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Research Article

Misdiagnosis of Dengue Fever as Malaria and Typhoid Fever and Their Co-infection in Rural Areas of Southwest Nigeria

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Lawrence Okoror[1], Emmanuel Olufemi Bankefa[1], Oluchi Mariam Ukhureigbe[1], Evelyn Olubumi Ajayi[1], Samuel Osanyilusi[1], Bryan Ogeneh[2]

1. Department of Microbiology, Federal University Oye-Ekiti, Oye-Ekiti, Nigeria; 2. Federal University Oye-Ekiti, Oye-Ekiti, Nigeria

Background: Misdiagnosis of dengue as malaria and typhoid fever can be a major source of public health concern in large areas of Nigeria, as dengue is not routinely screened for in health care settings and clinicians have to depend on empirical treatment, which may compromise patients' health. As studies have reported a high prevalence of the dengue virus in areas of Nigeria, and dengue and malaria have similar symptoms, the same arthropod vector, and the same mode of transmission, coupled with differential diagnosis. Though typhoid fever differs from dengue and malaria by not having an arthropod vector and having a different mode of transmission, it shares a differential diagnosis with dengue and malaria, which makes misdiagnosis possible. The misdiagnosis of these three diseases has since become a major concern towards therapeutic administration because of their co-occurrence in many cases and the same location.

Methods: A total of 1074 samples were taken from rural health facilities in Southwest Nigeria and tested for malaria and typhoid fever. Those testing positive were tested for the DENV NS1 protein, DENV IgM, DENV IgG, and RT-PCR.

Results: Of the 1074 samples, 714 were positive for malaria, and 333 were positive for typhoid fever. From this, 315 (29.4%) were positive for DENV NS1. Fifty (6.7%) and 13 (3.9%) of the 714 malaria samples and 333 typhoid samples, respectively, had dengue fever co-infection. Co-infection of the three types of pathogens occurred in 5 (0.5%) of the samples. A total of 54 (5%) DENV cases were wrongly diagnosed as malaria, while 14 (1.3%) DENV cases were wrongly diagnosed as typhoid.

Conclusion: Conclusively, there were a significant number of misdiagnosed cases of DENV as either malaria or typhoid; hence, it is recommended to include DENV screening in routine hospital tests, especially in cases of malaria and typhoid negative by rapid diagnostic testing.

Corresponding author: Lawrence Okoror, Lawrence.okoror@fuoye.edu.ng

Introduction

Dengue and Malaria fevers are the most common arthropod-borne diseases caused by mosquito bites,

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and they also have similar signs and symptoms. Typhoid fever, which is caused by *Salmonella typhi*, and 3 types of *Salmonella paratyphi* also share similar symptoms with both malaria and dengue fevers. Co-infection of Malaria and Dengue was first reported in 2005 [1], while Orhue et al. in 2003 [2] reported a co-infection of malaria and typhoid fever [2] and that the few cases that have been reported indicated that co-infections may be more severe than single infections (malaria or dengue fever) [3], although there is a paucity of reports for dengue and typhoid fever co-infection. In most cases, it is common to first think of a malaria diagnosis when feverish syndromes are observed in patients, and secondly, typhoid fever will be suspected and even treated empirically. Dengue virus infection is rarely taken into consideration by clinicians because the disease is not considered endemic, which could lead to fatal consequences. Hence, early diagnosis of dengue infection will not only prevent complications such as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) but will also curtail unnecessary consumption of antimalarial drugs and antibiotics, thereby reducing the menace of antimicrobial resistance. Dengue fever, also known as breakbone fever, is an infectious tropical disease caused by the Dengue virus, a member of the Flaviviridae family [4]. About 390 million cases of dengue infections are reported every year [5], and the virus is a cause of serious health problems in many tropical and subtropical areas of the world. Dengue hemorrhagic fever (DHF) first emerged as a public health problem in 1954. The first epidemics occurred in other regions of the world in the 1980s and 1990s, caused by all four serotypes of the Dengue virus [6]. DHF and DSS are major public health concerns because of their severe and often fatal disease in children, as approximately 90% of infections occur in children less than 15 years of age [7].

Dengue virus is transmitted to humans by the bite of an infected Aedes mosquito, mostly *Aedes aegypti* [8]. It primarily propagates in skin dendritic cells and replicates in target cells such as monocytes or macrophages [9]. Symptoms include fever, headache, muscle and joint pains, and a characteristic skin rash that is similar to measles. In a few cases, the disease progresses into life-threatening DHF, resulting in bleeding, low levels of blood platelets, or blood and plasma leakage, or into dengue shock syndrome, where low blood pressure occurs [10] and can lead to death. Malaria remains one of the deadliest infectious diseases in Africa, and its parasites belong to the genus Plasmodium. In humans, malaria is caused by *P. falciparum, P. malariae, P. ovale, P. knowlesi* [11]. Among the parasites known to transmit malaria, *P. falciparum* is the most common species identified (75%), followed by *P. vivax* (20%) [12][13]. Like in dengue, symptoms of malaria include fever, headache, rash, vomiting, and joint pain.

Typhoid fever also remains an important public health problem in many developing countries of the world, including Nigeria [14]. About 11 million cases of typhoid fever occur annually, with 600,000 deaths [2]. In tropical Africa and some other developing countries, enteric fever is rampant because of the low socio-economic status and poor hygienic conditions in these regions. During the past few years in Nigeria, there has been a high incidence of typhoid fever [14], creating fear or panic in any febrile illness, which has led to drug abuse among the populace, especially chloramphenicol. Typhoid fever, though it may not be a major etiology of fever in tropical countries, has several factors, which include likely misdiagnosis and drug abuse, that may have been responsible for the high prevalence reported in routine diagnosis.

Dengue, malaria, and typhoid are three major diseases of public health concern in tropical settings and developing countries. Few cases of Dengue-Malaria coinfection have been reported in Nigeria [15], and not much Dengue (DENV) and typhoid have been reported. Moreover, concurrent infection with the three different infective agents, especially DENV and malaria, leads to an overlap of their clinical features [15]. This can pose a diagnostic challenge to physicians, especially in endemic areas [16]. There have also been reports of misdiagnosis of malaria and DENV infections [17], which could be due to the similar symptoms of both infections. Although reports of DENV and typhoid infection are sparse, it is therefore imperative to investigate the co-occurrence due to their differential diagnosis. Though there may be cross-reactivity between DENV and other flaviviruses using ELISA as a testing procedure, this study included RT-PCR, which is more specific, and the NS1 protein gene is also more specific for DENV. This study is not aware of any study that investigated DENV, malaria, and typhoid co-infection and misdiagnosis in Nigeria. Hence, this study investigated the frequency of co-occurrence of dengue, malaria, and typhoid fevers in rural communities in Southwest Nigeria, as well as the possible misdiagnosis of dengue fever for malaria and/or typhoid fever.

Southwest Nigeria is one of the six geopolitical zones of Nigeria, representing a geographic and political region of the country's Southwest. The zone stretches along the Atlantic seaboard from the international border of Benin Republic in the West to the South in the East and North Central to the North (Nigeria Demographic and Health Survey of 2018). The Southwest is split by the Central African Mangrove in the coastal far south, while the major inland ecoregion is the Nigerian lowland forest (Nigeria Demographic and Health Survey of 2018). This location is an excellent breeding ground for mosquitoes of all species, and the lowland forest region also encourages the likely sylvatic transmission of dengue by species of Aedes mosquitoes. This study concentrated on locations along the lowland forest regions of Southwest Nigeria. Nigeria accounts for over 27% of malaria cases worldwide, and the Southwest states account for 16% of these cases (Nigeria Demographic and Health Survey of 2018). The Nigeria Demographic and Health Survey in 2018 also reports that the disease burden of typhoid fever is still high, with attendant complications, and Southwest Nigeria bears more than 11% of the total national burden. Though there is no clear-cut definition of the dengue fever burden in Nigeria, several dengue fever outbreaks have been reported in Nigeria, which are distributed across the Southwest states (Nigeria Demographic and Health Survey of 2018).

Methods

Study Design

This study is a cross-sectional research conducted in several health facilities in rural areas of Southwest Nigeria. A cross-section of the patients seeking diagnosis for malaria and typhoid were tested for dengue virus (DENV) NS1, IgM, and IgG using ELISA and Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR). The DENV RT-PCR test was done independently of whether the patients were positive for malaria and typhoid or not, and to capture any samples missed by the ELISA technique. The ELISA technique used will give us the adequate serological information needed for diagnosis. The NS1 gene that was included in the testing was to ensure that whatever serotype was missed by IgG and IgM would be captured by assaying for the NS1 gene. Samples were collected from the month of October to the end of September of the preceding year. Samples were collected from patients with the agreement of anonymity.

Inclusion Criteria

This study involved all consenting out-patients who reported to health facilities for malaria and typhoid fever while conducting the research. Any sample testing positive for the different types of malaria parasites was included in the study, and any sample testing positive for different causative agents of typhoid fever was included in the study, or both diseases. All collected samples must have been tested in the facility and results registered in the facility register. These samples were then tested for dengue NS1, used as a first-line marker for the dengue virus. Since the NS1 gene is more specific to the dengue virus, it will further reduce the chance of cross-reaction by ELISA with other flaviviruses. All samples testing positive for dengue NS1 were again tested for anti-DENV IgM, IgG, and RT-PCR. All samples tested must have been clinically confirmed by a clinician following laboratory diagnosis. The ethical committee of Joseph Ayo Babalola University approved this study.

Sample Collection

5 ml of blood was aseptically collected from patients (n=1074) seeking malaria and/or typhoid diagnosis in the health institutions in rural areas of Southwest Nigeria from October to September in the year sampled. The blood samples were collected into EDTA bottles from each participant by a trained phlebotomist using a needle and syringe and were immediately transported in the cold chain to the Microbiology Laboratory. Each bottle was labeled indicating the participant's age, sex, and location. Blood samples were divided into 2 EDTA bottles, one to be used for DENV ELISA and the other for malaria and typhoid.

Malaria

Malaria testing was done in the clinics using Rapid Diagnostic Testing (Diagreat Biotechnology, Beijing, China), and tests were carried out as recommended by the manufacturer, after which samples were immediately shipped to the laboratory and confirmed using the Giemsa staining technique through thin and thick film preparation.

Typhoid Fever

Typhoid fever was tested in the clinical laboratory using the slide agglutination and typhoid RDT (Diagreat Biotechnology, Beijing, China), and the tests were carried out as recommended by the manufacturer. Samples were shipped to the laboratory for confirmation using the tube agglutination technique as

adapted for the use of a microtiter plate, and all the patients were made to come back a week after the first test for a second sample collection used for paired sample testing [18]. All the samples testing positive for both malaria and typhoid fever were further tested for the dengue virus using the ELISA technique. Typhoid fever samples were later confirmed by blood culture onto molten tryptone soy agar; those that were negative were regarded as typhoid negative.

Enzyme Linked Immunosorbent Assay

Blood samples for ELISA were immediately centrifuged at 3000 rpm (Beckman Microfuge centrifuge) and sera separated from the whole blood, immediately used for ELISA. Sera stored in the refrigerator were brought out and allowed to attain room temperature, as well as all the reagents. Sera were dispensed into the antibody-impregnated ELISA microplate, and the tests were carried out as described by the manufacturer of the ELISA test kits (Melsin Medicals, China). ELISA kits used included ELISA NS1, IgM, and IgG for research. ELISA plates were loaded into the microplate reader (Molecular Devices, USA) at an optical density of 450 nm; absorbance was generated and analyzed using the myassays software to generate the concentration of each sample in each of the parameters for analysis.

RNA Extraction

RNA was extracted using the Norgen Biotek total RNA extraction kit. 100 μl of non-coagulating whole blood was collected into well-labeled RNAse-free microfuge tubes, and 350 μl of lysis buffer was added to the blood in the microfuge tubes. The extraction procedure was carried out as directed by the manufacturer (Norgen Biotek, Ontario, Canada). All samples testing positive for DV NS1, IgM, and IgG had their RNA extracted for RT-PCR.

RT- QPCR Procedure

RT-qPCR was carried out on all samples testing positive for dengue NS1 by the ELISA technique after RNA extraction. A PCR reaction mixture was set up using the MAXIMA SYBR Green with ROX RT-PCR master mix to achieve a total volume of 25 μl, using the hot start as described by the manufacturer. The primers used were obtained from already published research [19], which are universal primers targeting the 3′ untranslated region of all complete genome sequences of the dengue virus available in GenBank (n,305) (DENV_F-GCATATTGACGCTGGGARAGAC, DENV_R1-3 - TTCTGTGCCTGGAATGATGCTG, DENV_R4-YTCTGTGCCTGGATWGATGTTG) and the probe (DENV_P- CAGAGATCCTGCTGTC). Hence, all four types of DENV will be detected. The primers were sent to Inqaba Biotech, South Africa, for synthesis. The PCR mixtures were put into the thermocycler (Bio-Rad C1000 Touch Thermal Cycler, Bio-Rad, USA). The PCR program was as follows: UDG pretreatment at 50°C for 2 mins for 1 cycle, initial denaturation at 95°C for 10 mins for 1 cycle, denaturation at 95°C for 15 sec for 40 cycles, annealing at 60°C for 30 sec for 40 cycles, and extension at 72°C for 30 sec for 40 cycles. Data acquisition was then done and analyzed with the CFX Maestro Software for CT values.

Statistical analysis

The t-test was used to compare malaria infection with DENV infection and typhoid and DENV infection to establish co-infection between each of the infections and DENV. It was also used to compare the monthly distributions. The chi-square test was used to establish an association for misdiagnosed samples with the actual dengue-positive samples with malaria or typhoid fever and was done independently. Linear regression was used in the MyAssays software to obtain the concentration of the various antibodies used (IgM, IgG, and NS1).

Results

A total of 1074 blood samples were collected from different health facilities in rural locations in Southwest Nigeria, of which 714 (69%) blood samples were positive for malaria parasites, 333 (31%) were positive for typhoid fever, and from the malaria- and typhoid-positive samples, 315 (29.4%) were positive for dengue using the NS1 protein (Table 1).

Enzyme Linked Immunosorbent Assay

Linear regression of antibody concentration for samples tested across four, using absorbance measurement at an optical density of 450 nm, and analyzed using myassay microplate software, for NS1 protein, IgM, and IgG, respectively. Fig. 1 and 2 show the individual sample concentrations to DENV NS1 protein and DENV IgM, respectively. The frequency of sample concentrations is shown in Fig. 3, 4, and 5, respectively, for DENV NS1 protein, DENV IgM, and DENV IgG, respectively. From the results of individual sample concentration and frequency of sample concentration, it shows that the concentration of NS1 protein and IgM was evenly distributed across the samples, while IgG demonstrated high concentrations in some individual

samples. From DENV NS1 positive results, 80 (25.4%) of the total samples were positive for anti-DENV IgM, and 20 (6.3%) were positive for anti-DENV IgG.

RT-PCR

Of the 315 samples testing positive for DENV NS1 protein, 287 (91.1%) samples were positive for DENV by RT-PCR (Table I) confirmation. Of the 80 samples that tested positive for DENV IgM, 69 (86.3%) of the samples were positive for DENV using RT-PCR, while of the 20 that tested positive for DENV anti-IgG, 14 (70%) tested positive for DENV by RT-PCR.

Co-infection

Of the total number of 714 confirmed malaria cases, 50 (6.7%) also had DENV NS1 (co-infection) (t=5.8540), which was also confirmed by RT-PCR; there were 13 (3.9%) typhoid-DENV co-infections (t=9.3611). From the 333 samples positive for typhoid fever, malaria/typhoid co-infection with DENV had 5 (0.5%) samples positive (p=0.9296 and p=0.9432, respectively).

Misdiagnosis

A total of 54 DENV-positive samples were wrongly diagnosed as malaria parasites, while 14 (1.3%) samples positive for DENV were misdiagnosed as typhoid fever (χ=86.877, p=0.0000001), showing an association between all misdiagnosed samples, which is a confirmation that some samples were diagnosed as typhoid and malaria but were actually positive for dengue alone. Age group distribution shows that the age groups 21-26, 27-32, and 15-20 have the highest number of all samples positive for all infections (Table II).

Demographic distribution

The age group 39-44 years had the highest number of DENV-malaria co-infections. However, the age groups 27-32 and 33-38 were higher for DENV-typhoid co-infection, while the 27-32 and 33-38 years brackets had the highest positive cases for the 3 co-infections. These age brackets also had the highest number of DENV misdiagnoses with 15 cases each, after the age group 39-44, which had 20 cases (Table I). The age groups 27-32 and 33-38 had the highest number of misdiagnosed samples for DENV, with 6 samples each. Table 2 shows the sex distribution for malaria, typhoid, DENV, and all their co-infections. Males were more infected with malaria parasites, 412 (55.6%), while females were 329 (44.4%). For typhoid, 218 (65.5%) were females, while 115 (34.5%) males were positive. DENV NS1 had 200 (63.5%) positive males, and 115 (36.5%) positive samples were females. Other DENV parameters also followed the same trend.

Monthly distribution

The monthly distribution shows that the months of October, September, August, July, June, May, and April had the highest number of positive cases for malaria and DENV. Samples positive for typhoid fever did not show a particular distribution pattern (Table III). November and June had the highest number of co-infection cases with Malaria/DENV (t= -0.639, p=0.537) as compared to others, while September had the highest amount of co-infection for the 3 parameters (t=0.905, p=0.387), and October and March had the highest number of typhoid and DENV co-infections (t=1.008, p=0.537) (Fig. 6).

There was no significant difference between NS1 protein and RT-PCR results using the ANOVA statistic (F=2.3765, p=0.0883). However, there was a significant difference between NS1 protein, IgM, and IgG (F=3.5327, p=0.0452).

Age Group	Malaria	Typhoid	DENV NSI	DENV IgM	DENV IgG	DENV PCR	Malaria/ DENV	Typhoid/ DENV	Malaria/ DENV/Typhoid
15-20	110	53	48	21	9	46	0	0	0
21-26	135	49	48	27	5	48	10	5	1
27-32	115	61	36	10	0	34	9	5	2
33-38	101	61	37	8	2	34	9	0	1
39-44	97	44	45	8	3	40	22	0	1
45-50	86	25	39	6	1	30	0	0	0
51-56	62	21	41	0	0	36	0	2	0
57-62	35	19	21	0	0	19	0	1	0
Total	741	333	315	80	20	287	50	13	5

Table 1. Age group distribution (in years) of Dengue fever, Malaria, and typhoid fever with co-infection in some rural settlements in Southwest Nigeria.

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Age (years)	Malaria		Typhoid fever	
	Sex			
	Male	female	male	female
15-20	0	0	0	0
21-26	3	0	0	1
27-32	10	5	2	4
33-38	9	6	3	3
39-44	8	12	0	1
45-50	1	0	0	0
51-56	0	0	0	0
57-62	0	0	0	0
Total	**31**	**23**	**5**	**9**

Table 2. Age and sex distribution of DENV positive samples wrongly diagnosed as malaria and/or typhoid fevers.

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	No. of Dengue	No. of Typhoid	No. of Malaria
October	41	44	87
November	20	12	44
December	12	10	39
January	12	10	27
February	16	12	27
March	29	29	46
April	35	28	67
May	41	39	71
June	40	39	90
July	38	38	93
August	28	27	81
September	21	27	69
Total	**333**	**315**	**741**

Table 3. Monthly distribution of Dengue fever, Malaria, and typhoid fever in some rural settlements in Southwest Nigeria.

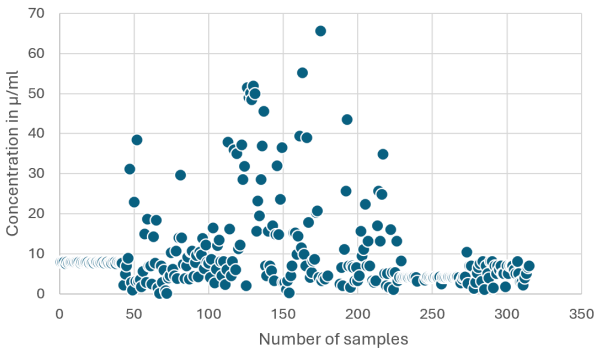

Fig. I. Individual concentration of NS1 protein. Approximately 200 samples had a high concentration of NS1 protein.

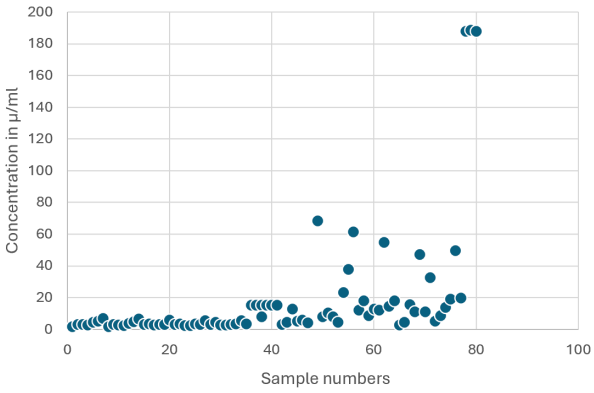

Fig. II. Individual Sample Concentration (IgM).

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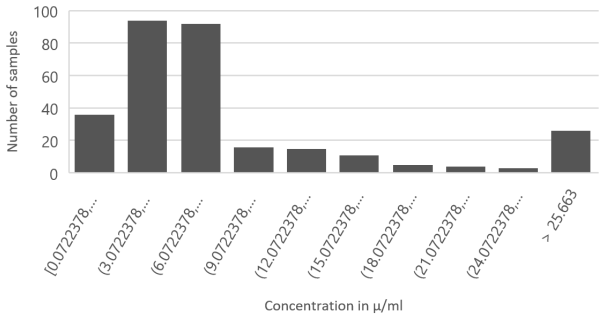

Fig. III. Frequency of sample concentrations to DENV NS1 protein showing the number of samples and concentration.

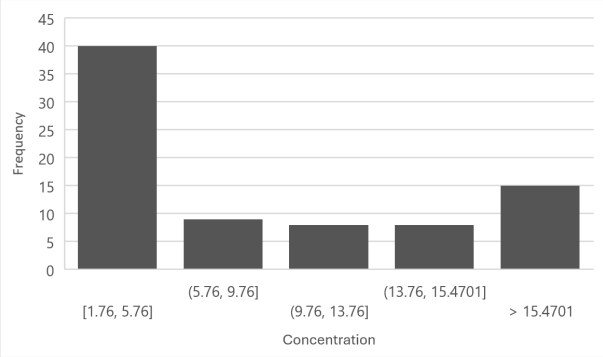

Fig. IV. Frequency of sample concentration with over 40 samples displaying a high concentration of anti-DENV IgM.

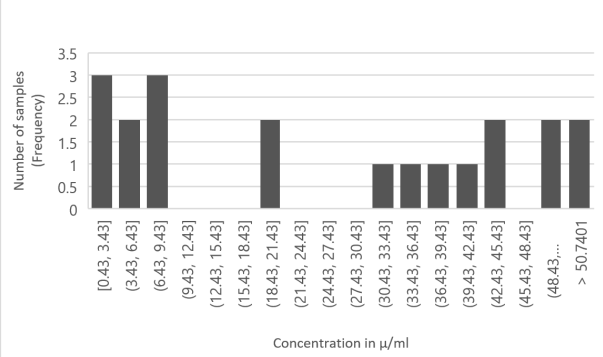

Fig. V. Frequency of concentration showing the number of samples with corresponding concentration to anti-DENV IgG.

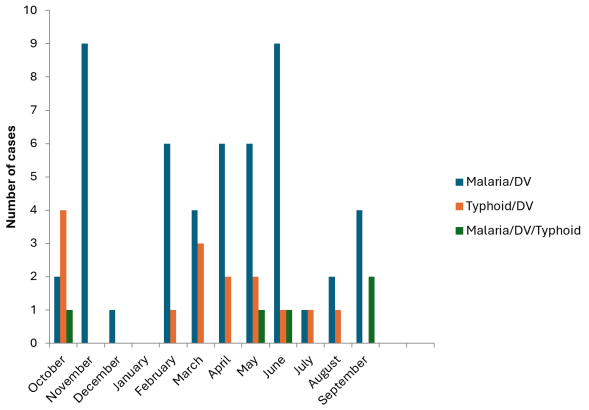

Fig. VI. Monthly Distribution of Co-occurrence of Dengue Fever with Malaria and Typhoid Fever in Rural Communities in Southwest Nigeria. Each bar represents the number of cases occurring at the same time in a particular month.

Discussion

This study is a cross-sectional and comparative analysis of DENV, malaria, and typhoid fever in co-infection in rural settings of Southwest Nigeria, with misdiagnosis of dengue fever for malaria or typhoid fever. DENV fever co-infection with malaria and typhoid fevers was analyzed using 4 different parameters, which included NS1, IgM, IgG antibodies, and later confirmed with RT-PCR. The NS1 protein is the first to be produced in the infection, which ensures the first early window is not missed in DENV infection; the IgM antibody is the first to be produced in response to an infection, while the IgG will remain even in convalescence. A combination of all these ensures that no case is missed during the period of the study. The RT-PCR is used to validate all the results from the serological analysis and that any DENV missed by the ELISA technique will be captured by RT-PCR, while at the same time quantifying the antigen in each sample. The potential source of bias in this study is the likelihood of false negative or false positive results, which has been taken care of by a combination of confirmatory tests carried out. The limitation in this study is that the period of onset of illness was not assessed, but the use of IgM and IgG antibodies to assess recent and convalescent infections provides for these limitations. The different seasons at which samples were collected may also be a source of limitation; while malaria may be more common during the rainy seasons along with dengue, typhoid fever may not be seasonal. However, statistical analysis was employed to take care of the differences in seasonality.

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Another limitation in this study is that results were not compared with data from other countries; however, this remains an area for further research.

Of the total 315 DENV NS1-positive samples, 287 samples were RT-PCR positive, thereby confirming that 28 samples, though they came in contact with DENV, had not yet established enough antigen to be detected by PCR, which thereby confirms the usefulness of the NS1 protein in detecting dengue infection.

This study generally reveals a high incidence of Malaria/DENV co-infection as well as Malaria typhoid, but the trio co-infection was not significant. A study of the prevalence of concurrent dengue and malaria was reported by Charrel et al. [1] to have a 0% prevalence in the Netherlands, which opposes the results from this study. The reason for this may be due to geographical variation and a high level of mosquito control in the Netherlands, which is lacking in Nigeria. In addition, the mosquito vectors for the two diseases have different habitats; the malaria mosquito vector has its habitat in the forest [16], while the dengue mosquito vector's main habitat is in the city [19]. Hence, overlapping of the habitats may not be easily available in the Netherlands, as is the case in Nigeria. A significantly low Malaria-Dengue concurrent rate can be expected [20]. Dengue-Malaria co-infection has been referred to as an uncommon phenomenon in temperate regions. However, reports showed that the highest dengue-Malaria co-infection rate was 23.21%, found in Pakistan, while as low as 0.01% was found in Senegal [21]. Another study conducted in Brazil recorded 2.8% [7]. The report from Pakistan was similar to that from this study, which is a result of the similar geographic conditions of both regions. The Senegal report also opposed results from this study, and a major factor responsible for that is due to the time of the year and the city where the Senegal sampling was done, since both countries are in sub-Saharan Africa, as indicated by the 2.84% Dengue virus and malarial concurrent infection within the Ilorin metropolis in Nigeria [22]. Okoror et al. [23] also reported a high prevalence of DENV infection (57.5%) in selected rural communities in Southwest Nigeria.

Since not all the hospitals officially screen for DENV, and the differential diagnosis of DENV, malaria, and typhoid despite similar clinical presentations makes misdiagnosis not unusual, co-infections may give rise to wrong diagnoses, especially in areas where clinicians depend on empirical treatment. Moreover, the treatment regimens for these coinfections are not the same as those for mono-infections. Hence, a delay in implementing the appropriate treatment regimen for these different infections due to poor diagnosis can result in fatal consequences, compromising the patient's health.

Age group distribution revealed that the age groups 21-26 through 39-40 have the highest prevalence of Malaria/DENV co-infection, and they were statistically significant (p=0.9296; CI=0.05). These age groups also had the highest prevalence of malaria alone as well as typhoid. The reason for this may be attributed to the active (working class) individuals who are found in this age range and their outdoor engagement on a day-to-day routine, making contact with mosquitoes unavoidable [24]. The study is also supported by Okoror et al. [25] , who reported the endemic nature of typhoid in Nigeria. In addition to this, the sex distribution shows that more males were infected with malaria as compared to females, and also that malaria DENV co-infection was more common in males compared to females, as well as typhoid DENV co-infection. The overall infections also show a similar pattern of sexual distribution. This was also reported by Dhanya et al. [26], though more males have been shown to have more outdoor activities than their female counterparts.

The distribution of Malaria/DENV and Typhoid monthly shows that the months of November and June had the highest Malaria/DENV co-infection distribution, while October and March showed the highest co-infection distribution for typhoid and DENV, with significant statistical differences from other months. This coincides with periods of high rainfall and progression to the dry season for malaria/DENV infection and the period of high breeding of mosquitoes. For typhoid/DENV co-infection, months of highest prevalence coincide with periods of high rainfall, as these are the periods of lowest hygiene due to high flooding, which increases the incidence of typhoid fever, and high proliferation of mosquitoes, leading to a high incidence of DENV. Some authors have also reported both seasonal and monthly distribution of Malaria/DENV co-infection. Dhanya et al. [26] , in an India study, reported a monthly distribution of Malaria and Dengue co-infection and reported that their highest prevalence was between July and December, which agrees with some of the months reported in this study. Savargaonkail et al. [24] also reported a similar distribution in India.

Total samples for malaria, typhoid, and DENV also follow a similar pattern of distribution. Other factors that may have influenced this distribution include rainfall fluctuation, humidity, and temperature [27][28].

The concentration of NS1 protein, which was evenly distributed across the study population, further goes to confirm the high prevalence of DENV in the population and the misdiagnosis of DENV for malaria and typhoid fever. This is more importantly noticed with the high anti-DV IgM and IgG, which, however, was distributed along sex and age. The high concentration noticed in males goes to establish the earlier claims that males are more involved with outdoor activities in the study population and therefore more exposed to the vector.

Conclusion

In conclusion, this research showed that many dengue-positive samples are misdiagnosed as malaria and typhoid fevers, and as such, dengue testing should be incorporated into the national testing scheme, especially in areas where high dengue infection has been reported, like in this study population, so as not to compromise the patients' health. The importance of this paper cannot be undermined because of the public health implications of undermining or misdiagnosing dengue as malaria or typhoid, a situation which could endanger the health of the population because of the likely progression of dengue fever to DSS, leading to death.

List of Abbreviations: NS1, DENV, DENV IgG, DENV IgM, RT-QPCR, DHF, DSS, ELISA, EDTA, RDT, RNA, PCR, MAXIMA, SYBR, ROX, RT-PRCR, DENV, UDG, CT

Statements and Declarations

Conflicts of Interest: We declare no conflict of interest. The authors did not receive any form of financial assistance whatsoever.

Ethical Clearance: Ethical approval was granted by the Ethical Review Committee of Joseph Ayo Babalola University, Ikeji-Arakeji, Nigeria (ref/2012/jabu0214/VC.12). All participants in this study agreed to participate by filling out questionnaires.

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Declarations

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