



SDI Review Form 1.6

PART 1:

Journal Name:	British Microbiology Research Journal
Manuscript Number:	2013_BMRJ_3708
Title of the Manuscript:	Detection of Mycobacterium tuberculosis Complex in Lung Specimen of Slaughtered Cattle and Goats By a DNA Based multiplex- Polymerase Chain Reaction and Ziehl-Neelsen Methods In Jos, Nigeria.

General guideline for Peer Review process is available in this link:

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

- This form has total 9 parts. Kindly note that you should use all the parts of this review form.



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PART 2: Review Comments

	Reviewer's comment	Author's comment (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)
Compulsory REVISION comments	The results reported in the manuscript could be valuable although, in my opinion, are not sufficient to be presented in a "research paper" format. I suggest include them in a "short communication" or a "short research article". Anyway, some issues should be changed before publication: - The origin (regions) of the slaughtered animals is not clearly stated and this matter should be taken into account in the discussion when results are compared with those obtained in other regions in Nigeria. - Only lungs were analysed and Tb lesions could be present in other organs. Tb lesions are frequently present in lymph nodes and the lungs are clear. This is an important limitation of the study and could affect seriously to the "prevalence" data reported. For this reason, it should be clearly stated in the discussion when results are compared with those obtained in other studies. Which organs were sampled in previous studies in Nigeria? - The term "prevalence" in the manuscript is confusing since it has been calculated based on data from animals from different regions of Nigeria. Moreover, data about the total number of animals slaughtered in the abattoir (cattle and goats) could be valuable. Fifty animals could be enough to calculate prevalence data from a specific region or herd (depending on the total population) but probably not if the animals are from different regions. In my opinion, data could be shown as results of surveillance in the slaughterhouse of a specific region of	-We would prefer our manuscript to be published as a full research paper -The origins of the slaughtered animals, was the northern region of Nigeria. We could not trace the herds because the butchers at the abattoir, could not provide that information. We were more concerned on the Tb statuses of the slaughtered cattle and goats in the study area - We collected lung samples only because, people in Nigeria do not consume the lymph nodes of animals but they eat the lungs as meat and use it to prepare various delicacies. The abattoir used as the study area, is central. Most consumers of meat in Plateau State Jos go there to buy meat(cattle or goat). -We do not agree with your statement.We



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	Nigeria and not in the present format (prevalence). - The usefulness of the multiplex PCR is questionable since the author remark that some <i>M. bovis</i> strains lacks one of the fragments amplified. This PCR seems only valid to detect <i>M. tuberculosis</i> complex members but the authors also said that some members could not be detected using this PCR (lines 335-336). - The inclusion of the isolates identification could add valuable information to the manuscript for publication (in special for the comparison with previous studies in the discussion). - No data about presence of Tb compatible lesions in the lungs is reported in the results section. Did you find lesions? If lesions were not observed, the sensitivity of ZN and DNA extraction decrease since small samples of tissue are selected to detect the mycobacteria. In contrast, if the tissues selected have lesions the sensitivity is higher. - The authors reported a prevalence of 0% in goats and suggested that goats could be resistant (line 306): The reference included in this line is about PCR, is it correct?. Tb in goats by <i>M. caprae</i>, <i>M. bovis</i> and even <i>M. tuberculosis</i> has been widely reported so susceptibility is clearly demonstrated. - Additional information about the fragments amplified should be included (gene?) and about primers used (previous references?). Moreover, inclusion of previous data regarding sensitivity of the DNA extraction method could be necessary. - The authors reported in the conclusions that cattle are more susceptible to Tb than goats. In my opinion, this study cannot conclude this. The study only showed that the cattle analysed was more heavily affected (in the lungs) than the goats but susceptibility should be studied using other kind of experiments. I have doubts about the	stated that, only the 245bp fragment was amplified which detects members of the MTBC. The 500bp fragment which is some times produced by the <i>M. bovis</i> species was not amplified by the JB21/JB22 primers. This failure, has also been documented by some authors as stated in our manuscript. They blamed this failure on mutations along the insertion sequence of <i>M. bovis</i>. Our study was a research to detect the presence of Tb agents. We used two pairs of primers which makes our PCR a multiplex PCR. The absence of the 500bp fragments or the inability of the INS1/INS2 primers to amplify the 500bp fragments should not make the m-PCR less useful in this study. - No, there was a mistake on the arrangement of the reference used in that line it is [11] not [12], in this study, no agent of Tb was seen in samples from goats. Some studies have stated that goats are immunologically, more resistant to diseases, including Tb than cattle. It is also possible that goats in the study area were not infected with Tb. - This study is also subject to further research, more research on this subject are ongoing, but as at when this research was done, the data we obtained showed the
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	statistical analysis to show the correlation between species and presence of infection. Obviously, attending to your results it seems that Tb affects more cattle than goats but is it real? Can you extrapolate this results knowing the limitations of your study? - A big section of the discussion is about in the potential Tb transmission between animals and humans (lines 368-392). Although transmission between cattle/goats and humans has been previously reported, this study is not focused on this matter since molecular characterization of animals and human isolates has not been performed. The English grammar should be revised throughout all the manuscript.	presence of Tb in slaughtered cattle and none in the goats. -We do not understand your point here, we have gone through the lines(368-392). In our review we mentioned, that some authors have reported transmission between humans and cattle.
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Minor REVISION comments	Line 29: the study showed the % of samples positive by ZN and not the % of bacilli present in the samples. Line 34: sample is more frequently used than specimen. Line 54: Mycobacteriaceae. Line 55: remove the “,”. Line 56: main etiological agent... Line 57: in my opinion “tuberculosis in birds” by M. avium is a mycobacteriosis since it differs from the tuberculosis caused by members of the M. tuberculosis complex. Line 58: the term Tb should be used throughout the manuscript instead of tuberculosis. Line 61: caprae and pinnipedii. Line 62: bacteria are closely related. Line 68-69: repetitive. Line 72: “to date” instead of “up till now” Line 75: complex. Lines 78-82: aerosol route should be included among the routes of infection between animals and humans and not only between humans. It is the most frequent in people (vets, farmers...) in close contact with infected animals in developed countries. Line 90: in the recent years, the scientists tend to define the bovine Tb as the Tb infection in cattle by any of the species included in the M. tuberculosis complex. In this line, Tb by M. bovis is a multi-host infection. Bovine Tb is the infection in cattle. Lines 97-107: inclusion of data about density of cattle and goats in Nigeria could be interesting. Lines 101-104: revise sentence construction. Line 122: UFC instead of organisms. Line 142 and lines 173-173: define the area of study more in depth. Lines 194-195: all kind of acid fast bacilli are detected using ZN (not only M. tuberculosis complex members).	We have seen these mistakes and have made the corrections to the best of our ability -In this line, we meant the number of bacterial cells per ml of sputum sample -It has been corrected on the text
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	Lines 223-227: references for the primers. Information about multiplex basis of the PCR should be included (M. tuberculosis complex members, M. bovis). Lines 242-257: include additional information about presence of lesions. Results of ZN and PCR can be indicated in the same table. All the figures are not necessary and could be removed. Line 248: "...sometimes...". The reason for this variability should be included. Why this fragment was chosen for the PCR if you know that some mutants are not detected? In my opinion, the term "prevalence" should not be used. Lines 256-257: The higher detection of infected cattle should be considered a descriptive finding. In my opinion, make a statistical analysis relating animal species and prevalence has no sense. Lines 304-306: include the author and the reference. In reference [12]: How many animals were tested? What samples were collected-analysed? Only lungs? Tuberculosis in goats has been widely reported so innate resistance is not clear (reference included seems no valid). Lines 307-311: How many goats were tested in the study of Ojo and collaborators? In what samples were M. bovis and M. tuberculosis isolated? Perhaps it is necessary to include these issues to remark that your results disagree with those obtained in that study. Line 331: include author and collaborators. Lines 332-336: in my opinion, considering that all samples positive by ZN were due to MTBC infection (did you find any Tb lesion?), a PCR that is less sensitive than the ZN seems not adequate for diagnosis. Among the reasons to the low rate of positives detected by PCR but confirmed by ZN could be also the limitations of the DNA extraction method. Anyway, results are not adequate for	It has been stated -We had to correct the reference, it is [11] not [12]. Lungs and associated lymph nodes were collected. 1,387 goats were tested, -Lungs and associated lymph nodes respectively - All the samples collected for both animals had lesions suggestive of Tb. - In our discussion, we stated that the ZN revealed all acid fast bacilli (which includes MTBC and non-MTBC mycobacteria, while the PCR was specific for only MTBC.
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	Tb prevalence estimations since almost the half of the samples detected by ZN (and not using PCR) could be due to atypical mycobacteria. Line 350: “risk” instead of “danger”? - Lines 357-358: Include author of the reference. Is the prevalence reported by [29] from humans infected with <i>M. bovis</i>?. In this case, results are not comparable. - Lines 412: this is an important issue, especially if Tb lesions were found (?). - Lines 414: Is the data lesion available? If positive samples are from lungs without Tb compatible lesions the results could be even more interesting.	Yes. We will remove the comparison - All lung samples used in this study had lesions suggestive of Tb. -We wanted our results to be as explicit as possible. Removing the figures will mean downgrading our m-PCR results - We chose this fragment, 500bp because many authors outside Nigeria have used it and have obtained good results , although one recorded 13.3% failure. We wanted to find out if we could use it in the study area to detect <i>M.bovis</i> . As we stated before, it was a research. - we disagree with you on this because, nomads in the northern region of Nigeria, often rear cattle and goats together, that is why we used the two species
<u>Optional/General</u> comments	Introduction is long in comparison with the other sections. The study could be focused on the using of ZN and direct PCR for detection of TB infected animals slaughtered in the abattoirs (no to estimate prevalence). Results section is poor for a research paper and could be more valuable in a short communication.	