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Research Article

Mosquito-borne Disease: A Review of the Possible Synergism Between Arboviral Infection and *Plasmodium* Infection in West Africa, Nigeria

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Abstract

Background: Mosquitoes are the most prevalent insect vectors in sub-Saharan Africa. These vectors are the leading cause of acute febrile infections within these regions and also a reservoir for many other microorganisms. This led to misdiagnosis and comorbidity with other diseases like Zika virus, dengue fever, and Japanese encephalitis infections. Due to various limitations of the Nigerian public health system, co-infections are not accurately assessed, and outbreaks of arboviral diseases are poorly reported and recorded.

Aims: We aim to offer an evidence-based approach to questions concerning the high mortality rate reported in cases of Malaria, especially in children. These reviewed techniques used in diagnosing malaria, proving it crude nature as an aid to misdiagnosis of malaria for viral diseases that shares overlapping symptoms**.** we described the different forms of interactions of arboviruses and *Plasmodium* in insect vectors, and indicated possible synergies. We analyzed various sero-epidemiological models that could aid efficient diagnosis and proposed the best technique for adoption.

Methods: A review of previous studies on the most prevalent febrile diseases in West Africa, Nigeria was conducted by consulting literatures from PubMed, Africa Journals Online, Google Scholar, and other databases to source studies within this niche in previous years. Relevant keywords such as mosquitoes, *Plasmodium* interaction, serological diagnosis, clinical signs of mosquitoes were used.

Result: The various publications consulted highlighted the possibilities and cases of malaria co-infection with several zoonotic arboviruses. There is good data to support the fact that arboviral infections have often been misdiagnosed as malaria and, in frequent cases, resulted in death reported as malaria mortality. Studies and findings on efficiently preventing misdiagnosis have been reported and discussed in various clinical trials, as presented in the reviewed articles.

Conclusion: The effective use of polymerase chain reactions (PCR, nested PCR, RT-PCR) as a serological model in malaria diagnosis is strongly recommended to completely exclude cases of arboviral infection in the diagnosis of malaria. In addition, adopting sero-epidemiological models will help forecast outbreaks of arboviral infections so that appropriate preventive measures are taken.

Keywords: Arbovirus; Malaria; Co-infection; RDT; PCV; *Plasmodium*; Mosquito; Dengue; Africa; Nigeria

Abbreviations

WHO: World Health Organization; TFM: Thick Film Microscopy; RDT: Rapid Diagnostic Test; PCR: Polymerase Chain Reaction; RNA: Ribonucleic Acid; RBC: Red Blood Cell; DENV2: Dengue Virus Type 2; JEV: Japanese Encephalitis Virus; CHK: Chikungaya; DEN: Dengue; IgM: Immunoglobulin M; IgG: Immunoglobulin G; TBS: Thick Blood Smear; ELISA: Enzyme-linked Immunosorbent Assay; RT-PCR: Real-time Polymerase Chain Reaction; ICS: Immunochromatographic Strip; MRDTS: Malaria Rapid diagnostic tests; HRP: Histidine-rich Protein; pHRP: *Plasmodium* Histidine-rich Protein; pLDH: *Plasmodium* Lactate Dehydrogenase; NAAT: Nucleic Acid Amplification Test

Introduction

According to the Journal of Egyptian Society of Parasitology, mosquitoes are undoubtedly the medically most important arthropod vectors of disease. The maintenance and transmission of various pathogens that cause malaria, lymphatic filariasis, and other numerous viral infections are absolutely dependent on the availability of competent mosquito vectors [1].

Malaria causes high morbidity and mortality in sub-Saharan Africa, especially Nigeria [2,3]. However, as the symptoms of malaria overlap with other tropical diseases, there is a hindrance to diagnostic efficiency using the clinical approach [4]. This compromise negatively impacts antimalarial drug use, particularly in children [5,6], and has resulted in death [7]. Previously, the World Health Organization (WHO) had recommended the use of presumptive diagnosis as a preliminary treatment in uncomplicated cases of malaria due to cost and time limitations in the time-consuming diagnosis [2,3]; unfortunately, this decision proved costly [4].

According to a research study in the Department of Medical Microbiology and Parasitology to determine the degree of sensitivity of various laboratory diagnostic methods (thick film microscopy (TFM), rapid diagnostic test (RDT), and polymerase chain reaction (PCR)): 217 children were admitted based on clinical diagnoses with malaria, and tests were conducted. 106 (48.8%) were positive by TFM, 84 (38.7%) by RDT and 125 (57.6%) by PCR. Using a reference method generated from the three diagnostic procedures, 71 (32.7%) patients were found to be truly infected, 90 (41.5%) were truly uninfected, and 56 (25.8%) were misidentified as infectious or non-infectious identified [8].

Recent advances in science provide good data supporting the treatment of malaria based on clinical diagnosis as a threat to overtreatment in people with low-grade parasitemia [9]. Accurate diagnosis is required as mosquitoes are also reservoirs for many other pathogens such as arbovirus [10].

Arboviruses, also known as arthropod-borne viruses, refer to a diverse group of viruses transmitted via mosquitoes, ticks, or sandflies. They are important causes of human diseases almost worldwide. This group of viruses belongs to the families *Togaviridae, Flaviviridae, Bunyaviridae, and Reoviridae*. These families have similar RNA genomes that allow for mutations that enable them to adapt to changing environments or host conditions. These viruses are unique because they require vectors for biological transmission to humans [11].

Arboviruses are maintained in nature primarily or to a significant extent by biological transmission between susceptible vertebrate hosts by hematophagous arthropods or through transovarial and possibly venereal transmission in arthropods; the viruses multiply and produce viremia in the vertebrates, multiply in the tissues of arthropods and, after a period of extrinsic incubation, are transmitted to new vertebrates by the bites of arthropods [14]. All arboviruses circulate among wildlife, and many cause diseases after spillover transmission to humans and agriculturally important domestic animals that are incidental or dead-end hosts [12]. Typical manifestations of an arbovirus infection range from asymptomatic to meningitis or encephalitis to death [13].

Due to the limited facilities in Nigeria and Africa, there are few reports on the possible co-infection between arboviruses and malaria. These health diseases are significantly under-reported due to limited facilities to diagnose arboviruses [15]. In Nigeria, malaria is consistently reported as a significant public health infection, while reports indicate that arboviruses might be leading the boat of morbidity and mortality in such cases [15]. Arboviruses are not systematically investigated and are generally only considered by clinicians, at best, when samples test negative for malaria, while studies have shown that a percentage of parasitemia is required for malaria infection. Consequently, this may result in a lack of appropriate preventive measures against arbovirus disease outbreaks [15].

Vector-host interactions

Plasmodium-mosquito interactions

Plasmodium is a genus of unicellular eukaryotes obligate parasites of vertebrates and insects. The malaria parasite's life cycle is extremely complex and is shared between two hosts – humans and the female Anopheles mosquito [16]. Egg production by female mosquitoes requires a blood meal. In principle, the feeding and breeding process can be repeated every 3-4 days for the duration of the female mosquito's lifespan. *Plasmodium* uses this cyclic feeding behavior to transmit from one vertebrate host to the next. The vast majority of parasites circulating in an infected human are asexually dividing merozoites. These parasites play no role in the transmission and die after being ingested by the mosquito [16]. However, a small fraction of circulating merozoites enter a terminal differentiation pathway (developmental switch) that culminates in the production of male and female gametocytes. These non-dividing sex forms are solely responsible for establishing the parasite life cycle in the mosquito vector and ultimately for transmission to a new vertebrate host [16].

Female Anopheles mosquitoes become infected after taking a blood meal from humans carrying *Plasmodium* sex stages, male and female gametocytes. Upon entering the midgut lumen, the male and female gametocytes differentiate into mature extracellular male and female gametes, respectively. The product of fertilization (sexual reproduction), the zygote, transforms into an invasive, motile ookinete within 24 hours [17].

Once formed, the ookinete migrates from the central blood bolus in the midgut and faces one or two critical physical barriers that block its path to colonizing the midgut. The first is a de novo formed chitin and protein-rich peritrophic matrix that variably assembles in response to feeding surrounding the blood bolus. The second is the epithelium of the midgut itself. The ookinete traverses the intestinal epithelium once these significant barriers are overcome [18].

After crossing the intestinal epithelium, the ookinete develops into an oocyst, which undergoes mitosis over the following week and releases sporozoites into the hemolymph. Sporozoites reach the salivary glands about 10-14 days after ingesting the infected blood meal. The mosquito then becomes infectious and will inject parasites into humans with its saliva during subsequent bites for the rest of its life. Ingestion of an infectious blood meal will result in malaria transmission only if the parasite makes it through bottlenecks in the gut and salivary glands and if the mosquito bites humans after the extrinsic incubation period, and whether the time it takes for the parasite to become infectious is reached [19].

Plasmodium-human interactions

The malaria parasite has a complex heteroxenous life cycle involving humans [20]. The six species of malaria parasites that infect humans include *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale wallickeri, Plasmodium ovale curtisi, Plasmodium malariae, and Plasmodium knowlesi* [21]. *Plasmodium vivax* is the most widespread species, and *Plasmodium falciparum* is the deadliest to humans [22]. The parasite is injected with the saliva during mosquito feeding and first undergoes a round of merogony in the liver, followed by several rounds of merogony in the erythrocytes. Gametogony begins in the erythrocytes of the vertebrate host and is completed within the mosquito, where sporogony takes place. This life cycle exhibits the general features of other apicomplexan parasites, characterized by asexual replication and the formation of invasive stages with typical apical organelles [23].

Liver stage

Plasmodium sporozoites are transmitted by female anopheline mosquitoes, which inoculate the parasites predominantly into the avascular portion of the skin while probing for a blood meal [24]. The sporozoites migrate through blood vessels to the liver (30-60 minutes) and then transverse liver sinusoidal endothelial cells or Kupffer cells to infect hepatocytes [22]. As with all *Apicomplexa*, the apical organelles facilitate entry into the host cell [23]. Those not blocked by antibodies invade the liver and begin dividing within hepatocytes [20].

The sporozoites then undergo asexual replication leading to the production of schizonts. The hepatocytes burst after five days at the earliest and release merozoites into the bloodstream [24].

Blood stage

The merozoites released into the bloodstream then invade the red blood cells. Within the erythrocyte, the parasite grows and multiplies cyclically. In each cycle of its growth within the erythrocyte, the parasite infects the erythrocyte as a merozoite,

builds a vacuole around itself as a ring stage, matures into a metabolically active trophozoite, and replicates as a schizont [16].

New infective merozoites are formed by mitosis in the schizont stages, after which the RBCs rupture and release the daughter merozoites into the circulation to establish fresh red blood cell infection [16]. As the number of infected red blood cells increases, some parasites develop into sexual forms, the micro- (male) and macro- (female) gametocytes. Gametocytes develop through five distinct stages, with only mature stage V parasites can undergo sexual reproduction when ingested by a feeding mosquito taking a blood meal [26].

Virus-mosquito interactions

Infection of an arthropod vector, e.g., mosquitoes, is typically required to maintain the transmission cycle of arboviruses [27]. A naïve mosquito acquires these viruses from an infected host through blood meals [28].

After ingestion of a viremic blood meal from a vertebrate host, the infection begins in the mosquito's midgut, during which virions come close to the epithelial cell lining and need to penetrate through the microvilli into the epithelial cells before the blood meal is surrounded by the peritrophic matrix, a chitinous sac formed during the blood digestion that is secreted by the epithelium of the midgut into the intestinal lumen [29].

The infection patterns of the midgut epithelial cells vary depending on the virus-mosquito-species combination. For example, dengue virus type 2 (DENV2) was not detected in the anterior portion of the midgut of *Aedes albopictus*, while Japanese encephalitis virus (JEV) infected the entire midgut of *Culex tritaeniorhynchus*. Only a small number of cells appear to be receptive to viral infection at this stage. Once the virus has entered an epithelial cell of the midgut, viral RNA replication occurs at the endoplasmic reticulum membrane. However, the site of viral maturation may vary depending on the virus and mosquito species [29].

Virions then enter the hemolymph through the basal lamina and are distributed throughout the mosquito body, where the virus amplifies (replicates) in secondary tissues. The presence of the virus in the salivary gland and ducts results in horizontal transmission to an uninfected vertebrate host while the mosquito is ingesting a blood meal [29]. To be transmitted to the next vertebrate host, the virus must infect the salivary glands [30] and sometimes the reproductive tissues for vertical transmission to the offspring.

Synergism between malaria and arboviruses

Overlapping symptoms between *Plasmodium* and arboviral infection in humans

A recent study surveyed the incidence of malaria and some common arboviruses in Africa, including chikungunya and dengue infections, in some suspected patients at Simawa Health Centre, Ogun State, Nigeria. The data collection included blood samples from 60 febrile patients (age 3-70 years) between April and May 2014. The Rapid Diagnostic Test (RDT) was used to detect the presence of Chikungunya (CHK) antibodies (IgM), Dengue (DEN) virus, and antibodies (NS1, IgM, and IgG) and malaria parasites *Plasmodium falciparum* and *Plasmodium vivax*. Malaria confirmatory tests were performed by microscopy and polymerase chain reaction (PCR). The result showed that mosquito-borne infections were observed in 63% (38) of the patients. The prevalence of CHK, DEN, and malaria infection individually was 11%, 0%, and 63%, respectively, while malaria with either CHK or DEN infection was 24% (9) and 3% (1), respectively. Malaria microscopic confirmation was positive in 94% (32) of malaria RDT samples, PCR successfully analyzed 50% (17). The study reports the presence of some arboviral infections with similar symptoms during co-infection with malaria [15]. This report further supports the demand for the proper diagnosis of such infectious diseases.

Presentations of *Plasmodium* and arboviral infection

Given the similar clinical presentations of arboviral infections and malaria and the absence of pathognomonic signs and symptoms for either disease, it is difficult to determine which pathogen is responsible for the clinical signs and symptoms in the co-infections [31]. In certain cases, patients dual-infected with malaria parasites and arboviruses have had relatively mild and nonspecific syndromes, including fever, headache, and vomiting [31]. Because the clinical features of arbovirus infections are generally nonspecific, most healthcare providers misdiagnose them as malaria or other febrile diseases [31]. Symptom severity associated with arboviral infections ranges from no symptoms to mild flu-like symptoms to very severe symptoms [32]. Most

infections caused by arboviruses are asymptomatic. However, symptoms can range from mild flu-like illness to severe encephalitis when they do. Clinical presentations are divided into two groups:

- Neuroinvasive
- Non-neuroinvasive

Neuroinvasive diseases have symptoms that suggest the disease can infect the nervous system, while the latter has features that do not indicate neural infiltration [32]. Neuroinvasive arboviral conditions cause meningitis or encephalitis, accompanied by rapid onset fever and symptoms such as headache, stiff neck, muscle pain (myalgia), disorientation, weak limbs, and seizures [32]. However, non-neuroinvasive arboviral diseases differ slightly in their symptoms [32]. The nervous system is unaffected, so no altered mental states are seen. However, non-neuroinvasive arboviruses present the following symptoms in association with a rapid onset fever; Headache, muscle pain (myalgia), joint pain, upset stomach, nausea, vomiting or diarrhea, and skin rash.

Symptoms	Arbovirus-infected patients n (%)	Co-infected patients n (%)	p value
Headache	16 (76)	19 (95)	0.18
Eye pain	00 (00)	02 (10)	0.23
Myalgia	12 (57)	06 (30)	0.12
Arthralgia	13 (62)	06 (30)	0.06
Rash	01 (05)	00 (00)	1.00
Vomiting	07 (33)	10 (50)	0.35
Diarrhoea	01 (05)	04 (20)	0.18
Chills	11 (52)	11 (55)	1.00
Cough	07 (33)	04 (20)	0.48

Table 1: Similar symptoms seen in patients infected with an arboviral infection and in patients infected with both malaria and arbovirus infections.

Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4730666/table/Tab3/?report=objectonly

Clinical effects of misdiagnosing arboviruses

In an article published in the Journal of pediatrics, the author acknowledged that dengue fever is a severe public health problem, particularly in tropical and subtropical regions, the most common arbovirus worldwide [33]. In the past five years, the incidence of dengue fever has increased 30-fold, with the highest rates in young adults and the higher mortality rates in the elderly; However, children represent a special group as they are at higher risk of developing the severe form of the disease [33].

Diagnostic steps in differentiating malaria and arboviral infection

The diagnosis of *Plasmodium* and arboviral infections share similar serology and overlapping manifestations, making clinical diagnosis very difficult [34]. Scientists are continuously working to develop a successful control and elimination technique that reduces complications from misdiagnosis [35]. In particular, the primary diagnostic approach for malaria infections is through the use of thick blood smear (TBS) and Rapid Diagnostic Tests (RDT), while arbovirus infections are tested by IgM antibody detection (ELISA) and RT-PCR assays [31].

Current malaria diagnostic options

Microscopy

Blood smear microscopic examination is a standard test performed on blood from an EDTA bottle if not obtained directly from a venipuncture. Blood is applied to a clean, grease-free slide, air dried (thick film), and fixed in 70% methanol (thin film). It is then stained with 10% Giemsa stain for 30 minutes, rinsed, air-dried, and then viewed under the microscope [36]. This method detects parasitemia by lysing the blood, which allows the release of different stages of malaria parasites – trophozoites, gametocytes, and schizonts [35]. However, this method is limited as it can only detect about 50-200 parasites per liter of blood [37,38]. Thin smears are used to detect the morphology of the parasite species and are prepared by spreading a drop of blood across a slide to create a feathered edge containing a single cell layer [39].

Figure 1: Microscopy of a thin smear of P. falciparum-infected erythrocytes stained with Giemsa. (A) A red blood cell infected with two malaria parasites in the “ring” stage as seen under a microscope at 100× oil immersion. (B) A normal uninfected red blood cell. (C) A normal leukocyte.

Link: tropicalmed-05-00102-g002.png

Disadvantages and Limitations of Microscopy in the diagnosis of malaria

Despite the simplicity of this method, the disadvantages of diagnosing malaria with this method are enormous [40]. One includes the inability of the techniques to adapt to rural settings, where most malaria patients seek healthcare with little basic medical infrastructure [41]. The diagnostic specificity of this technique is also sub-par compared to other techniques. However, the sensitivity varies greatly from region to region; by the relative skill of smear-readers and the magnitude of parasitemia. In the best of conditions, it's no better than average [6].

Malaria rapid diagnostic test (MRDT)

MRDTs are mostly kits for the detection of *Plasmodium* antigens. They involve using an immunochromatographic strip (ICS) [41]. The results were recorded by line depiction on the strip surface [41]. Current MRDT methods are specified to detect three different types of Plasmodia antigens [42]. The respective antigens; *Plasmodium* histidine-rich protein (HRP) 2 (pHRP-2) may be specific for *P. falciparum or P. vivax*. The second is *Plasmodium* lactate dehydrogenase (pLDH), which may be specific to P. falciparum or *P. vivax* or maybe a variant common to all *Plasmodium* species. The last is *Plasmodium* aldolase, which is pan-specific. By combining the detection of these three antigens in an immunochromatographic strip test (ICS), MRDTs can be used to detect any malaria species*; P. falciparum* alone, *P. vivax* alone, or any combination has been developed (Table 2) [42].

Types	Description
1	HRP-2 (*Plasmodium falciparum* specific)
2	HRP-2 (*P. falciparum* specific) and aldolase (panspecific)
3	HRP-2 (*P. falciparum* specific) and pLDH (panspecific)
4	pLDH (*P. falciparum* specific) and pLDH (panspecific)
5	pLDH (*P. falciparum* specific) and pLDH (*Plasmodium vivax* specific)
6	HRP-2 (*P. falciparum* specific), pLDH (panspecific), and pLDH (*P. vivax* specific)
7	Aldolase (panspecific)

Table 2: Types of Malaria Rapid diagnostic test.ns: HRP: Histidine-Rich Protein; pLDH: Plasmodium Lactate Dehydrogenase.

Modified from reference [31]. Source: Oxford Academic.

Disadvantages and limitations of the malaria rapid diagnostic test (MRDT) in diagnosing malaria

Although this diagnostic method is very effective and does not require skilled personnel to handle and manipulate, the level of sensitivity and specificity during co-infection is relatively low. In addition, it does not show low-level parasitemia [43]. This led to the need for a more accurate diagnostic method such as a serological approach and molecular-based techniques such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) to detect malaria [44]. However, these adopted techniques are standardized for diagnosing arboviral infections [45]. If used successfully, errors caused by a misdiagnosis of arboviral malaria diseases can be prevented and contained. In addition, many new diagnostic options for malaria are being developed and tested [35].

Polymerase chain reaction (PCR)

PCR-based tests are uniquely helpful in identifying asymptomatic and submicroscopic patients who miss microscopy and RDTs [11]. These procedures identify the presence of malaria genes in a blood sample. It includes the various use of antigen chain reactions such as nested PCR, real-time multiplex PCR, and reverse transcriptase PCR [48]. The sensitivity and specificity for the different types of PCR range from 98% to 100% and 88% to 94%, respectively, when microscopy was used as the gold standard [47].

Limitations of the polymerase chain reaction (PCR) in the diagnosis of malaria

Although PCR-based tests can be used qualitatively to test for *Plasmodium* antigens in initially suspected cases of malaria and parasitic species; However, despite advances in PCR, it does not show the extent of parasitemia [47].

Current arbovirus diagnostic options

Antibody detection (ELISA)

The antibody test detects targeted arboviral antibodies in response to infection. There are mainly two classes.

- **IgM antibodies:** Performed on symptomatic people's blood or cerebrospinal fluid [48]. They are produced first and are present within 1-2 weeks of infection. The blood values rise for a few weeks and then subside. After a few months, the IgM antibodies fall below the detection limit.

- **IgG antibodies:** Produced after IgM antibodies. Typically, the level rises with an acute infection, stabilizes, and persists long-term. IgG tests may be ordered after IgM testing to help diagnose a recent or previous arbovirus infection. Sometimes, testing is done by collecting two samples 2 to 4 weeks apart (acute and convalescent samples) to determine whether antibodies are from a recent or past infection [48].

Disadvantages and limitations of antibody detection (ELISA) in the diagnosis of arboviral infections

Antibody tests are often performed with similar viruses, so this method is not 100% accurate [48]. In such cases, a second test using a different approach, such as a nucleic acid amplification test (NAAT) or a neutralization assay, can be used to confirm positive results [48].

Real time-polymerase chain reaction assay (RT-PCR Assay)

RT-PCR Assay is the same as the Nucleic Acid Amplification Test (NAAT). It amplifies, measures and quantifies the arboviral genetic material in the blood to detect the presence of the virus. It is a standardized method that can detect current infection with the virus, often before antibodies against the virus are detectable [48].

Conclusion

Arbovirus and malaria infections are easily confused with one another. This problem does not arise from the incompetence of doctors but the lack of appropriate diagnostic tools. In particular, when making the diagnosis, the use of only clinical symptoms and rapid diagnostic techniques should be avoided.

To ensure that sick patients receive appropriate treatments, suspected malaria infections must be thoroughly investigated in well-equipped diagnostic laboratories to rule out the possibility of co-infection with arboviruses. Given the small number of recorded in-depth clinical examinations in West Africa, Nigeria, we advise that this observation be taken seriously. As a result, this article recommends that relevant stakeholders in the Nigerian public health sector and the general public adopt a holistic approach to prevent malaria misdiagnosis and outbreaks of arbovirus infection.

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Limitation

Cases of co-infections of malaria with arboviruses are poorly recognized with limited number of reported clinical cases in Nigeria.

Conflict of Interest

The authors declare no competing interest.

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