Author's response to reviews

Title: Two stages PCR assay for detection of human brucellosis in endemic area

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Author's response to reviews: see over
Dear Dr. Neil,
Many changes were made to the new revised manuscript according to editorial and referees requirements. I’ll go through the concerns point by point. Author’s responds are in bold.

**TITLE:**

Referee number 3 commented:
Title is too broad the authors should narrow their title to what has been investigated

The title has been changed in the new revised manuscript.

**BACKGROUND:**

The first sentence copied from the abstract (referee number 1 comment).

This sentence has been deleted and the background part stated the aim. Question, type and aim were stated in the last part of the background in the revised manuscript. Authors did not evaluate Real-Time PCR since it is not available in many laboratories especially in the suburban areas which were selected for the study. Most laboratories do have the conventional PCR facility (referee number 2 and 3 commented about the study type). Authors wanted to emphasize the use of two stages PCR for Brucella diagnosis particularly in the suburban endemic areas.

**METHODS:**

Referee number 1 commented:
AFI patients
• How did you select the study area? What is the base of selection?

The study locations were selected according to the population life style of each. The Northern area is a suburban area and most of the population are Bedouin where they consumes unpasteurized dairy products and in direct contact with animal harboring *Brucella.* The Central city is much modernized urban city and almost no Bedouin live in it.

• How did you collect the samples? Who collected the samples and Where (Name of hospitals if present)?

Samples were collected by certified clinicians and nurses in two local hospitals. Hospital names are not necessary since the location’s names are not mentioned in the study.

• What do you mean by the “healthy individuals”, How did you detect the healthy one?
Healthy individual volunteers were selected by the clinicians with criteria mentioned in the subject part.

Referee number 2 commented on the healthy control subject’s number.

The 20 healthy control subjects were volunteers and that was the maximum numbers we succeeded to convince to participate. Convincing Arabian people (Bedouin and others) to participate in a scientific cooperation is too hard especially in the suburban areas.

Referee number 3 has similar comments on patients and control subjects too.

All patients were selected according to the AFI criteria as mentioned in the manuscript and according to the physician screening. All the control subjects were selected at the same time as patients. Control subjects have no previous history of unknown febrile illness and no animal contacts as where screened by physicians. All personal data are not shared for this study.

Referee number 1 commented:
• What do you mean by the “minor modifications“?

Minor modifications were limited to incubation time or temperature for some samples.

RESULTS:

Referee number 1 commented:
• You mentioned “All samples were subjected to Brucella genus amplification using B4/B5 primers which amplify a conserved region in all Brucella species to detect the presence of Brucella DNA as the cause for the Acute Febrile Illness (AFI)” is this a result?

Yes, it was part of the results, since the positive samples of the first PCR test were subjected to the species-specific second PCR step.

Referee number 2 commented on figure’s quality:

I’m so sorry for this bad quality. I think it’ll be much better to have only one figure (# 1 in the revised manuscript). Figure #1 from the un-revised manuscript was deleted and the new revised manuscript has only one figure which is the species-specific amplification.

DISCUSSION:
Referee number 3 commented:
The discussion should provide a clear interpretation of the findings, specifically: the results suggest that Brucella infection could be associated to a large % of AFI cases in the Northern region but not in the Central region. How can the very large difference be explained? What are the implications? Also, what are the reasons why a very large proportion of individuals react to both B. abortus and B. melitensis?

The percentage of Brucella case was expected to be high in the Northern Bedouin area than the Central urban (non-Bedouin) area. The double infection may exist because Bedouins herds include different animals like sheep, goats, camels and cattle. B. melitensis infection arises from sheep and goats while B. abortus infection can arise from cattle.

Hopefully some improvements were done and the entire referee’s comments were fulfilled. This manuscript was revised by a fluent English speaking colleague for language issues. Please accept my apologies for any inconvenience and for my bad English. Thanks again for you concerns and considerations.