A non-invasive method to investigate the wildlife reservoir of African sleeping sickness in Serengeti National Park, Tanzania

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Human African Trypanosomiasis (HAT), also known as African Sleeping Sickness, causes serious disease in humans. It is caused by subspecies of the parasite *Trypanosoma brucei* and carried by the tsetse fly vector. There are three species of *T. brucei,* two of which occur in Tanzania. *T. b. rhodesiense* causes acute human disease, 'rHAT'. *T. b. brucei* is morphologically identical to *T. b. rhodesiense* but is not human infective. There is a single gene difference between these subspecies, the serum-resistance-associated gene (SRA), which makes *T. b. rhodesiense* human infective. Recombination can occur between these two subspecies, resulting in the SRA gene being transferred to new genetic backgrounds.

rHAT is a zoonotic disease with a wildlife reservoir. Cases of rHAT occur regularly but infrequently in Serengeti, although in general HAT is known to be underdiagnosed. In 2000/2001, an epidemic occurred, with cases reported in park staff, local populations and tourists. *T. brucei s.l.* has been identified in a diversity of wildlife species in Serengeti National Park, Tanzania, using opportunistic sampling and PCR. However, previous studies relied on opportunistic wildlife sampling, and therefore there were limited by a small sample size, because wildlife samples are challenging to obtain. Increasing the sample size will enable more detailed investigation into the wildlife reservoir of rHAT. Faecal sampling is a possible method to increase the sampling capacity. It has been validated in experimental infections in cattle and mice, with some preliminary work has been done in wildlife. It will provide a non-invasive method to increase sample size considerably, and avoid issues of logistics, ethics and cost that come with live wildlife sampling.

This study aims to first validate faecal sampling as a suitable method for investigating the wildlife reservoir of *T. brucei.* This is being done by obtaining matched blood and faecal samples from individual animals. These samples are then analysed by PCR to compare *T. brucei* detection between blood and faeces. Once validated, sampling can then be used to identify wildlife risk factors for infection, such as species, age, tsetse density and proximity to human habitation. Fieldwork is being undertaken in Serengeti National Park, which is an historical and ongoing rHAT focus. The ongoing research projects taking place here will enable opportunistic sampling. There is also availability of historical data, and an abundance of wildlife, making this a very suitable location for this study.

Analysing samples by PCR soon after collection will show which samples contain *T. brucei* s.l and therefore guide more targeted sampling, for example to certain species. Lab capacity for DNA extraction and PCR has been set up in the park, for the first time. This involves the use of small, compact and robust 'lab-in-a-suitcase' type equipment.

Overall, *T. b. rhodesiense* and *T. b. brucei* have been shown to exist in many wildlife species, but previous studies were limited by sample size. Obtaining a large sample set through fecal sampling will provide understanding of the wildlife factors that increase the likelihood of *T. brucei* prevalence.

Future work will involve whole genome sequencing of *T. b. brucei* and *T. b. rhodesiense* samples obtained from the wildlife. This will enable a thorough investigation into the prevalence and phylogenetic relationships between *T. brucei subsp.* in wildlife. This will give insight into how human infectivity emerges in wildlife and spreads to human populations and help to explain why there can be sudden increases in numbers of human cases, resulting in epidemics.