Investigating *Entamoeba* species infecting wild and semi-wild orangutans

P. Stuart[1], E. Yalcindag[1], H. Morrogh-Bernard[2], C. Nguyen[3], W. Nurcahyo[4],

R. Pecková[1], M. van Noordwijk[5], I. Foitová[1]

Contact: peterdstuart@hotmail.com ivona_foi@hotmail.com

Masaryk University, Czech Republic[1], The Orangutan Tropical Peatland Project, Indonesia[2], UMI-Saving of the Pongidae Foundation, Czech Republic[3], Universitas Gadjah Mada, Yogyakarta, Indonesia[4], University of Zürich, Switzerland[5]

Introduction

The aim of this study is to identify *Entamoeba* species infecting wild and semi-wild Sumatran (*Pongo abelii*) and Bornean (*Pongo pygmaeus*) orangutans. For the continued survival of orangutans, intensive management of the remaining populations is required, including preventing the introduction of infectious diseases to the remaining populations. Infection with *Entamoeba* is of interest as orangutans have been observed to be infected with *Entamoeba* species and infection has been linked to morbidity and mortality in primates. However, it remains to be determined if the species infecting orangutans is the pathogenic species *Entamoeba histolytica*.

Figure 1. Sumatran orangutans currently listed as critically endangered
© OHP

Materials and methods

Orangutan faecal samples have been collected from orangutans from 2004 to 2011 in three different sites in Sumatra (354 samples from 105 individuals), Bukit Lawang, Ketambe and Suaq and two sites in Borneo (219 samples from 114 individuals), Sebangau and Tuanan. All samples were collected from wild orangutans, except for 105 samples collected from semi-wild orangutans in Bukit Lawang.

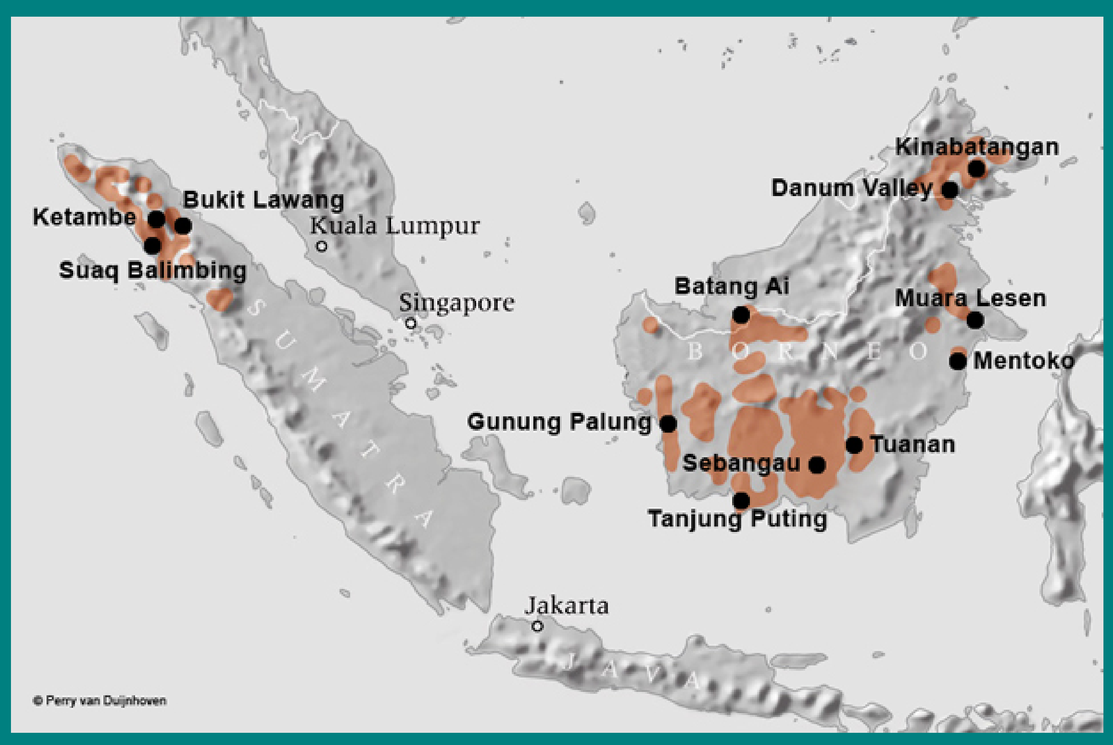

Figure 2. Sampling sites Bukit Lawang, Ketambe, Suaq, Sebangau and Tuanan

DNA was extracted using the Qiagen DNA stool kit. A single round PCR assay based on the analysis of 18S rRNA (Santos *et al.* Par Res 2010, 106:883-888) was used to identify all species of *Entamoeba* (662-667 bp) that might be present and a nested multiplex PCR based on 16S rRNA was used to differentiate *E.dispar* (174 bp), *E. histolytica* (439 bp) and *E. moshkovskii* (553 bp) (Khairnar and Parija 2007, BMC Mic.). HM-1IMSS *Entamoeba histolytica* trophozoites were used as a positive control and nuclease free water as a negative control.

Results

38 samples tested positive for *E. histolytica* (19 individuals), 3 for *E. dispar* (3 individuals) and 3 using the single round PCR assay for *Entamoeba*, demonstrating a low sensitivity for the single round assay. The PCR products of 25 of the samples have been sequenced. The single round PCR product and *E. dispar* positives did not produce sequences of good quality. By comparison with published sequences using NCBI BLAST the *E. histolytica* postives showed a 97% similarity to *E. histolytica,* 96% similarity to *Entamoeba nuttalli* and *E. dispar* and a 94% similarity to *E. moshkovskii*. The sequences showed no variation.

35 of the *E. histolytica* samples (16 individuals) came from Sumatra in comparison to 3 from Borneo (3 individuals). The majority of these came from semi-wild orangutans in Bukit Lawang. Significantly more semi-wild Sumatran orangutans were infected in comparison to wild Sumatran orangutans (χ^2= 5.679, 1df; P=0.02).

Conclusions

•Our results suggest wild and semi-wild orangutans are being infected with *E. histolytica* and interactions with humans may be a key factor.

•Nested PCR assays have a greater sensitivity for the detection *Entamoeba*, when working with faeces, than single run assays, as has been found in studies on humans.

•Due to genetic similarity between *E. histolytica*, *E. dispar* and *E. nuttalli* further tests are needed to confirm that the infections are *E. histolytica*. This will be done targeting the SSU rDNA, SREHP genes and a tRNA gene-linked short tandem repeat (STR) loci in nested PCR formats. These tests are on polymorphic regions and will also confirm no contamination took place.

•This will further enable us to identify the source of infection of orangutans. Also if any of the *E. histolytica* observed to be infecting them are different from those infecting humans.

Acknowledgements

Authors would like to thank the State Ministry of Research and Technology (RISTEK) and Directorate General for Natural Conservation (PHKA) for granting permission to conduct research in Indonesia. The study was financially supported by the Foundation “UMI – Saving of Pongidae” with partial support by Czech Academy of Sciences Grants P505/11/1163 and by the Program of “Employment of Best Young Scientists for International Cooperation Empowerment” (grant number CZ.1.07/2.3.00/30.0037) co-financed from European Social Fund and the state budget of the Czech Republic.

MASARYK UNIVERSITY
FACULTY OF SCIENCE