SUMMARY RESEARCH REPORT:
BRITISH VETERINARY ASSOCIATION

Prevalence and molecular identification of helminths in wild and captive Sri Lankan Elephants, *Elephas maximus maximus*.

Introduction
The Asian elephant (*Elephas maximus* L., 1798) is listed as ‘endangered’ on the IUCN Red List of Threatened Species. There are three recognized subspecies: Indian (*E. m. indicus*, or mainland subspecies), Sumatran (*E. m. sumatranus*) and Sri Lankan (*E. m. maximus*- EMM). EMM was once found throughout Sri Lanka, but is now restricted mainly to the lowlands in the dry zones. There are an estimated 3,500–6,000 EMM thought to be left in the wild (Choudhury et al., 2008).

Within Sri Lanka, EMM have great cultural and economic importance (Fernando et al., 2011). Elephants are used in religious festivals, and in the past were invaluable in the transport of timber. They still are of great economic value, mainly through tourism (Figure 1).
Helminth are ubiquitous in many wild animal species, and infections are often sub-clinical. However, when the host-parasite equilibrium is disrupted, disease may become apparent. While research has discovered the parasites that affect the Indian subspecies (Bhalerao, 1933, Bhalerao, 1935, Chandrashekar et al., 1979, Gupta, 1974), similar studies on helminths of EMM have not been conducted in detail.

Molecular techniques allow rapid, accurate identification of helminths from a small number of eggs to species level. The use of the first or second internal transcribed spacer (ITS) sequences of the nuclear ribosomal DNA (rDNA) has allowed genetic identification of strongyle species as well as other helminth groups (Gasser et al., 1993, Bott et al., 2009).

Scientific summary
This study investigates the prevalence of helminth parasites in wild, captive and released E. m. maximus, as well as comparing helminth prevalence between different demographic groups (age and gender). Samples were collected from elephants from Colombo Zoo, Elephant transit Home (ETH) near Udawalawe National Park, and from Udawalawe National Park (Figure 2). Samples were processed at the University of Peradeniya, in Kandy.
A total 83 faecal samples from *E. m. maximus* were studied and 67.5% positive for helminth ova. All positive samples were positive for strongyle eggs (Figure 3). Wild elephants had the highest prevalence of helminth infection (83%), followed by captive elephants (53%) and lastly released elephants (44%). These differences were found to be statistically significant (using a chi-squared test, $p=0.0068$, significance defined as alpha<0.05). No statistically significant differences were found in helminth prevalence compared to age or between genders.
Sequencing Polymerase Chain Reaction (PCR) products, generated from 34 elephants faecal samples using a generic nematode-specific protocol, revealed multiple copies of three different sequences. Comparison of sequence similarity using Basic Local Alignment Search Tool (BLAST) analysis showed these three sequences to be similar, but not identical, to sequences of strongyles from African Elephants (*Murshidia linstowi, Murshidia longicaudata* and *Khalilia sameera* respectively) (McLean et al., 2012). Phylogenetic analysis supported annotation of two putative *Murshidia* species and one *Khalilia* species circulating within the *E. m. maximus* population (Figure 4). The strongyles sequenced in this study could either be new, undescribed species or the same species as described in the African elephants which have diverged due to geographical isolation.

**Figure 4:** Genetic comparison of sequence results of strongyle nematodes present in Asian elephants with their closest matched identified species, using *Oesophagostomum radiatum* (also in the Strongyloidae family) as an outgroup. A maximum likelihood/ neighbour joining/ maximum parsimony tree was derived from the three sequence types detected. The percentage of trees in which the associated taxa clustered together is shown next to the branches (1,000 bootstrap replicates). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

**Personal Experience**
This project gave me the unique opportunity to live and work in Sri Lanka. I felt very privileged to see wild elephants and to experience first hand the wonderful work of the Elephant Transit Home in rehabilitating and releasing orphaned elephants. Working at the University of Peradeniya was a great experience- everyone was so welcoming and I improved and learnt many new lab techniques. During my stay I was fortunate enough to be invited to a Sri Lankan wedding of colleagues in the lab- and experience that I (and my sister who came out to visit me for two weeks after my placement) will never forget!
Sri Lankan wedding, with me (Liliana Heinrich) in the middle.

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All pictures are my own.
References


