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Comparative Analysis of the Epidemiological Dynamics of Malaria in the Health Districts of Koulikoro and Timbuktu in Mali 2018-2022

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SUMMARY AND KEYWORDS

In Mali, malaria remains a major public health challenge with heterogeneous transmission patterns. This study compared malaria morbidity in two distinct geographical contexts: Koulikoro (Sudanian zone) and Timbuktu (irrigated Sahelian zone). It was a descriptive, analytical study based on data from the DHIS2 (2018–2022). The variables analyzed included prevalence, seasonality, gender, age, and methods of biological confirmation (RDT, thick blood smear). Comparative statistical analysis used chi-square tests and logistic regressions to identify factors associated (age, sex, seasonality) with the observed variations. In 2021, Koulikoro recorded 58,999 confirmed cases of malaria. The region followed a strict rainfall pattern with a peak in the third quarter (T3). While Timbuktu experienced off-season peaks linked to irrigation (peaking in Q1 2021 with 13,883 cases), children under 5 were the most affected in Koulikoro (56.5% in 2022), whereas adults (≥ 5 years) bore 80% of the burden in Timbuktu. Thick-droplet irrigation use increased from 18.2% to 59.2% in Koulikoro, while Timbuktu remained 97% dependent on rapid diagnostic tests (RDTs), a statistically significant difference ($p<0.001$). In Koulikoro, the prevalence of malaria increased significantly, rising from 4.99% in 2018 to a peak of 19.21% in 2021. In Timbuktu, the prevalence more than doubled between 2018 (8.04%) and 2020 (18.225%), before gradually declining to 14.87% in 2022. The study demonstrated the need to decentralize control strategies. Strengthening microscopy in the North and adjusting the schedule of the Integrated Malaria Control Program (IMCP) in irrigated areas are essential for sustainably reducing the burden of malaria in Mali.

Keywords : Malaria, Epidemiology, DHIS2, Koulikoro, Timbuktu, Mali.

INTRODUCTION

Although the pathophysiology of malaria (a parasitic infection caused by protozoa of the genus Plasmodium and transmitted by the mosquito vector of the genus Anopheles) has been scientifically understood for over a century, this disease remains a major global public health emergency. Despite technological and therapeutic advances, malaria retains its devastating power, hindering the socio-economic development of many regions (1) . Sub-Saharan Africa remains the epicenter of this endemic disease, disproportionately bearing approximately 95% of cases and 96% of deaths worldwide. By 2025, the epidemiological situation had become considerably more complex. This dynamic is exacerbated by the convergence of several critical factors: the increasing resistance to artemisinin-based combination therapies (ACTs) in East and Central Africa, the expansion of invasive vectors such as Anopheles stephensi, and climate change that is altering transmission zones (2–4) . Indeed, the region now records approximately 263 million cases annually. This resurgence is explained by a critical convergence of factors: climate change altering transmission zones, the emergence of resistance to insecticides and treatments, and chronic underfunding of control programs (5,6) .

Analysis of WHO data for 2024 reveals Mali's particular vulnerability to malaria (1) . With 8.15 million cases and over 14,000 deaths, the country contributes significantly to global morbidity. Among the eight nations that experienced a major resurgence (+1.4 million cases over four years), Mali exemplifies the health crisis affecting sub-Saharan Africa, the region where 95% of mortality related to the parasite occurs (1,7) .

Despite the implementation of key strategies, including seasonal malaria chemoprevention (SMC) and the mass distribution of long-lasting insecticidal nets (LLINs) (8,9) , the path to elimination remains fraught with challenges (8,9) . The persistence of pockets of high transmission and the emergence of resistance to insecticides and antimalarial drugs threaten the progress made over the past decade. Its prevalence varies considerably across areas, depending on epidemiological, ecological, and socioeconomic factors. Malaria stratification in Mali has classified areas based on incidence adjusted for population density. Transmission zones range from hyperendemic (south, prevalence > 50%) to hypoendemic (urban areas such as Bamako, prevalence < 10%) (8)

In Mali, this complexity is manifested by a marked spatio-temporal heterogeneity. The persistence of malaria morbidity, despite the intensification of control strategies, underlines the need to analyze the local specificities of transmission, particularly in contrasting geographical contexts such as those of the districts of Koulikoro and Timbuktu (10) .

The effectiveness of national disease control programs (NDCPs) relies on the ability to adapt interventions to local epidemiological realities, a concept known as epidemiological stratification (11) . In Mali, geographical heterogeneity is exacerbated by anthropogenic and environmental factors. Districts cannot be treated uniformly (11) . In particular, areas benefiting from hydro-agricultural projects, such as irrigated perimeters, are suspected of acting as permanent or prolonged transmission hotspots, providing long-term larval habitats for vectors (11,12) .

Understanding this epidemiological dynamic requires a detailed analysis of environmental and hydro-climatic factors. In Mali, the contrast between southern and northern zones offers a unique framework for study. On the one hand, the Koulikoro district, located in the Sudanian zone, exhibits transmission classically correlated with the seasonal rhythm of rainfall. On the other hand, the Timbuktu district, although situated in an arid Sahelian environment, is characterized by the influence of the Niger River and vast irrigated rice-growing areas.

These hydro-agricultural developments introduce a major ecological variable: they create permanent larval habitats that are unaffected by rainfall, thus promoting the persistence of malaria transmission even during the dry season (13) . The existence of such environmental refuges in desert areas poses specific challenges to community-based control strategies. Therefore, comparing surveillance data between these two districts is essential to assess the effectiveness of current interventions and adapt public health policies to the country's micro-ecological realities.

In Mali, previous work has often focused on the effectiveness of LLINs and SPCs in children, leaving an analytical gap regarding variations in incidence among adolescents and adults in areas of modified transmission. The present study seeks to fill this gap using a long-term comparative approach (2018–2022) based on routine data, thus providing a crucial epidemiological and programmatic perspective.

METHODOLOGY

Type and period of study

We conducted a cross-sectional study with comparative and analytical aims. This research is based on the use of epidemiological surveillance data for malaria collected over a five-year period, from January 1, 2018 to December 31, 2022.

Places of study

The study took place in two health districts in Mali, selected for their socio-ecological and climatic heterogeneity. *The Koulikoro health district (Southern Zone)* : Characterized by a Sudanese climate and a peri-urban area influenced by its proximity to Bamako. Malaria transmission there is typically seasonal and linked to rainfall, with moderate agricultural activity. The *Timbuktu health district (Northern Zone)* : Located in the Sahel region, this district is distinguished by the presence of vast irrigated rice-growing areas. This hydro-agricultural infrastructure creates a microclimate favorable to the persistence of Anopheles mosquito breeding sites, potentially extending transmission beyond the usual rainy season.

Study population and sampling

The study population comprised all suspected and confirmed cases of malaria recorded during consultations at health facilities in the two districts. We conducted exhaustive sampling by extracting all data reported in the weekly database of the District Health Information Software 2 (DHIS2). Inclusion criteria were based on the consistent presence of the case in the database throughout the study period, regardless of age or sex.

Operational definitions and variables

The variables of interest were defined as follows: *Hospital prevalence:* defined as the ratio between the annual number of confirmed cases (by RDT or thick blood smear) and the total estimated population for the year in question. *Biological variables:* results of rapid diagnostic tests (RDTs) and thick blood smears (TBS). *Temporal variables:* analysis by quarters (T1, T2, T3, T4) to capture seasonality. *Sociodemographic variables:* sex and age, the latter stratified into two classes according to the priority targets of the National Malaria Control Program (NMCP): under 5 years and 5 years and over.

Data collection and analysis

The data were extracted from DHIS2, then consolidated and cleaned using Microsoft Excel® 2016. Statistical analysis was performed using R software (version 4.x) via the RStudio interface. Descriptive analysis focused on categorical variables, which were presented as absolute frequencies and proportions (percentages). Inferential analysis, comparing proportions between the two districts and different time periods, involved applying a Z-test. The threshold for statistical significance was set at $p < 0.05$. The Stats Tester tool was used to validate the comparison tests.

Ethical and professional considerations

This research protocol was submitted to and approved by the health authorities of the Koulikoro and Timbuktu districts. Access to the DHIS2 data was subject to prior authorization from the relevant regional health directorates. To guarantee patient confidentiality and anonymity, all extracted data were anonymized before analysis; no personally identifiable information (names, surnames, or precise addresses) was collected or processed. The study was conducted in strict compliance with the principles of the Declaration of Helsinki and the rules of medical ethics in force in Mali. The results of this research are intended solely for improving malaria control strategies and strengthening the community health system.

RESULTS

Distribution of suspected malaria cases by age group

A total of 398,408 suspected cases of malaria were reported during the study period. The Koulikoro district recorded the highest number of suspected cases (n = 258,880). A notable difference was observed in the age structure: specifically, the proportion of children under 5 years of age was higher in Koulikoro (33.94%) than in Timbuktu (23.53%). Comparative analysis revealed a highly significant difference in the age structure of

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suspected cases between the two districts ($p < 0.001$). The suspected disease burden among children under 5 years of age was significantly higher in the Koulikoro district (33.94%) compared to the Timbuktu district (23.53%). This disparity suggests different transmission dynamics or healthcare-seeking behaviors depending on the geographical context.

Comparative analysis reveals a statistically highly significant difference in the age structure of suspected cases between the two districts ($p<0.001$). The suspected disease burden among children under 5 years of age is significantly higher in the Koulikoro district (33.94%) compared to the Timbuktu district (23.53%). This disparity suggests different transmission dynamics or healthcare-seeking behaviors depending on the geographical context.

Table 1: Distribution of suspected malaria cases by age group in the districts of Koulikoro and Timbuktu (2018-2022)

Age range (years)	Koulikoro n (%)	Timbuktu n (%)	Total n (%)	p-value
< 5 years	87,868 (33.94)	32,826 (23.53)	120,694 (30.29)	0.0001
≥ 5 years	171,012 (66.06)	106,702 (76.47)	277,714 (69.71)	
Total	**258,880 (100)**	**139,528 (100)**	**398,408 (100)**	

Distribution of confirmed malaria cases by age group and year

Across the entire study area, the number of confirmed cases increased from 27,358 in 2018 to 80,976 in 2022, marking a substantial improvement in the reporting system. In Koulikoro, a dramatic reversal in the morbidity pattern was observed. While children under 5 years of age represented only 29.90% of cases in 2018, their proportion increased steadily to reach 56.54% in 2022. This shift has now positioned malaria as a predominantly childhood disease in this district. Conversely, the Timbuktu district shows remarkable stability in its age distribution. Throughout the period, the 5 and older age group remained by far the largest, consistently representing between 73.86 % (2018) and 80.35% (2022) of reported cases. The epidemiological profile there therefore remains predominantly adult, contrasting with the dynamics observed in Koulikoro.

Table 2: Annual evolution of confirmed malaria morbidity by age group in the districts of Koulikoro and Timbuktu (2018-2022)

Year	Age range	Koulikoro n (%)	Timbuktu n (%)	Total n (%)
2018	< 5 years	4219 (29.90)	3463 (26.14)	7682 (28.08)
	≥ 5 years	9891 (70.10)	9785 (73.86)	19676 (71.92)
	Subtotal	**14,110 (100)**	**13,248 (100)**	**27,358 (100)**
2019	< 5 years	10664 (27.59)	2956 (20.87)	13620 (25.79)
	≥ 5 years	27989 (72.41)	11211 (79.13)	39200 (74.21)
	Subtotal	**38,653 (100)**	**14,167 (100)**	**52,820 (100)**
2020	< 5 years	16086 (39.95)	6279 (19.74)	22365 (31.03)
	≥ 5 years	24179 (60.05)	25529 (80.26)	49708 (68.97)
	Subtotal	**40,265 (100)**	**31,808 (100)**	**72 073 (100)**
2021	< 5 years	20138 (34.13)	7000 (22.76)	27138 (30.24)
	≥ 5 years	38861 (65.87)	23757 (77.24)	62618 (69.76)
	Subtotal	**58,999 (100)**	**30,757 (100)**	**89,756 (100)**
2022	< 5 years	30303 (56.54)	5381 (19.65)	35684 (44.07)
	≥ 5 years	23292 (43.46)	22000 (80.35)	45292 (55.93)
	Subtotal	**53,595 (100)**	**27,381 (100)**	**80,976 (100)**

Distribution of confirmed malaria cases by sex

A reversal in the sex ratio was observed in Koulikoro. In 2018, men were in the majority (51.41%). This trend

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gradually reversed, reaching a female predominance of 52.12% in 2022. In Timbuktu, the district stands out with a consistent and increasingly pronounced female predominance throughout the study period. The proportion of women among confirmed cases increased from 52.23% in 2018 to 53.98% in 2022, indicating greater vulnerability (or increased use of healthcare) among women in the irrigated Sahelian zone.

Table 3: Annual distribution of confirmed malaria cases by sex in the districts of Koulikoro and Timbuktu (2018-2022)

Year	Sex	Koulikoro n (%)	Timbuktu n (%)	Total n (%)
2018	Male	7,258 (51.41)	6,329 (47.77)	13,587 (49.65)
	Female	6,859 (48.59)	6,919 (52.23)	13,778 (50.35)
	Subtotal	**14,117 (100)**	**13,248 (100)**	**27,365 (100)**
2019	Male	19,320 (49.90)	6,623 (46.75)	25,943 (49.06)
	Female	19,397 (50.10)	7,544 (53.25)	26,941 (50.94)
	Subtotal	**38,717 (100)**	**14,167 (100)**	**52,884 (100)**
2020	Male	26,339 (49.49)	14,881 (46.78)	41,220 (48.48)
	Female	26,882 (50.51)	16,927 (53.22)	43,809 (51.52)
	Subtotal	**53,221 (100)**	**31,808 (100)**	**85,029 (100)**
2021	Male	26,279 (47.57)	14,432 (46.92)	40,711 (47.34)
	Female	28,960 (52.43)	16,325 (53.08)	45,285 (52.66)
	Subtotal	**55,239 (100)**	**30,757 (100)**	**85,996 (100)**
2022	Male	28,884 (47.88)	12,601 (46.02)	41,485 (47.30)
	Female	31,441 (52.12)	14,780 (53.98)	46,221 (52.70)
	Subtotal	**60 325 (100)**	**27,381 (100)**	**87,706 (100)**

Distribution of confirmed cases by age group and sex

Cross-analysis of the data (Table IV) reveals gender disparities that are reversed according to patient age. Among children under 5 years of age, males predominate overall, representing 52.87% of confirmed cases. This male predominance is more pronounced in Koulikoro (53.51%) than in Timbuktu (50.91%). Among individuals aged 5 years and older, a systematic reversal of the trend is observed, with females accounting for 53.97% of all cases (53.70% in Koulikoro and 54.38% in Timbuktu). It is worth noting that the number of cases in those ≥ 5 years (237,992) is more than twice that of those under 5 years (100,988), highlighting the continued significant burden of disease among adults in both districts.

Table 4: Distribution of confirmed malaria cases by age group and sex (2018-2022)

Age range	Sex	Koulikoro n (%)	Timbuktu n (%)	Total n (%)
< 5 years	Male	40,621 (53.51)	12,768 (50.91)	53,389 (52.87)
	Female	35,288 (46.49)	12,311 (49.09)	47,599 (47.13)
	Total < 5 years	**75,909 (100)**	**25,079 (100)**	**100,988 (100)**
≥ 5 years	Male	67,459 (46.30)	42,098 (45.62)	109,557 (46.03)
	Female	78,251 (53.70)	50,184 (54.38)	128,435 (53.97)
	Total ≥ 5 years	**145,710 (100)**	**92,282 (100)**	**237,992 (100)**

Comparison of the annual prevalence of malaria in the two health districts

The study presented the evolution of the prevalence of confirmed malaria in the populations of Koulikoro and Timbuktu. A statistically significant difference ($p<0.001$) was observed between the two districts each year. In Koulikoro, the prevalence increased dramatically, rising from 4.99% in 2018 to a peak of 19.21% in 2021. In 2022, a slight decrease was noted, with a prevalence of 16.97%. In Timbuktu, the profile is more contrasting. The prevalence more than doubled between 2018 (8.04%) and 2020 (18.225%), before gradually decreasing to 14.87% in 2022. Overall, despite a larger population in Koulikoro, the relative parasite pressure (prevalence)

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remained comparable to or higher than that of Timbuktu over the last three years, indicating an intensification of transmission in the south. There was a significant difference between the prevalence rates of 2018 and 2022 in the health districts of Koulikoro and Timbuktu.

Table 5: Comparison of the annual prevalence of malaria in the health districts of Koulikoro and Timbuktu from 2018 to 2022

Year	Koulikoro		Timbuktu		p-value
	Population	n (%)	Population	n (%)	
2018	282570	14110 (4.99)	164797	13248 (8.04)	0.001
2019	290688	38653 (13.30)	169547	14167 (8.36)	0.001
2020	298894	40265 (13.47)	174297	31808 (18.25)	0.001
2021	307187	58999 (19,21)	179230	30757 (17.16)	0.001
2022	315742	53595 (16.97)	184163	27381 (14.87)	0.001

Distribution of confirmed malaria cases using thick blood film (EBF)

The study highlighted a fundamental difference in biological diagnostic practices between the two districts ($p<0.001$). In Koulikoro, the use of thick blood smears saw a linear and massive increase, rising from 18.20% in 2018 to 59.20% in 2022. Conversely, in Timbuktu, confirmation by thick blood smears remains marginal, not reaching 8% and falling to 3% in 2022. In this district, almost all diagnoses rely exclusively on rapid diagnostic tests (RDTs).

Table 5: Comparison of the proportions of thick smear use in the health districts of Koulikoro and Timbuktu by year

Year	Koulikoro		Timbuktu		p-value
	Confirmed case	n (%)	Confirmed case	n (%)	
2018	14110	2568 (18.20)	13248	212 (1.60)	0.001
2019	38653	8070 (20.88)	14167	1091 (7.70)	0.001
2020	40265	9019 (22.40)	31808	2385 (7.50)	0.001
2021	58999	25900 (43.90)	30757	2183 (7.10)	0.001
2022	53,595	31729 (59.20)	27381	821 (3.00)	0.001

Distribution of confirmed malaria cases using Rapid Diagnostic Tests (RDTs)

Following the evolution of the proportion of cases confirmed by rapid diagnostic tests (RDTs) in the two study districts, a statistically significant difference ($p = 0.001$) was consistently observed between 2018 and 2022. In Koulikoro, a gradual and marked reduction in reliance on RDTs was noted. While they accounted for 81.79% of confirmations in 2018, this proportion fell to 40.84% in 2022. This decrease is inversely proportional to the increase in the use of thick blood smear microscopy observed previously in this district. In Timbuktu, the district maintains an almost exclusive reliance on RDTs for case confirmation. The proportions remained very high and stable, fluctuating between 92.30% and 98.40% over the entire period. In 2022, 97% of cases there were still confirmed by RDTs.

Table 6: Comparison of the proportions of RDT use in the Koulikoro and Timbuktu health districts by year

Year	Koulikoro		Timbuktu		p-value
	Population	n (%)	Population	n (%)	
2018	14110	11541 (81.79)	13248	13036 (98.40)	0.001
2019	38653	30574 (79.10)	14167	13076 (92.30)	0.001
2020	40265	31246 (77.60)	31808	29422 (92.50)	0.001
2021	58999	33098 (56.10)	30757	28573 (92.90)	0.001
2022	53,595	21886 (40.84)	27381	26559 (97.00)	0.001

Quarterly breakdown confirmed cases of malaria in Koulikoro according to the years of study

The study observed a consistent peak in morbidity during the third quarter (July-September) for each study year. In 2021, this peak reached its highest level with 32,716 cases, representing a 54% increase compared to the third quarter of 2020 (21,248 cases). The fourth quarter (October-December) also remains a period of high transmission, with the number of cases often exceeding the combined total of the first two quarters. Between 2018 and 2021, the district experienced continuous growth in the number of reported cases, rising from an annual total of approximately 14,110 cases in 2018 to nearly 59,000 in 2021. The year 2022 marked a slight stabilization with a persistent seasonal pattern.

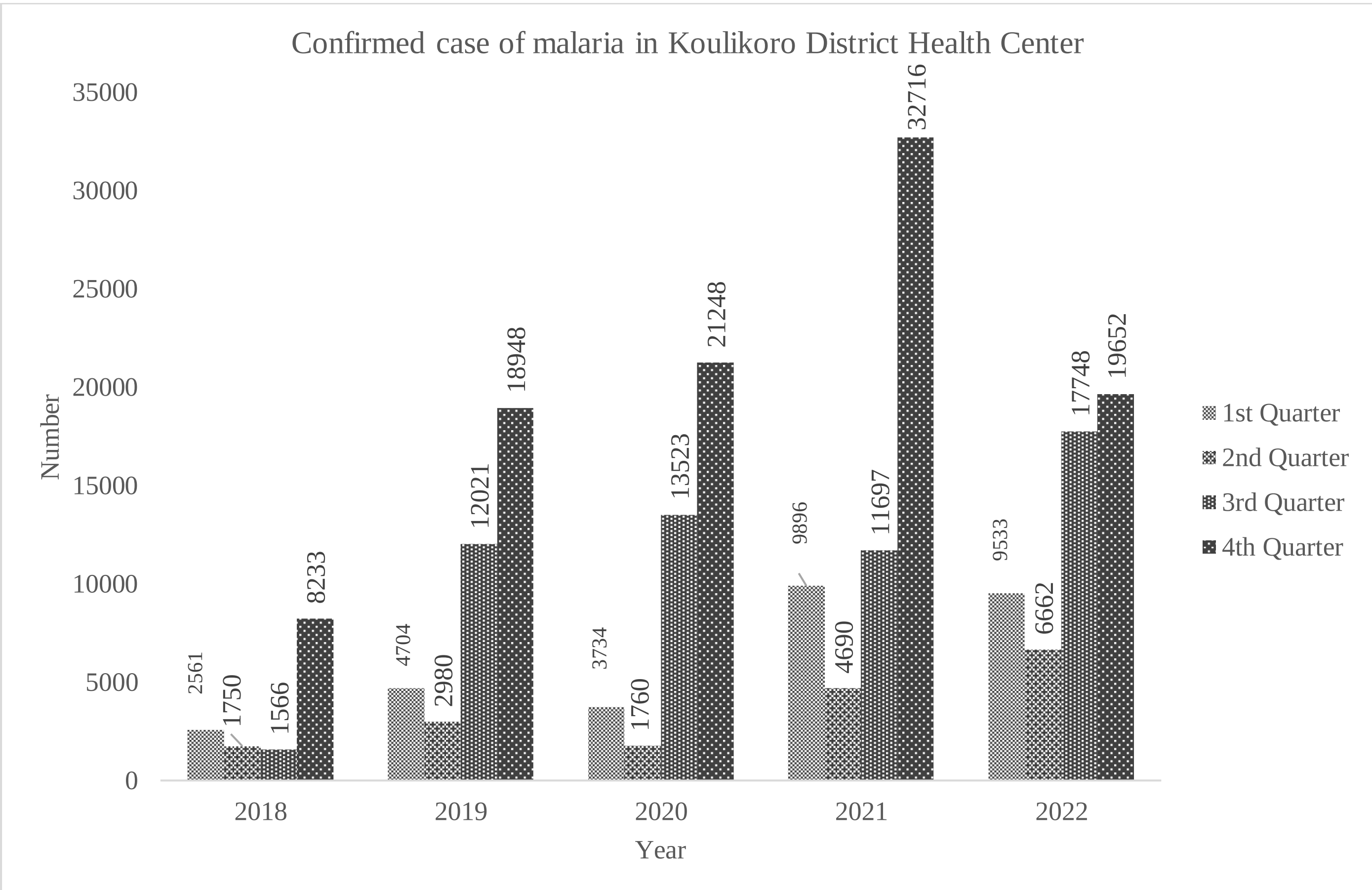

Figure 1: Quarterly comparison of confirmed malaria cases by year in the Koulikoro health district

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Quarterly distribution of confirmed malaria cases in Timbuktu by year

Unlike typical savannah profiles, Timbuktu exhibited atypical and fluctuating seasonality. While a peak was observed in the third quarter of 2018 and 2019, 2021 saw an exceptional surge in cases as early as the first quarter (13,883 cases), representing the highest volume for this district during the entire study period. The number of cases rose from a base of approximately 3,000 cases per quarter in 2018 to peaks exceeding 11,000 cases in 2020 (fourth quarter) and 2021 (first quarter). The year 2022 was characterized by a late resurgence, with the peak in morbidity shifting towards the second quarter (11,028 cases) and the fourth quarter (10,184 cases), suggesting a change in the usual transmission patterns.

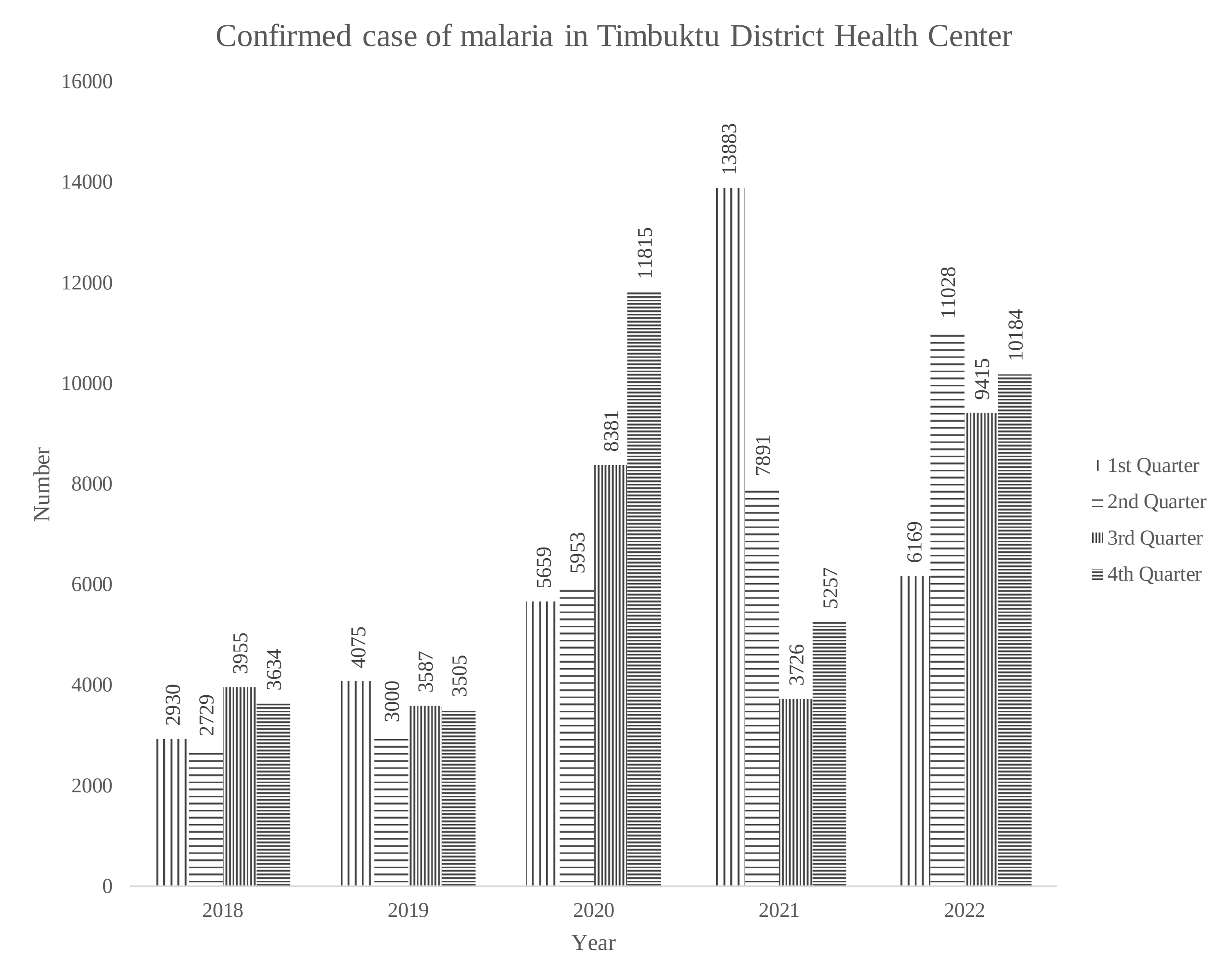

Figure 2: Quarterly comparison of confirmed malaria cases by year in the Timbuktu health district

Cumulative quarterly distribution of confirmed cases by district (2018-2022)

Figure 3 presents the overall volume of confirmed malaria cases aggregated by quarter for the two health districts over the entire study period. The Koulikoro district exhibited a strongly seasonal transmission pattern with a gradual increase throughout the year. The number of cases peaked dramatically in the fourth quarter (100,797 cases), followed by the third quarter (56,555 cases), totaling 205,622 cases for the period. In contrast to Koulikoro, Timbuktu showed a more even distribution across the quarters, although a higher volume was noted in the fourth quarter (34,395 cases) and the first quarter (32,716 cases). The cumulative total reached 126,776 cases. Although the two districts had different annual totals, the fourth quarter represented the period of highest malaria pressure for both. However, Koulikoro records a total morbidity burden 62% higher than that of Timbuktu.

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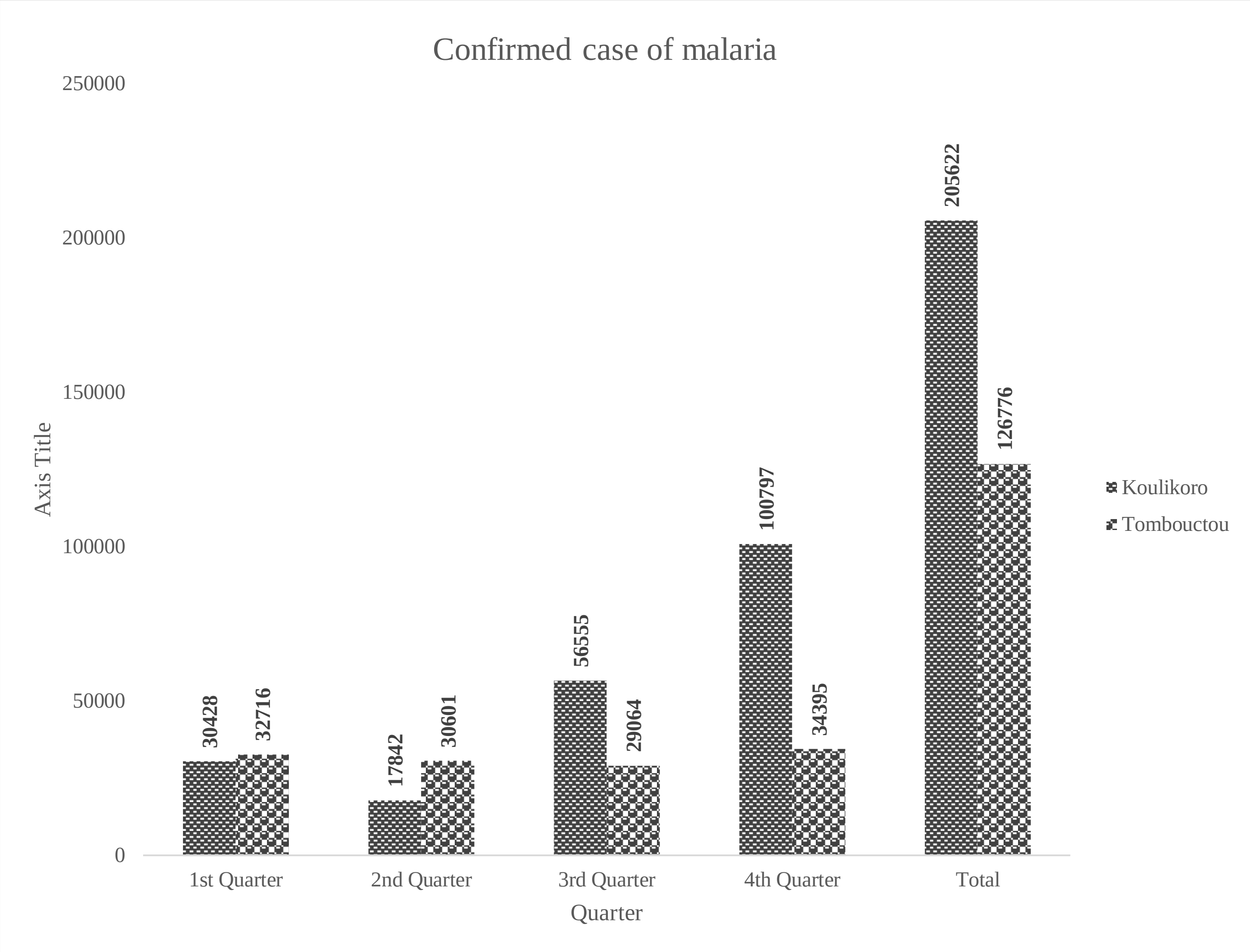

Figure 3: Quarterly comparison of confirmed malaria cases in the Koulikoro and Timbuktu health districts from 2018 to 2022 (in number)

DISCUSSION

Distribution of suspected malaria cases by age group

Analysis of the distribution of suspected cases reveals a striking disparity between the two districts. While in Koulikoro, children under 5 years of age account for more than a third of consultations (33.94%), this figure drops to 23.53% in Timbuktu. This statistically significant difference ($p < 0.0001$) can be interpreted by the specific micro-ecological characteristics of the North. In Timbuktu, the presence of vast irrigated rice-growing areas alters the typical transmission dynamics. Unlike savannah areas where malaria primarily affects children, irrigated agricultural areas in the Sahel promote prolonged exposure for adults working in the rice paddies. According to Sissoko et al. in 2004, the constant humidity maintained by irrigation allows for increased vector survival, thus increasing the risk of bites for agricultural workers, even outside the rainy season (13) .

Furthermore, the predominance of malaria cases in adults (≥ 5 years) in Timbuktu (76.47%) could reflect slower acquisition of malaria immunity or constant infection pressure related to developments along the Niger River. Conversely, in Koulikoro, the peri-urban profile and more strictly seasonal transmission concentrate morbidity among young children, the biologically most vulnerable population before the acquisition of protective immunity (1,10) . These results underscore the importance of decentralizing control strategies: while seasonal malaria chemoprevention (SMC) remains vital in the South, strengthening individual protection among workers in irrigated areas in the North appears to be a complementary public health priority.

Distribution of confirmed malaria cases by age group and year

The divergence in age profiles between Koulikoro and Timbuktu is one of the most salient findings of this study. In Timbuktu, the persistence of high morbidity among adults (≈ 80%) confirms the specific characteristics of transmission in the irrigated Sahelian zone. Unlike classic savanna models, the irrigation of the Niger River creates microclimates that allow for perennial transmission. Adults, due to their occupational exposure in rice-growing areas, constitute a high-risk group, an observation consistent with the work of Sissoko et al. (2022) on the impact of hydrological changes in Mali (14) . The increase in the proportion of cases among children under 5 years of age in Koulikoro (56.54% in 2022) suggests a transition to an intense transmission pattern where biological vulnerability once again becomes the determining factor. This finding underscores the vital importance of seasonal malaria chemoprevention (SMC). As highlighted in the WHO's 2024 World Malaria Report, the effectiveness of community-based interventions can be influenced by urbanization and vector density (1) . This predominance of cases among children in Koulikoro could reflect either the success of adult protection efforts or the persistence of peri-urban mosquito breeding sites that primarily affect children. The overall increase in reporting (tripling in 5 years) is not a sign of an uncontrolled epidemic, but rather reflects the maturation of the DHIS2 system in Mali and the systematic use of RDTs and EGs for biological confirmation. This trend toward "epidemiological transparency" aligns with the CDC's recommendations (2025), which advocate the use of real-time data to optimize the allocation of resources (15) . According to Kaboré's work (2019), the disease burden is more critical in this age group due to the lack of protective immunity (16) . The difference in proportion between Koulikoro (33.94%) and Timbuktu (23.53%) for this age group could suggest earlier parasite pressure in Koulikoro, a rice-growing area conducive to permanent larval habitats.

Distribution of confirmed malaria cases by sex

The increasing predominance of females within the cohort of confirmed cases (exceeding 53% in Timbuktu) is a major public health indicator. This finding can be interpreted from two complementary perspectives. Environmental and occupational exposure in Timbuktu: the persistence of a high female prevalence could be linked to domestic and market gardening activities near the Niger River. Permanent irrigation creates ecological niches where the risk of mosquito bites is constant, particularly impacting women during morning or dusk work. This observation is corroborated by the work of Sissoko et al. (2022) on hydrological changes in Mali (17) . Access to and use of healthcare: statistics concerning women raised in the DHIS2 often reflect better adherence to basic health services, particularly through prenatal consultations or child follow-up, thus facilitating their own diagnosis. As highlighted in the 2024 Global Malaria Report, improved access to community-based care facilitates the capture of morbidity data among previously underreported populations (2) . Epidemiological surveillance and system performance may explain the massive increase in cases between 2018 and 2020, reflecting the ramp-up of the DHIS2 platform and the strengthening of biological confirmation (RDT/Thick Blood Smear). This trend aligns with the CDC's (2025) recommendations, which advocate for data-confirmed surveillance to avoid wasting ACTs (Artemisinin-based Combination Therapy) (18) .

Our results show a male predominance among children under 5, which corroborates several studies in sub-Saharan Africa suggesting increased biological vulnerability of young boys to parasitic infections during early childhood. However, the reversal observed among adults (5 years and older) in favor of females (54%) is a major public health indicator. This phenomenon can be explained by two key factors: women's increased use of healthcare, as they are often responsible for family health and tend to attend primary healthcare facilities (CSComs) more regularly, thus facilitating their reporting in the DHIS2 system; and environmental exposure in the North: In Timbuktu, the female predominance among adults could be linked to domestic and agricultural activities near irrigated areas of the Niger River, where the risk of Anopheles mosquito bites is constant. Temporal Dynamics and Enhanced Surveillance: The massive increase in the number of confirmed cases between 2018 and 2022 (from 27,365 to 87,706) does not necessarily reflect a worsening of the epidemic, but likely indicates a substantial improvement in data completeness in the DHIS2 and better implementation of the policy of systematic biological confirmation (RDT/Thick Blood Smear) before treatment in Mali. This observation is consistent with data from the Malaria Indicator Survey in Mali (EIPM, 2021), which highlights increased use of health services by women (8) . Factors that may explain this disparity include the fact that in Mali, pregnant women benefit from free malaria diagnosis and treatment, as well as the free distribution of insecticide-treated bed nets (ITNs) during prenatal consultations. This targeted approach leads to better malaria case cap-

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ture among women in the DHIS2 system compared to adult men. Morning and evening domestic activities (water fetching, preparing meals outdoors) expose women more to Anopheles mosquito bites during peak vector activity hours. Our figures are similar to those reported by Tapily et al. (2020) in a study conducted in Bamako, which found a female predominance of 54% (19) .

Distribution of confirmed cases by age group and sex

The male predominance among young children (< 5 years) observed in both districts is a documented phenomenon in sub-Saharan Africa. This increased vulnerability of boys could be linked to early biological and immunological factors, as suggested by Snow's (2023) work on malaria control milestones (3) . However, the female predominance among adults (≥ 5 years) in almost identical proportions (approximately 54%) in Koulikoro and Timbuktu suggests two major determinants: access to care, as adult women, the linchpins of family health, tend to attend primary healthcare facilities (CSComs) earlier, facilitating their reporting in the DHIS2 (2,10) . Exposure related to activities in Timbuktu: In the rice-growing areas of Timbuktu, women are particularly exposed during domestic and market gardening activities carried out at times of high vector activity (dusk) near permanent larval breeding sites created by irrigation (13) . The persistence of morbidity among adults (the fact that those over 5 years of age represent nearly 70% of the overall disease burden confirms this) demonstrates that malaria is no longer an exclusively childhood disease in Mali. While seasonal malaria chemoprevention (SMC) effectively protects children under 5, these results argue for strengthening individual protection (insecticide-treated bed nets, repellents) for the working population, particularly in the rice-growing district of Timbuktu where transmission is perennial (13, 20) .

Comparison of the annual prevalence of malaria in the two health districts

The overall increase in prevalence between 2018 and 2021 in both districts reflects a shift towards improved case reporting by the health system. This trend is explained by the widespread use of laboratory testing, reducing the historical "epidemiological silence." The decrease in prevalence observed in 2022 in Timbuktu (14.8%) and Koulikoro (16.9%) could signal the initial impacts of integrated control strategies, particularly seasonal malaria chemoprevention (SMC) and the distribution of long-lasting insecticidal nets. As highlighted in the 2024 Global Malaria Report, stabilizing prevalence is a key step before initiating the pre-elimination phase (2) . The high prevalence in Timbuktu in 2020 (18.2%) illustrates the continued risk in Sahelian areas dependent on the Niger River. Variations in prevalence are closely linked to rainfall and water management for irrigation, factors that directly influence Anopheles mosquito density. These results suggest that malaria control in Mali must remain flexible and adapted to the hydroclimatic specificities of each district (13,20) .

Distribution of confirmed malaria cases using thick blood film (EBF)

The increased use of microscopy in Koulikoro (59.2%) reflects a strengthening of the technical infrastructure and improved availability of qualified personnel in areas near the capital. This ability to perform thick blood film is crucial, as it allows not only confirmation of the Plasmodium species, but above all, the quantification of parasitemia, essential for monitoring severe cases (21) . In Timbuktu, the near-total reliance on rapid diagnostic tests (RDTs) is explained by the logistical constraints inherent in conflict zones or areas with difficult access, where maintaining functional microscopes and reagents is a constant challenge. However, this situation is concerning at a time when the WHO is reporting the emergence of pfhrp2/3 gene deletions in West Africa, rendering some RDTs ineffective. As the CDC (2010) points out, surveillance based solely on RDTs could mask an underestimation of cases in these contexts (18, 20) .

Your results paint a contrasting picture of epidemiological transition. Koulikoro is evolving towards a profile of intense malaria transmission, predominantly among children under five (56.5% in 2022), and high-quality diagnosis. Timbuktu maintains a profile as a rice-growing area where the burden falls on the working-age population (80% adults), with diagnosis dependent on community mobility (RDT) (2.20) .

Distribution of confirmed malaria cases using Rapid Diagnostic Tests (RDTs)

The disparity observed in the use of rapid diagnostic tests (RDTs) illustrates the structural challenges of medi cal biology in Mali. In Koulikoro, the decline in RDT use in favor of thick blood smears (from 81.8% to 40.8%) demonstrates a successful transition to a gold standard diagnostic test. This shift allows for better clinical management, particularly for monitoring parasite density. Conversely, the situation in Timbuktu (>97% RDT use in 2022) underscores the vital importance of this tool in areas with difficult access or limited resources. While RDTs guarantee rapid care at the community level, this over-reliance poses two major risks identified by the WHO (2024) (1) . Deletions of the pfhrp2/3 gene: These genetic mutations can cause false-negative RDTs, a phenomenon increasingly documented in West Africa (20) . Antigenic persistence: RDTs can remain positive for several weeks after effective treatment, potentially leading to an overestimation of current morbidity (18) .

Cross-analysis of confirmation methods (RDT vs. EG) suggests that the National Malaria Control Program (NMCP) should adopt differentiated strategies: strengthening microscope maintenance and technician training in Timbuktu, while consolidating the achievements in Koulikoro. Epidemiological surveillance based on DHIS2 becomes more reliable when biological diagnostics are diverse and of high quality.

Distribution quarterly confirmed cases of malaria in Koulikoro according to the years of study

The concentration of cases during the third and fourth quarters coincides with the rainy season in Mali (June to October), the period of maximum proliferation of Anopheles gambiae breeding sites. This finding is perfectly consistent with the 2024 Global Malaria Report, which emphasizes that rainfall variations remain the main driver of transmission instability in the Sudanian savanna zone (2) . The massive increase in the number of cases between 2018 and 2021 (a fourfold increase) should not be interpreted solely as a failure of control measures. It primarily reflects an improvement in the performance of the DHIS2 surveillance system and the systematic use of rapid diagnostic tests (RDTs) and thick blood film, in accordance with the guidelines of the Mali National Malaria Control Program (PNLP Mali) (2024). Although the CPS (Community-Based Prevention) program is deployed during the high transmission period (July-October), the persistence of high peaks in the third quarter in Koulikoro suggests a possible extension of the transmission period beyond the CPS cycles. There is a need to strengthen effective coverage among children under 5 years of age, whose share of total morbidity is steadily increasing, reaching 56.5% in Koulikoro in 2022.

Quarterly distribution of confirmed malaria cases in Timbuktu by year

Figure 2 reveals an epidemiological profile that deviates from the purely rainfall-driven transmission patterns observed in Koulikoro. In Timbuktu, the presence of the Niger River and irrigated rice-growing areas creates conditions favorable to perennial transmission or off-season peaks (such as the peak in the first quarter of 2021). Irrigation allows for the maintenance of permanent larval habitats for Anopheles pharoensis or Anopheles gambiae sl, even during the dry season. This phenomenon, described by Sissoko et al. (2022), explains why the adult population (5 years and older), very active in irrigated fields, bears approximately 80% of the disease burden in this district (17) . The massive peak in Timbuktu at the beginning of 2021 (13,883 cases in Q1) could be correlated with exceptional environmental factors or disruptions in control activities (net distribution or SMC) linked to the unstable security situation in the region. As highlighted in the 2024 Global Malaria Report, conflict zones in the Sahel are facing a resurgence of malaria due to the disorganization of health systems and population mobility. The quarterly variability in Timbuktu suggests that the standard seasonal malaria chemoprevention (SMC) schedule, generally aligned with the rainy season (July-October), may be insufficient or out of sync with local realities. These data argue for a spatially targeted control strategy adapted to the hydro-agricultural context, integrating vector control in irrigated areas throughout the year.

Cumulative quarterly distribution of confirmed cases by district (2018-2022)

Analysis of Figure 3 highlights two distinct epidemiological realities in Mali. In Koulikoro, the massive concentration of cases in the fourth quarter (October-December) immediately follows the rainy season, reflecting a classic biological lag between peak rainfall and peak parasite transmission. Conversely, the more diffuse distribution observed in Timbuktu, particularly the continued high volume of cases in the first quarter, confirms the influence of irrigated rice-growing areas and the Niger River. These ecosystems maintain productive larval habitats even in the absence of rain, promoting perennial transmission. As the WHO (2024) emphasizes, these irrigated areas require continuous rather than purely seasonal vector control strategies (2) . The predominance of the fourth quarter as the overall peak period (135,192 cumulative cases for the two districts) underscores the period of maximum stress for primary healthcare facilities. This period often coincides with the end of seasonal malaria chemoprevention (SMC) campaigns, suggesting a possible "rebound" or significant residual transmission among age groups not covered by SMC, particularly those over 5 years old, who represent a major proportion of morbidity in Timbuktu. The reliability of these quarterly data, from the DHIS2 system, now allows for predictive planning of input needs (ACTs, RDTs), essential to avoid stockouts during these critical peaks identified in both areas.

Summary of epidemiological dynamics (2018-2022)

Cross-analysis of the data reveals a fundamental divergence between Koulikoro and Timbuktu. In Koulikoro, the increase in morbidity is accompanied by a shift towards high-quality diagnosis (59.2% of cases are diagnosed by epidemiological studies). This area, although following a classic rainfall pattern with a massive peak in the fourth quarter (100,797 cumulative cases), sees its morbidity burden concentrated on children under 5, who represented 56.5% of cases in 2022. Conversely, Timbuktu presents a profile of an irrigated Sahelian zone where transmission is less dependent on rainfall than on hydro-agricultural infrastructure. This results in a more diffuse quarterly distribution and a burden that falls 80% on those over 5. However, the persistent reliance on rapid diagnostic tests (RDTs) (97% in 2022) remains a major vulnerability for surveillance in this district.

Evaluation of control strategies: successes and limitations

The contrasting results suggest that current national strategies are producing varying effects. Seasonal malaria chemoprevention (SMC): Highly effective in Koulikoro for protecting young children, it appears to reach its limits in Timbuktu, where the majority of cases occur in adults and outside the usual rainy season. The increase in cases reported in both districts via DHIS2 demonstrates improved reporting completeness but also underscores that malaria remains in a state of sustained endemicity despite mass interventions.

This comparative study (2018-2022) highlights a marked heterogeneity in the epidemiological dynamics of malaria between the districts of Koulikoro and Timbuktu, emphasizing the need for interventions adapted to the context.

In Koulikoro, where morbidity follows a classic rainfall-dependent pattern, with a pronounced peak in the fourth quarter (100,797 cumulative cases), and primarily affects children under five (56.5% in 2022), it is recommended to strengthen seasonal malaria chemoprevention (SMC) and vector control measures before periods of peak transmission. Furthermore, increased use of microscopic diagnostics, beyond the current 59.2% use of thick blood smears, would improve diagnostic accuracy and case confirmation.

In Timbuktu, where malaria transmission is influenced by irrigated rice cultivation and characterized by off-season peaks, particularly in the first quarter, interventions must be adapted to the local transmission dynamics rather than relying on traditional seasonal approaches. Targeted strategies for populations aged five and older, who represent 80% of the disease burden, should be prioritized. Furthermore, it is essential to reduce the near-total reliance on rapid diagnostic tests (97%) by integrating confirmatory microscopy and quality assurance systems to improve the reliability of surveillance.

In both districts, the increased use of the DHIS2 platform offers the opportunity to implement real-time, data-driven decision-making. National malaria control strategies must evolve from uniform frameworks to precision public health approaches that are spatially stratified and adapted to local hydroclimatic and socio-ecological conditions. These adaptive, evidence-based interventions will be essential to accelerating progress toward malaria elimination goals in Mali.

CONCLUSION

This comparative study, conducted from 2018 to 2022, highlights a marked heterogeneity in the epidemiologi cal dynamics of malaria between the districts of Koulikoro and Timbuktu. In Koulikoro, morbidity follows a classic rainfall pattern with a massive peak in the fourth quarter (100,797 cumulative cases) and primarily affects children under 5 years of age (56.5% in 2022). The district has successfully made a qualitative transition to the reference diagnostic method, with thick blood film use reaching 59.2%. In Timbuktu, the pattern is influenced by irrigated rice-growing areas, leading to off-season peaks (particularly in the first quarter) and a morbidity burden borne by the population aged 5 and over, accounting for 80%. The near-total reliance on rapid diagnostic tests (RDTs) (97%) remains a major challenge for the accuracy of surveillance. While the performance of the DHIS2 system has enabled better visibility of the disease, the uniformity of national strategies must now give way to a precision approach, spatially distributed and adapted to local hydro-climatic contexts in order to achieve the elimination objectives in Mali.

Limitations of the study

Despite the relevance of the observed trends, this study has certain limitations inherent to the use of secondary data . *Potential underreporting* : The DHIS2 data only include patients attending public health facilities. *Environmental factors* : The lack of precise meteorological (local rainfall) and entomological data in this study prevents the establishment of direct causal correlations with vector densities. *Security context* : Instability in the Timbuktu region may have influenced population access to healthcare and, consequently, the reporting of surveillance data.

Research perspectives

To further explore these results and address the identified limitations, the following avenues are proposed:

1. Molecular Biology Studies (Focus Timbuktu) : The excessive reliance on RDTs in Timbuktu necessitates research on the prevalence of pfhrp2/3 gene deletions. These mutations can cause false negative RDTs, thus masking the true circulation of the parasite in the north of the country.

2. Entomological Surveillance and Resistance : It is crucial to study the dynamics of larval populations in the irrigated areas of Timbuktu. Insecticide susceptibility tests should be carried out to verify whether current long-lasting insecticidal nets (LLINs) maintain optimal efficacy against local vectors in irrigated areas.

3. CPS Evaluation "Extent" : Considering that those over 5 years old are the most affected in Timbuktu (80.3% of cases in 2022), a pilot study evaluating the impact of extending Seasonal Malaria Chemoprevention (SMC) to school-aged children and adolescents in this specific district would be of great operational interest.

4. Analysis of the impact of climate change : Conduct a study correlating DHIS2 morbidity time series with satellite data on precipitation and temperature to model the risks of epidemic resurgence in Koulikoro.

End page

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Conflicts Of Interest

There are none: The authors declare that they have no conflicts of interest related to this article.

Ethical approval and consent to participate

Ethical approval: The study was approved by the technical committee of the Faculty of Medicine and Odonto-stomatology of Bamako at the University of Sciences, Techniques and Technologies of Bamako (FMOS /USTTB). Authorization was obtained from the health authorities of the Koulikoro and Timbuktu health districts before the start of the study.

Consent to participate : The data was collected anonymously, so that the identity of the study participants could not be identified or identified within the meaning of the applicable law relating to the protection of natural persons with regard to the processing of personal data.

Consent to publication

General: Not applicable. This manuscript does not contain any individual data (images, videos or personal details) that could identify the participants.

Availability of data and materials

Data available: The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

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