**Author's response to reviews**

**Title:** Analysis of risk factors for T. brucei rhodesiense sleeping sickness within villages in SE-Uganda

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**Author's response to reviews:** see over
Dear BMC Infectious Diseases,

Many thanks for the reviewer’s comments relating to the above manuscript, and for your understanding in giving us a somewhat extended period to address these. Please find a point-by-point reply to the comments from each reviewer below.

We look forward to hearing from you in due course.
Many thanks.

Eric Fèvre (and colleagues).
“If left untreated, it is invariably fatal”. Authors have to moderate this since no data are available on the “natural evolution” of the disease. Long term asymptomatic carriers have been described (see Sternberg 2004 Parasite Immunol. 26, 469-476), spontaneous cures and human “trypanotolerance” are suspected (see Garcia et al., 2006, Trends Parasitol).

The phrase was modified accordingly, and the reference to Sternberg 2004 has been added.


We have added a section to the introduction to address this point, as follows:

“An estimated 300,000 new human infections occur annually, causing an estimated 46,000 deaths per year (Mathers, 2007 #2168)[World Health Organization, 2006 #1684]. A recent programme set up in the year 2000 including intensified surveillance and control measures, training of health personnel and access to drugs has made significant contributions to the recently observed decline in the number of newly reported cases, mainly for T.b. gambiense [World Health Organization, 2006 #1684]{Jannin, 2005 #2228}.

“Whereas in West-African HAT humans are the reservoir for the parasites...”. While its epidemiological significance is still under debate, the existence of an animal reservoir of T b gambiense has been proven.

We have modified the relevant statement to read:

“Although human-infective parasites have also been found in animals in West Africa [Simo, 2006 #2205], humans are thought to be the principal reservoir hosts for T.b. gambiense HAT. East-African HAT is a zoonosis {Fèvre, 2006 #1424} and transmission requires the presence of suitable reservoir animals (e.g. domestic livestock, wild bovids).

It is said in this part that “There were only slight differences between women and man with regard to occupation and activities involving potential risks for HAT infection (see Table 2)”. Examining table 2 shows that they differ significantly for almost all activities presented (P=0.001; P=0.007; P<0.001) except for visiting the cattle market. This is in total contradiction with the sentence above. Furthermore, it appears that in this area (given the data in LIRI) males are more affected than females (62% versus 38%). This deserves to be discussed in the discussion part.

This problem results from a manuscript version conflict, for which we apologise. We are grateful to the referees for drawing our attention to this problem. A paragraph describing and interpreting the information given in table 2 was added to the results and discussion section, as follows:

Eg Results:

“In the study population, we observed relevant and significant differences between women and men with regard to occupation and activities involving potential risks for HAT infection (e.g. herding cattle, collecting water or firewood, for details see Table 2).

Behavioral and socio-demographic risk factors. This part is presented in two sections: univariate analysis and multivariate analysis. Description of the variable included in the analysis is given in the multivariate part. This should rather be done in the first part and focus in the second part on only those variables which remain significant in the multivariate analysis.

The paragraphs in this section have been modified accordingly.

Table 3: Presentation of this table is not satisfactory and authors should find a way of making it more readable (studied variable should be written on only one line); 0.053 and 0.05 are not <0.05.
Studied variables are presented in one line. Spaces within the table were increased to improve readability, and errors have been corrected. Importantly, we have now separated the variables from the univariate and multivariate analyses in to 2 separate tables, increasing, we hope, the overall clarity of the presentation of these results.

In their analysis authors included all variables in the multivariate analysis by conditional logistic regression (22 variables). This number is far too high, especially since the number of individual included in the study is low and can cause statistic instability. In order to calculate more accurate OD ratio and provide a better model authors have to conduct a stepwise conditional logistic regression analysis were only variable with suggestive P values (<0.2) in the univariate analysis are selected and entered one by one in the model until no significant increase in the likelihood of the model is observed.

After a thorough re-evaluation of the statistical methods used for fitting the model for the multivariate analysis, a stepwise backward selection procedure was used, where only variables with a p<0.1 were retained for the final model. Variables had to be plausibly associated on theoretical grounds with HAT. A description of the procedure has been included in the results section.

Authors should read their discussion and rewrite it to make it fit to the data presented in the result section. For example, in the second paragraph: “In our case-control study, having a family member with history of HAT was the strongest risk factor associated with HAT infection, followed by the need for regularly leaving the village for work outside the village” No mention is made here about cassava crops (P=0.021) whereas regular work outside the village is only orderline significant (P=0.049).

The discussion section has been comprehensively revised, in part to reflect these comments.

As stated by the authors the main finding of this study is the presence of familial aggregation of HAT cases, “an established feature in HAT”. The fact that in their work author make this observation in the frame of a study of environmental and behavioral risk factor should allow them to better discuss this point. In fact in this study, no such factor were able to explain the familial aggregation (having a previous case of HAT in the family remains the most significant factor even after introducing other environmental and behavioral risk factors in the multivariate model). Thus either this familial aggregation is due to none evaluated environmental risk factors or it could be due to factors from the host itself, i.e. genetic factors of resistance/susceptibility. Familial aggregation is a common feature in many infectious diseases (malaria, tuberculosis, leprosy, leishmaniasis, schistosomiasis....). For these diseases it could be demonstrated that it was not only due to shared environmental factors but to the genetic background of the host. Considering this point authors should note that although few work has been carried out on host genetic factors in THA, several studies indicate their importance in human whereas it has been clearly demonstrated in animal models such as mice and bovine (see review in Garcia et al., 2006, Trends Parasitol).

The discussion has been modified in order to better explore the interpretation of the data, particularly with regard to the possible association between spatial and behavioural risk factors, and we continue to highlight the issue of familial aggregation as suggested. We have added the Garcia 2006 reference.

“The poorest villages, and within those the poorest inhabitants, are therefore most at risk of contracting sleeping sickness.” The authors evoke some socio-economic factors while no mention of such factors is made in the methods nor in the results section.

This statement has been omitted.

Minor revisions:
1. “by Okia et al. in 1994”, add [17]
Reference was inserted.

2. Reference 14 is not essential and should be removed.
The reference was removed.
Reviewer #2: Marleen Boelaert

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1. P7 Results ln 6 'there were only slight differences between women and men (see Table 2)'. In fact table two shows men's involvement in cattle herding is twice as frequent as women's. This is not what I would qualify a slight difference.

The text has been modified accordingly, (see also reply to comments of reviewer #1)

2. Table 2 P18 would be more informative if the denominators of the categories Total/Men/Women would be mentioned.

The information was included in the table.

3. Table 3 Please include sample size and number in categories

We feel that it would be unusual and would negatively influence readability of the table to include information about sample size and numbers per category in logistic regression results. We do, however, present these data in the “methods” and “study population” section of the manuscript.

4. P5 ln 10 between wetlands AND the homesteads

“and” was inserted
Reviewer #3: Grébaut Pascal

Discretionary Revisions (which the author can choose to ignore)

The observations that were done in this study are specific to the study area. As it is outlined in this article, HAT transmission patterns are complex, in particular the social organization that modulates the relationship that man can have with his environment, and consequently with the vector and the parasite. For instance, knowing that the relationship of a non-native population with its environment can differ from the natives's one, subsistence agriculture does not necessarily imply the same relationship with the environment than cropping. This was described in the HAT foci in Western Africa (Hervouet & Laveissière, 1987). Conversely, in some situations the familial use of some usual places can prevent HAT from a larger expanding (Grébaut et al., 2001), limiting it to familial aggregation. Concerning your last sentence, I can not agree with the recommendation of altering the individual behaviour and use of the landscape, if people don't want it will you oblige them? Wouldn't it be easier to disrupt HAT transmission by simple vector control? In fact I do not really understand the operational improvement that these informations could bring to classical survey (diagnosis, treatment and vector control), could you be clearer about it?

We do not recommend altering people’s behaviour or use of the landscape. Rigorous and sustained vector control in a resource-limited setting such as in the study region for a disease affecting only a comparatively low number of individuals is very difficult given the poor existing knowledge about tsetse habitat as well as the nature of tsetse habitat (swamps or bushes around usually inaccessible wetland). Knowledge of risk factors (e.g. almost all cases of HAT occurred in homesteads located in proximity to wetlands) may give important information to regional and local planning committees and help to reduce the risk in existing and particularly in new settlements. We have edited the final statement to better reflect our intended meaning, as follows:

“The results presented will be useful for the design of specific, targeted control interventions and the implementation of preventive measures (e.g. in land-use planning) in order to reduce the risk of HAT for village inhabitants in south-eastern Uganda”
Reviewer #4: Pere Simarro

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Sleeping Sickness have different patterns of transmission according to: human activities, sex and distribution of daily work, environment and other factors which determine the contact within the vector and the reservoir including humans. In some foci sex is a risk factor, where man’s main activity is either fishing or hunting. In other foci, women are the most affected group of population when their main activities revolves around washing clothes and other household chores out of the village. While in some other foci, children involved in cattle raring or water collection are more at risk etc. As the authors themselves indicated, in other studies the proximity to cattle markets, coffee plantations, etc was also seen as risk factors Authors have developed interesting methods to study various factors influencing HAT transmission. Nevertheless they have to provide more details regarding the area where this study took place including GIS coordinates. Likewise they have to discuss the importance of local factors in each foci and the danger to generalize finding regarding risk factors transmission from one focus to all HAT foci.

The message should be that each foci should be investigated regarding transmission factors in order to plan control activities rather that imply in the conclusion that “the analysis of both spatial and behavioural risk factors has provided within-village data at risk that can be used for the design of control and intervention programmes”

During comprehensive revision of the manuscript, we have pointed out more clearly that results only apply to the the HAT focus of the study area, e.g. in the discussion and the conclusion section. Data from other HAT foci (e.g. Busoga district) has been comparatively discussed. A description of local factors (e.g. presence and distribution of cattle) has been included and described in more detail.

The last part of the above comment was implemented particularly in the revised conclusion, and we have added the following final statement:

“We would recommend studies at an equivalent scale in other T.b. rhodesiense foci to better understand risk factors and to improve HAT management elsewhere.”

We have added the co-ordinates of the boundary of the study region () to aid in placing this study in the context of other foci in Africa, and have highlighted a separate paper describing the general characteristics of the focus in more detail:

“The study was undertaken in Tororo District, south-east Uganda (longitude 33.8-34.0; latitude 0.5-0.9). The study area has been previously described in detail elsewhere (Odiit, 2004 #986)."

Local planning authorities and village leaders were also provided with detailed maps of swamp boundaries and other features digitised during the fieldwork, for planning purposes at their own discretion.

Discretionary Revisions (which the author can choose to ignore)

Background
Authors have to include Central Africa since currently it is the most affected region in Africa.

The manuscript describes the occurrence of HAT “across 36 countries in sub-Saharan Africa” and then focuses on the specific situation in SE-Uganda and the study area.

In West-Africa, some authors have published papers about the role of the animal reservoir in HAT transmission. Hence the phrase “Whereas in West-Africa HAT humans are the reservoir
for the parasite" should be modified accordingly. Probably adding "humans are the main reservoir" could be enough.

This was modified accordingly, see also reply to reviewer #1.

Methods
This does not appear clear, what happens when in the process to identify controls, the selected household had previously had an HAT case ? In this case, was the household selected and subsequently have the control included or not ? Clarification needs to be provided regarding this topic.

Cases as well as controls were asked the same set of questions including history of HAT in a household member. The difference between cases and controls in terms of the frequency of occurrence of HAT in a household member was, in fact, one of the variables under study. Controls were included irrespective of the history of HAT in a household member – the study showed that significantly more cases than controls had a household member with previous HAT infection.
Reviewer #5: Florence Fournet

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

**Why do authors choose a 3 years period before the study to select the villages (limiting the size of the cases sample)?**

The sample size was limited by the number of cases available for study from the case records of LIRI hospital. On one hand, the quality of records kept for cases older than three years did not appear to be sufficient and on the other hand, the investigators wanted to ensure that changes in village structure and land use over time did not affect the results. Therefore, we chose a limit of 3 years for the occurrence of cases of HAT prior to the beginning of the study.

**Distribution of the HAT cases has to be studied in the whole district and then hypothesis have to justify the choice of only certain villages. The criterions upon which these villages have been chosen have to be clarified.**

For logistic constraints, it was not possible to study all villages in the district (for the spatial analysis, every single household of a village had to be visited). Therefore it was decided – as stated in the manuscript – to carry out the study in those villages most affected (highest number of cases per village among all villages) by the disease in order to increase the probability of reaching statistical significance in the spatial risk factor analysis. A detailed study of district-level factors has been previously published and we make reference to that paper in our manuscript (1. Odiit M, McDermott JJ, Coleman PG, Fèvre EM, Welburn SC, Woolhouse MEJ: Spatial and temporal risk factors for the early detection of *T. b. rhodesiense* sleeping sickness patients in Tororo and Busia districts, Uganda. *Trans R Soc Trop Med Hyg* 2004, 98:569-576).

**A map of all the villages of the Tororo district would be useful to better understand the geographical situation. It may be interesting to give also a prevalence map of the HAT at the village scale for the whole district. The map may explain better why the authors have chosen the 17 villages of the study and then the 4 other ones.**

For the mode of selection of villages for the spatial analysis, please see reply to the comment above. Analysis of the HAT distribution in the entire district is outside of the scope of this study, and has already been carried out in our earlier work:


**Why cases detection was only passive?**

In the study area, no active case-detection programme is in place. A retrospective analysis of case records was the only source of information available about HAT cases in the study area at the time of the study. The authors did not have an influence on the acquisition of hospital data, and an active screening programme would have been outside the scope of the present study.

**Why do they chose villages for the spatial analysis upon the basis of 5 to 8 cases? It would be important to give first the number of the cases within each of the 17 villages and to justify this choice.**

This decision was taken mainly on logistic grounds.
The authors must explain why do they choose a radius range from 400 m to 5000 m between the wetland and the homestead? Presence of the wetland appears at a risk factor but is distance the only determinant of HAT to be considered?

In the manuscript, we state that: “Buffers with a radius ranging from 400m up to 5000m were used representing the range of the daily spatial activity patterns of the inhabitants.” and “Proximity of a homestead to the wetlands as a possible risk factor was assessed by the proportion of the buffer area intersecting with the wetland.” In fact, direct distance to the nearest wetland is not the risk factor investigated – we investigate a more representative measure for such an area consisting of branched and irregular shape of the wetlands – proportion of landcover within the radius in question. Due to the scale of the geographical features in the study zone, with buffers less than 400m no homestead – regardless of case or control status – will intersect with the swamp, and the contrary is the case for buffers over 5000m where all buffers will intersect with the wetlands.

Are entomological data available which may be included the regression model?

The regression model includes human behavioural factors and type of land use around homesteads. Both types of risk factors investigated serve as surrogate markers to determine the intensity of man-fly contact. Unfortunately, reliable entomological data of a quality sufficient to parameterise such a model in the study region are very sparse.

Why spatial data like villages localization, demographic data (population density) were not included into the regression model as well as the villages?

Villages were selected according to the procedure described. The study was designed specifically to analyse risk factors for individual village inhabitants. Other risk factors such as population density are appropriate for an analysis at larger geographical scales – see Odiit M, McDermott JJ, Coleman PG, Fèvre EM, Welburn SC, Woolhouse MEJ: Spatial and temporal risk factors for the early detection of T. b. rhodesiense sleeping sickness patients in Tororo and Busia districts, Uganda. Trans R Soc Trop Med Hyg 2004, 98:569-576.

Differences between males and females are not slight as they are significant for the majority of the tested variables.

This has been addressed in relation to comments from the other reviewers.

What proximity to homestead means for cassava crops? Is cassava consumed by the homestead or is it sold on the market, inducing an increase of the displacements to the markets?

We state that: “the presence of cassava as a crop type around the homestead (OR 1.93, CI95 1.01-3.68, p=0.046) was found to be significantly associated with sleeping sickness. Activities associated with cassava cropping in themselves were not investigated, but it is likely that a proportion is kept for home consumption and excess sold on the open market.

The authors should link the behavioral risk factors to the spatial risk factors and should give hypothesis to explain why there is a positive correlation between the positive status of the homestead and the wetland.

The discussion was comprehensively revised paying attention to this point.