Author’s response to reviews

Title: Biosafety and biosecurity requirements for Orientia spp. diagnosis and research: Recommendations for risk-based biocontainment, work practices and the case for reclassification to Risk Group 2.

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Editor Comments:

1. Please rewrite your discussion section in original language. It currently contains an unacceptable overlap in text with previously published sources. Although we acknowledge that these may be your own previous work, the present submission should be in original language.

Response: Amended – thank you for pointing out this matter. We have used our own software to detect where there was any duplication with our previous work and we have rewritten the following sentences in the discussion section.

“This has resulted in the erroneous requirement for BSL3 biocontainment for all Orientia spp. manipulations, while individual procedural risks are not considered.”
“BSL3 biocontainment laboratories fundamentally differ from BSL2 in that they provide protection to the outside environment from accidental aerosol contamination by directional airflow via a cascading negative pressure gradient and HEPA filtered exhaust air.”

“The risk-averse approach by administrators to manipulation and propagation of Orientia spp. and similar rickettsial pathogens, even for low-risk activities, has resulted in the proliferation of high-biocontainment laboratories in both relatively-wealthy and developing countries.”

“With regard to this evidence, we believe the culture of Orientia spp. and Rickettsia spp. requires a risk-based approach to biological safety, and therefore suggest that the blanket risk classification of this pathogen as RG3 coupled with BSL3 biocontainment requirement for all manipulations should be revised.”

“This is supported by the fact that scrub typhus LAIs have not been reported for nearly 20 years [13], and exposure of laboratory staff to infectious materials and their products are ameliorated by rigorous biological safety mitigation strategies through administrative controls including a focus on good microbiological practises and procedures, competency assessment, was well as judicious selection of PPE.”

Reviewer reports:

Ratree Takhampunya, Ph.D. (Reviewer 1):
Comments
2. P8, line50 to P9, line21: Under recommended risk assessments for Orientia spp. research activities. The scale of scrub typhus culture from clinical specimen/animal/arthropod: When stating the small-scale of the in vitro culture to be considered as LOW risk activities, more definition (maybe volume or quantity of viable rickettsia pathogens) should be added. This can be used as guidelines or reference for research lab (not clinical lab) when assessing risk-management.

Response. Amended as suggested. The section has been completely rewritten along the lines of the reviewer’s suggestion. The section now reads as follows, “LOW risk activities for both laboratory staff and environmental contamination in case of an accident would be small-scale processing of suspected scrub typhus patient samples such as initial in vitro inoculation or subsequent passage of patient samples on to cell cultures with a guideline of a total of ≤100 ml (in a single or multiple culture vessels) at a concentration of ≤106 cfu/ml Orientia spp. organisms. This may also include small-scale passaging of reference cultures.

MEDIUM risk activities may include those such as medium-scale passaging of reference cultures or medium-scale passage of clinical Orientia spp. isolates to increase the viable organism concentration thereby increasing the risk to the laboratory staff whilst maintaining a low risk of escape to the environment in case of an accident. Suggested guidelines for medium risk activities would be incubation and manipulation guideline of a total of ≤500 ml (in a single or multiple culture vessels) at a concentration of ≤106 cfu/ml Orientia spp. organisms

HIGH risk activities involve increased volume and concentration of the Orientia spp. organisms manipulated such as in the case of reagent production which may involve large-scale production of highly concentrated Orientia spp. as well as manipulation with increased inherent risk such centrifugation and concentration techniques, as there is an increased risk to the laboratory staff and of
escape to the environment in case of an accident. Suggested guidelines for medium risk activities would be incubation and manipulation guideline of a total of \( &gt;500 \text{ ml} \) (in a single or multiple culture vessels) at a concentration of \( &gt;10^6 \text{ cfu/ml} \) Orientia spp. organisms.”

3. Likewise, when stating that the significantly increase the volume and concentration of the culture materials (ml? or viable cells) such as in the case of reagent production then the risk would increase to MEDIUM or HIGH. If possible, please clearly define the volume and concentration.

Response. Amended as suggested. See above.

4. Adding which group the risk is referring to when stating the "risk" in this paragraph, on what (environment/community) or whom (laboratory personnel) the risk would have impact?

Response. Amended as suggested. See above.

5. Again in Table 4: Please define "low concentration, low volume" and "high concentration, high volume". These criteria can be used to distinguish in-house reagent production (such as for IFA or ELISA) and industrial production (mass production).

Response: Amended as suggested.

2) BSL-3 biocontainment cannot prevent the incident of parental inoculation (bite, cut, and inoculation), so the requirement of BSL-3 biocontainment for some research work such as cultivation or some small animal work (even the works performed under class II BSC) seems to be overstated. Although high volume, high concentration production can generate an infectious aerosol; however, working under class II BSC should successfully prevent the transmission to laboratory personnel who works with Orientia spp. Therefore, defining the scale of cultivation should allow some in-house antigen production activity be performed in class II BSC under BSL-2 facility.

Response: We thank the reviewer for their response. We believe we have covered this in the above revision.

John Antony Jude Prakash, MD (Reviewer 2):
This is a very well written article on an issue which is of great importance to many researchers in rickettsia.

My only comment is that animal studies for identification of the pathogen and for studies on pathogenesis, vaccine response be included clearly as requiring ABSL2 precautions. I request the authors to re-word their abstract to include the activities allowed at ABSL2.

Response: Amended as suggested. We thank the reviewer for their comment. This is covered this already in the section entitled “Risk associated with Orientia spp. during experimental animal activities” and we have added the following sentence to the paragraph, “For MEDIUM risk procedures where there is minimal aerosol generation then Animal Biosafety Level 2 (ABSL2) is recommended however where HIGH risk activities are performed including necropsies, Animal Biosafety Level 3 (ABSL3) is recommended.” And have also amended the abstract accordingly.

In Table 3 it is clearly stated that for experimentally-infected animals that ABSL2 is sufficient with the exception of when performing necropsy procedures which require higher levels of containment.