SUMMARY RESEARCH REPORT:
BRITISH VETERINARY ASSOCIATION

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

Working at the University of Perdenyia, Parasitology Department. From right to left: Amal Dharmapriya, Kaushalya Karunathilake, Liliana Heinrich and Dr. Jayanthe Rajapakse.

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).

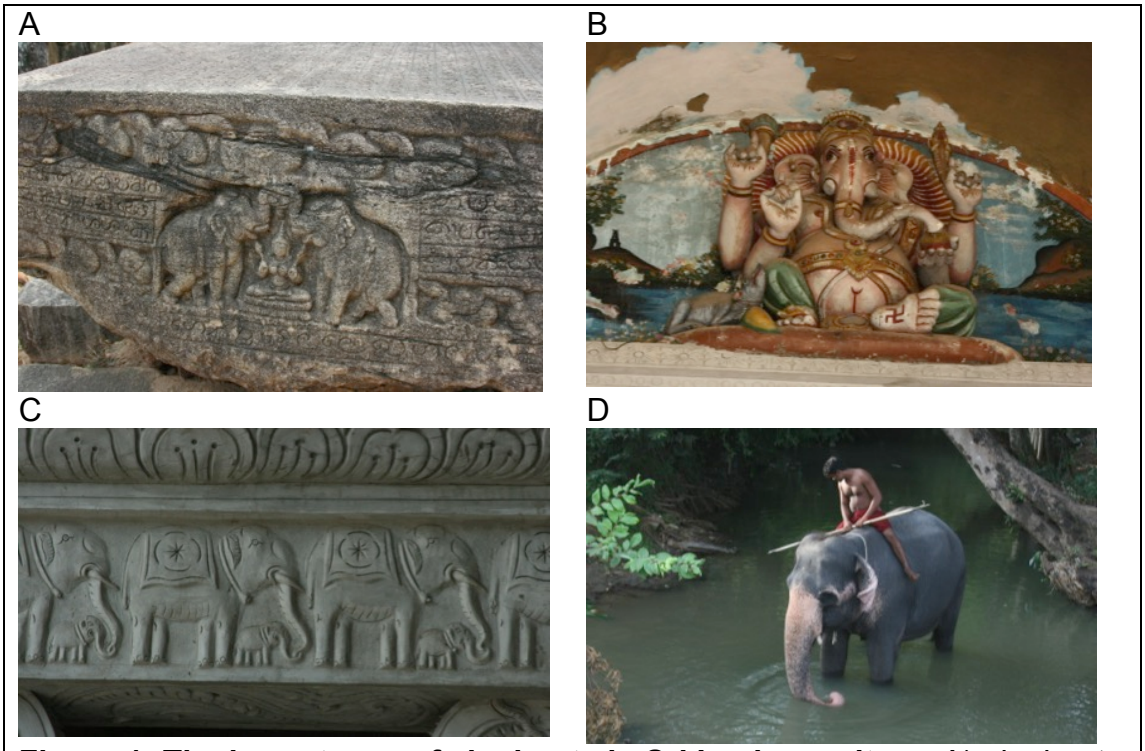

Figure 1: The importance of elephants in Sri Lankan culture. A) elephant engravings on Gal Potha (stone book) in the ancient city of Polonnaruwa. B) Temple decoration of Ganesh the God of wisdom and learning in a Hindu temple in Batticaloa. C) Elephant engravings in a modern Hindu temple in Batticaloa. D) A working elephant used for manual labour (log lifting) having a bath at the end of his working day near Sigiriya. (All images by Liliana Heinrich).

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, Chandrashekaran 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

B

Figure 2: Sri Lankan elephants: A) Young captive elephants at the ETH being bottle fed. B) A wild 'tusker' in Udawalawe National Park (pictures by the author).

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.

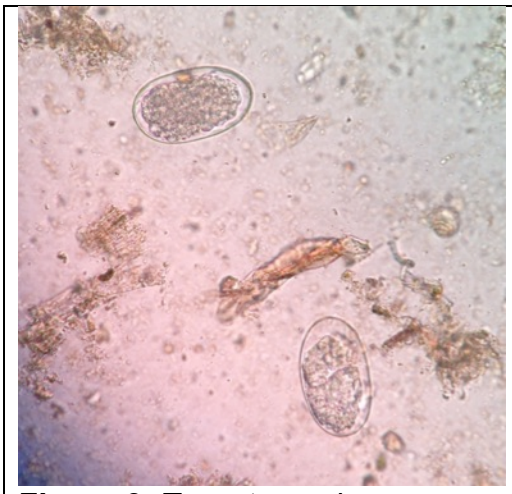

Figure 3: Two strongyle eggs viewed under 40x magnification using a light microscope.

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.

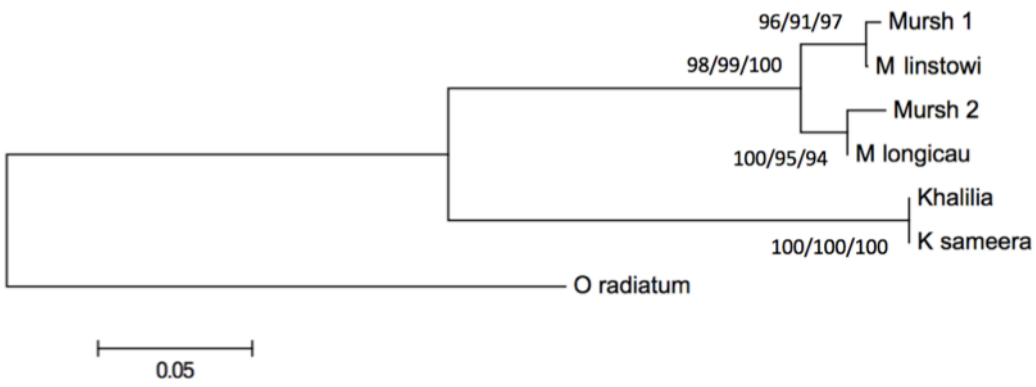

Figure 4: Genetic comparison of sequence results of strongyle nematodes present in Asian elephants with their closest matched identified species, using *Oesophagostomum radiatum* (also in the Strongyloidae family) as an outgroup. A maximum likelihood/ neighbour joining/ maximum parsimony tree was derived 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.

Acknowledgements
I would like to thank my supervisor at the Royal Veterinary College, London, Dr. Ayona Silva-Fletcher for her ongoing help and support throughout this project. Also to Dr. Damer Blake, my co-supervisor, for his indispensable help with the molecular work carried out here in the UK.

I am also grateful to Dr. Jayanthe Rajapakse, my supervisor at the University of Peradeniya, Sri Lanka, for not only helping me with the lab work in Sri Lanka but also for making me feel so welcome in his department. A massive thank you to the whole parasitology group for being so welcoming and helping me out whenever possible. Special mention to Kaushalya Karunathilake and Amal Dharmapriya for helping me in the lab and translating endless elephant labels for me.

Thank you to Dr. Dinusha de Silva and the rest of the staff at the Elephant Transit Home for the sample collection. Thank you to Colombo Zoo for also providing us with samples from their elephants.

Thank you to Prabath Niroshana and his wonderful family for letting me stay in your house and for immersing me in your culture.

For generous grants and their votes of confidence I would like to thank the British Veterinary Association, the British Veterinary Zoological Society Zebra Foundation and the Royal Veterinary College Zoological Society. Without these funds I would not have been able to conduct this project.

All pictures are my own.

References

BHALERAO, G. 1933. The trematode parasites of the Indian elephant, Elephas Indicus. *Indian J. Vet. Sci. Anim. Husb,* 3**,** 103-105.

BHALERAO, G. 1935. Helminth parasites of the Indian elephant from Andaman and Burma. *Indian J. Vet. Sci. Anim. Husb,* 5**,** 35-45.

BOTT, N. J., CAMPBELL, B. E., BEVERIDGE, I., CHILTON, N. B., REES, D., HUNT, P. W. & GASSER, R. B. 2009. A combined microscopic-molecular method for the diagnosis of strongylid infections in sheep. *Int. J. Parasitol.,* 39**,** 1277-87.

CHANDRASHEKARAN, K., RAJMOHAN, K. & SUNDARAM, R. 1979. A case of cestode infection in an Indian elephant. *Kerala J. Vet. Sci,* 10**,** 157-158.

CHOUDHURY, A., LAHIRI CHOUDHURY, D. K., DESAI, A., DUCKWORTH, J. W., EASA, P. S., JOHNSINGH, A. J. T., FERNANDO, P., HEDGES, S., GUNAWARDENA, M., KURT, F., KARANTH, U., LISTER, A., MENON, V., RIDDLE, H., RÜBEL, A. & WIKRAMANAYAKE, E. 2008. *Elephas maximus. The IUCN Red List of Threatened Species* [Online]. Available: http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T7140A12828813.en [Accessed 23 February 2016].

FERNANDO, P., JAYEWARDENE, J., PRASAD, T., HENDAVITHARANA, W. & PASTORINI, J. 2011. Current status of Asian Elephants in Sri Lanka. *Gajah,* 35**,** 93-103.

GASSER, R. B., CHILTON, N. B., HOSTE, H. & BEVERIDGE, I. 1993. Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. *Nucleic Acids Res.,* 21**,** 2525-6.

GUPTA, M. 1974. A preliminary report on diseases and parasites of zoo animals, birds and reptiles. *Indian J. Anim. Health,* 13**,** 15-24.

MCLEAN, E. R., KINSELLA, J. M., CHIYO, P., OBANDA, V., MOSS, C. & ARCHIE, E. A. 2012. Genetic identification of five strongyle nematode parasites in wild african elephants (Loxodonta africana). *J. Wildl. Dis.,* 48**,** 707-16.