemic curves visualizing the outbreak's progression.

Ethical Considerations and Collaboration:

This study adhered to ethical research principles and received approval from the Director General for Health Affairs in North Kordofan State and the University of Kordofan. The research benefitted from collaboration with various stakeholders involved in planning and implementing the survey phases. These stakeholders included staff from the Federal and State Ministries of Health, faculty members, and students from the University of Kordofan's Faculty of Medicine.

Results

Education levels varied between male and female household heads. Over half of the male heads had limited education, while females generally had relatively higher levels of education.

The survey revealed a potential risk factor for mosquito breeding: unreliable access to clean water. Only 63.8% of participating households received piped water from the municipality, and the supply was often irregular, pumped intermittently every 7-10 days. Residents relied on alternative sources like donkey-delivered water (14.6%), water trucks (10.8%), and borehole wells (28.1%) to meet their needs. Furthermore, a concerning 8.7% of surveyed houses experienced severe water shortages exceeding four days in the two weeks prior to the survey. The survey identified leaky pipes around 76 houses, resulting in small collections of stagnant water. Additionally, stagnant water was found near 10% of the houses.

The survey identified a total of 1,348 water containers across the visited households, with an average of 2.4 containers per household. Clay pots (Zeers), found in 7.94% of households, were the most common container type. These clay pots also had the highest infestation rate; 35.1% of examined zeers contained *Aedes aegypt*i mosquitoes, the primary dengue fever vector. Overall, a significant proportion (33.3%) of all containers examined were positive for *Aedes aegypt*i mosquitoes. The infestation rates were 26% for barrels, 46.8% for other pots, and a concerningly high 79.4% for street clay pots (Sabeel). Ground water tanks and roof water tanks were less prevalent and had lower infestation rates. Details of these

breeding sites, including household indices (HH), container indices (CI), and pupae indices (PI), are summarized in Table 1.

Entomologists in El-Obeid conducted a survey on October 3rd, 2023, to assess mosquito breeding in water containers. A total of 3,221 containers were examined, revealing a concerningly high infestation of 1,805 containers, the Container Index (CI) reached 56%, indicating that over half of the examined containers were breeding grounds for mosquitoes. The Pupal Index (PI) was reported as 14.5.

On exploring participants' experiences with mosquito bites, nearly a quarter (24.2%) reported bites primarily during mid-day, while another 20.7% experienced bites throughout the day. Day and night biting activity was reported by 13.6% of participants. The remaining participants (around 41.5%) provided unclear information about when they were bitten. Only 1.8% of surveyed households reported being visited by a vector control team before the survey.

North Kordofan Vector Control Program: Performance Evaluation

Table 2 assesses a vector control program in North Kordofan, Sudan, focusing on its effectiveness in preventing dengue fever. The table assigns scores (percentages) to various program areas, revealing both strengths and weaknesses. Overall, the program demonstrated a mixed performance, with some aspects requiring significant improvement. The program excels in information sharing (54.38%), ensuring effective communication about dengue fever and prevention strategies. Additionally, good coordination and collaboration (47.5%) exist between different stakeholders involved in vector control efforts. Several areas require improvement for the program to function more effectively. Strategic planning (31.88%) needs strengthening to establish a clear long-term vision for vector control. Investing in staff training and development (34.69%) is crucial to enhance staff capacity. More efficient program management practices (38.33%) are necessary to optimize program effectiveness. The program would also benefit from ongoing research and development (39.25%) to improve control strategies. Finally, clearer performance indicators (40.35%) are needed to track progress and measure program impact.

High staff turnover and lack of incentives (26.13%) can hinder program effectiveness and staff motivation. Limited budget and resources (13.31%) might restrict program activities. Additionally, improving data collection and reporting practices (49.25%) is crucial for informed decision-making.

Discussion

Water Insecurity and Dengue Risk in El-Obeid City

This study investigated the association between water insecurity and potential breeding sites for *Aedes aegypt*i, the primary vector of dengue fever, in El-Obeid City. Survey data revealed that households relied on diverse water sources (municipal supply, donkey carts, tankers, boreholes), suggesting potential water insecurity. Likewise, the irregularity of the municipal supply or high costs for buying water from alternative sources are likely to contributed to this insecurity. Water scarcity may led to household water storage practices, and inadvertently created breeding sites for *Aedes aegypt*i. Improving access to clean water through infrastructure repairs and development of alternative water sources is crucial. By addressing water insecurity, enhancing vector control efforts, promoting community participation through education, and closing knowledge gaps, El-Obeid City can significantly reduce its vulnerability to dengue fever outbreaks.

Entomological Indices

The entomological survey identified *Aedes aegypt*i as the primary vector, breeding predominantly in artificial containers within and around households. Uncovered clay pots (zeers) used for water storage were particularly attractive, providing suitable breeding sites both indoors and outdoors. These findings align with previous research highlighting clay pots and barrels as common breeding grounds for *Aedes aegypt*i [13, 14]

Analysis of entomological indices revealed a significant mosquito population in El-Obeid City. House Index (HI), Container Index (CI), and Breteau Index (BI) values all exceeded World Health Organization (WHO) thresholds **[15].** The BI demonstrated a strong correlation with dengue transmission. The Container Index (CI) provided a broader perspective on potential breeding sites beyond houses. It is important to acknowledge that the commonly used Stegomyia indices (HI, CI, and BI) focus on identifying potential breeding sites through the detection of immature mosquitoes in water containers. However, these indices do not directly translate into the level of vector control needed to prevent dengue transmission. A survey conducted in El-Obeid City, North Kordofan State, during September to November 2018 (about the same period of the 2022 outbreak), identified *Aedes aegypt*i as the sole mosquito species breeding in water containers. The survey revealed high infestation rates, as indicated by the House Index (HI), Container Index (CI), and Breteau Index (BI) values, suggesting a significant risk of dengue transmission **[16]**. Yet, there was no reported outbreak of dengue in El-Obeid in 2018.

Established research demonstrates a clear link between seasonal rainfall patterns, mosquito population levels, and dengue case incidence, although potential inconsistencies in inspection methods exist. This study mirrored this trend, with peak dengue cases coinciding with the rainy season (September) followed by a decline. The rainy season likely contributes to increased mosquito density by creating additional temporary breeding sites. As expected, *Aedes aegypt*i density exhibited seasonal fluctuations, peaking during the rainy season and dropping to zero in dry months (January-February). Seasonal variations were also observed in mosquito indices, with HI peaking in November and BI and CI peaking in August. Rainfall, high humidity, and ambient low temperatures in autumn were positively correlated with the density of females Ae. aegypti collected in the residences. [17]

Unique Challenges of Dengue Control

In Sudan, the training of vector-control workers is essentially geared to control malaria. Dengue fever control requires a distinct strategy compared to malaria control due to the different mosquito species involved. *Aedes aegypt*i, the primary dengue vector, thrives in small, man-made containers around homes, unlike Anopheles mosquitoes that breed in larger stagnant water bodies. This necessitates a shift from targeting large water sources to eliminating small, artificial containers. Public education campaigns, community clean-up

drives, and improved waste management practices are crucial to achieve this.

The study revealed a discrepancy between the known biting patterns of *Aedes aegypti* and reported mosquito bites, suggesting potential variations in local mosquito behavior or a lack of public awareness. This knowledge gap, coupled with the limited education of a significant portion of the male population, underscores the need for targeted public education campaigns emphasizing daytime biting habits.

Little education levels could hinder individual and community-level efforts to combat dengue fever. It is essential to address this knowledge gap through targeted public education campaigns that emphasize the daytime biting habits of *Aedes aegypti* mosquitoes. This renders traditional bed nets, designed for night-time malaria prevention, less effective against dengue. However, bed nets can still play a complementary role in hospitals by isolating patients and preventing nosocomial transmission. Understanding the preferred micro-environments of these mosquitoes within hospitals is essential; especially for patients admitted with dengue fever [18]. On the other hand, traditional methods like fumigation and bed nets are largely ineffective against *Aedes aegypti* due to their unique breeding habits and daytime biting activity.

Additionally, *Aedes aegypti* require multiple blood meals per egg-laying cycle, increasing their potential for disease transmission compared to Anopheles mosquitoes; controlling *Aedes aegypti* involves eliminating breeding sites, encouraging larviciding to significantly reduce mosquito populations before they reach adulthood. A sustainable solution hinges on empowering the community. Public education campaigns play a vital role in raising awareness about mosquito behavior and preventive measures [19]. Engaging communities fosters ownership and long-term program sustainability; and empowers individuals to participate in ongoing prevention efforts [20]. Strategies may need to be adapted to social and cultural norms for community acceptance. Development and implementation of appropriate supportive legislation for resource allocation and waste disposal regulations would be necessary.

Assessment of the North Kordofan Integrated Vector Control Program

The evaluation of the North Kordofan Integrated Vector Control (IVC) program revealed a mixed performance. While the program demonstrated strengths in information sharing, collaboration, outreach, and data management, it also exhibited critical weaknesses in areas such as budget, human resources, and strategic planning. Insufficient funding severely limits the program's capacity, hindering essential activities like insecticide spraying and habitat management. Additionally, high staff turnover and a lack of incentives negatively impact program continuity and effectiveness. The program's emphasis on data management is commendable, however, greater investment in data collection, research, and analysis is necessary to inform decision-making.

The program's outreach interventions, while demonstrating some success, can be further enhanced through improved communication and social mobilization strategies to address knowledge gaps within the community regarding mosquito behavior and preventive measures. To improve program effectiveness, substantial investments in budget, human resources, and data management are required. Strengthening outbreak monitoring, research, and development capabilities will also be crucial.

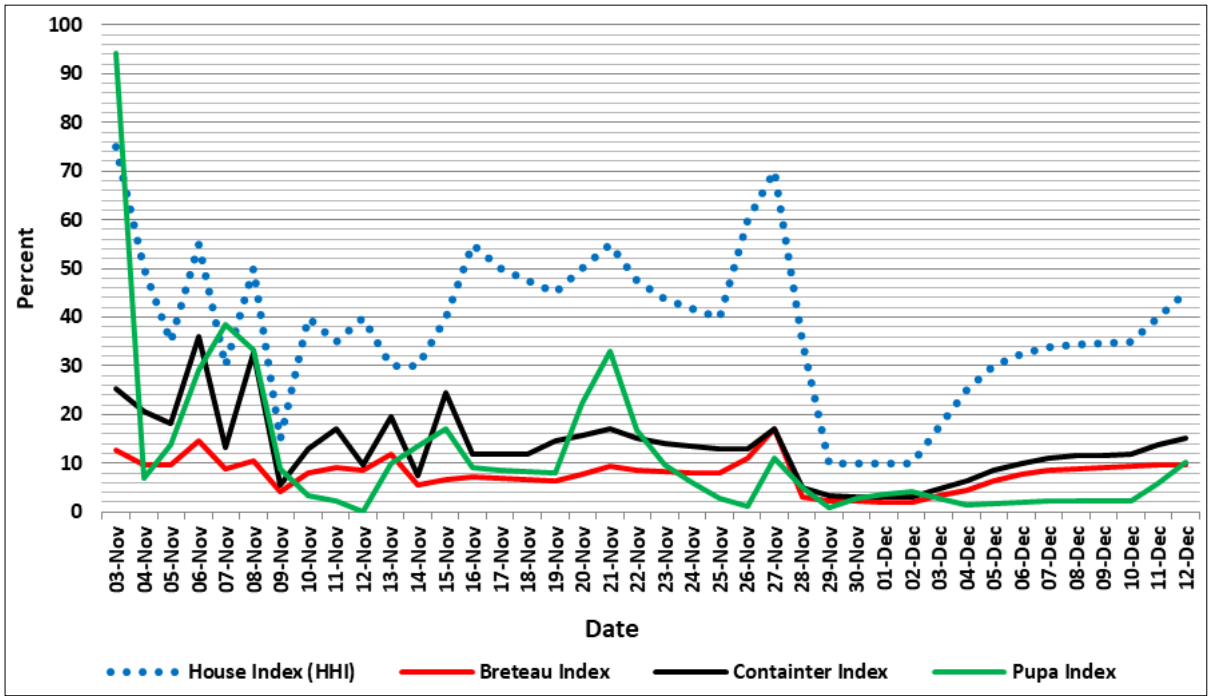

Fig 1: Using random selection, 80 of these squares were chosen

Table 1: Shows visited houses prior to and after the teams visits

Water Reservoir	Number of Houses visited	Percent	Number of containers per a household	Container examined	Number of containers Positive for *A. eygpti*	Percent
Clay pot (zeer)	107	7.94	7.7	821	288	35.1
Barrels	528	39.17	1.8	939	244	26
Ground water reservoir (Tanks)	385	28.56	0.5	189	43	22.8
Roof water reservoir (Upper water tabk)	64	4.75	1.0	64	No Data	No data-
Other pots (e.g. flower pots)	72	5.34	7.7	555	260	46.8
Street clay pots (Sabeel)	20	1.48	6.3	126	100	79.4
Other water holding containers	172	12.76	3.4	591	138	23.4
Total	1348	100.00	2.4	3221	1073	33.3

Table 2: Assessment of the North Kordofan Integrated Vector Control Program, November, 2022

Activity	Score (%)
Information Sharing	54.4
Data Management and Reporting	49.3
Outreach intervention Programs	48.3
Coordination and Collaboration:	47.5
Performance Indicators	40.4
Research and Development	39.3
Program Management	38.3
Staff Capacity and Training	34.7
Strategic Planning	31.9
HRM staff turnover, incentives)	26.1
Outbreak Monitoring	21.3
Communication and Social Mobilization	20.5
Data and Resources3	18.3
Budget and Resources	13.3

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