Author's response to reviews

Title: Recurrent wheezing is associated with intestinal protozoan infections in Warao Amerindian children in Venezuela: a cross-sectional survey

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Author's response to reviews: see over
Rebuttal

Comments on the manuscript of the Associate editor

- Page 5: "consistent protective effect of infection with Ascaris lumbricoides, Trichuris trichiura, hookworm and Schistosomiasis. Should read Schistosoma sp instead of schistosomiasis. It is a disease not a parasite."
  Response:

- Page 6: "Stool samples were preserved in sodium acetate-acetic-formalin (SAF) preservative [29] and stored at 4°C?? Why the samples are stored in 4C after SAF preservation?"
  Response:

- Page 6: "Pure polymerase chain reaction (PCR) template preparation kit (Roche, Germany). Real time PCR of fecal samples was performed on the Roche LightCycler 480 system for detection of Dientamoeba fragilis, Giardia lamblia, Cryptosporidium parvum, Ascaris lumbricoides, Strongyloides stercoralis, Ancylostoma duodenale and Necator americanus. [30-32]"
  > The authors should provide more details (rewrite this section) about their Real time PCR methods (DNA extraction, primers sequence, probe, cycle, etc). The references provided are not accurate.
  > Reference 30: the authors used a single organism D. fragilis and used ABI 7500 Real Time detection system
  > Reference 31: The authors did not use the same PCR machine as described in this manuscript. They focused on E histolytica, Giardia and Cryprosporidium and they used iCycler Real Time PCR detection system.
  > Reference 32: the authors focused on soil helminth parasites and used Rotor Gene 6000 real-time PCR system (Rotorgene-Q).

  Since each Real time PCR system is different, the authors should provide in details their approach.

  Response:

- Page 6: "The diagnosis of parasitic infections was based on both microscopy and PCR? Please delete this sentence. It was already mentioned above."
  Response: We deleted the sentence which was mentioned above.

- Page 6: "Definitions? The authors should be clearer. Definitions of what?"
  Response: We agree that it is a short term and a single word is not very transparent for the reader. These are the definitions of the study objectives atopic eczema, recurrent
wheezing and malnutrition. We changed the word ‘definitions’ to ‘Definitions of atopic eczema, recurrent wheezing and malnutrition’.

- Page 7: ?Recurrent wheezing was only assessed in children aged 12 months and above?. Please give the number of children. It is not so obvious.
  Response: OPZOEKEN IN DATABASE of table 2.

- Page 7: ?From August to November 2012, 229 children 0 to 24 months of age were included?.
  The number in Table 1 =228. Please consolidate number.
  Response: OPZOEKEN IN DATABASE.

- Page 8: ?Fecal samples were collected from 100 children (44%). Children from whom stool samples were obtained were more likely to suffer from stunting than children from whom stool samples were not obtained (56 % vs. 40%, p=0.013) ?
  Why stools samples were not taken from all children? Some children might be asymptomatic or might be in incubation time during the visits of the team. Please comment on this.
  Response: The approach was to collect stool samples from all participants. Due to different possible causes only 100 samples were collected. In every village we were staying a maximum of 5 days and after that we had to go to the next village. A lot of the parents with their children visited the research team by boat and some were not able to return for a stool sample the next days. Other parents did not understand the reason to collect a stool sample if their children weren’t visible ill or malnourished. In the discussion section we suggest this: ‘The prevalence of stunting was higher in children from whom stool samples were obtained than in children without a stool sample. A possible explanation for this finding is that parents of malnourished children were more likely to collect stool samples because of concerns about the state of health of their children.’
  To be more clear about the fact that parents did not always hand in stool samples and that it was not a preconceived choice we changed a sentence in the discussion section:

  ‘In this survey a stool sample was collected in 100 of the 229 subjects (44%).’ was changed into ‘VERANDEREN ZIN IN MS EN HIER IN REBUTTAL’

- Page 9: ?The prevalence of atopic eczema in the study population was 19% (n=43) and recurrent wheezing was observed in 23% (n=28).? Please give the total number of children with recurrent wheezing since this observation was only made on children >12 months?
  Response: opzoeken in database wat totale prevalentie van recurrent wheezing was.

- Page 9: ?The overall gastrointestinal parasitic infection prevalence in children 0 to 2 years of age in our study was 70%?.
  This number might be lower if the stools samples were taken in all children (229
instead of 100). I am not totally convince with the approach taken by the authors, collecting only 100 stools samples.

Response:

- Table 2 is not clear and difficult to understand. The authors should simplify it and add it in the manuscript instead of supplementary Table.

- The authors should clarify the source of Figure 1.