Author's response to reviews

Title: Outcome of acute East African trypanosomiasis in a Polish traveller treated with pentamidine

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Author's response to reviews: see over
Dear Editor,

I enclose a new version of a manuscript No 6367901541061967 for a consideration for publication in the BMC Infectious Diseases with some corrections according to suggestions and remarks proposed by both reviewers. The changes in the manuscript have been made as follows:

Reviewer no. 1

1. The Discussion has been shortened significantly. Three references have been removed from Discussion: Bukachi et al., 2009; Mugasa et al, 2008, 2009. The short paragraphs: “Uganda is the unique country (…)”, and “HAT caused by T. b. gambiense (…)”, as well as “Suitable Glossina spp. vectors abound in the Queen Elizabeth National Park (…)” have been moved to Introduction.

2. Summary, Case presentation, line 7: “veinpuncture” has been changed to “venipuncture”, the same editorial error has been corrected in Case presentation, 4th paragraph, line 8.

3. Spelling errors have been removed: Summary, Case presentation, line 11 & Case presentation in the main text, 5th paragraph, line 1: “leucopoenia” has been corrected as “leucopenia”.

4. Summary, Conclusion, line 1, and Discussion, 1st paragraph, line 1 & 2nd paragraph, line 3, and Conclusions, line 3: terminology has been corrected as “Sleeping sickness” instead of “African sleeping sickness”.

5. A short version of the case report has been sent to ProMed during the patients’ hospitalisation in the Department, when his clinical status was severe and some epidemiological data were difficult to be evaluated. Some more precise details as the travel schedule (which has been changed by organizers) were possible to be collected afterwards, and with a help of his wife. So “Six days after a whole day spent … a painless skin lesion appeared on the left arm (…)”, as mentioned in the 3rd paragraph of the Case presentation section, there is a correct version.
6. The data concerning Glasgow Coma Scale, blood pressure and respiratory rate on admission were not previously described as not significant and being within a normal range. Values of Glasgow Coma Scale, oxygen saturation and urine output have been added in the Case presentation, 4\textsuperscript{th} paragraph: “On admission (...) dyspnoea, hypoxaemia (oxygen saturation 78.9%), generalised oedema and oliguria (urine output 250 ml). (...)The patient was conscious and very well orientated (15 points in Glasgow Coma Scale) (...)

7. The additional information on the number of blood transfusions, plasma, platelets, anti-thrombin III and albumin has been added in the Case presentation section, 8\textsuperscript{th} paragraph: “(...) repeated transfusions of blood (2 units), plasma (9 units), platelets (50 units), anti-thrombin III (500 units) and 20\% albumins (400 ml) (...)

8. Case presentation, 9\textsuperscript{th} paragraph, line 9: “4rd” has been corrected to “4\textsuperscript{th}”.

9. An additional information concerning home-made CATT, IFAT and ETAT has been completed in Case presentation, the last paragraph: “The serological tests, which are produced under full quality control, are currently manufactured only at the Institute of Tropical Medicine in Antwerp (Belgium) from where field kits containing reagents, control sera, and test accessories can be obtained (http://www.itg.be/itg/GeneralSite/default.aspx?W PID=471&MIID=433&L=E; contact Philippe Büscher; pbuscher@itg.be)

10. Introduction, 3\textsuperscript{rd} paragraph (formerly Discussion, 5\textsuperscript{th} paragraph): references to Glossina spp. distribution in Uganda have been added [5-8]

11. Discussion, 5\textsuperscript{th} paragraph (formerly 8\textsuperscript{th} paragraph): references to prognosis has been added [27-29]

12. Discussion, 6\textsuperscript{th} paragraph (formerly 10\textsuperscript{th} paragraph), line 4: “rhodesience” has been corrected to “rhodesiense”.

13. The phrase: “On admission, Trypanosoma-specific antibodies were not yet detectable, but elevated concentration of total immunoglobulin M (IgM) was shown” has been deleted from the text, as a detailed information concerning levels of total IgM in mg/l is presented in Table 1: 1610 mg/l on the day 0, 3530 mg/l on the day 7, 7030 mg/l on the day 15, 5050 mg/l on the day 30, 3370 mg/l on the day 60, 3120 mg/l on the day 90, and 2170 mg/l on the day 120. The normal range is important to evaluate the laboratory data, but if confusing, it has been finally removed from the chapter.

14. Discussion, the last paragraph: the citation “A 2-fold reduction in serum antibody titre (...)” has been re-edited and an appropriate reference has been added as follows: “A 2-fold reduction in serum antibody titre may be interpreted as a sign of
at least temporary parasite reduction, but it cannot be used to ascertain definitive cure [36].”

15. Titles of Figures have been shortened according to Reviewers’ suggestions. Figures 2 and 3 have been combined like 2A and 2B.

16. Concentrations of bilirubin, fibrinogen and creatinine from Table 1, all have been corrected to mg/dl as mentioned in the main text of the manuscript.

17. Figure 5 has been removed.

18. The Discussion has been significantly shortened, and some small parts have been moved to the Introduction chapter. Three reference following appropriate paragraphs have been removed from Discussion: Bukachi et al., 2009; Mugasa et al, 2008, 2009.

19. Discussion, confusing paragraph “After the first dose of suramin, the blood stage parasites disappeared in less than 48 hours (…)” has been corrected and appropriate references have been added: "After prescription of suramin, the blood stage trypomastigotes disappeared within 3 days in all recently imported cases [13,16]. This was finally observed in this patient treated with pentamidine alone”.

20. We agree with the Reviewer that pentamidine was given against generally accepted recommendations. It has been described in Introduction: “In many instances, the recommended first line treatment with suramin is not readily available (…)”. But the aim of the study is to strongly emphasize that there are some T. b. rhodesiense strains which were so far unknown to be sensitive to pentamidine. Our patient was saved as receiving the medicine as urgently as possible, in less than 2 hours after admission to the emergency room, just after evaluation of peripheral blood films. A sanitary transportation from a distant infectious diseases department within Poland has been delayed of 24 hours in this case (!), so there was no option to wait another day for medicines from a foreign country. Moreover, blood films can confirm Trypanosoma brucei infection but not a subspecies classification. Molecular and serological analysis were performed retrospectively, after therapy. A paragraph concerning classical treatment and an alternative therapy has been re-edited and clarified in Discussion (on page 11): “Pentamidine is recommended to be the standard therapy for acute West African trypanosomiasis. Suramin is generally considered as the drug of choice for an early stage of Rhodesian sleeping sickness; melarsoprol alone or in a combination with nifurtimox is proposed for a late stage of the illness [3,13,31,32]. A combined therapy with suramin and eflornithine seems to be very promising [32,33]. In case of unavailability of suramin, treatment
with pentamidine plays a crucial role in the prevention of severe complications of second-stage HAT, characterised by poor clinical prognosis [1]. In a large study of 56 travellers from non-endemic countries infected with *T. b. rhodesiense*, 7% were treated with pentamidine alone [34]. In some other patients with a recent stage of Rhodesian sleeping sickness, anti-protozoan therapy with pentamidine has been initiated and then switched to suramin upon availability [15]. Therefore, pentamidine is accepted to be an alternative drug for the management of an early phase of *T.b. rhodesiense* infection [35].

21. The paper indicates a possibility of existence of some *T.b. rhodesiense* strains sensitive to pentamidine, which has not been demonstrated before; related to the point no. 20 and 22.

22. According to the Reviewers’ suggestion, the 4th conclusion has been added in the last chapter to eliminate some confusing feelings about a promotion of treatment with pentamidine in *T.b. rhodesiense* infections: “In *T. b. rhodesiense* infections without a central nervous system involvement, treatment with pentamidine may be considered an option until access to suramin has been achieved”.

**Reviewer no. 2**

1. We agree with the Reviewer, it is important to stress why a described case is unusual or exceptional. For this reason we suggest to change the title of the study like: “Outcome of acute East African Trypanosomiasis in a Polish traveller treated with pentamidine”. The name of the medicine is crucial in the described case, as *T. b. rhodesiense* infections were so far unknown to be sensitive to pentamidine. This important information has been removed from the title during the pre-review process.

2. Pentamidine is not officially available in Poland and it requires a special procedure of importing from abroad, including an agreement of the Ministry of Health which takes approximately 6-8 weeks from application. So, this is never available in urgent situations outside specialised centres in Poland. Concerning suramin, it has been described in Introduction: “In many instances, the recommended first line treatment with suramin is not readily available (…)”. Moreover this is stressed in the Case presentation (8th paragraph, page 6): “Because of the severe clinical condition of the patient, and the non-availability of suramin on the day of admission, intensive anti-parasitic treatment with intravenous pentamidine isethionate (…) generally considered as the standard therapy for only acute West African trypanosomiasis (…) was immediately
instituted”. We decided to add the next conclusion (conclusion no. 4) concerning possibility of treatment with pentamidine if suramin is not available: “In *T. b rhodesiense* infections without a central nervous system involvement, treatment with pentamidine may be considered an option until access to suramin has been achieved”. Moreover, the additional citation concerning treatment has been added (Discussion, page 11): “In case of the unavailability of suramin, treatment with pentamidine plays a crucial role in the prevention of severe complications of second-stage HAT, characterised by poor clinical prognosis [1]. In a large study of 56 travellers from non-endemic countries infected with *T. b. rhodesiense*, 7% were treated with pentamidine alone [34]. In some other patients with a recent stage of Rhodesian sleeping sickness, anti/protozoan therapy with pentamidine has been initiated and then switched to suramin upon availability [15]. Therefore, pentamidine is accepted to be an alternative drug for the management of an early phase of *T.b. rhodesiense* infection [35]”.

3. Molecular diagnosis of *T.b. rhodesiense*, including differential diagnosis between both *Trypanosoma* subspecies has been made retrospectively after treatment in the Institute of Tropical Medicine in Antwerp. The additional clarification has been added in the chapter on Diagnostic differentiation of *Trypanosoma brucei* complex: “Antigenic diagnosis of East African trypanosomiasis was done retrospectively after anti-parasitic therapy using (…) (Institute of Tropical Medicine, Antwerp)”. The geographic distribution of *T. brucei* protozoans and their co-existence in Uganda, with appropriate references, is widely described in Introduction, 3rd paragraph. In Introduction (the last paragraph), we have written: “This is probably the first case of imported severe Rhodesian trypanosomiasis with extremely high intensity of infection and sensitive to pentamidine that has been so far described in literature [1,9]”.

4. In Discussion (the 1st paragraph): “Moreover, this was the first recorded case since the past 25 years of acute-stage East African trypanosomiasis occurring in a European traveller infected in Uganda (Eastern Africa)”. A short overview on single previous cases treated with pentamidine has been added in Discussion, page 11 (see point 2 above).

5. Evaluation of genetic markers of resistance is undoubtedly of a great experimental and scientific value, and should be performed for a series of cases or groups of patients in given geographic areas for a more accurate and objective assessment. In clinical practice, genetic markers not always correlate with clinical signs of sensitivity or resistance, they can only determinate a genetic
predisposition for a high probability of resistance. So, in a single case we based on a real clinical sensitivity to pentamidine confirmed by parasitological, biochemical and serological analysis. We wrote in Discussion, the last paragraph: Further analysis of some genetic markers of sensitivity and/or resistance to pentamidine in T. b. rhodesiense strains from Uganda has been promising.

6. The Discussion has been significantly shortened, and some small parts have been moved to the Introduction chapter, as proposed by the both Reviewers.

7. The information about pentamidine has been removed from the title during a pre-review process (see point 1). We propose the title: “Outcome of acute East African trypanosomiasis in a Polish traveller treated with pentamidine”.

8. The titles of Figures have been shortened. Figures 2 and 3 have been combined as 2 A & B. Figure 5 has been removed, so the total number of figures has been shortened to 3.

9. The Figure 5 (6th picture) has been removed (see above).

10. Table 1 allows to eliminate a wordy description of laboratory markers in the main text, and shows a progression or regression of different parameters during a course of infection, very difficult to be clearly or more compactly presented as a simple description in a chapter.

11. The English style of the manuscript has been checked by a native English speaking physician (see Acknowledgements section).

Yours sincerely,

On behalf of all Authors
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Poznań, Poland, November 11, 2013