UMYU Scientifica, Vol. 3 NO. 3, September 2024, Pp 333 – 339
https://doi.org/10.56919/usci.2433.035
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Influence of Altitudinal Gradient on Human Malaria Prevalence in Plateau State, Central Nigeria: A Longitudinal Study

Abba Joseph Ogwuche[1,2], Mwansat Samuel Georgina[1], Adeiyongo Celine M[1], Akindgh Terver Mark[1], Samson, Daan[1], Nanvyat, Nannim[1], & Oguche, Stephen[3]

[1]Department of Zoology, Faculty of Natural Sciences, University of Jos, Jos Plateau State, Nigeria
[2]Infectious Disease Unit, Jos University Teaching Hospital, Jos, Nigeria
[3]Department of Paediatrics, Faculty of Medical Sciences, University of Jos, Nigeria

ARTICLE HISTORY

Received March 15, 2024
Accepted September 26, 2024
Published September 30, 2024

KEYWORDS

Altitudes, malaria transmission, parasite density, seasons

ABSTRACT

This longitudinal study assessed the prevalence of human malaria infection across different altitudinal zones in Plateau State, North-Central Nigeria, aiming to identify the impact of altitude on malaria transmission dynamics. Malaria is a potentially lethal disease caused by protozoan parasites called *Plasmodium*. Abiotic factors are known to be the main factors influencing the epidemiology of the disease. In 2018 and 2019, blood samples were taken and examined for the presence of *Plasmodium falciparum* malaria using the SD-PAN Ag mRDT. After that, blood film slides were created in order to calculate the parasite density for each of the four seasons' worth of positive samples. The prevalence rates of malaria in the three locations vary significantly, with the highest rates observed at lower altitudes (Pangwasa 211m) above sea level and the lowest at the highest altitude (Vwang 1330m) above sea level. Vwang has the lowest malaria prevalence of 16.25%, Jing (663m) has a higher prevalence of 40.0%, and Pangwasa has the highest prevalence at 46.9.

INTRODUCTION

The African Plateaus are known to be hypo-endemic for malaria, primarily due to abiotic factors like low temperatures and relative humidity, which are unfavorable for anopheline mosquitoes' growth and reproductive success (Clements, 2000; Matsushita et al., 2019). A commonly held misconception is that the main abiotic factor limiting *Plasmodium falciparum* transmission in the African highlands is low ambient temperature. Slight temperature fluctuations are thought to potentially create temporarily favorable conditions for unstable transmission within populations with low functional immunity (Wanjala & Kweka, 2016; Matsushita et al., 2019). The expanding threat is indicated by the increased frequency of malaria outbreaks in Kenya's highlands (above 1,500 m asl), where the disease was formerly uncommon (Shanks et al., 2000).

Current global data indicates that malaria remains a significant public health issue, with 249 million cases reported in 2022, a slight increase from 244 million in 2021. The disease is most prevalent in tropical regions, with sub-Saharan Africa bearing the brunt, accounting for 94% of all malaria cases and 95% of malaria-related deaths. Children under five are particularly vulnerable, comprising 78% of all malaria deaths in the region. Despite some progress, such as a 27% reduction in malaria case incidence globally between 2000 and 2015, the burden remains disproportionately high in Africa (WHO, 2022), with Plateau State being one of the most affected regions (MIS, 2021). The State's unique geography, with varying altitudes, may influence malaria transmission dynamics. Altitudinal gradients have been shown to impact mosquito distribution, behavior, and malaria transmission in other regions (Oyewole et al., 2010). However, the relationship between altitudinal gradient and malaria prevalence in Plateau State is not well understood. Plateau State is located in the central region of Nigeria, with a diverse geography that includes highlands, lowlands, and plateau areas. The State has a high malaria burden, with a prevalence rate of 48.1%, and 21.4% of children between the ages of 6 and 59 months had malaria (MIS, 2021). Since there is currently a dearth of knowledge regarding vector bionomics and malaria transmission in the highlands of Jos Plateau State, Nigeria, malaria control initiatives aimed at these regions are likely to be ineffective. Current control measures, such as insecticide-treated bed nets and indoor residual spraying,

Correspondence: Abba, Joseph Ogwuche. Department of Zoology, Faculty of Natural Sciences, University of Jos, Jos Plateau State, Nigeria. ✉ josephabba89@gmail.com
How to cite: Abba, J. O., Mwansat, S. G., Adeiyongo, C. M., Akindgh, T. M., Samson, D., Nanvyat, N., & Oguche, S. (2024). Influence of Altitudinal Gradient on Human Malaria Prevalence in Plateau State, Central Nigeria: A Longitudinal Study. *UMYU Scientifica*, 3(3), 333 – 339. https://doi.org/10.56919/usci.2433.035

have shown limited success in reducing malaria transmission (Chaccour et al., 2019). While studies have investigated the impact of altitudinal gradient on malaria transmission in other regions (Martens, Jetten & Rotmans, 1995; Bodker et al., 2003; Castro, 2017), there is a limited understanding of its influence in Plateau State. In order to provide light on the epidemiology of malaria in the region and guide control measures, a longitudinal study was done to examine the relationship between altitudinal gradient and malaria prevalence over time during four distinct seasons where blood samples were collected in four distinct seasons defined as early wet (April-June), late wet (July-September), early dry (October-December), and late dry (January-March). Consistent methodologies were employed across all seasons to ensure comparability.

MATERIALS AND METHODS

Study area

The study was carried out in Vwang of Jos South LGA (1330m, S-N 9⁰43, 44.76), Jing in Pankshin LGA (211m, S-N 9⁰13, 009⁰18), and Pangwasa in Shendam LGA, and (633m, S-N 9⁰43'44.76) respectively all in Plateau State, Central Nigeria. The communities have a unique and beautiful climate that is conducive to the production of cotton, groundnuts, rice, Irish potatoes, Maize, and soya beans. December and January experience temperatures of below 15^{o} C. During February and March, temperatures rise again to about 30^{o}C in the hottest months of the year. Temperatures fall during the rainy season to about 20^{o}C. Vwang, Jing, and Pangwasa of Plateau State have a tropical climate consisting of wet and dry seasons driven by the movement of the two dominant winds—the rain-bearing southwesterly winds and the cold, dry, and dusty Northeasterly winds, usually referred to as the Harmattan. The dry season occurs from November to March, with a spell of coolness accompanied by the dry, dusty Harmattan wind, felt mostly in the North in December and January. The wet season occurs from April to October. The majority, if not all, of the inhabitants are farmers and animal breeders, resulting in the year-round movement of cows, sheep, and goats in the villages. Animal sheds are often situated within the courtyard, and at night, small ruminants are usually kept inside the dwellings. The houses are basically of three types (mud with thatch roof, mud with corrugated iron roof, and cement block with corrugated iron roof).

Ethical Consideration and Approval

The Jos University Teaching Hospital institutional ethics (JUTH) committee with reference number JUTH/DCS/ADM/127/XXVIII/1280 and Plateau State Health Ministry of Nigeria Ethics Committee (PHMEC) with reference number MOH/MIS/202/VOL.T/X approved the research protocol and provided ethical consideration and approval for the implementation of the study. Similarly, the house owners gave their informed consent to partake in the study after due explanation of the objectives and collection methods through individual discussions and group meetings that took place. All participants' data were anonymized to maintain confidentiality, and only aggregated data were used in the analysis.

Sample collection and Preparation for *Plasmodium falciparum* Detection

Blood samples were collected in four distinct seasons defined as early wet (April-June), late wet (July-September), early dry (October-December), and late dry (January-March). Consistent methodologies were employed across all seasons to ensure comparability. 1ml of intravenous blood was collected into sterile ethylene diamine tetraacetic acid (EDTA) bottles in accordance with standard clinical and laboratory procedures (Lewis *et al.,* 2016, WHO, 2020). The SD-PAN Ag mRDT was used to screen the samples for the presence of the malaria parasite *P. falciparum*. Microscopy was performed on thick (6µl) and thin (2µl) blood film slides using 10% Giemsa stain. Parasite density was evaluated for all positive samples at the APIN Public Health Initiative-supported HIV clinic (Malaria unit), Jos University Teaching Hospital (JUTH), Jos, Nigeria.

Statistical Analysis

Data entry was conducted using Excel 16, and statistical analyses were performed using SPSS v22 (IBM Chicago). The chi-square test was employed to assess associations, with a significance level set at $p < 0.05$.

RESULT

Pangwasa, at 211 meters above sea level, had a malaria prevalence of 47.7%; Jing, at 663 meters above sea level, had a prevalence of 40.5%, and Vwang, at 1330 meters above sea level, had an 18.8% prevalence, according to the human blood malaria microscopic parasitological results across the study sites. Seasons at the three sites revealed that the late wet (July-Sept) had the greatest percentage of malaria cases (65, or 33.6%), followed by the early wet (April-June), with 51, or 26.4%, cases (Figure. 1). Additionally, there was a significant difference between the lowest altitude (211 m asl) and the highest altitude (1330 m asl) at the three sites along the altitudinal gradient for the mean human blood malaria microscopy results throughout seasons (Chi-Square, p=0.027). Multivariate logistic regression analysis assesses the impact of altitude and season on malaria prevalence; the intercept (43.133) represents the expected malaria prevalence at 0 masl altitude and Late Wet season, coefficient for altitude (-

0.023) indicates that for every 1 masl increase in altitude, malaria prevalence decreases by 0.023%. The coefficient for the season (-7.253) indicates that the Early Wet season has a 7.253% lower malaria prevalence compared to the Late Wet season. R-squared: 0.854 (the model explains 85.4% of the variation in malaria prevalence). F-statistic: 24.31 (p-value < 0.001). The result suggests that Altitude has a significant negative impact on malaria prevalence (p-value = 0.003), while the season has a significant impact on malaria prevalence (p-value = 0.014), with the Early Wet season having lower prevalence.

Male blood parasite density varied significantly throughout the transmission season in late dry (ldm) seasons (P<0.05), whereas female blood parasite density did not significantly differ among the three locations in late dry (ldf) seasons (P>0.05) (Figure 2).

Age group differences in the mean malaria parasite density across the three altitudinal gradients were statistically significant (P<0.05). The highest mean parasite density was seen in adults between the ages of 51 and 60 and children between the ages of 0 and 10 (Figure 3).

Between the three altitudinal research locations, there was a significant difference in mean malaria parasite densities, P<0.05 (Figure 4).

Table 1: Number of individuals involved in the determination of malaria parasites in Vwang, Jing, and Pangwasa, respectively.

	Vwang	Jing	Pangwasa	Total/Year 2018 to 2019
No. Houses sampled	40	40	40	120
Female	117(31.4)	133(35.8)	122(32.8)	372(100.0)
Male	63(37.5)	47(27.9)	58(34.5)	168(100.0)
No. of people tested	180(100.0)	180(100.0)	180(100.0)	540(100.0)

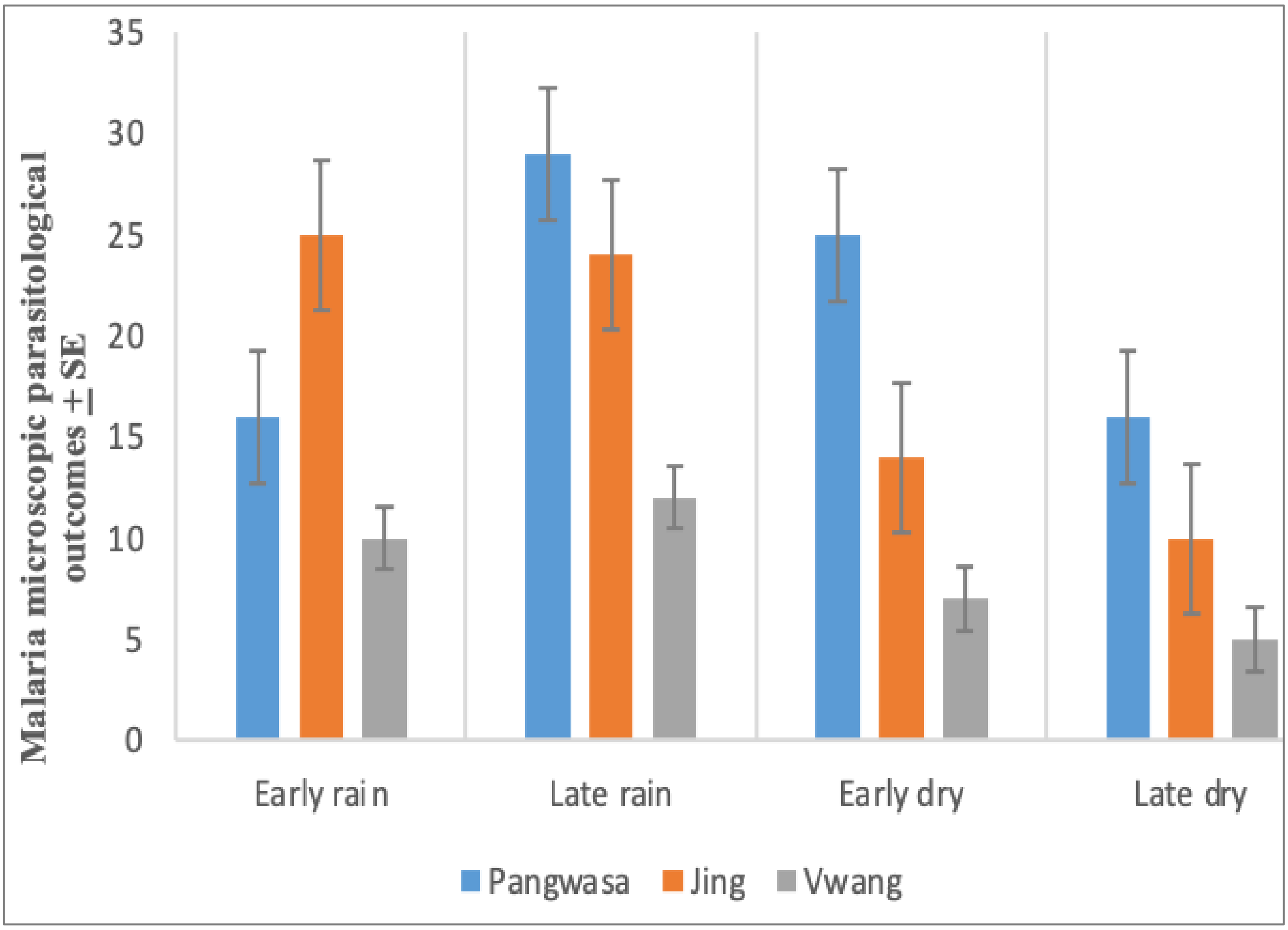

Figure 1: Human blood malaria microscopic parasitological outcomes across the transmission seasons in the three different altitudes (Pangwasa 211m asl, Jing663m asl, Vwang1330m asl).

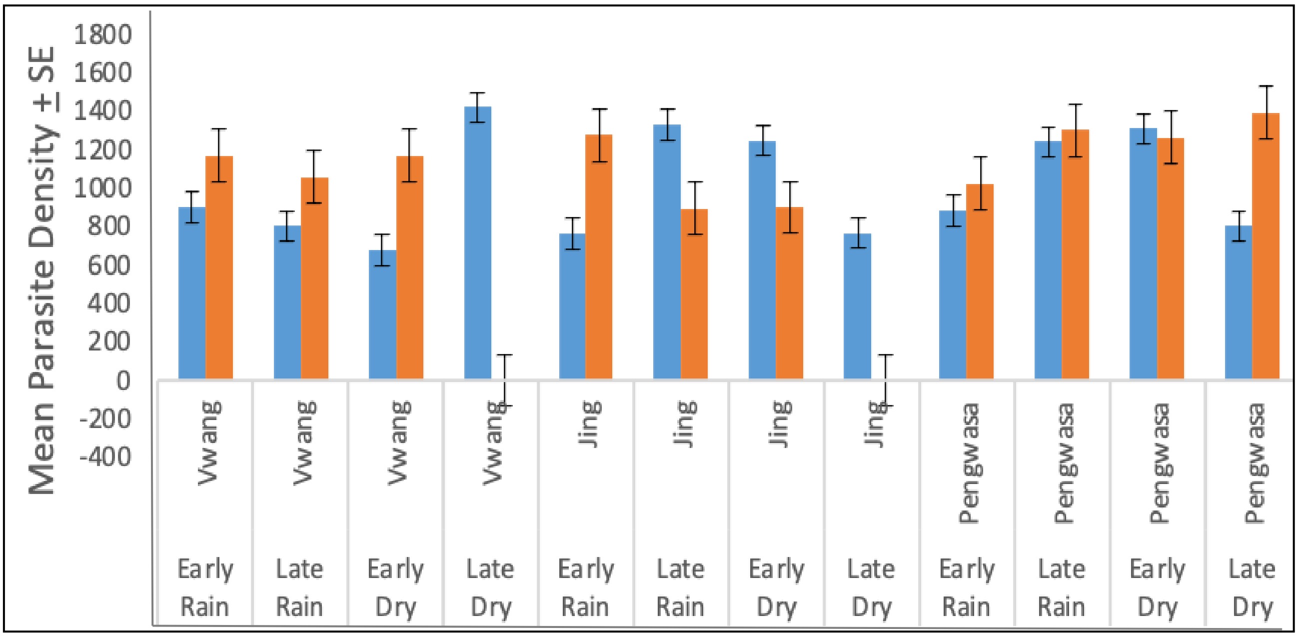

Figure 2 Mean parasite density in the human blood of inhabitants by gender across transmission seasons. Key: (Blue=Males, Red=Females).

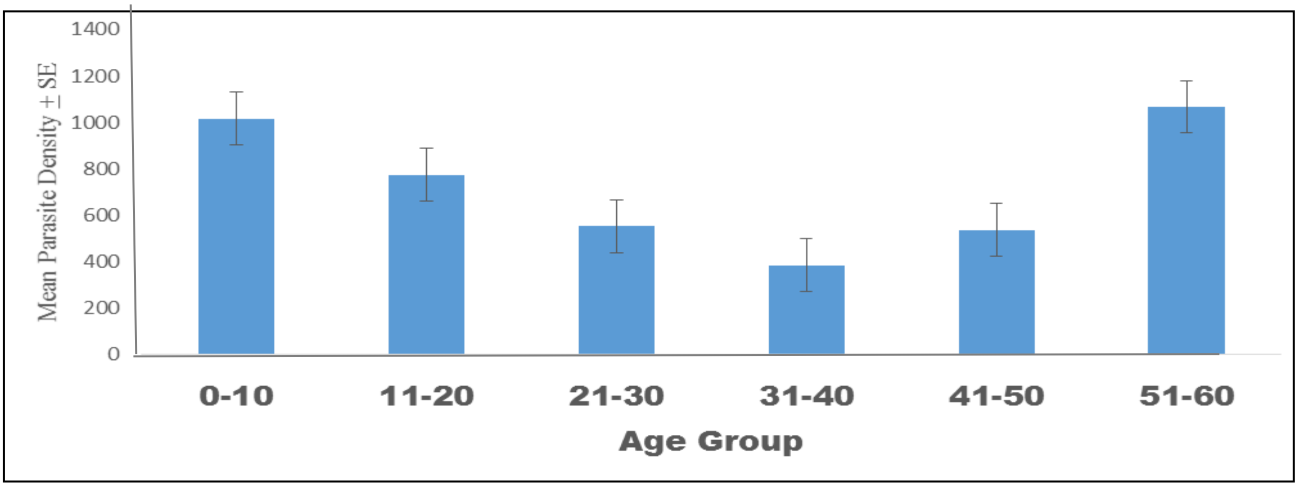

Figure 3: Mean Parasite Density across the sites by age group (P<0.05)

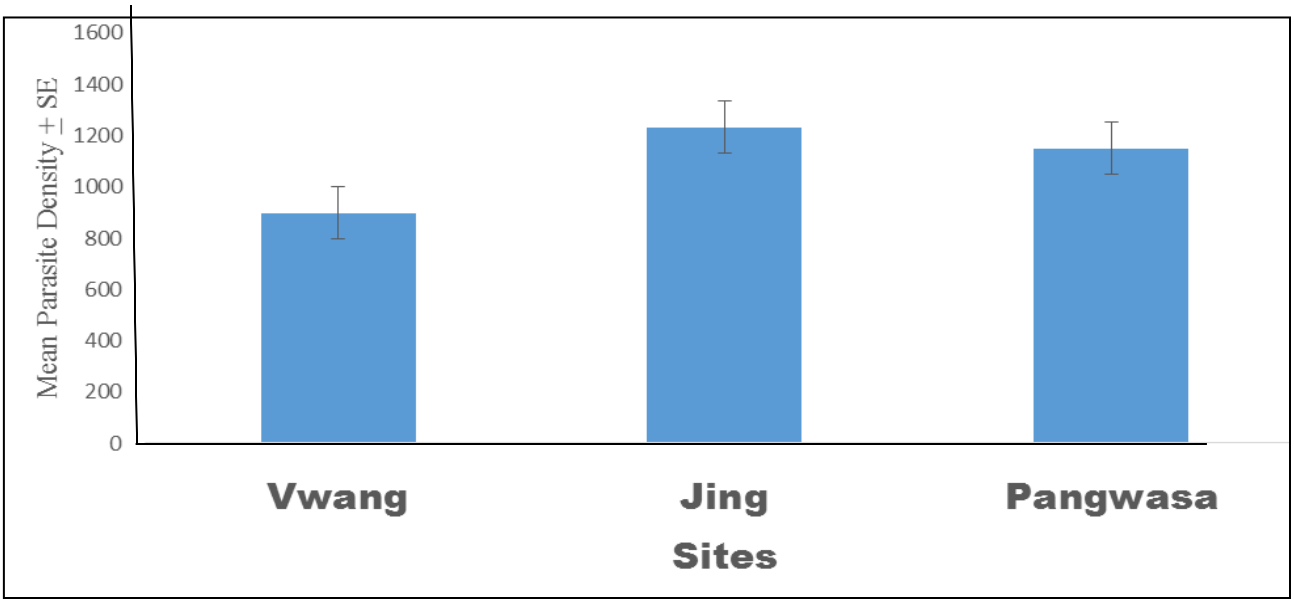

Figure 4: Mean Parasite Density across the sites (P<0.05)

DISCUSSION

The lower prevalence of malaria at higher altitudes, (Vwang (18.8% at 1330 meters), aligns with the well-documented inverse relationship between altitude and malaria transmission likely due to lower temperatures reducing mosquito activity (Tonnang et al., 2010; Gething et al., 2011), also studies conducted in 2016 by Wanjala & Kweka, 2016, which show that abiotic factors stop *Plasmodium falciparum* from spreading over Africa's highlands. Himeidan & Kweka, 2012 also highlight the effect of environmental factors like altitude and seasonal changes on malaria incidence in the East African highlands, demonstrating that higher altitudes have lower malaria prevalence due to cooler temperatures.

The African highlands are also known to be hypoendemic for malaria due to their low temperatures and high relative humidity (Clement, 2000). Most malaria epidemics in the African highlands are thought to be caused by *Plasmodium falciparum*, the most prevalent and lethal malaria parasite on the continent (Wirtz et al., 1987). There are clear seasonal differences in the prevalence of malaria; over the altitudinal gradient, the early wet, late wet, and early dry seasons all show considerable prevalence rates of malaria. Multivariate logistic regression analysis assesses the impact of altitude and season on malaria prevalence; the intercept (43.133) represents the expected malaria prevalence at 0 masl altitude and Late Wet season, coefficient for altitude (-0.023) indicates that for every 1 masl increase in altitude, malaria prevalence decreases by 0.023%. The coefficient for the season (-7.253) indicates that the Early Wet season has a 7.253% lower malaria prevalence compared to the Late Wet season. R-squared: 0.854 (the model explains 85.4% of the variation in malaria prevalence). F-statistic: 24.31 (p-value < 0.001). The result suggests that Altitude has a significant negative impact on malaria prevalence (p-value = 0.003) while the season has a significant impact on malaria prevalence (p-value = 0.014), with the Early Wet season having lower prevalence. The findings in this study agree with Caminade et al., 2014 which discusses the influence of various climatic factors, including altitude and seasonality, on malaria transmission and prevalence. It provides evidence that temperature changes associated with altitude have a significant impact on the distribution and transmission rates of malaria.

The availability of nesting places and greater contact between human-carrying vectors may be the reason for this. Both the recently published 2018 (WHO, 2018) and the Nigeria Demographic Health Survey 2019 agree that there was a significant variation in the mean parasite density across the sites by age groups, with the younger and older having substantially greater parasite densities. All genders may be similarly exposed to and at risk from the disease because the mean parasite density by gender did not exhibit statistical significance across transmission seasons. In addition to malaria, abiotic factors (temperature and relative humidity) have a significant influence on the number of malaria vectors at each of the three study sites. Temperature has a significant but nonlinear impact on mosquito population size; in each of the three study sites, values at 20°C and 27°C were noted, albeit with two peaks at these temperatures. Anopheles mosquito populations are highly dependent on temperature; these insects respond to variations in temperature (Beck-Johnson et al., 2017). The incidence of human malaria infection in the Plateau State in Central Nigeria along an inclined gradient. Because Anopheline mosquitoes are sensitive to temperature throughout their life cycle, low temperatures hinder them from maturing or reproducing (Paaijmansn et al., 2009).

Furthermore, between 16°C and 19°C, *Plasmodium falciparum* cannot develop (Nanvyat et al., 2018). These results imply that abiotic variables are important in the spread of malaria in Plateau State. Because individuals living in the house are able to stabilize the relative humidity to 51-60%, which favors mosquito survival, the number of mosquitoes trapped also increased as the relative humidity increased, peaking at 51-60. Following this, the mean number of mosquitoes trapped decreased (Hay et al., 2010). The number of mosquitoes captured rose as the relative humidity increased, peaking at between 51 and 60 percent, since humans can stabilize the relative humidity in their houses to between 51 and 60 percent, which is excellent for mosquito survival. Outside, relative humidity can vary greatly (Hay et al., 2010). In the African highlands, devastating epidemics can break out when populations of certain Anopheles species exceed critical thresholds (Abeku et al., 2004). According to Boedker, et al. (2003), these species were also the most prevalent anopheline in Ethiopia's highlands. There are serious health repercussions because these *Anopheles* species have been connected to malaria and lymphatic filariasis in Nigeria (Aigbodion & Uyi, 2013). This is particularly true considering that Sub-Saharan Africa has a high malaria incidence rate, and Nigeria is one of the five countries that account for over half of all malaria cases worldwide (WHO, 2020). The high species abundance found at low and intermediate altitudes has important epidemiological implications (Pangwasa and Jing). Pangwasa and Jing are surrounded by large rivers that supply water and more favorable environmental conditions for both adult development and the parasite's stage of development inside the vector. There may be more malaria-risk populations in Pangwasa and Jing due to the high concentration of malaria vector species found there.

CONCLUSION

The study demonstrates that altitudinal gradients significantly influence malaria prevalence, with lower altitudes experiencing higher transmission rates. These findings provide critical insights for targeted malaria control strategies, particularly in lowland regions. Future research should explore the role of microclimatic variations in shaping malaria transmission dynamics.

LIMITATIONS OF THE STUDY

The following are the limitations of the study.

Multivariate Regression Analysis to assess the impact of multiple factors (e.g., altitude, season, demographic variables) on malaria prevalence was not adequately done.

Also, Logistic Regression to predict the probability of malaria infection based on altitude and other covariates as well as Analysis of Variance (ANOVA) to compare mean parasite densities across different altitudes could be done in the future.

ACKNOWLEDGMENT

We want to express our gratitude to the Infectious Disease Unit at Jos University Teaching Hospital for lending us their lab space. Vilji Shefe, Mathias Bewuji, Iliya Balak, and the research team for their technical assistance during the fieldwork. We also thank Miskom Stephen Longla the Dachin Shendam, Miskom Panshir Shefe of Jing, Gwom Rwey Vwang, and Da Gyang Gutt Balak of the research sites for their invaluable assistance.

DISCLOSURES

There are no disclosed conflicts of interest for the writers.

REFERENCES

Abeku, T.A., Hay, S.I., Ochola, S., Langi, P., Beard, B., De Vlas, S., Cox, J. (2004). Malaria epidemic early warning and detection in African highlands. *Trends of Parasitology*; 20:400-405. **[Crossref]**

Aigbodion, F.I., & Uyi, O.O. (2013). Temporal distribution of and habitat diversification by some mosquitoes (Diptera: Culicidae) species in Benin City, Nigeria. *Journal of Entomology,* 10:13-23. **[Crossref]**

Beck-Johnson, L.M., Nelson, W.A., Paaijmans, K.P., Read, A.F., Thomas, M.B., Bjørnstad, O.N. (2017). The importance of temperature fluctuations in understanding mosquito population dynamics and malaria risk. *Royal Society Open Science;* 4(3):160969. **[Crossref]**

Bødker, R, Akida, J, Shayo, D, Kisinza, W, Msangeni, H.A., Pedersen, E.M., Lindsay, S.W. (2003). Relationship between altitude and intensity of malaria transmission in the Usambara Mountains, Tanzania. *Journal of Medical Entomology* 40(5), 706-17. **[Crossref]**

Caminade, C., Kovats, S., Rocklov, J., Tompkins, A. M., Morse, A. P., Colón-González, F. J., & Lloyd, S. J. (2014). "Impact of climate change on global malaria distribution." *Proceedings of the National Academy of Sciences*, 111(9), 3286-3291. **[Crossref]**

Castro, M.C. (2017). Malaria transmission and prospects for malaria eradication: The role of the environment. In D.F. Wirth & P.L. Alonso (Ed) Malaria: Biology in the era of eradication. Cold Spring Harbor Laboratory Press. (pp. 183-191). **[Crossref]**

Chaccour C, Zulliger R, Wagman J, Casellas A, Saifodine A, Candrinho B, et al. (2019). Combining long-lasting insecticidal nets (LLINs) with third generation-indoor residual spraying (IRS) provides significant added protection compared to LLINs alone in children under five years of age in a high-transmission area of mozambique. *American Journal of Tropical Medicine and Hygiene* ;101(5):534.

Clements, A.N. (2000). *The biology of Mosquitoes: development, nutrition and reproduction Volume 1*. 2nd edition. CABI Publishing, London School Hygiene Tropical Medicine.

Gething, P. W., Van Boeckel, T. P., Smith, D. L., Guerra, C. A., Patil, A. P., Snow, R. W., & Hay, S. I. (2011). "Modelling the global constraints of temperature on transmission of Plasmodium falciparum and P. vivax." *Parasites & Vectors*, 4, 92. **[Crossref]**

Hay, S.I., Sinka, M.E., Okara, R.M., Kabaria, C.W., Mbithi, P.M., Tago, C.C., Benz, D., Gething, P.W., Howes, R.E., & Patil, A.P. (2010). Development global maps of the dominant *Anopheles* vectors of human malaria. *PLOS Medicine* 7:e1000204. **[Crossref]**

Himeidan, Y. E., & Kweka, E. J. (2012). "Malaria in East African highlands during the past 30 years: Impact of environmental changes." *Frontiers in Physiology*, 3, 315. **[Crossref]**

Jetten, T.H., Martens, W.J.M., Takken, W. (1996). Model simulation to estimate malaria risk under climate change. *Journal of Medical Entomology;* 33:361-371. **[Crossref]**

Lewis, S. M., Bain, B. J., & Bates, I. (2016). "Dacie and Lewis Practical Haematology." 12th Edition.

Lindsay, S. W. & Martens, W. J. (1998). Malaria in the African highlands: past, present and future. *Bulletin World Health Organization*, 1998; 76(1): 33–45.

Martens, W.J.M., Jetten T.H, Rotmans J. (1995). Climate change and vector-borne diseases: A global modelling perspective. *Science direct Journals.*5, (3), 195-209. **[Crossref]**

Matsushita, N., Kim, Y., Sheng Ng, C.F., Moriyama, M., Igarashi, T., Yamamoto, K., Otieno, W., Minakawa, N., & Hashizume, M. (2019). Differences of rainfall- malaria associations in lowland and highland in western Kenya. *International Journal of Environmental Research and Public Health*, 16, 3693; **[Crossref]**

Nanvyat, N., Mulambalah, C.S., Barshep, Y., Ajiji, J.A., Dakul, D.A., Tsingalia, H.M. (2018). Malaria transmission trends and its lagged association with climatic factors in the highlands of Plateau State, Nigeria. *Tropical Parasitology*; 8:18-23. **[Crossref]**

National Malaria Elimination Programme (NMEP) Nigeria, National Population Commission (NCP) Nigeria, and ICF. 2022. Nigeria Malaria Indicator Survey 2021 Final Report Abuja, Nigeria, and Rockville, Maryland, USA: NMEP, NPC, and ICF

National Population Commission (NPC) [Nigeria] and ICF. (2019). *Nigeria Demographic and Health Survey* 2018. Abuja, Nigeria, and Rockville, Maryland, USA: NPC and ICF.

Oduola, A.O., Olojede, J.B., Oyewole, I.O., Otubanjo, O.A., Awolola, T.S. (2013). Abundance and diversity of Anopheles species (Diptera: Culicidae) associated with malaria transmission in human dwellings in rural and urban communities in Oyo State, Southwestern Nigeria. *Parasitology Research*.;112(10):3433–3439. **[Crossref]**

Oyewole, I.O., Ibidapo, C.A., Okwa, O.O., Oduola, A.O., Adeoye, G.O., Okoh, H.L., & Awolola, T.S., (2010). Species composition and role of Anopheles Mosquitoes in malaria Transmission Along Badagry Axis of Lagos Lagoon, Lagos, Nigeria International Journal of insect science (2): 51-57. **[Crossref]**

Paaijmans, K.P., Read, A.F., Thomas, M.B. (2009). Understanding the link between malaria risk and climate. *Proceeding of the National Academic of Sci*ence U S A.106(33):13844–13849. **[Crossref]**

Shanks, G.D., Biomndo, K., Hay, S.I., Snow, R.W. (2000). Changing patterns of clinical malaria since 1965 among a tea estate population located in the Kenyan highlands. *Society of Tropical Medicine and Hygiene*; 94(3):253–5. **[Crossref]**

Tonnang, H. E., Kangalawe, R. Y., & Yanda, P. Z. (2010). "Predicting and mapping malaria under climate change scenarios: The potential redistribution of malaria vectors in Africa." *Malaria Journal*, 9, 111. **[Crossref]**

Wanjala, C.L., & Kweka, E.J. (2016). Impact of Highland Topography Changes on Exposure to Malaria Vectors and Immunity in Western Kenya. *Frontiers in Public Health* 4:227. **[Crossref]**

Wirtz, R.A., Zavala, F., Charoenvit, Y., Campbell, G.H., Burkot, T.R., Schneider, I., Esser, K., Beaudoin, R.L., & Andre, R.G. (1987). Comparative testing of Plasmodium falciparum sporozoite monoclonal antibodies for ELISA development. Bulletin World Health Organization, 65, 39-45. Change (Bull. WHO, 1998) to Wirtz, et al., 1987

World Health Organization (2018). World Malaria Report. www.who.int

World Health Organization (2019). World Malaria Report. www.who.int

World Health Organization (2020). World Malaria Report. www.who.int

World Health Organization (WHO). "WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy." Geneva: World Health Organization, 2010.

World Health Organization. (2022). World malaria report. Geneva: https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022

World Health Organization: Basic malaria microscopy, part 1:2010, pp iii+80.