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

Elpidius Rukambile[1]

1 Central Veterinary Laboratory

General comments

This manuscript, titled "**Misdiagnosis of Dengue Fever as Malaria and Typhoid Fever and Their Co-infection in Rural Areas of Southwest,"** is a very informative manuscript, the data of which may play a great role in assisting policymakers to make informed decisions, including the inclusion of dengue fever in routine diagnosis. Despite this importance, there are several areas which need the authors' attention. The entire manuscript needs grammar work; I failed to grasp some concepts simply because they were presented in poor grammar. The materials and methods miss some important information to make the section self-explanatory. Presentation of the results needs extensive work, especially in tables and figures, which miss self-explanatory titles and captions, respectively. There are statistical analyses using software; I expected to see the analysis outputs rather than seeing only the significant results being mentioned. The authors failed to present the study results in a proper way, which made this beautiful study less informative than it was supposed to be. The specific comments for each section are narrated below.

Introduction

"…four serotypes of the Dengue virus." There is a need for mentioning all four types of serotypes.

"…. its parasites belong to the genus Plasmodium." I think it should be "vector," not "parasite."

"Among the parasites known to transmit…." Refer to the above comment.

I suggest adding information on the mode of transmission and a short description of risk factors."

I think there is a need for providing a snapshot of the available reliable and commonly used diagnostic tests for these diseases.

Methods

"……..patients seeking diagnosis for malaria and typhoid….." I think they don't seek; normally, the request for the test is initiated by the doctor/clinician, etc.

"The ELISA technique used will give us the adequate serological information needed for diagnosis." When? But also, the

sentence is not self-explanatory.

"Since the NS1 gene is more specific…." We target a protein in ELISA, not a gene.

"The DENV RT-PCR test was done independent of whether the patients were positive for malaria and typhoid or not, and to capture any samples missed by the ELISA technique." This could have been stated earlier in the introduction section that the former is superior to the latter; that is why a snapshot of the common diagnostic methods is important.

"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" Work on grammar

"…………who reported to health facilities for malaria and typhoid fever……" Not clear

"Any sample testing positive for the different types of malaria parasites…..." Work on grammar

"……………and any sample testing positive for different causative agents………..." Work on grammar

"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" Re-write this sentence.

"………...seeking malaria and/or typhoid diagnosis in the…..." Please look for the best way to present this.

"………...collected into EDTA bottles…………." I am not sure if this is the correct name.

"………...into 2 EDTA bottles, one to be used for DENV ELISA……" I am wondering how the blood collected in EDTA can be used for ELISA.

"Malaria testing was done in the clinics using Rapid Diagnostic Testing…..." I think this test has its generic name, not just rapid test.

"……………and all the patients were made to co'"me back a week after the……..." Work on grammar.

"Typhoid fever samples were later to be confirmed by blood culture……" "Slide agglutination and typhoid RDT samples were later confirmed by blood culture…………"

"……………sera separated from the whole blood………" How did you manage to separate the sera from the blood collected in EDTA?

"……...350………" 350 what? Litres, microliters, etc.

"RT-qPCR was carried out on all samples testing positive for dengue NS1 by the ELISA technique after RNA extraction" Work on grammar.

"Hence, all four types of DENV will be detected." When?"

"Hence, all four types of DENV will be detected." When?

"The primers were sent……" "The primer sequences……………"

Results

"A total of 1074 blood samples were collected from……………" Repetition, does not belong to this section.

The reader will also be interested to know how many samples were positive for the rapid test but negative for Giemsa staining, positive for slide agglutination and typhoid RDT but negative for culture, and vice versa.

"…………714 (69%) blood samples were positive for malaria parasites, 333 (31%) were positive for typhoid fever…………..." Based on which tests?

"Linear regression of antibody concentration for samples tested across four, using………." Four what?

"…………. analyzed using myassay microplate software, for NS1 protein, IgM, and IgG, respectively." The word "respectively" is wrongly used

"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   " These are presented as the captions of the figures instead of being used as supporting evidence of what is being described

"From the results of individual sample concentration and frequency of sample concentration……..." Work on grammar

"From DENV NS1 positive results…." Why are you using "From" in this sentence?

"Of the 315 samples testing positive for………" Tested

"Of the 80 samples that tested positive for DENV IgM, 69 (86.3%) of the samples were positive for….." Work on grammar

"………(Table I) confirmation." No such table in your manuscript

"From the 333 samples positive for typhoid fever, malaria/typhoid co-infection with DENV had 5 (0.5%) samples positive……." Positive regarding which test?

"A total of 54 DENV-positive samples were wrongly diagnosed as malaria………." Include the percentage in brackets

"Age group distribution shows that the age groups 21-26, 27-32, and 15-20…..." Nowhere in the Materials and Methods section did you mention collecting this information

"…………21-26, 27-32, and 15-20……" Were these ages in months or years?

"……...infections (Table II)." No such table in this manuscript

"……...while the 27-32 and 33-38 years brackets………" What does the word "brackets" mean here?

"These age brackets………..." "age group?"

"…………misdiagnoses with 15 cases respectively …..." The word "respectively" does not belong here

"These age brackets also had the highest number of DENV misdiagnoses with 15 cases respectively, after the age group 39-44, which had 20 cases (Table I)." "…had the highest…..." then how come you said "after the age group 39-44"?

I think you need to include the table with demographic information such as age, sex, positive samples for each disease, etc., otherwise it will be difficult to understand the other tables.

'Table 1". Mixed infection issues: on which test method are you basing your conclusions for all three diseases? I am asking this because different tests were used for each of these three diseases.

"………samples for DENV, with 6 samples respectively……." The word "respectively" does not belong here.

"Table 2 shows the sex distribution for malaria, typhoid, DENV, and all their co-infections" Used as caption instead of being used to support the description

"Table 2 shows the sex distribution for……" Nowhere in the Materials and Methods section did you mention collecting this information.

Table 2. Age and sex distribution of DENV-positive samples wrongly diagnosed as malaria and/or typhoid fevers. "Wrongly diagnosed by whom?

"Males were more infected with malaria parasites, 412 (55.6%), while females were 329 (44.4%)" Work on grammar.

"For typhoid, 218 (65.5%) were females, while 115 (34.5%) males were positive." Work on grammar.

"DENV NS1 had 200 (63.5%) positive males, and 115 (36.5%) positive samples were females." Work on grammar.

"Other DENV parameters also followed the same trend." What does this sentence mean?

"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." You have mentioned more than half of the months in which you conducted the study, and then you said "highest"? You can do that if they have the same value.

"Samples positive for typhoid fever did not show a particular distribution pattern (Table III)." How? The table shows the number of positive samples in each month. Why are you saying that?

"…………..distribution pattern (Table III)." No such table in this manuscript.

"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). " Based on which tests?

"There was no significant difference between NS1 protein and RT-PCR results………" Results in regards to what?

"However, there was a significant difference between NS1 protein, IgM, and IgG" Not clear?

"Table 1. Age group distribution (in years) of Dengue fever, Malaria, and typhoid fever with co-infection in some rural settlement in Southwest Nigeria" Not clear.

"Table 1" This table is difficult to comprehend. How do the numbers in the columns for malaria and typhoid differ from the numbers in the test methods columns?

"Table 2. Age and sex distribution of DENV-positive samples wrongly diagnosed as malaria and/or typhoid fevers." Based on which tests? The title of the table should be self-explanatory.

"Table 3." I suggest this table be converted to a chart.

"Table 3. Monthly distribution of Dengue fever, Malaria, and typhoid fever in some rural settlement in Southwest Nigeria." Based on which test methods?

"Fig. I. Individual concentration of NS1 protein. Approximately 200 samples had high concentration of NS1 protein" What do you mean by high? What was your cut off points for high and low? Also, the caption should be self-explanatory

"Individual Sample Concentration (IgM)" Caption is not self-explanatory

"Figure III" The horizontal axis should be at one decimal place otherwise it is difficult to comprehend

"Fig. IV. Frequency of sample concentration with over 40 samples displaying a high concentration of anti-DENV IgM" What do you mean by high concentration? Refer my previous comments

"Figure IV" What is the measurement of concentrations

"Figure V" Why you included the concentration with 0 number of samples. Also, I am not comfortable on how the value on horizontal axis were presented

Discussion

"…………………...with misdiagnosis of dengue fever for malaria or typhoid fever….." I am not sure how you arrived at this, I think there is information missing in Materials and Methods sections.

"…………...to assess recent and convalescent infections provides for these limitations. "………to assess recent and convalescent infections address these limitations."

"However, statistical analysis was employed to take care of the differences in seasonality" How?

"Another limitation in this study is that results were not compared with data from other countries; however, this remains as an area for further research." Why? This mean the authors did not take a trouble to visit available literature.

"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." Not clear re-write

"…………………..but the trio coinfection was not significant." How?

"………………...high level of mosquito control in the Netherlands……………………" Reference?

"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" Work on grammar.

"The report from Pakistan was similar to that from this study, which is a result of the similar geographic conditions of both regions" Work on grammar

"Since not all the hospitals officially screen for DENV……" "Since not all the hospitals routinely screen for DENV…………..."

"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)" I did not see statistical analysis output anywhere, please work on this in the Materials and Methods section

"These age groups also had the highest prevalence of malaria alone as well as typhoid" Work on grammar.

"The reason for this may be attributed to the active……..." Work on grammar.

"……...with significant statistical differences from other months" I don't remember if I saw this in the Results section, please check.