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Exposure and Recent Arbovirus Infection in Children Suspected for Malaria Attending the Sentinel Site for Malaria Surveillance in Libreville, Gabon

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ABSTRACT

Background: *Acute febrile illnesses remain a major diagnostic challenge in tropical regions where malaria and arboviral infections coexist. This study assessed the prevalence and interaction of Plasmodium falciparum and arbovirus infections among febrile patients in Gabon.*

Methods: *A total of 524 patients presenting with acute fever were enrolled. Demographic, clinical, and biological data were collected. Malaria diagnosis was performed by microscopy, and arboviral infections (Zika, dengue, and chikungunya viruses) were screened using rapid diagnostic tests (RDTs).*

Results: *The median age of participants was 60 months. Malaria prevalence was 38.0% (199/524), with P. falciparum as the sole species detected. Arbovirus RDT positivity was observed in 39.1% (205/524) of cases, mainly due to recent Zika virus infection (35.1%; 184/524), while dengue and chikungunya IgM seropositivity rates were 8.0% (42/524) and 0.6% (3/524), respectively. Suspected coinfections were frequent (21.2%), predominantly P. falciparum-Zika virus (17.3%; 39/225). Parasite density was significantly higher in coinfected patients ($p < 0.01$). Coinfection was associated with lower haemoglobin levels ($p = 0.03$) and a higher risk of thrombocytopenia ($p < 0.01$). Older age and absence of insecticide-treated net use were significant risk factors ($p < 0.05$). Arboviral seropositivity increased with age and was highest among urban residents, whereas malaria was more prevalent in rural areas.*

Conclusion: *The coexistence of malaria and arboviral infections is common among febrile patients in Gabon, with coinfection associated with haematological alterations and higher parasite loads. These findings highlight the need for integrated diagnostic approaches and improved vector control strategies in endemic settings.*

Keywords

Malaria, Arboviruses, *Plasmodium falciparum,* Zika virus, Dengue virus, Chikungunya virus, Coinfection, Febrile illness, Gabon, Haematological parameters.

Introduction

In sub-Saharan Africa, children mortality is strongly associated with infectious diseases whose most common symptom is fever [1]. Malaria remains among the top ten causes of death in many African countries, but non-malarial acute febrile illnesses (NMFI)

are also a common clinical presentation in most in and outpatient clinics [2]. Clinicians face febrile illness management issues due to the wide range of aetiologies and the similarities of symptoms observed between malaria and other infectious diseases.

Indeed, the differential diagnosis of the infectious agents responsible for fever is not common in many countries due to lack of diagnostic tools or accessibility [3,4]. The presumptive treatment of patients with a negative malaria results with an antimalarial drugs or an antibiotic is common [5]. This leads to the rapid emergence of drug resistant strains and increases mortality among febrile patients because the real etiology remains untreated [6].

According to the malaria world report 2023 in 2022 almost 70% of febrile patients attending care in health centers benefit from parasitological confirmation by microscopy or the use of rapid diagnostic tests (RDT) before treatment. Indeed, in Gabon where six sentinel sites for malaria surveillance have been set-up for the prompt management of febrile patients, each one receives a free diagnosis of malaria with microscopy or RDT. Data from the main sentinel site of the Hospital of Melen which receive 3000 to 4000 children per year, highlighted a substantial reduction of malaria case. The frequency of positive blood smear ranges between 20 to 45% [7-9]. Thus 55 to 80% of the febrile case do not have malaria, should benefit for the detection of causative infections agents. In most of malaria endemic settings, the sentinels' sites are in semi-urban and rural areas, where apart from the malaria test, hematological measurements and chest radiography, other diagnostic stools such as hemoculture, procalcitonin, C reactive protein and molecular test for the identification of viral agents are not always available. Identifying the causes of acute non-malarial fevers is of great importance to improve patient management, but also for more effective surveillance systems that include detection of emerging pathogens and to guide the implementation of diagnostic and prevention tools [10]. A recent study performed at Hospital of Melen sentinel site highlighted the high frequency of antibiotic or antimalarial to patients with a negative malaria test [11]. Arthropod-borne viruses, also known as arbovirus, are one of the predominant causative agents of acute febrile illness in areas where high temperatures, humidity, and poor sanitation contribute to the proliferation of mosquito vectors, particularly in Africa. Arboviruses are not systematically mentioned or sought by clinicians, even when a malaria test is negative [12]. Moreover, arboviral infections are often poorly diagnosed as malaria due to the similarity of the clinical presentation [13]. Even in the absence of specific or localized symptoms, the suspect etiology of a non-malarial febrile illness is a bacterial infection [11].

In Gabon, arbovirus, such as dengue virus (DENV), Chikungunya (CHIKV) and Zika virus (ZIKV), have been circulating in the country since 2007 and detected, reported during outbreaks [14,15]. Two years ago, outbreak was observed in Lambaréné [14]. Moreover, all the favorable conditions for the mosquito vector abondance are present in the capital city of Libreville were unplanned urbanization, especially in shanty towns, which are also overcrowded, favorable climates, poor management of waste which is often stored near the dwellings. High prevalence of arbovirus vectors such as Aedes mosquito as well as proof of continuous circulation of VDEN, VCHIK and VZIK were recently reported [16,17].

The involvement of these viruses in acute febrile states is unknown in Gabon, these viruses share many epidemiological and clinical characteristics with other acute non severe febrile illness including malaria. The diagnosis of these agents is confirmed by RT-PCR. The diagnosis of these viruses is not routinely available in the country's various health centers. Thus, for a rapid screening or triage of patients, serological tests can be useful. Combined with malaria RDT and the interpretation of the hematological measurements, the serological detection of three arbovirus can first confirm the exposure of the population, thus conforming the circulation of DENV, CHIKV and ZIKV. Secondly in, patients with a recent acute febrile illness, the detection of IgM or NS1 Ag can provide a first screening and triage of the patients. Having these information's will contribute to fill the gap on the possible contribution of arbovirus on acute febrile illness, on the recognition of the circulation of these viruses in study setting.

Thus, the aim of this study was to determine frequency of malaria and/or DENV, CHIKV, ZIKV RDT positivity among children and adolescents with acute febrile illness in Libreville, the capital city of Gabon.

In sub-Saharan Africa, child mortality is strongly associated with infectious diseases, the most common symptom of which is fever [1]. Malaria remains among the top ten causes of death in many African countries, but non-malarial acute febrile illnesses (NMFIs) are also a common clinical presentation in both outpatient and inpatient settings [2]. Clinicians face challenges in the management of febrile illnesses due to the wide range of aetiologies and the similarity of symptoms observed between malaria and other infectious diseases.

Indeed, the differential diagnosis of the infectious agents responsible for fever is not commonly performed in many countries due to the lack of diagnostic tools or limited accessibility [3,4,18]. The presumptive treatment of patients with negative malaria results using antimalarial drugs or antibiotics is widespread ([5]). This practice contributes to the rapid emergence of drug-resistant strains and increases mortality among febrile patients because the true aetiology often remains untreated [19].

According to the *World Malaria Report 2023*, in 2022, almost 70% of febrile patients attending health centres benefited from parasitological confirmation by microscopy or rapid diagnostic tests (RDTs) prior to treatment [20]. In Gabon, where six sentinel sites for malaria surveillance have been established for the prompt management of febrile patients, each patient receives a free malaria diagnosis using microscopy or RDT. Data from the main sentinel site at Melen Hospital, which receives between 3,000 and 4,000 children annually, have shown a substantial reduction in

malaria cases. The frequency of positive blood smears or positive RDTs ranges between 20% and 45% [8,9,21]. Thus, 55% to 80% of febrile cases are non-malarial and should benefit from the detection of the true causative infectious agents.

In most malaria-endemic settings, sentinel sites are located in semi-urban and rural areas where, apart from malaria testing, haematological measurements and chest radiography, other diagnostic tools such as blood culture, procalcitonin, C-reactive protein, and molecular assays for the identification of viral agents are not always available. Identifying the causes of acute non-malarial fevers is therefore crucial, not only to improve patient management but also to strengthen surveillance systems for emerging pathogens and to guide the implementation of diagnostic and preventive tools [10].

A recent study conducted at the Melen Hospital sentinel site highlighted the high frequency of antibiotic or antimalarial prescription among patients with a negative malaria test [11]. Arthropod-borne viruses, also known as arboviruses, are among the predominant causative agents of acute febrile illness in areas where high temperatures, humidity, and poor sanitation favour the proliferation of mosquito vectors, particularly in Africa. Arboviruses are not routinely suspected or tested for by clinicians, even when malaria tests are negative [12]. Moreover, arboviral infections are often misdiagnosed as malaria due to the similarity in clinical presentation [13]. Even in the absence of specific or localised symptoms, the suspected aetiology of a non-malarial febrile illness is often bacterial infection [11].

In Gabon, arboviruses such as dengue virus (DENV), chikungunya virus (CHIKV), and Zika virus (ZIKV) have been circulating since 2007 and have been detected and reported during several outbreaks [14]. Two years ago, an outbreak was observed in Lambaréné [14]. Furthermore, all the conditions favourable to mosquito vector abundance are present in the capital city, Libreville, including unplanned urbanisation—particularly in overcrowded shanty towns—favourable climatic conditions, and poor waste management, with rubbish often stored close to dwellings. A high prevalence of arbovirus vectors, such as *Aedes* mosquitoes, as well as evidence of the continuous circulation of DENV, CHIKV, and ZIKV, have recently been reported [16,22].

The involvement of these viruses in acute febrile illness remains poorly understood in Gabon, even though they share many epidemiological and clinical characteristics with other non-severe acute febrile illnesses, including malaria. The diagnosis of these viral agents is confirmed by RT-PCR, which is not routinely available in most health centres across the country. Therefore, for rapid screening or triage of patients, serological tests can be useful. Combined with malaria RDTs and the interpretation of haematological parameters, the serological detection of the three arboviruses can first confirm population exposure thus confirming the circulation of DENV, CHIKV, and ZIKV and secondly, in patients with recent acute febrile illness, the detection of IgM or NS1 antigen can serve as an initial screening and triage tool. Having this information will help fill the gap regarding the possible contribution of arboviruses to acute febrile illnesses and improve recognition of their circulation in the study area.

Thus, the aim of this study was to determine the frequency of malaria and/or DENV, CHIKV, and ZIKV RDT positivity among children and adolescents presenting with acute febrile illness in Libreville, the capital city of Gabon.

Methodology

Study design and site

A cross-sectional study was conducted from July 2022 to December 2022 at the malaria sentinel site in Melen Regional Hospital, located at 12 km from Libreville, the capital city of Gabon. The main activity of this sentinel site consists in screening febrile patients for malaria before consultations at the hospital.

Study population

The study population was composed by children presenting at the sentinel site for malaria surveillance an acute febrile illness. Inclusion criteria were all outpatient children and adolescents with a fever or history of fever during the last 7 days, who agreed to participate in the study children's parents, or legal guardians gave a written informed consent and/or assent for participation. Patients who refused to sign the consent form and presenting severe illnesses were not included and managed according to the routine protocol.

Data collection

Children and parents were approached by the study team each morning and the project was presented and explained to them. The following information were recorded on a case report form: socio-demographics, temperature. After the physician physical examination, five ml of venous blood was collected for malaria diagnosis, arbovirus NS1 and/or IgM detection and hematological measurements.

Laboratory procedures

Malaria and arbovirus infection diagnosis

Each sample were systematically tested for malaria and arbovirus infections. Malaria diagnosis was performed using thick and thin blood smears as previously described [23].

We used IgM/IgG antibody or NS1 antigen RDTs of Biopanda Reagents Kit for the detection Dengue (DENV), ZIKA (ZIKV) and Chikungunya (CHIKV) detection. The Biopanda Rapid Test detects qualitatively NS1 antigen, IgG, and IgM antibodies of arbovirus in human whole blood, serum, or plasma samples. This test applies lateral flow immunochromatography and is a tool to assist in the diagnostic of arboviral infections.

Thus, the ZIKA combo Rapid test (RAPG-Zika-003) detects antibody and the NS1 antigen of the ZIKA virus. The RDTs for dengue (DENGUE IgG/IgM rapid test RAPG-DEN-001) and chikungunya (Chikungunya IgG/IgM Rapid test RAPG-CHK-001) were used for the detection of IgM. All tests and interpretation

of results were performed according to the manufacturer's instructions.

A recent suspected arboviral infection was defined as any acute febrile illness (fever<7 days) that tested positive by RDT with the detection of IgM and/or AgNS1 of ZIKV, and IgM of DENV and IgM of CHIKV, while a confirmed malaria case for patients with a positive blood smear. A suspected malaria and arboviruses co-infection was defined for any acute febrile illness with a positive blood smear and at least one positive arbovirus RDT result for both arbovirus and malaria infections.

Haematological parameter measurement

Haemoglobin and platelets levels were measured using a Sysmex XN 350 automate analyser. The definition of anaemia was based on the WHO criteria. Haemoglobin level of ≥11g/dL was considered as normal, 10-10,9 g/dL as mild, 5.0-9.9g/dL as moderate, and <5.0g/dL is regarded as severe malaria [24]. Thrombocytopenia was defined when the platelet count was below 150000/mm3.

Statistical analysis

All data were entered and recorded in an Excel sheet, continuous data are summarized as median (25e-75e interquartile range), and qualitative data as proportion. Differences across groups were analyzed using the Chi-squared test, Fisher's exact test, the Kruskall-Wallis test or Mann-Whitney test. All analyses were performed using Statview 5.0 software.

Ethics considerations

This study was approved by the regulatory authority the Ministry of Health (0072/P/COPIL-CS-COVID-19), the Director of Medical Affairs of the Melen Regional Hospital and the head of pediatrics department. Written informed consent was obtained from all parent or legal guardians of children and adolescent, these letters also provided their assent to participate.

Definition

- A suspected recent arboviral infection was defined for any RDT positive test (detection of IgM and/or NS1 Antigen)
- Malaria was defined for any patient with a positive microscopic test.
- A suspected co-infection was defined when *Plasmodium sp* and IgM or NS1 Ag were detected in the same sample.

Results

Characteristics of study population

A total of 524 acute febrile cases were included, their median age was 60 [24.0-132.0] months, and the male/female sex ratio was 1.06. More than half (54.4%; n=285/524) were children over 5 years old, and less than 20% slept regularly under a mosquito net. Residence was recorded for 497 patients, most of them (64.8%; n=322/497) lived in the peri urban site. (Table 1).

Seroprevalence of VZIK, VDEN, VCHIK and frequency of malaria

The prevalence of malaria was 38.0% (n=199/524), *P. falciparum* was the only plasmodial species detected. Arbovirus RDT positive rate was 39.1% (n=205/524). Overall, 184 (35.1%) participants were tested positive for recent Zika virus infection, 86 for NS1Antigen, 72 for ZIKV specific IgM and 26 for both NS1 Antigen and ZIKV specific IgM. DENV specific IgM was detected in 42 (8.0%) patients while CHIKV IgM seropositivity rate was 0.6% (n=3). Globally considering the number of positive tests, 187 (35.7%) patients were probably infected by one pathogen, 92 (17.6%) by two and 19 (3.6%) by three pathogens (Figure 1).

Table 1: General characteristics of study population.

Variables		N	%
Gender			
	Male	271	51.7
	Female	253	48.3
Age group in years	< 5	238	45.4
	5-10	135	25.8
	11-15	73	13.9
	>15	78	14.9
Living area (N=497)			
	Urban	140	28.2
	Peri urban	322	64.8
	Rural	35	7.0
ITN use		79	15.2
Fever duration in days		3.0	[1.0-4.0]
T° [IQR]		38.0	[37.1-38.7]

ITN: Insecticide treated net, T°: Temperature

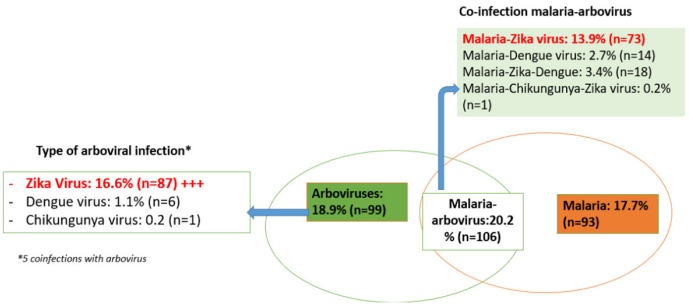

Figure 1: Seroprevalence of arboviruses and frequency of malaria, mono and co-infection.

Suspected coinfections were frequent, Malaria-Zika virus predominated (17.3%; n=92) followed by Malaria-VDENG (5.9%; n=31) (Figure 1).

The *Plasmodium falciparum* parasite density was significantly higher in case of coinfection (10325 [894-50540] *vs* 882 [179-14425] p/µL, *p< 0.01*).

Malaria and arbovirus seropositivity rates according to the demographic and biological data

No association was found between the presence of single or coinfection and gender (p=0.20), median temperature (p=0.83)

and median fever duration (p=0.15) (Table 2). Nevertheless, a trend toward a higher rate of patients with a fever duration of more than 3 days was observed in participants with either arbovirus (57.0%), malaria (51.8%), malaria-arbovirus (61.1%) compared to those without arbovirus or *P.falciparum* infection (44.6%) (p=0.07). There was a strong association with age. Indeed, patients with suspected malaria-arbovirus coinfection were significantly older than patients with single infections (Table 2). As observed in Table 2, all participants without arbovirus (48[21-120] months), all those with other causes of fever (36[14.4-96.0] months) were significantly younger than all those with *P. falciparum* single or coinfection (60[24-126] months) or a suspected recent arbovirus infection (72[31-144] months) (p=0.03). Sleeping under a bednet was common in the groups of patients with negative test result (17.3%; n=39/225) or those with arbovirus positive RDT only (19.2%; n=19/99) compared to *P. falciparum* infected patients (10.7%; n=21/197) (p<0.01) (Table 2).

The median Hb level was different according to the type of infection; it was the lowest in case of suspected co-infection (p=0.03). Likewise, a trend toward a higher frequency of moderate to severe anemia (52.1%; n=50/96) was also noticed in the group of participants with both malaria and RDT positive results (Table 2).

In the same way, the median platelet count was significantly lower in case of coinfection in that situation, patients were at 8 fold at higher risk of having thrombocytopenia (8.7 [5.3-14.2]; *p<0.01*) (Table 2).

Seroprevalence of arboviruses and frequency of malaria according to the age group and residence site

While the rates of arbovirus RDT positivity infections (19.3%, n=46), malaria (18.9%; n=45) and malaria-arbovirus RDT positivity (13.9%; n=33) were comparable among children younger than 5 years old (*p=0.47*). Suspected coinfection predominated among the in 5-10 years old (27.4% vs 14.8% and 17.8%) for suspected recent arbovirus infection and malaria respectively (*p<0.01*); and 11-15 years old group (35.6% vs 17.8% and 20.5% respectively; *p<0.01*) (Figure 2a). The frequency of participants with detected arbovirus IgM or NS1 Antigen was significantly higher in participants older than 15 years old (25.6%; p<0.01) (Figure 2a).

Likewise, malaria (18.6%; n=60/322), arbovirus IgM and/or NS1Ag (18.3%; n=59) and suspected coinfection (20.2%; n=65) rates were comparable in the peri urban site. The figure 2b shows that single malaria was more frequent in rural areas, where the lowest prevalence of arbovirus RDT positivity (14.3%; n=5/35) was also recorded, while the highest one (24.3%; n=34/111) was recorded in the urban area (*p<0.01*). The coinfection rates were comparable between sites, ranging from 22.9% in the rural site to 18.6% in Libreville, the urban site. (Figure 2b).

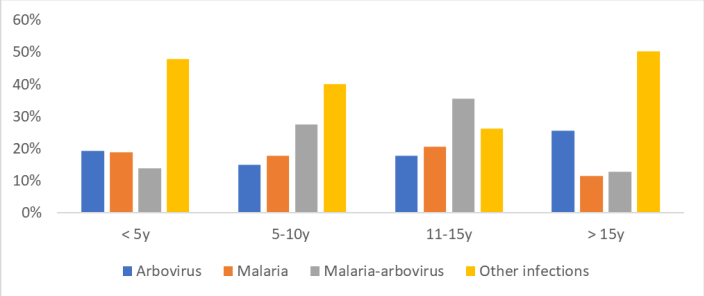

Figure 2a: Frequency of infections according to the age group.

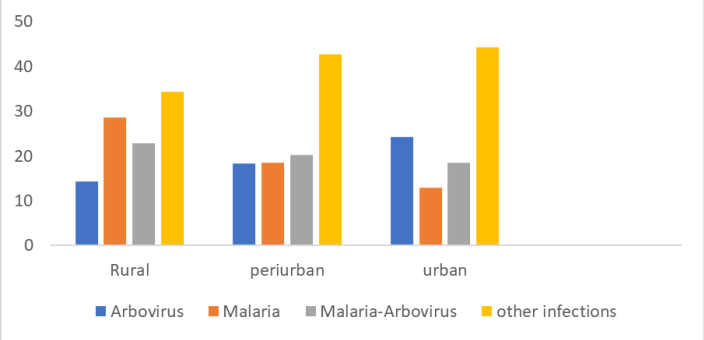

Figure 2b: Frequency of infections according to the residence site.

Number of pathogens and studied variable

Residents from the rural sites were more likely to carry one pathogen (n=15/35; 42.9%) compared to those from the urban (35.7%) and the peri urban (36.4%) site. Only one (0.7%) participant living in Libreville was tested positive for three pathogens. This was the case for 14 (4.4%) out of the 322 inhabitants of the semi urban site. All these differences were not statistically significant. Furthermore, none of the participant with a least three detected pathogens slept under an ITN, those with no detected pathogen more often used it (17.3%; n=39/225).

Table 2: Frequency and median of studied variables according to the type of infections.

Variables	Malaria	ArboV IgM or AgNS1	ArboV-Malaria	Other	*p*
Age, [IQR]	54[23.0-120.0]	60 [24.0-156.0]	84 [36.0-138.0]	36.0[14.4-96.0]	<0.01
T° [IQR]	38.0[37.0-39.0]	38.0 [37.0-38.5]	38.0 [7.1-39.0]	38.0[37.2-38.5]	0.83
Fever duration,	3.0[1.0-4.0]	3.0 [1.0-5.0]	3.0[2.0-4.0]	2.0[1.0-3.0]	0.15
ITN use, n (%)	11(11.8)	19 (19.2)	10 (9.6)	39(17.3)	<0.01
Hb, [IQR]	10.1[7.9-11.3]	10.4 [8.5-11.4]	9.8 [8.0-11.0]	10.5[8.9-11.6]	0.03
Severe anemia, n (%)	21 (24.1)	17 (19.0)	23 (24.0)	31(15.7)	0.06
Platelets, [IQR]	195[11.8-276]	278 [168.8-345.3]	115 [69.0-199.0]	296[218-380]	<0.01
Thrombocytopenia, n(%)	28 (32.2)	16 (18.6)	62 (64.6)	21(10.4)	<0.0001

IQR: interquartile range, T°: Température

The median Hb level was significantly lower in participants with two pathogens detected (9.5 [7.8-11.0] g/dL; p=0.01). Consistent with this finding, anemia rate was higher among infected cases (87.7%; n=236/269; OR: 1.6 [1.0-2.6]; p=0.05) compared to those with no pathogens detected (82.1%; n=36:201; p=0.02).

Thrombocytopenia was also more likely be associated with the number of detected pathogens (p<0.01). While only 10.4% (n=21/201) of persons with no detected infection had low platelet count, thrombocytopenia rate was 26.2%, 56.0% and 88.2% in patients positive for one, two and three pathogens respectively.

Discussion

The aim of this study was not to assess the effectiveness of these RDTs, but rather to analyze the added value of introducing other fever discrimination tools, to help practitioners' better guidance for practitioners in adapting management. The aim is to rationalize antibiotic prescribing in patients with a negative blood smear. In addition, patients with less than 7 days of fever were included. According to the kinetics of antibodies to arboviruses, it is possible to consider, the presence of IgM and/or the presence of IgM and/or NS1 antigen as an indicator of current or recent infection. It has revealed the high prevalence of arboviruses, with significant circulation of arboviruses in rural, urban and semi-urban areas in Gabon.

This study revealed high prevalence's of malaria (38.9%) and arbovirus (39.1%), while the malaria-arbovirus coinfection was 20.2%, with ZIKV being the most prevalent arbovirus in the mono or coinfection with malaria. According to the temperature no association was found between the presence of single or coinfection. Sow and *al*, reported twice the frequency of co-infections in Kedougou in southeastern Senegal, that concurrent infections of malaria and arbovirus were detected in more than 40% patients and ZIKV was the most prevalent arbovirus in the co-infection with malaria, but this author reported a high-grade fever (>40°C) was associated with patient having dual infection compared to this with single infection [12]. These results can be explained by the concurrent circulation of vector borne diseases such as malaria and arthropod-borne viral diseases (arbovirus) in tropical regions [25] as is the case in Gabon and Senegal. An individual can become co-infected when bitten by a mosquito harboring both the malaria parasite and an arbovirus. In fact, malaria vectors have previously been found infected with arbovirus, emphasizing the plausibility of the dual infection of malaria parasites and an arbovirus within the same mosquito [12]. *Anopheles (An.) funestus* and *An. gambiae* are a major malaria vector in Gabon [26], in Kedougou, Diallo and al have been found that *An.funestus* was infected with CHIK and YFV. It would be necessary to carry out entomological studies to confirm this co-infection parasite-arbovirus in these vectors in our country. Another mechanism by which a patient may become infected by both *Plasmodium spp,* and arbovirus is consecutive bites from two different infected mosquitoes. In our study, children aged 5 to 15 years had significantly more co-infection than malaria or arbovirus single. This may be because children spend more time outdoors and are not adequately protected against infectious mosquito bites (*Anopheles spp* for malaria and *Aedes spp* for arboviruses). These mosquitoes are particularly active at dusk when children are often outside playing or walking around. Secondly, this human-vector contact could also be explained by the cohabitation of these two vectors in the same environment but also have the same means of multiplication and feeding. Moreover, As far as malaria is concerned, prevention strategies are more focused on children under 5 years of age, they are more protected by being more covered and sleeping under ITNs, this has led to the notion of epidemiological transition observed in Gabon since 2008 where children over 5 years of age are more likely to get malaria because they are less protected than younger children [7,21,27].

The higher prevalence of malaria found in our study is comparable to that reported by Lendongo-Wombo et al., in the southeastern city of Franceville-Gabon [28]; but also, in accordance with global data that showed an increase in malaria cases [29]. These results could be explained by the lack of awareness campaigns and the absence of vector control methods during the time of COVID-19 pandemic.

The prevalence of anti-arboviral IgM (ZIKV and DENV) was high among febrile patients consulting at the malaria sentinel site. These infections, although endemic in our context, are not often mentioned by health personnel and are often misdiagnosed as malaria because of the similarity of symptoms. Presumptive treatment of fever with antimalarial is widely practiced reducing malaria attributable mortality. This practice means that ill patients may be inappropriately treated, particularly where rapid diagnosis test kits are not readily available, or if the opportunity to test for arboviral infections is missed [30]. In this study antigen NS1 and specific IgM ZIKV was the most prevalence (35.1%) arbovirus detected by RDT followed by DENV specific IgM (8.0%). ZIKV is an emerging arthropod-borne virus related to the DENV; both viruses have similar epidemiology and transmission cycles in urban environments [31]. These arboviruses have been circulating in Gabon since 2007, with the CHIKV and DENV outbreaks [14]. Other authors in Gabon, notably in Lambaréné, have reported the presence of these viruses in serum samples from febrile patients between 2020 to 2021, although the prevalence is significantly lower than those reported in our study [22]. On the other hand, high frequencies of ZIKV (60.7%); CHIKV (61.2%) and DENV (40.3%) in a seroprevalence study from healthy individuals were reported by the same team [22]. This could be explained by the methodology used, the study area and the population studied. Patients infected with arboviruses was recorded more frequently in the urban areas, this could be due to anarchic urbanization, because urban areas are often densely populated, thus offering a favorable habitat for mosquito breeding. Several factors such as stagnant water (natural or artificial breeding sites), waste and poorly managed landfills and poorly maintained green spaces, but also environmental conditions such as heat and humidity can favor the survival of mosquitoes and therefore the transmission of these pathogens. Urban areas may offer favorable conditions for the transmission of arboviruses, which would contribute to the increase in cases of vector-borne diseases in these areas. It is therefore

important to set up programs for the prevention and control of these diseases through monitoring, education, and awareness of the population, but also the promotion of environmental hygiene.

Laboratory test results showed the median Hb level was significantly lower in patient with two pathogens detected and that thrombocytopenia rate increased with the number of detected pathogens. Thrombocytopenia has also been commonly reported in cases of dengue [32] and it is a laboratory finding was associated during Zika virus infection reported by others authors; and there are correlation between low platelet counts and malaria [33]. The three pathogens frequently associated were *Plasmodium spp*, DENV and ZIKV; this explains why thrombocytopenia was aggravated in patients with these three pathogens.

This study highlights similar prevalence of ZIKV (16.6%) and malaria (17.7%) in acute febrile illness; ZIKV infection usually manifests with nonspecific clinical symptoms; therefore, it most misdiagnosed as malaria or dengue. It is important to diagnose this infection, because ZIKV infection is associated with morbidity, especially in pregnant women [34]. Moreover, serological cross-reactivity between ZIKV and DENV has been observed in earlier studies [34]. This cross-reactivity leads to the misdiagnosis of dengue because of the similarity of the clinical symptoms in addition to serological cross-reactivity. It is crucial to implement an effective clinical and diagnostic management strategy, especially in cases of infections by emerging arthropod-borne viruses. In this study we detected these viruses using immunochromatography tests, this allowed us to make the epidemiology of pathogens circulating in malaria site surveillance. Nevertheless, Patient sera were conserved to confirm this data from RT-PCR for the detection of ZIKV, DENV and CHIKV genome and ELISA serology.

Conclusion

Therefore, the study highlights the importance of establishing a specific diagnosis of malaria and arbovirus infections among patients with an acute febrile illness, especially in our context where *Plasmodium spp* and arboviruses co-circulating. It is essential for public health systems to be able to better manage these diseases and set up a surveillance program for these viruses with epidemic potential.

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