Journal of Helminthology (2016) **90,** 434–440
doi:10.1017/S0022149X15000541

Molecular identification of the strongyloid nematode *Oesophagostomum aculeatum* in the Asian wild elephant *Elephas maximus*

O. Phuphisut[1], W. Maipanich[1], S. Pubampen[1], M. Yindee[2], N. Kosoltanapiwat[3], S. Nuamtanong[1], A. Ponlawat[4] and P. Adisakwattana[1]*

[1]Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Ratchathewi, Bangkok 10400, Thailand: [2]Department of Clinical Sciences and Public Health, Faculty of Veterinary Sciences, Mahidol University, Phuttamonthon, Nakorn-pathom 73170, Thailand: [3]Department of Microbiology and Immunology, Faculty of Tropical Medicine, Ratchathewi, Mahidol University, Bangkok 10400, Thailand: [4]Department of Entomology, Armed Force Research Institute of Medical Sciences, Ratchathewi, Bangkok 10400, Thailand

(Received 27 March 2015; Accepted 1 June 2015; First Published Online 27 July 2015)

Abstract

The transmission of zoonoses by wildlife, including elephants, is a growing global concern. In this study, we screened for helminth infections among Asian wild elephants (*Elephas maximus*) of the Salakpra Wildlife Sanctuary, Kanchanaburi, Thailand. Elephant faecal samples (45) were collected from the sanctuary grounds during January through November 2013 and assayed individually using the tetranucleotide microsatellite technique. Microscopic examination indicated a high prevalence of strongylids (93.0%) and low prevalences of trichurids (2.3%) and ascarids (2.3%). To identify the strongylid species, small subunit (SSU) rDNA sequences were amplified from copro-DNA and compared with sequences in GenBank. The generated SSU-rDNA sequences comprised five distinct haplotypes that were closely related to *Oesophagostomum aculeatum*. A phylogenetic analysis that incorporated related nematodes yielded a tree separated into two main clades, one containing our samples and human and domestic animal hookworms and the other consisting of *Strongyloides*. The present results indicate that *O. aculeatum* in local elephants is a potential source of helminthiasis in human and domestic animals in this wild-elephant irrupted area.

Introduction

The disruption and clearing of forested areas by activities such as residential construction, agriculture, industrial operations and public utility development are occurring worldwide. This disturbance of wildlife habitats has led to an increase in various socio-economic problems, including emerging and re-emerging zoonoses. In Thailand, elephants (*Elephas maximus*) have been seriously affected by increasing fragmentation of their forest habitat and decreasing habitat quality, forcing them to appear frequently at forest edges and to trespass in agricultural and residential areas. In addition to these threatening and destructive activities, wild elephants are reservoirs of diseases, such as helminthiasis, that cause health problems in both humans and domestic animals (Horak *et al.*, 1988). Parasitic nematodes that have been frequently observed in previous studies of Thai

*Fax: 66 (0) 2643-5600
E-mail: poom.adi@mahidol.ac.th

and African elephants include *Murshidia falcifera*, *M. neveulemairei*, *Murshidia* sp., *Leiperenia leiperi*, *Khalilia buta*, *K. sameera*, *Quilonia* spp., *Parabronema africanum*, *P. rhodesiense*, *Parabronema* spp., *Oesophagostomum mocambiquei* and *O. mwanzae* (Greve, 1969; Basson *et al.*, 1971; Sikes, 1971; Soulsby, 1982; Horak *et al.*, 1988; Boomker *et al.*, 1991; Carreno *et al.*, 2001; du Toit, 2001; Boomker, 2014). Interestingly, no eggs or larvae of *Oesophagostomum* spp. have been reported in Thai wild elephants.

Oesophagostomum, or nodule worm, is a parasitic nematode belonging to the family Chabertiidae. Infection with these helminths can cause granulomas in the walls of the small and large intestines. *Oesophagostomum bifurcum* is a common species that has been detected in livestock, non-human primates and humans (Gasser *et al.*, 2006), while *O. aculeatum* has been reported in the Japanese macaque and is also potentially transmitted to humans (Arizono *et al.*, 2012; Ghai *et al.*, 2014). We thus screened faecal samples of wild elephants for *Oesophagostomum*, using the small subunit ribosomal DNA gene (SSU rDNA) as a genetic marker. This marker has been widely used to clarify the taxonomy and molecular relationships of many parasite groups (Dorris *et al.*, 2002; Marigo *et al.*, 2011; Anderson *et al.*, 2012). The present results indicate that *Oesophagostomum* found in wild elephants may pose a potential health risk to local inhabitants and domestic animals.

Materials and methods

Collection and examination of faecal samples

Forty-five faecal samples from wild elephants were collected from the grounds of the Salakpra Wildlife Sanctuary, Kanchanaburi Province, Thailand during January through November 2013. All samples were observed, characterized and graded for freshness by veterinarians. Geographic coordinates of each sample were recorded (table 1).

To identify helminth eggs and larvae, samples were subjected to faecal concentration techniques, including sedimentation and flotation methods (Setasuban, 1989). All samples were examined for helminth eggs and larvae using a light microscope (Carl Zeiss, Jena, Germany). To prepare faecal samples for extraction of copro-DNA, 2 g of each sample was dissolved in 200 ml of distilled water, filtered through two layers of wet cotton gauze, and left undisturbed for 1–2 h to obtain precipitates. After two additional cycles of dissolution and filtration, the precipitates were combined and stored at −70°C for future molecular analysis.

Molecular analysis

Precipitates of faecal samples were snap-frozen in liquid nitrogen and then ground with a plastic pestle. The homogenization was repeated three times to ensure complete breakage of helminth eggs and larvae. The copro-DNA was then extracted using a FavorPrep Stool DNA Isolation Mini kit (Favorgen, Ping-Tung, Taiwan) according to the manufacturer's instructions.

To confirm and determine the quality of wild elephant DNA, a portion of the cytochrome *b* (Cytb) gene was amplified in all faecal samples. A portion of the SSU rDNA gene was amplified for identification of intestinal nematode species. Sequences of primers used to amplify Cytb and SSU rDNA genes, which followed Fernando *et al.* (2000) and Dorris *et al.* (2002), respectively, are shown in table 2. Amplifications of both genes were carried out in 50-μl reaction volumes containing 1 μl of DNA template, 0.5 μM of each primer, and 1× Top*Taq* Master Mix (Qiagen, Hilden, Germany). The amplification profile used for Cytb was as follows: 95°C for 5 min; followed by 35 cycles of 95°C for 60 s, 60°C for 60 s and 72°C for 60 s; and a final extension of 72°C for 5 min. For SSU rDNA, the amplification profile consisted of 95°C for 5 min; followed by 35 cycles of 95°C for 30 s, 53°C for 30 s and 72°C for 30 s; and a final extension of 72°C for 5 min. All polymerase chain reaction (PCR) products were analysed by 1% agarose gel electrophoresis and submitted to Macrogen (Seoul, Republic of Korea) for sequencing.

Tetranucleotide microsatellite markers were used to determine the number of individual elephants, according to previous studies (Archie *et al.*, 2003; Suwattana *et al.*, 2010). In particular, three tetramicrosatellite loci (LaT05, LaT08 and LaT26) were amplified in 50-μl reaction volumes containing 1× Top*Taq* Master Mix (Qiagen), 0.5 μM of each primer (table 2), and 1 μl of DNA template. Amplification conditions for LaT05 and LaT08 consisted of 95°C for 10 min, followed by one cycle of 95°C for 30 s, 66°C for 30 s and 72°C for 30 s. Ten subsequent cycles were performed under identical conditions, except that the

Table 1. Collection sites, with coordinates, of elephant faecal samples from Kanjanaburi Province, Thailand.

Sample ID	Collection sites	Sample ID	Collection sites
SP6	47P0529930X UTM1585949Y	SP-B6	47P0529349X UTM1585656Y
SP7	47P0529990X UTM1586008Y	SP-B7	47P0529320X UTM1585581Y
SP16	47P0531131X UTM1587578Y	SP-B9	47P0529245X UTM1585528Y
SP1-3	47P0530735X UTM1585879Y	SP-B10, B11	47P0529337X UTM1585463Y
SP1-4	47P0531533X UTM1586303Y	SP-B12	47P0529325X UTM1585456Y
SP-A9	47P0531084X UTM1590481Y	SP-B13	47P0529304X UTM1585425Y
SP-A10	47P0530694X UTM1586731Y	SP-B14, B15, B16	47P0529256X UTM1585320Y
SP-A13	47P0530719X UTM1586716Y	SP-B22	47P0528762X UTM1591716Y
SP-A16, A17	47P0530591X UTM1587182Y	SP-C8	47P0525217X UTM1584921Y
SP-A18, A19	47P0530556X UTM1587152Y	SP-C9	47P0525198X UTM1584937Y
SP-A25	47P0530577X UTM1587045Y	SP-C14	47P0535121X UTM1581921Y

https://doi.org/10.1017/S0022149X15000541 Published online by Cambridge University Press

Table 2. Oligonucleotide primers used for PCR amplification of tetramicrosatellite loci and Cytb and SSU rDNA genes.

No.	Locus/position	Primer sequences (5′–3′)	Observed size (bp)
1	LaT05*	F: CAC-CAC-CCA-TCC-ATC-TGT R: TGG-CTT-CTG-TGA-GTT-CAC-C	120–280
2	LaT08*	F: ATG-GAC-AGG-CAG-AAA-GAT-TT R: TCC-CAA-TAA-CAG-GAT-AGC-ATT	110–300
3	LaT26*	F: AAC-CCA-GGC-TAA-AGC-ACC-AA R: TTT-CCT-GCT-TGA-GAG-CCA-AA	110–240
4	MDL3 (Cytb) MDL5 (Cytb)	F: CCC-ACA-ATT-AAT-GGG-CCC-GGA-GC R: TTA-CAT-GAA-TTG-GCA-GCC-AAC-CA	~630
5	SSUA (SSU rDNA) SSU22R (SSU rDNA)	F: AAA-GAT-TAA-GCC-ATG-CAT-G R: GCC-TGC-TGC-CTT-CCT-TGG-A	~370

*Microsatellite markers; F, forward; R, reverse.

annealing temperature was decreased by 1°C per cycle; this was followed by 30 cycles of 95°C for 30 s, 56°C for 30 s and 72°C for 30 s; with a single extension of 72°C for 5 min. For LaT26, amplification conditions were 95°C for 10 min; followed by 40 cycles of 95°C for 30 s, 53°C for 30 s and 72°C for 30 s; and a single extension step of 72°C for 5 min. PCR products were analysed by 6% polyacrylamide gel electrophoresis.

Data analysis

The obtained partial SSU rDNA sequences were compared with related nematode sequences (table 3) to identify species and haplotypes, using the multiple alignment program Clustal X version 2.0 (Thompson *et al.*, 1997). A neighbour-joining phylogram based on *p*-distances was constructed in MEGA version 5.0 (Tamura *et al.*, 2011). Bootstrap analyses were conducted using 1000 replicates.

Results

Microscopic examination of elephant faeces revealed helminth eggs and larvae in 40 of the 45 samples, and these stages were categorized into three nematode groups, including the strongylids, trichurids and ascarids. Strongylid eggs measuring 80–85 × 42–47 μm were found in all 40 infected samples, compared to trichurid eggs, measuring 55–62 × 27–31 μm, which were found in one sample (fig. 1A and B). Ascarid eggs measuring 58–65 × 43–47 μm were also found in one sample (fig. 1C), which was concurrently infected with strongylid eggs. Additionally, eight samples contained rhabditiform larvae with buccal canal and rhabditiform oesophagus lengths of 15–19 μm and 100–110 μm, respectively. Their tails were slightly long, 90–95 μm from the anus to the tip of the tail. Larval bodies in entirety were 590–640 × 17–21 μm (fig. 1D), with unclear genital primordia.

An accurate determination of helminth prevalence and genetic diversity was impeded by the difficulty in identifying the source of each faecal sample. Tetranucleotide microsatellite analysis was therefore performed to identify the actual number of distinct faecal samples. The Asian elephant Cytb gene was first amplified in all samples by conventional PCR to determine the specificity and quality of extracted DNA. All samples yielded PCR products of the expected size for the genus *Elephas* (elephant) – approximately 630 bp long. Sequences generated from these products closely matched DNA sequences of *E. maximus* (Asian elephant) in GenBank (data not shown). Samples were further analysed for individual differences using three tetramicrosatellite loci. This analysis yielded 8–10 alleles per locus and 11–15 haplotypes (data not shown). The analysis results indicated that the 45 faecal samples originated from 43 individual wild elephants. Consequently, the actual distribution of nematodes in our group of samples was 93.0% strongylids, 2.3% trichurids and 2.3% ascarids.

Table 3. Accession numbers of sequences downloaded from GenBank for use in the present study.

Species	GenBank number	Species	GenBank number
Oesophagostomum aculeatum	AB677956.1	*S. fuelleborni kelleyi*	AJ417029
Necator americanus	AJ920348.1	*S. fuelleborni fuelleborni*	AJ417030
Ancylostoma caninum	AJ920347	*S. venezuelensis*	AJ417026
A. ceylanicum	AB683978	*S. westeri*	AJ417032
A. duodenale	EU344798.1	*S. suis*	AJ417028
Ascaris lumbricoides	X06713.1	*S. papillosus*	AJ417027
Strongyloides stercoralis	AF279916	*S. ratti*	AF036605
S. cebus	AJ417025		

https://doi.org/10.1017/S0022149X15000541 Published online by Cambridge University Press

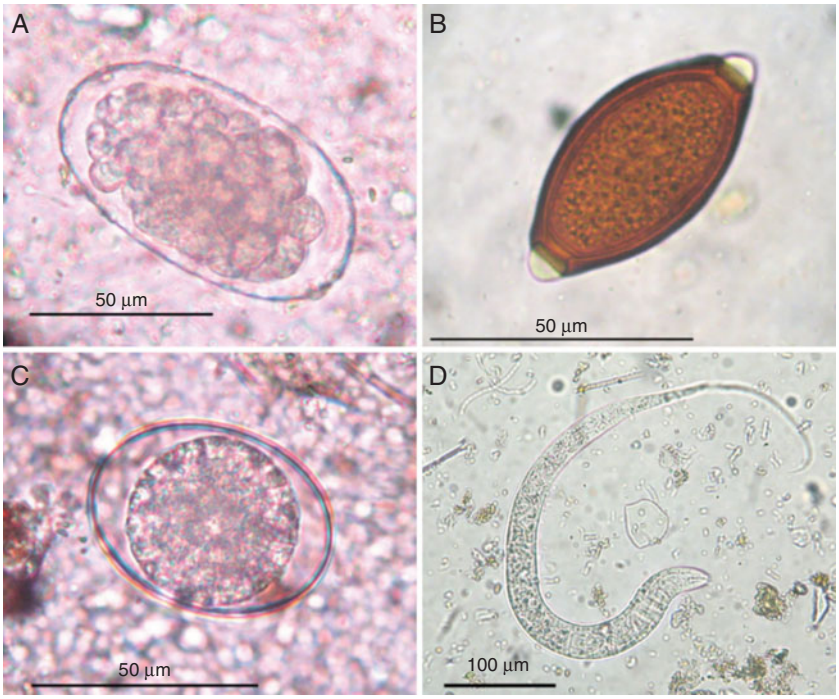

Fig. 1. Egg and larval stages identified in faecal samples of wild elephants, including eggs of (A) *O. aculeatum*, (B) *Trichuris* sp., (C) *Ascaris* sp. and (D) the rhabditiform larva of *O. aculeatum*.

Microscopic examination revealed that 40 faecal samples were positive for strongylid eggs or larvae but did not allow species determination. SSU rDNA was therefore used as a marker for species identification. All samples were amplified with specific primers and gave products of the expected size, 370 bp. All amplicons were subsequently sequenced, but good-quality sequences were only obtained from 27 of the 40 samples. Alignment and comparison of the 27 sequences in Clustal X revealed five distinct haplotypes (A, B, C, D and E) (table 4). Thirteen DNA sequences belonging to haplotype A showed 100% identity with *O. aculeatum* SSU rDNA sequences from the Japanese macaque (*Macaca fuscata yakui*). Another 13 sequences, consisting of two haplotype B, eight haplotype C and three haplotype D sequences, were 99% identical to *O. aculeatum*. The sole haplotype E sequence matched *Ancylostoma caninum* (dog and cat hookworm), showing 99% identity.

Phylogenetic analysis of the SSU rDNA sequences yielded a tree composed of two main clades (fig. 2): clade I containing all helminths sequenced in this study as well as *O. aculeatum* and the hookworm species *N. americanus*, *A. caninum* and *A. ceylanicum*, and clade II comprising various *Strongyloides* species. Among the five helminth haplogroups, *p*-distances ranged from 0.004 to 0.024 (data not shown). Haplotype A was the same haplotype as *O. aculeatum* and was very closely related to *N. americanus* (0.004). Haplotypes C and D were sister to each other;

Table 4. Identified haplotypes and variable nucleotide positions of the partial SSU rDNA gene (370 bp) and frequencies of these haplotypes in 27 *O. aculeatum* samples.

	Variable nucleotide positions								
Haplotype	059	107	111	123	142	149	156	163	Haplotype frequency
A	G	A	A	C	G	T	T	A	13
B	T	·	·	·	A	·	·	·	2
C	·	·	·	T	·	·	·	·	8
D	·	G	·	T	·	·	·	·	3
E	·	·	T	·	·	C	C	G	1
Total									27

Dots correspond to nucleotides identical to the corresponding nucleotide in the haplotype A sequence. Position numbers refer to positions 1–895 of the SSU rDNA gene partial sequence (895 bp) of *O. aculeatum* (GenBank number AB677956).

https://doi.org/10.1017/S0022149X15000541 Published online by Cambridge University Press

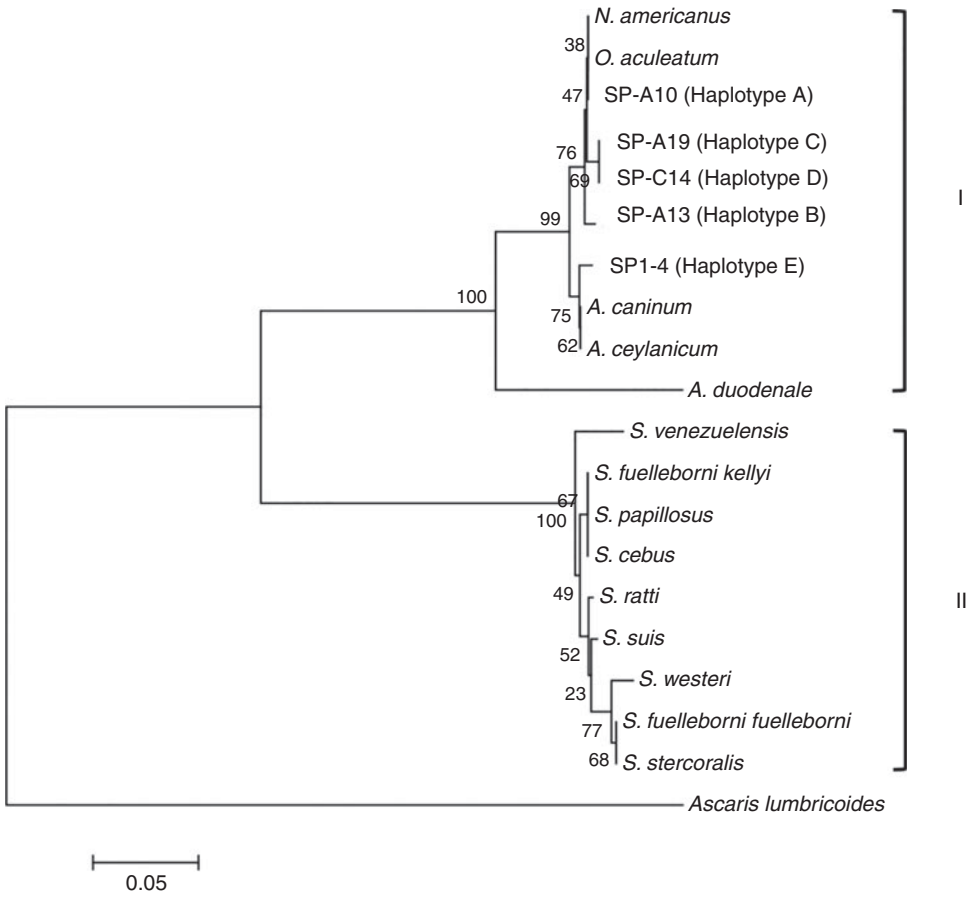

Fig. 2. Phylogenetic tree of *O. aculeatum* and related species based on partial SSU rDNA gene sequences; genetic relationships were inferred by the neighbour-joining method using *Ascaris lumbricoides* as an outgroup, and the scale bar indicates the number of nucleotide substitutions per site. Clade I comprises *O. aculeatum*, hookworm species and all haplotypes in the present study; see also table 4 for haplotypes A–E. Clade II comprises *Strongyloides* species.

these two haplotypes were in turn sister to haplotype A, *N. americanus* and *O. aculeatum* (0.004–0.012). Haplotype B was in the same group as haplotypes A, C, D, *N. americanus* and *O. aculeatum*, but was genetically the most distinct of these members (0.008–0.016). Haplotype E was distinct from the other four haplotypes (genetic distance $\geq$0.016) and was more closely related to *A. ceylanicum* and *A. caninum*. *Strongyloides* species were clearly separate, being restricted to clade II. Genetic distances between the two clades ranged from 0.311 to 0.452, with an average genetic distance of 0.333.

Discussion

In this study, we investigated the prevalence of helminth infections in wild elephants in Thailand. Our microscopic examination detected three groups of helminths – strongylids, trichurids and ascarids – in the faeces of wild elephants. These groups have been found frequently in both Asian and African elephants (Greve, 1969; Basson *et al.*, 1971; Sikes, 1971; Soulsby, 1982; Carreno *et al.*, 2001; du Toit, 2001). Strongylids were the most common, being present in more than 90% of collected samples. The taxonomic characteristics used to identify this group – morphology of eggs and rhabditiform larvae (buccal canal and genital primordium) – were insufficient to allow species-level differentiation, with one exception – the tails of rhabditiform larvae of *Oesophagostomum* were slightly longer (from anus to the tip of the tail) than those of hookworms and *Strongyloides* (Blotkamp *et al.*, 1993; Bowman *et al.*, 2003).

To identify nematode species, we used an alternative DNA-based approach involving SSU rDNA. Previous research has found that SSU rDNA can resolve deep phylogenetic relationships and shed light on the evolution of nematodes (Holterman *et al.*, 2006). SSU rDNA markers have also been used to identify successfully nematode species in genera such as *Strongyloides* (Dorris *et al.*, 2002), *Mexiconema* (Mejia-Madrid & Aguirre-Macedo, 2011) and *Angiostrongylus* (Constantino-Santos *et al.*, 2014). Comparison of SSU rDNA sequences of nematodes infecting elephants with their orthologues indicated that 26 sequences matched *O. aculeatum* from the Japanese macaque (AB677956). Faecal examination of

https://doi.org/10.1017/S0022149X15000541 Published online by Cambridge University Press

long-tailed macaques in Thailand has also uncovered *Oesophagostomum* spp. infections (Malaivijitnond *et al.*, 2006). The monkeys inhabiting the same area as the elephants in this study, however, have neither been identified to species nor examined. A future investigation at this study site addressing these issues might provide evidence of *O. aculeatum* transmission between monkeys and elephants.

When we compared SSU rDNA sequences of our samples with sequences available in GenBank, we found that 13 were 100% identical to the SSU rDNA sequence of *O. aculeatum* (AB677956). Another 13 were 99% identical. The high similarity of these SSU rDNA sequences may not be sufficient for confirm the identity of the isolated rhabditiform larvae. This identification should be verified by morphological observation and sequencing of adult worms. To obtain worms at the adult stage, however, wild elephants must be treated with an anthelmintic drug – a logistically impossible situation. Future application of additional genetic markers may be a good option to verify the species identification.

Our phylogenetic analysis of the order Strongylida placed *O. aculeatum* from various hosts, including wild elephants, into the same clade as human and domestic animal hookworms (*N. americanus* and *Ancylostoma* spp.). The close relationship of these species suggests that *O. aculeatum* can potentially infect humans and domestic animals. The fact that this parasite infects non-human primates (Ross *et al.*, 1989; Karim & Yang, 1992; Kamel *et al.*, 1995) in addition to elephants also supports this hypothesis. Nevertheless, information about *O. aculeatum* host specificity and adaptation is still limited and this issue needs clarification.

In the phylogenetic tree, several *Strongyloides* spp. were included to compare with our obtained sequences to avoid misidentification. As mentioned elsewhere, SSU-rDNA sequences differ significantly in the genus *Strongyloides* – an advantage for species identification (Dorris *et al.*, 2002). Thus, we used several *Strongyloides* spp. to ensure that our obtained DNA sequences were not any other *Strongyloides* spp.

Using tetramicrosatellites, we were able to distinguish faecal samples from individual elephants and thus could determine the frequency of helminth infection accurately. The tetramicrosatellite technique has been demonstrated to be effective for identification of individual Asian and African elephants (Archie *et al.*, 2003; Suwattana *et al.*, 2007, 2010), as well as individuals of other organisms (Kaul *et al.*, 2001; Behl *et al.*, 2002; Itoh *et al.*, 2009; Costa *et al.*, 2012). In addition to their use for determination of actual sample numbers and thus helminth frequency, microsatellites can reveal the relationships and diversity of local wild elephants, which may be useful for their control and management.

In conclusion, faecal examination combined with copro-PCR amplification and sequencing of SSU rDNA identified *O. aculeatum* in wild elephants. Although no evidence of transmission of this helminth between elephants and humans or domestic animals has been observed to date, further investigation is warranted to determine the likelihood of risk. In the meantime, healthcare workers, veterinarians and other persons living in the elephant-incursive area should be aware of the possibility of health problems caused by *O. aculeatum* infection.

Acknowledgements

We express our gratitude to the wildlife rangers of the Salakpra Wildlife Sanctuary, Kanchanaburi, Thailand for their help during sample collection. We thank Miss Arissara Pongsiri, Department of Entomology, Armed Forces Research Institute of Medical Sciences for geographic information system (GIS) training. We also thank the Faculty of Tropical Medicine, Mahidol University, for funding the proofreading and editing of this manuscript.

Financial support

This work was supported by the Faculty of Tropical Medicine Research Fund for fiscal year 2012 through the Tropical Medicine One Health team.

Conflict of interest

None.

References

Anderson, J., Upadhayay, R., Sudimack, D., Nair, S., Leland, M., Williams, J.T. & Anderson, T.J.C. (2012) *Trichuris* sp. and *Strongyloides* sp. infections in a free-ranging baboon colony. *Journal of Parasitology* **98**, 205–208.

Archie, E.A., Moss, C.J. & Alberts, S.C. (2003) Characterization of tetranucleotide microsatellite loci in the African Savannah elephant (*Loxodonta africana africana*). *Molecular Ecology Notes* **3**, 244–246.

Arizono, N., Yamada, M., Tegoshi, T. & Onishi, K. (2012) Molecular identification of *Oesophagostomum* and *Trichuris* eggs isolated from wild Japanese macaques. *Korean Journal of Parasitology* **50**, 253–257.

Basson, P.A., McCully, R.M., de Vos, V., Young, E. & Kruger, S.P. (1971) Some parasitic and other natural diseases of the African elephant in the Kruger National Park. *Onderstepoort Journal of Veterinary Research* **38**, 239–254.

Behl, R., Kaul, R., Sheoran, N., Behl, J., Tantia, M.S. & Vijh, R.K. (2002) Genetic identity of two Indian pig types using microsatellite markers. *Animal Genetics* **33**, 158–159.

Blotkamp, J., Krepel, H.P., Kumar, V., Baeta, S., van't Noordende, J.M. & Polderman, A.M. (1993) Observations on the morphology of adults and larval stages of *Oesophagostomum* sp. isolated from man in northern Togo and Ghana. *Journal of Helminthology* **67**, 49–61.

Boomker, J. (2014) *Helminth infections of wildlife*. Licensed under a Creative Commons Attribution license. Available online at http://www.afrivip.org/education/wildlife-health/helminth-wildlife/2014 (accessed February 2015).

Boomker, J., Horak, I.G. & Flamand, J.R.B. (1991) Parasites of South African wildlife XII. Helminths of

Nyala, *Tragelaphus angasii*, in Natal. *Onderstepoort Journal of Veterinary Research* **58**, 275–280.

Bowman, D.D., Lynn, R.C., Georgi, J.R. & Eberhard, M.L. (2003) *Georgi's parasitology for veterinarians*. 8th edn. 422 pp. St. Louis, Missouri, Saunders.

Carreno, R.A., Neimanis, A.S., Lindsjo, J., Thongnoppakun, P., Barta, J.R. & Peregrine, A.S. (2001) Parasites found in faeces of Indian elephants (*Elephas maximus*) in Thailand following treatment with mebendazole, with observations on *Ptenderius papillatus* (Cobbold, 1882) Stiles and Goldberger, 1910 by scanning electron microscopy. *Helminthologia* **38**, 75–79.

Constantino-Santos, D.M.A., Basiao, Z.U., Wade, C.M., Santos, B.S. & Fontanilla, I.K.C. (2014) Identification of *Angiostrongylus cantonensis* and other nematodes using the SSU rDNA in *Achatina fulica* populations of Metro Manila. *Tropical Biomedicine* **31**, 327–335.

Costa, V., Pérez-González, J., Santos, P., Fernández-Llario, P., Carranza, J., Zsolnai, A., Anton, I., Buzgó, J., Varga, G., Monteiro, N. & Beja-Pereira, A. (2012) Microsatellite markers for identification and parentage analysis in the European wild boar (*Sus scrofa*). *BMC Research Notes* **5**, 479.

Dorris, M., Viney, M.E. & Blaxter, M.L. (2002) Molecular phylogenetic analysis of the genus *Strongyloides* and related nematodes. *International Journal for Parasitology* **32**, 1507–1517.

du Toit, J.G. (2001) *Veterinary care of African elephants*. pp. 1–59. Pretoria, Republic of South Africa, Novartis.

Fernando, P., Pfrender, M.E., Encalada, S.E. & Lande, R. (2000) Mitochondrial DNA variation, phylogeography and population structure of the Asian elephant. *Heredity* **84**, 362–372.

Gasser, R.B., de Gruijter, J.M. & Polderman, A.M. (2006) Insights into the epidemiology and genetic make-up of *Oesophagostomum bifurcum* from human and non-human primates using molecular tools. *Parasitology* **132**, 453–460.

Ghai, R.R., Chapman, C.A., Omeja, P.A., Davies, T.J. & Goldberg, T.L. (2014) Nodule worm infection in humans and wild primates in Uganda: cryptic species in a newly identified region of human transmission. *PLoS Neglected Tropical Diseases* **8**, e2641.

Greve, J.H. (1969) *Strongyloides elephantis* sp. n. from an Indian elephant. *Elephas indicus. Journal of Parasitology* **55**, 498–499.

Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J. & Helder, J. (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution* **23**, 1792–1800.

Horak, I.G., Boomker, J., De Vos, V. & Potgieter, F.T. (1988) Parasites of domestic and wild animals in South Africa, XXIII. Helminth and arthropod parasites of warthogs, *Phacochoerus aethiopicus*, in the eastern Transvaal Lowveld. *Onderstepoort Journal of Veterinary Research* **55**, 145–152.

Itoh, T., Sato, Y., Mano, T. & Iwata, R. (2009) Estimating a suitable microsatellite marker set for individual identification and parentage tests of brown bear (*Ursus arctos*) in the Akan-Shiranuka Region, eastern Hokkaido, Japan. *Journal of Forest Research* **14**, 117–122.

Kamel, A.G.M., Yang, C.O. & Norazah, A. (1995) *Oesophagostomum aculeatum*, the nodular worm causing helminthoma in man in Malaysia. *Internal Medicine Journal* **2**, 287–290.

Karim, N. & Yang, C.O. (1992) Oesophagostomiasis in man: report of the first Malaysian case with emphasis on its pathology. *Malaysian Journal of Pathology* **14**, 19–24.

Kaul, R., Singh, A., Vijh, R.K., Tantia, M.S. & Behl, R. (2001) Evaluation of the genetic variability of 13 microsatellite markers in native Indian pigs. *Journal of Genetics* **80**, 149–153.

Malaivijitnond, S., Chaiyabutr, N., Urasopon, N. & Hamada, Y. (2006) Intestinal nematode parasites of long-tailed macaques (*Macaca fascicularis*) inhabiting some tourist attraction sites in Thailand. *Proceedings of the 32nd Congress on Science and Technology of Thailand*, Bangkok, Thailand. 1–3 November. pp. 73–77.

Marigo, J., Thompson, C.C., Santos, C.P. & Iñiguez, A.M. (2011) The *Synthesium* Brachycladiidae Odhner, 1905 (Digenea) association with hosts based on nuclear and mitochondrial genes. *Parasitology International* **60**, 530–533.

Mejia-Madrid, H.H. & Aguirre-Macedo, M.L. (2011) Systematics of *Mexiconema cichlasomae* (Nematoda: Daniconematidae) based on sequences of SSU rDNA. *Journal of Parasitology* **97**, 160–162.

Ross, R.A., Gibson, D.I. & Harris, E.A. (1989) Cutaneous oesophagostomiasis in man. *Journal of Helminthology* **63**, 261–265.

Setasuban, P. (1989) *Soil-transmitted helminthiasis and laboratory diagnosis*. Bangkok, Thailand, Mahidol University.

Sikes, S.K. (1971) *The natural history of the African elephant*. London, UK, Trinity Press.

Soulsby, E.J.L. (1982) *Helminths, arthropods and protozoa of domesticated animals*. 7th edn. 809 pp. London, Baillière Tindall.

Suwattana, D., Koykul, W., Jirasupphachok, J. & Kanchanapangka, S. (2007) Microsatellite polymorphism and parentage control in Thai domestic elephants (*Elephas maximus*). *Thai Journal of Veterinary Medicine* **37**, 33–38.

Suwattana, S., Jirasupphachok, J., Kanchanapangka, S. & Koykul, W. (2010) Tetranucleotide microsatellite markers for molecular testing in Thai domestic elephants (*Elephas maximus indicus*). *Thai Journal of Veterinary Medicine* **40**, 405–409.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731–2739.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**, 4876–4885.