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Journal Name:	British Microbiology Research Journal
Manuscript Number:	2013_BMRJ_7851
Title of the Manuscript:	Molecular Characterization of hepatitis B virus circulating in asymptomatic and symptomatic carrier in Côte d'Ivoire
Type of the Article	

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



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PART 1: Review Comments

	Reviewer's comment	Author's comment <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
<u>Compulsory</u> REVISION comments	RED=ADDITION; BLUE =DELETE No doubt, this is a very GOOD STUDY; however, it was inadequately designed and executed. Firstly, permit me to say that reviewing this study was highly time-demanding because it is fraught with grammatical errors; almost every other line has, at least, one spelling error or scientific error. More time-demanding because I accepted to review it as my work and field of interest, but this time I am already racing against time to finish 8 chapters I vowed to contribute to a book, the deadline of which is 6th of December, 2013. I have only finished 3 chapters. So reviewing this work did not make it easy at all! In order to fulfill my obligation, I had to overlook the grammatical errors, and review the scientific work. My observations are hereunder: TITLE	Thanks for manuscript critical. All your suggestion will be corrected in the text before sending.



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	Line 5: No period/full stop after title ABSTRACT Line 10: These deaths are due to complications such as cirrhosis and hepatocellular carcinoma Line 16: “.....symptomatic carrier and to establish eventual correlation between genotypes found and clinical outcome in Côte d’Ivoire. Line 17: Côte d’Ivoire is a country, how did you select 139 samples to represent a country? Otherwise you let the title reflect the locale from where you obtained the 139 samples. Line 17: The experiment was consisted of 139 samples Lines 17-18: A studysystem based on serology and molecular testing. Line 19: Genotypes G and H were not determined. Line 23: This study revealed a predominance of genotype E of HBV and revealed that genotype E was associated (P=0.044 or 0.03) with clinical outcome.	Line 17: This error has been corrected in the text. The study has been carried out on 754 samples but only 139 were HBs Ag positive for the further study.
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	Line 24: However, the high number of non-typable HBV DNAs probably required amplification of the whole genome. Line 20-23 and results section: Your estimated proportions are not clearly stated, one would wish to know whether you are referring to overall proportions or group-specific proportions because you have 2 groups (symptomatic and asymptomatic); your proportions are not clearly delineated. More so in the results sections, you used different denominators, though correct, but it is confusing as one would want to see the same values you stated in the abstract section in your resultsection. They are all different making the whole results very confusing. For instance, in the abstract section, you stated 97.1% genotype E and 2.9% genotype B; but in the results section, these figures are missing, I only saw genotypes E (62.95%), 1 genotype B (1.85%). Even careful reader may not easily understand these. You must be consistent from beginning to the end. INTRODUCTION Line 43: More than 4 billion people had contact with Hepatitis B virus worldwide (Ying-Hui Shi &	Line 20-23: In the abstract the proportion based on the typeable (only typeable); there are 35 of 54 HBsAg positive which are typeable. In the 35 typeable we are 34 genotype E (97.1%) and 1 genotype B (2.9%). In the results, the proportion based on the 54 HBsAg positive (Typeable and non-typeable) with 34 genotype E (62.95%), 1 genotype B (1.85%) and 19 non typeable (35.2%)
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	al. 2012), correct this reference style!!! Line 44: including 350 million who are chronic carriers (Mulyanto& al. 2012) All the initials of authors in the within text references should be deleted (e.g. Lock AS et al. 2001) Line 46: Africa and Asia remains the continent most affected..... Line 53: Previous studies showed that genotype A virus was more virulent than genotype C virus which was also more virulent than genotype B virus Line 56: What about the role of the others genotypes in clinical outcome of viral hepatitis B? I would suggest you cast this thus “there is dearth of information on the roles of other genotypes in the clinical outcome of hepatitis B viral infection”. Line 61: Only few data exist on the genotypes of circulating Hepatitis B virus Line 64: “....to establish a possible association between HBV genotype and clinical outcome”	
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MATERIALS AND METHODS

Line 71: Reference Center for Viral Hepatitis of the Institute de Pasteur, Côte d'Ivoire.

Line 71: The study involved 2 groups of asymptomatic participants in whom HBV detection was fortuitous and symptomatic carriers comprising chronic carriers and liver cancer patients.

Critical to this study is method of sample selection; it is necessary you indicate what characterized the symptomatic group as such; I mean what symptoms did you see in them for inclusion in the study? And what made those asymptomatic such? (What are your inclusion and exclusion criteria?)

The selection method for participants must be clearly stated to make anyone see that they were representative of Côte d'Ivoire.

Line 74: The participants gave written informed consents; the informed consent forms were obtained from all of them.

For symptomatic: Asthenia, jaundice, Hepatitis B chronic, liver cancer.

For asymptomatic: Patients coming in Hepato gastro enterology for investigation, patients presenting some risk of transmission as polytransfused.

All the participants are agreeing to participate to the study. Those who refuse are not included to the study.



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	Line 76: National Committee of Ethics and Research (NCER or CNER, check this abbreviation for correctness). The assay section was poorly written, it is also replete of wrong spellings. Which did you use for the analyses, serum or plasma? As you said “Serum separated and stored at -20°C for serological testing; and plasma at -80°C for molecular testing. Of course you could not have obtained serum and plasma from one blood sample! One cannot but ask whether you obtained two different samples from each patient, one with and one without anticoagulant to make plasma and serum respectively, however, this is unlikely to be the case! So let me know the one you used, serum or plasma. If you did obtain two blood samples per participant, then your methodology here and in the abstract should state so. RESULTS I observed that your statistical analysis to establish whether or not HBV genotype was associated with	I used serum for serological assay and plasma for molecular testing to avoid congelation and decongelation. For each participant two aliquots have been done: one with and one without anticoagulant. It will be mentioned in the text. I also used plasma for further study on hepatitis C.
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clinical outcome is apparently wrong. From the table below, I used CHI^2 and obtained $P=0.044$ (Fisher's exact 2-sided) or $P=0.027$ (Fisher's exact 1-sided) with Odds Ratio of 3.63. When I did it with binary logistic regression, I got $P=0.03$ and Odds Ratio of 3.60 (95%CI: 1.129-11.478). Using the two approaches, I got similar results, each one different from your result. So you need to re-analyze your data. Though truly, genotype E of HBV was significantly associated with clinical outcome, i.e. those with genotype E of HBV were more likely to have had symptomatic HB; and that they had 3.6 times greater likelihood of being symptomatic than the asymptomatic patients in your study.

	Genotype E	OTHERS (non-typable +/- Genotype B)
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	Symp	24	8	I used this table to do my statistical analysis: <table border="1"> <tr> <td data-bbox="1205 495 1362 532"></td> <td colspan="2" data-bbox="1362 495 1892 532">Population</td></tr> <tr> <td data-bbox="1205 532 1362 609"></td> <td data-bbox="1362 532 1549 609"></td> <td data-bbox="1549 532 1724 609">Asymptomatic</td> <td data-bbox="1724 532 1892 609">Symptomatic</td> </tr> <tr> <td data-bbox="1205 609 1362 685" rowspan="2">Genotype E</td> <td data-bbox="1362 609 1549 649">Present</td> <td data-bbox="1549 609 1724 649">10</td> <td data-bbox="1724 609 1892 649">25</td> </tr> <tr> <td data-bbox="1362 649 1549 685">Absent</td> <td data-bbox="1549 649 1724 685">12</td> <td data-bbox="1724 649 1892 685">7</td> </tr> </table> It is the reason why I obtained: $p = 0,01$ and Odds ratio=0,23 But I think that you have done the best one, so it will be corrected in the text. Thank you for your professionalism and expertise.		Population				Asymptomatic	Symptomatic	Genotype E	Present	10	25	Absent	12	7
		Population																
		Asymptomatic	Symptomatic															
Genotype E	Present	10	25															
	Absent	12	7															
Asymp	10	12																

Most importantly, the size (or base pair) markers are not indicated on the ethidium bromide-stained gel, absence of which drowns the validity of this feat. You only have arrow pointing to 167 bp, but there are no markers with which one can compare this to lend credence to the genotype E band. So let some values of bp be indicated on the gel for reference or guide!

How did you ascertain that the 54 samples had HBV DNA?

DISCUSSION

Line 152: One good point you seemingly overlooked is the observations that only 54 of the 139 HBsAg- positive participants had HBV DNA; and only 35 of the 54 with HBV DNA were typable. What could be responsible for these observations? Why would samples with HBV DNA end up not typable? Are there any previous studies to support these observations?

The discussion is not as logical as I would actually



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	wish; kindly tailor your discussion toward your objectives and your other pertinent observations. CONCLUSIONS You need to make this brief and concise, and valid. Your conclusions are not readily clear and not all derived from your observations. PARDON ME: I did not review the reference list. DECISION The manuscript is not yet publishable; accept it for publication after all the corrections have been effected. The English must be free of grammatical errors.	I have done a thurst PCR HBV DNA only to determine the positive samples. And these samples have been used to follow the study. This situation could be explained by our methodology (Naito 2001); the condition of working; the problem of conservation due to the problem of electricity.... But the study is following with and other methodology (Touraj 2012) to identify all the strains. OK.
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<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		