Author's response to reviews

Title: Progressive dementia associated to ataxia and obesity in patients with Tropheryma whipplei encephalitis

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Author's response to reviews:

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To the Editor in Chief
BMC Infectious Diseases

Dear editor,

Please find our answers (in bold) to the reviewers for the manuscript of “Obesity and progressive dementia associated with Tropheryma whipplei” from Fenollar et al. for BMC Infectious Diseases. The modifications are marked in yellow in the text.

Reviewer's reports:

Reviewer 1: Lynne Sloan

1. Interesting work. Based on the few patients and the limited literature, I am not certain I would conclude this is a clinical entity of middle aged men.

Minor revisions:

1. pg. 4, line 78 - change send to sent
We have performed the modification.

2. pg.9, line 198 - remove a - should read developed hepatitis and obesity.
We have removed “a”.

3. pg. 11, line 248-249 - The sentence starting with, "Several viruses have been..., does not make sense. Please revise.
As suggested, we have revised our sentence, page 13, line 309: Several viruses have been already reported to contribute to weight gain in animals. In humans, one adenovirus has been also implied to contribute to weight gain.

4. Pg 12 conclusion - consider removing the statement about a clinical entity in middle-aged men. There is limited data to support this statement. As suggested, we have deleted “in middle-aged men”.

5. pg 27, table 2, patient 4 - under follow-up, what is lost of vue?
“Lost of vue” means that we were not able to be in contact with this patient anymore. We have deleted “lost of vue” and instead we have written: “We were not able to be in contact with this patient anymore”.

6. pg 27, table 2, patient 5 - under follow-up - should be after, not fater
We have made the corrections.

7. The legends under the tables should indicate what patients were tested at your facility and which patients were from your literature review. It is not clear what data is from the literature review and what data is your work.
As suggested, we have added in the legend of Table 1:
*: All the samples from these patients were analyzed with our facilities.
1: Patients were seen and followed by one of us (DR) in consultation.
As suggested, we have added in the legend of Table 2:
1: Patients were seen and followed by one of us (DR) in consultation.
2: Patients were from the literature review.

Reviewer 2: A. von Herbay

1. Major Compulsory Revisions
1.1. PCR testing was in the centre of this study, but the manuscript provides hardly any information to the reader about the PCR assay. While the authors feel this is stuff published elsewhere previously, essential information on methods must be included in the manuscript in sufficient details. It appears unlikely that there should not have been changes in PCR testing over the years (2001-2009). If so, those changes should be made transparent accordingly.
All the changes that we have performed on our molecular assays have been systematically previously published. As suggested, page 5, line 109, we have added the main changes to make them transparent: “PCR assays were performed as previously reported. Two hundred µl of cerebrospinal fluid specimens were submitted for DNA extraction using QIAamp DNA MiniKit (Qiagen, Hilden, German) according to the manufacturer’s recommendations. PCR mixes were prepared using a Fast-Start DNA Master SYBR Green kit (Roche, Mannheim, Germany) and following the manufacturer’s instructions. Quantitative PCR (qPCR) was performed in a LightCycler thermocycler (Roche biochemicals, Mannheim, Germany). For each assay, positive and negative
controls were used. At intervals of 5 samples, negative controls (water, PCR mix and human samples) were evaluated. A tenfold dilution of a standard suspension of 106 T. whipplei strain Marseille-Twist was used as a positive control and for quantification, as previously reported. The quality of DNA extraction from samples was estimated by PCR targeting a housekeeping gene coding #-actin. If a first PCR assay was positive, the result was systematically confirmed by a second PCR assay using a second set of primer pairs. In case of discrepancies between the two PCR assays or incorrect controls, samples were submitted to new DNA extraction and/or new qPCR assays.

The PCR primers that we have used have evolved with improvement in knowledge about T. whipplei. From January 2001 to February 2004, all samples were tested using our regular qPCR, targeting a 489-bp fragment of the 16S–23S rRNA gene intergenic spacer using the primers tws3f (5’-CCGTTGACTTAAACCTTTTTGAGA) and tws4r (5’-TCCCGAGGCTTATCGCAGATTG), as previously reported. If this assay was positive, a 650-bp fragment of the rpoB gene using the primers TWRPOB.F (5’-TTTTTCCGCGTGCCTCAA) and TWRPOB.R (5’-TTTCTCCGAGGTTGCCTGAC) was performed. For all of the assays, when an amplified product was detected, the identification of T. whipplei was systematically confirmed by sequencing, as previously reported. Since October 2003, the availability of the T. whipplei genome has offered the possibility of choosing repeated sequences of T. whipplei to perform highly sensitive and specific PCR assays. Thus, a PCR targeting repeated sequences of T. whipplei has been developed with, when an amplified product was detected, identification of T. whipplei confirmed by sequencing (between October 2003 to March 2004) and using specific oligonucleotide Taqman* probes (since April 2004). From October 2003 to March 2004, T. whipplei qPCR targeting a 164-bp sequence of the bacterium incorporated the primers pairs, 53.3F (5’-AGAGAGATGGGGGTGCAGGAC) and 53.3R (5’-AGCCCTTCTGCCAGACAGAC) in the reaction mix. If this assay was positive, the result was confirmed by a second assay using a second set of primer pairs 342F (5’-AGATGATGGACATCTGCTCTTCTATCATC) and 492R (5’-AACCCTGTCCTGCACCCCC), targeting a different DNA sequence. Since April 2004, T. whipplei qPCR targeting a 155-bp sequence of the bacterium, incorporated the primer pair TW27F (5’-TGTTTTGTACTGCTTGTACAGGATCT) and TW182R (5’-TCCTGCTCTATCCCTCCTATCATC), and a Taqman* probe (27F-182R, 6-FAM-AGAGATAACTCTGTGTTAAGTTGCACCA-TAMRA) was used in the reaction mix. If this assay was positive, the result was confirmed by a second assay using a second set of primer pairs TW13F (5’-TGAGGTGATCGTAGCAGATGT) and TW163R (5’-TCCAATACAAAGACAAACCAACAC), and a Taqman* probe (13F-163R, 6-FAM-AGAAGATGATTGCGGTGTTG-TAMRA) targeting a different 150-bp sequence. Finally, all the cerebrospinal fluid specimens prior to October 2003 were retrospectively tested using PCR targeting repeated sequences and the specimens sampled from October 2003 to February 2004 were tested using both regular and repeat-PCR.”
1.2. Data about the success of DNA extraction from CSF samples, and from control PCR are lacking. This needs to be given in the results section. We have added these data in the methods and the results sections. Page 5, line 114: “For each assay, positive and negative controls were used. At intervals of 5 samples, negative controls (water, PCR mix and human samples) were evaluated. A tenfold dilution of a standard suspension of 106 T. whipplei strain Marseille-Twist was used as a positive control and for quantification, as previously reported. The quality of DNA extraction from samples was estimated by PCR targeting a housekeeping gene coding # -actin.” Page 6, line 121: “In case of discrepancies between the two PCR assays or incorrect controls, samples were submitted to new DNA extraction and/or new qPCR assays.” Page 11, line 258: “Negative and positive controls were correct including the amplification of the gene coding #-actin.”

1.3. It appears inappropriate to ban data about inconclusive findings in many patients to a supplementary table (#2). These conflicting data must be presented in the results section. Given the absence of PAS positive particles and negative IHC, any positive PCR should be interpreted cautiously. Some readers might consider the author’s diagnosis in theirs cases #1 and #5 as “not certain”. As suggested, we have added this Table in the results section. Of course any positive PCR should be interpreted cautiously and we have added several parts in our revised manuscript about this topic. However, it is also highly important to underline that positive PAS-staining in brain biopsies are absolutely not specific of Whipple’s disease. We have added also recently published data showing that T. whipplei infection can be detected in skin biopsies of patients with classic Whipple’s without the positivity of PAS-staining (Angelakis et al. Journal of Infection 2010). In the discussion part, page 12, line 296, we have added: “However, it has been also recently reported that T. whipplei DNA is highly frequent in the skin biopsies of patients with classic Whipple’s disease whereas the PAS staining is significantly less sensitive in the same samples. Besides, these bacteria are alive, as a positive culture from a skin specimen has been obtained. Overall, these data show that PAS-staining, which is considered for a long time as the gold standard technique for the diagnosis of T. whipplei infection, is frequently negative despite the presence of T. whipplei infection.”

1.4. It is difficult to understand why in all patients with positive PCR results, cultures consistently failed to cultivate Tropheryma. This issue needs to be commented in the results section.

As suggested, we add comments about this in the result section. Page 11, line 265, we added : “We hypothesized that this difference maybe linked to a lower amount of bacteria present in the cerebrospinal fluid specimens of patients with T. whipplei encephalitis.”

1.5. The author’s criteria how to define “certain” and “possible” WD, and how to exclude WD among published cases, needs to be reconsidered. How is it justified to restrict “certain” cases to those with PCR+, but to designate patients with electron microscopy as “possible”?
PCR allows the detection of a DNA sequence that is specific of T. whipplei and allows a certain diagnosis. Electron microscopy allows the detection of structures resembling the bacterium; even if highly suggestive of T. whipplei, it will never allow an accurate, specific and certain identification. We have made modifications according to this remark, page 4, line 94: “The diagnosis was considered to be certain only for patients with positive T. whipplei PCR, that allows a specific identification of the bacterium…” and page 5, line 102: “Patients for whom an electron microscopy detected structures suggesting the presence of the bacterium in association with PAS-staining on brain biopsies were classified as possible T. whipplei infection…”

1.6. It is difficult to appreciate the reportedly low prevalence of positive PCR findings among the many CSF samples tested, as long as no information is provided about the patients under study. To comply with standards of good practice, the authors must provide at least basic clinical data about their patients age and gender. In addition, they should provide information about the status of antibiotic treatment prior to CSF sampling, as this might explain some negative PCR results.

The reviewer is right for the lack of information, mainly about antibiotic treatment that can explain negative results. However, we have not all the information for the 824 cerebrospinal fluid specimens. Thus, modifications have been performed according to these remarks. We have modified our discussion, page 13, line 318: “This clinical entity seems apparently rare… Besides, the lack of information about the status of antibiotic treatment prior to cerebrospinal fluid samplings for most of the patients, must also lead us to take this prevalence cautiously, as that might explain some negative PCR results.”

1.7. The report raises the question whether PCR testing of CSF in neurological patients is clinically reasonable, and whether a positive PCR result is sufficient to reach a diagnosis. This issue should be covered in the discussion section.

As suggested, we have added in the discussion, page 14, line 333: “PCR testing of cerebrospinal fluid specimens for T. whipplei in neurological patients is clinically reasonable as the lack of a specific treatment is fatal. As usual, for all the diagnosis performed using PCR, it is necessary to be cautious and apply a rigorous strategy when performing and interpreting the analyses due to the risk of false-positive results. A carefully checked positive PCR in cerebrospinal fluid specimens is sufficient to reach a diagnosis. It is important to remember the history of this patient for whom the diagnosis of Whipple’s disease was ruled out in front of negative PAS-staining in spite of positive PCR and finally confirmed using both techniques at the autopsy of the patient.”

1.8. From a clinical perspective, the antibiotic regimen used to treat symptomatic cerebral Whipple’s disease is of interest. Unfortunately, this topic is not considered in the discussion section. This should be added in a revised manuscript.

As suggested, we have added, page 14, line 341: “Another question concerns the management of this entity. The advances in knowledge with in vitro tests and
full genome sequencing of T. whipplei has shown that the usual long-term treatment based on sulfomethoxazole-trimethoprim is a sulfonamide monotherapy and that an alternative may be doxycycline and hydroxychloroquine, an alkalinizing agent. However, it is noteworthy to remember that sulfomethoxazole-trimethoprim have replaced cyclines, that had the reputation, not yet confirmed for doxycycline, of not crossing the blood–brain barrier in adequate amounts. It is why we have first used an association of doxycycline, hydroxychloroquine and sulfomethoxazole-trimethoprim for the management of T. whipplei encephalitis but recent evidence suggests that it is better to use sulfadiazine as its efficacy is comparable to that of the sulfomethoxazole, but a higher dose of sulfadiazine can be used to improve its ability to cross the blood–brain barrier. Antibiotics had a spectacular effect. However, three out of our 5 patients presented with clinical relapse despite therapy for at least 18 months. When the treatment was reintroduced, a spectacular effect was again observed. As for classic Whipple disease, the optimal treatment of T. whipplei encephalitis is not yet been determined. Overall, the data emphasize the necessity to perform a long-term treatment and lifetime follow-up."

2. Minor Essential Revisions

2.1. Information given on the index patient and the other four patients is out of proportion. A more balanced presentation of the five patients should be attempted.

Since the beginning, we have made the choice to make a detailed presentation in the manuscript of our index patient because his history is very intriguing and rich in lesson and all our study was initiated on the basis of this observation. An exhaustive presentation of all the patients is performed in Table 2 that includes all the crucial information: Age, sex, neurologic signs (first symptoms, all the symptoms), extraneurologic signs (those previous the apparition of the extraneurologic signs as well as those contemporary), the results from all the specimens to perform the specific diagnosis of T. whipplei encephalitis (cerebrospinal fluids, brain biopsies, digestive biopsies, blood, saliva, and stools), the other examinations (blood abnormalities if present, cerebrospinal fluids—with data about blood cells, protein and glucose levels—, brain CT scan, brain-MRI), the treatment (drugs, drugs dosage, length of treatment) and the follow-up (including the effect of the treatment, the presence of sequelaes, the length of follow-up). Thus, we think that the addition of the 4 other patients in the text will not give more information and will impose a deleterious reduction of the report of our index patient.

2.2. The authors do not make use of the common designation “cerebral Whipple’s disease” for patients without intestinal Whipple’s disease. They prefer to designate their patients as “T. w. encephalitis”. They may have a point to do so, but they should outline this in more detail, and also why they consider a diagnosis of encephalitis, rather than cerebral infection.

We do not used the common designation of “cerebral Whipple’s disease” mainly because T. whipplei is responsible not only of Whipple’s disease (characterized
by positive PAS-staining of small-bowel biopsies) that can be associated to various manifestations (including neurological) but also of other chronic infections without gut lesions (mainly endocarditis and T. whipplei encephalitis) as well as acute infections such as pneumonia and gastroenteritis. We added these explanations, page 3, line 56: “The well-known and classic form of Whipple’s disease, which is characterized by periodic acid-Schiff (PAS)-stained bacilli in infected small-bowel macrophages, represents only one rare clinical form of infection that can be caused by T. whipplei. Indeed, the bacterium has been also involved in subacute or chronic infections without gut lesions such as endocarditis, encephalitis, uveitis, adenopathy, pulmonary and osteoarticular infections. The diagnosis and the management of these infections are different. Finally, recently acute infections with T. whipplei were reported such as pneumonia, gastroenteritis or transit bacteremia.”

We consider that the diagnosis of encephalitis is more precise than a diagnosis of cerebral infection that may include not only the diagnosis of encephalitis but also those of meningitis.

3. Discretionary Revisions
3.1. The title should be reconsidered. The present title sounds a bit like a single case report.

As suggested, we have changed our previous title “Obesity and progressive dementia associated with Tropheryma whipplei” for “Progressive dementia associated to ataxia and obesity in patients with Tropheryma whipplei encephalitis.”

3.2. The introduction section (about Whipple’s disease) is somewhat biased, and all references point toward the authors own publications.

As suggested, we have included several references from other authors and we have also updated the references according to the most recent publications about T. whipplei and Whipple’s disease.

3.3. The authors do not give reference to other’s experience with PCR tests applied to CSF samples in patients without intestinal Whipple’s disease. This should be added.

We give reference to 29 manuscripts about T. whipplei and neurologic involvements. We summarize the cases of 15 patients from the literature with certain or possible T. whipplei encephalitis. We make an exhaustive comparisons between the different entities. We try to propose a clinical picture of T. whipplei encephalitis as well as a diagnosis strategy (showing major pitfalls for PAS-staining) and a management of this entity. It will be of course interesting to discuss about other’s experience with PCR tests applied to CSF samples in patients without intestinal Whipple’s disease but it was not the aim of this study.

Yours sincerely,
Prof. Didier RAOULT, corresponding author