Reviewer’s report

Title: Factors associated with tungiasis among primary school children: a cross-sectional study in a rural District in Rwanda

Version: 1 Date: 28 Jun 2019

Reviewer: Marlene Thielecke

Reviewer's report:

Many of my concerns have been addressed adequately and most of the points are well-solved. Thus, I still have some comments.

- It always should read: Tunga penetrans (not tunga penetrans). The genus name is written with a capital letter while the species name is written in small letters.

Background:
Page 3-4: The new part with general information about the biology, development and transmission which you added in the section "Background" has to be rewritten. There are some imprecisely and/or unclear information. Some sentences are grammatically incorrect. The structure is confusing, maybe you should try to start with explaining that the life cycle consists of an on-host and an off-host part. Then you describe the on-host part clearly, then the off-host part. You speak about the biology in a very detailed way. It this really relevant for your study? I think you should focus more on the pathology so that the reader can imagine why it has an impact on the school performance of affected children. Describe symptoms like itching, pain, impairment of walking... acute and chronic pathology. How long remains the flea in the epidermis? Please indicate co-infections like tetanus, gangrene or even HBV. (You describe some aspects later in the method section. I think I would fit better to give the information in the section background).

- Female of sand flea enters the host through the skin (-> "enters the host through the skin" could mean everything. Does the flea enter the body until the gut? Better: female sand fleas penetrate into the epidermis of its host) in unfertilized stage and copulation with the male occurs at that level (-> what level do you mean? Better: Copulation with the male sand flea occurs on the host after penetration). (Please add an appropriate reference).
- The fertilised (-> choose if you write fertilised or fertilized) female become hypertrophic (-> why? What happens? -> intestines grow, eggs mature) and expel eggs (-> to where? -> to the outside). (Please add an appropriate reference).
- The unfertilized eggs stop developing while those fertilized progress to development and become larvae 1 within 6 days (-> Leave this out because it is not relevant/interesting: The unfertilized eggs stop developing while; the supplement that it is larvae 1).
- These larvae feed on organic material in place (-> Please add information about the favored places for larvae to feed and develop like cracks and gaps in houses, shady places etc).
- The development of larvae 1 to larvae 2 takes a minimum of one day, and this larva will start pupation
stage within 4 to 10 days or more (leave this out because it is not relevant/interesting: The supplement that there is larvae 1 and 2).
- The adult fleas developed inside the pupation within 9 to 15 days (it should read: develop).
- Once the adult female fleas reach the skin of the host begin to suck the blood within 5 minutes and prepare to penetrate (it should read: Once the adult female fleas reach the skin of the host they begin to suck blood ...).
- Two thirds of the body of the flea is inside the skin after 24 hours of penetration, while complete penetration will be after 40 hours (leave this out because it is not relevant/interesting: Two thirds of the body of the flea is inside the skin after 24 hours of penetration).
- By feeding, there is enlargement of the flea (the enlargement of the flea is not due to feeding alone, see above), which is complete on the sixth day and then the eggs are expelled (for a period of several days).
- Ejected eggs reach the ground and are developed into larvae, pupae and then immediate adult form (it is not true that they develop immediate to adults - larvae and pupae can last for a long period, up to months until they hatch to adults under good conditions/circumstances).
- The life cycle of sand flea is partially or totally complete in human and domestic or wild animals depending on the tropical surroundings (it cannot be true that the cycle is totally completed in humans or animals as there is an on-host and off-host part in the life cycle of sand fleas! Obviously the off-host part take outside the host).
- This risk is increased in some countries like Uganda, where domestic animals such as goats, sheep, and pigs are kept in the same house with family members over night for preventing them to be stolen (it would be nice to add information in this regard about the situation in Rwanda; do people there keep animals inside their house?).
- Sand flea is among the parasites that can complete its life cycle when the person is sleeping (this sentence is very wired.. of course a complete life cycle does take a lot of more time! You are speaking about the place of transmission and not about time. Please make this clear).
- Afterwards, eggs are transported to the fissure and cracks when the floor is clean (you mean cleaned?).
- The adult sand flea emerges from pupae and penetrates the skin of the person when he deposes the naked feet on the ground (if you want to keep the right order, you first have to speak about the larvae which feed from the organic material in the fissures and cracks).

Background, Page 4:
- concerning the programs aiming preventing at treating tungiasis: You cited two publication, but you did not wrote any word about the content.

Methods, Design:
- What do you mean with "over time"?

Methods, Setting:
- it should read: primary schools located in a remote rural area ...
- All three schools had cemented floors and walls. (this information is a contradiction to the information you gave just before. Please give more information about the condition of the floors. Did they have a lot of fissures, cracks and holes?)

Methods, Data collection:
- ... children, who were included in the study were clinically assessed by inspection on the whole body to ascertain whether they had skin lesions suggesting tungiasis infestation. (is this true? How did you observe the whole body? In a class room? Were other pupils present by that time? Did the pupils take
all the clothes off?)
- loss of toenails and deformed nails as signs of chronic tungiasis...
- .. children who had any body part embedded by sand flea but without clinical manifestation were not considered as cases. (-> this does not really make sense as each embedded sand flea causes clinical signs sooner or later. In a very early stage (in the first few days) an embedded sand flea may not cause any symptoms but in the course of time the show a clinical manifestation. So, you did not count children with early stages as cases? Even if clinic will have developed later? Please, indicate how many embedded sand fleas were necessary for the case definition. At least one or more?)

Methods: Outcome and independent variables:
- In a Rwandan educational system, secondary school last 6 years. After that, a student may pursue studies in undergraduate and postgraduate program... (-> Leave at least the last sentence out, because not really relevant).
- Situation at school was assessed through school attendance (regular attendance, missing less than two days per week, missing more than two days per week). (-> please leave the information in brackets out. Otherwise it is doubled).

Results:
- ..those whose families shared houses with domestic animals, especially goats... (-> very interesting. Are there studies about tungiasis in goats?)

Comparison with other studies:
- Poor hygienic and housing conditions as major predictors of tungiasis infestation were echoed by other authors. (-> the content of this sentence is found in the sentence before and after. So it is a doubled information.)
- Increasing body of literature... -> what does this mean?

Conclusion:
- it should read: ...including dirty feet, wearing dirty clothes, ...

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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