RESEARCH NOTE

GENETIC DIFFERENTIATION OF *STRONGYLOIDES STERCORALIS* FROM TWO DIFFERENT CLIMATE ZONES REVEALED BY 18S RIBOSOMAL DNA SEQUENCE COMPARISON

Wallop Pakdee, Urusa Thaenkham, Paron Dekumyoy, Surapol Sa-nguankiat, Wanna Maipanich and Somchit Pubampen

Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. Over 70 countries in tropical and subtropical zones are endemic areas for *Strongyloides stercoralis*, with a higher prevalence of the parasite often occurring in tropical regions compared to subtropical ones. In order to explore genetic variations of *S. stercoralis* form different climate zones, 18S ribosomal DNA of parasite specimens obtained from Thailand were sequenced and compared with those from Japan. The maximum likelihood indicates that *S. stercoralis* populations from these two different climate zones have genetically diverged. The genetic relationship between *S. stercoralis* populations is not related to the host species, but rather to moisture and temperature. These factors may directly drive genetic differentiation among isolated populations of *S. stercoralis*.

Keywords: *Strongyloides stercoralis*, 18S ribosomal DNA, Thailand, Japan

INTRODUCTION

Strongyloides stercoralis is a species of medically important parasites that are pathogenic to humans. It belongs to the genus *Strongyloides*, which includes several other soil-transmitted nematodes (Coker *et al*, 2000). *S. stercoralis* is distributed around the world and is believed to infect around 30-100 million people. It is most commonly found in tropical and subtropical climate zones, which include over 70 different countries (Concha *et al*, 2005). However, the prevalence of the parasite is usually higher in tropical zones (ranging from 2% to 25%) than subtropical zones (0.4%-4.0%) (Afzal and Steven, 2001). Factors such as high temperature, high moisture and poor sanitation, which exist in many tropical countries, may enhance the prevalence of *S. stercoralis* (Nilforoushan *et al*, 2007; Koosha *et al*, 2009).

In general, the genetic makeup of parasite populations in the tropics is different from those in the subtropics, presumably to allow for different adaptations

Correspondence: Wallop Pakdee, Department of Helminthology, Faculty of Tropical Medicine, 420/6 Ratchawithi Road, Bangkok 10400, Thailand.
Tel/Fax: +66 (0) 2643 5600
E-mail: wallop.pak@mahidol.ac.th

to hosts and processes of geographical dispersal (Hasegawa *et al*, 2009). If this is universally true, then populations of *S. stercoralis* from different climate zones should present genetic variations.

Thailand and Japan present an ideal model to prove this hypothesis. Thailand is a tropical country endemic to *S. stercoralis*, while Japan is in the subtropics, where the parasite is also prevalent in sporadic areas (Vadlamudi *et al*, 2006). In this study, we explored the genetic differences of *S. stercoralis* from both countries using 18S rDNA as a genetic marker.

MATERIALS AND METHODS

Worm collection

Stool samples from patients living in the provinces of Nakhon Si Thammarat and Tak were examined. Filariform larvae were collected after specimens were cultured on filter paper for 7 days and identified as *Strongyloides* (Sa-nguankiat *et al*, 2011). The larvae were harvested and preserved in 100% ethanol at -20ºC until used. The study protocol was approved by the Scientific Ethics Committee of Mahidol University, MUTM 2012-012-01.

DNA analysis

Genomic DNA was extracted from three individual larvae isolated from the two provinces using a Tissue Genomic DNA mini kit (Geneaid, New Taipei City, Taiwan), according to the manufacturer's protocol. From the genomic DNA samples, partial segment of the small subunit ribosomal DNA (18S rDNA) was amplified using primers SS-F: 5′AACAGCTATAGACTACACGGTA 3′ and SS-R: 5′ TTTATGCACTTGGAAAGCTGGT 3′. The two primers were designed from the complete sequence of ribosomal DNA (18S rDNA) of *S. stercoralis* in GenBank (AB_453316, AB_453315, AJ_417023, AJ_279916). PCR was conducted in a total volume of 50 l, composed of 2xTop*Taq*™ Master Mix Kit, which contains Top*Taq* DNA Polymerase, PCR buffer, 1.5 mM $MgCl_2$ and 200 M each dNTP (QIAGEN, Hilden, Germany). PCR amplicons were obtained under the following thermocycling conditions: 94ºC for 2 minutes, 30 cycles at 94ºC for 30 seconds, 64ºC for 30 seconds, and 72ºC for 30 seconds, and a final step at 72ºC for 8 minutes. PCR amplicons were electrophoresed in 1.0% agarose gel, stained with ethidium bromide and visualized with a UV-transilluminator. Each PCR amplicon was sequenced using the amplification primers in an ABI Prism 377 DNA sequencer (Macrogen, Seoul, Korea). The sequences obtained were checked individually by BLAST program (McGinnis and Madden, 2004) to confirm the correct target. The electropherogram of each sequence was examined for sequence accuracy by BioEdit version 7.0 (Hall, 1999).

The 18S rDNA sequences were aligned with the other sequences of *Strongyloides* spp deposited in GenBank (Table 1), using CLUSTAL X (Thompson *et al*, 1997); all gap sites in the sequences were excluded by BioEdit (Hall, 1999). The pairwise genetic distance between the sequences of *Strongyloides* were estimated using the *p*-distance method. The sequences were constructed into a phylogenetic tree using the maximum likelihood method. The best fit model was determined as Tamura 3-parameter + gamma distribution of rate parameters (G). Support for tree topologies was assessed with 1000 bootstrap replicates. These analyses were carried out using MEGA 5.05 program (Tamara *et al*, 2011).

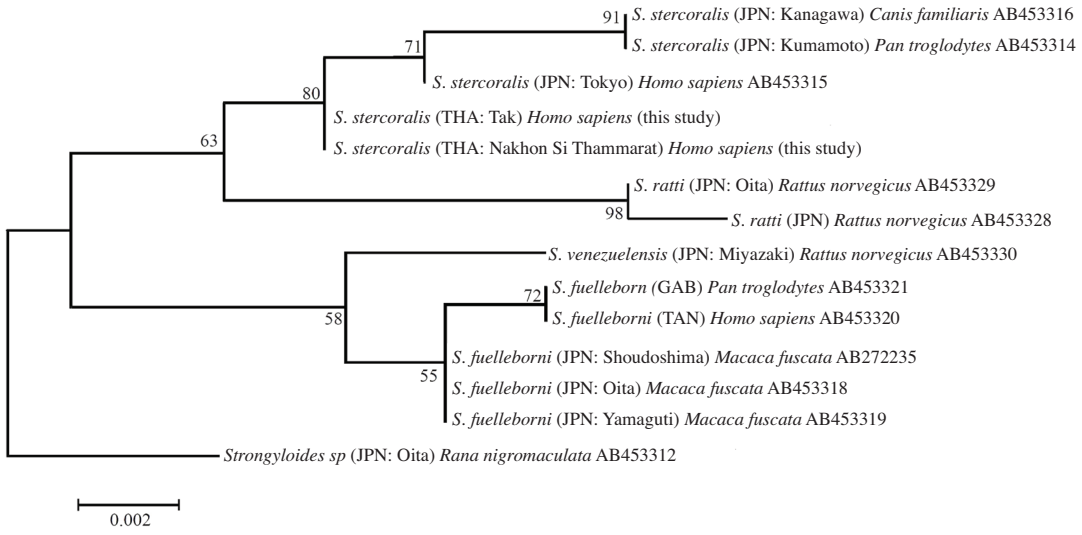

Fig 1–Phylogenetic relationship of *S. stercoralis* from different climate zones revealed by using 18S rDNA sequence. The phylogenetic tree was constructed by maximum likelihood method. Bootstrap proportion was used to determine the confidence level of relationship at each internal node.

Table 1
Source of *Strongyliodes* spp.

Species	Locality	Host	18S rDNA sequence accession number
S. stercoralis	JPN; Kanagawa	*Canis familiaris*	AB453316
S. stercoralis	JPN; Kumamoto	*Pan troglodytes*	AB453314
S. stercoralis	JPN; Tokyo	*Homo sapiens*	AB453315
S. stercoralis	THA; Tak	*Homo sapiens*	This study
S. stercoralis	THA; Nakhon Si Thammarat	*Homo sapiens*	This study
S. ratti	JPN; Shimane	*Rattus norvegicus*	AB453329
S. ratti	JPN; Lab strain	*Rattus norvegicus*	AB453328
S. venezuelensis	JPN; Miyazaki	*Rattus norvegicus*	AB453330
S. fuelleborni	GAB	*Pan troglodytes*	AB453321
S. fuelleborni	TAN	*Homo sapiens*	AB453320
S. fuelleborni	JPN; Shoudoshima	*Macaca fuscata*	AB272235
S. fuelleborni	JPN; Oita	*Macaca fuscata*	AB453318
S. fuelleborni	JPN; Yamaguti	*Macaca fuscata*	AB453319
Strongyloides sp	JPN; Oita	*Rana nigromaculata*	AB453312

GAB, Gabon; JPN, Japan; TAN, Tanzania; THA, Thailand

Table 2
Pairwise genetic distance estimated from 18S rDNA sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	-													
2	0.000	-												
3	0.002	0.002	-											
4	0.002	0.002	0.000	-										
5	0.002	0.002	0.000	0.000	-									
6	0.008	0.008	0.006	0.006	0.006	-								
7	0.010	0.010	0.012	0.012	0.012	0.014	-							
8	0.016	0.016	0.014	0.014	0.014	0.020	0.016	-						
9	0.016	0.016	0.014	0.014	0.014	0.020	0.016	0.000	-					
10	0.012	0.012	0.010	0.010	0.010	0.016	0.012	0.004	0.004	-				
11	0.010	0.010	0.008	0.008	0.008	0.014	0.010	0.006	0.006	0.002	-			
12	0.010	0.010	0.008	0.008	0.008	0.014	0.010	0.006	0.006	0.002	0.000	-		
13	0.020	0.020	0.018	0.018	0.018	0.016	0.016	0.016	0.016	0.012	0.010	0.010	-	
14	0.022	0.022	0.020	0.020	0.020	0.018	0.018	0.018	0.018	0.014	0.012	0.012	0.002	-

1, *S. fuelleborni* (GAB; *Pan troglodytes*); 2, *S. fuelleborni* (TAN; *Homo sapiens*); 3, *S fuelleborni* (JPN, Shoudoshima; *Macaca fuscata*); 4, *S. fuelleborni* (JPN, Oita; *Macaca fuscata*); 5, *S. fuelleborni* (JPN, Yamaguti; *Macaca fuscata*); 6, *S. venezuelensis* (JPN, Miyazaki; *Rattus norvegicus*); 7, *Strongyloides* sp (JPN, Oita; *Rana nigromaculata*); 8, *S. stercoralis* (JPN, Kanagawa; *Canis familiaris*); 9; *S. stercoralis* (JPN, Kumamoto; *Pan troglodytes*); 10, *S. stercoralis* (JPN, Tokyo; *Homo sapiens*); 11, *S. stercoralis* (THA, Tak; *Homo sapiens*); 12, *S. stercoralis* (THA, Nakhon Si Thamarat; *Homo sapiens*); 13, *S. ratti* (JPN, Shimane; *Rattus norvegicus*); 14, *S. ratti* (JPN, lab strain; *Rattus norvegicus*)

RESULTS

Genetic variation between *S. stercoralis* specimens of Thailand and Japan were analyzed using the highly conserved 18S rDNA. The results indicated that *S. stercoralis* from these two different climate zones had a genetic difference of around 0.002 (Table 2), the same value found for the genetic differences in *S. fuelleborni* populations from Japan and Tanzania (Table 2). The genetic relationship between the two *S. stercoralis* populations did not appear to be related to the host species (Fig 1). This same conclusion was reached for *S. fuelleborni* in Japan and Tanzania using cytochrome c oxidase subunit 1 (Hasegawa *et al*, 2010).

DISCUSSION

S. stercoralis is a species that has a worldwide distribution (Becker *et al*, 2011). It is capable of infecting primates and several other mammals (Junior *et al*, 2006). Our study indicates that, similar to *S. fuelleborni*, *S. stercoralis* does not have genetic variations correlating with different hosts. Genetic variation in these two species is mostly influenced by geographical differences. Moisture and temperature are two factors that directly affect the *Strongyloides* life cycle and these two factors are responsible for the distinct differences of *Strongyloides* spp in tropical versus subtropical zones (Hasegawa *et al*, 2009). This suggests that not only geographical location, but also moisture and temperature may directly affect genetic differentiation between isolated populations of parasites.

This study presents evidence of genetic variation of *S. stercoralis* from only two geographic locations. *S. stercoralis* isolated from Japanese patients has small genetic differences from specimens obtained from dogs and chimpanzees in Japan (Hasegawa *et al*, 2010), but comparatively large differences from specimens isolated from Thai patients. More research exploring the phylogeny of *S. stercoralis* is required to see if parasites isolated from other geographical areas and climate zones, especially in temperate zones, are consistent with our findings. Additionally, studies using other different genetic markers will also enable a clearer depiction of the phylogeny of *S. stercoralis* populations.

ACKNOWLEDGEMENTS

The authors thank the staff of the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Thailand for assistance and Tim Jackson for editing the English language of this manuscript. This study was made possible through funding from the Faculty of Tropical Medicine, Mahidol University.

REFERENCES

Afzal AS, Steven LB. Diagnosis of *Strongyloides stercoralis* infection. *Travel Med* 2001; 33: 1040-7.

Becker SL, Sieto B, Silué KD, *et al*. Diagnosis, clinical features, and self-reported morbidity of *Strongyloides stercoralis* and hookworm infection in a co-endemic setting. *PLoS Negl Trop Dis* 2011; 5: e1292.

Coker AO, Isokpehi RD, Thomas BN, Fagbenro-Beyioku AF, Omilabu SA. Zoonotic infections in Nigeria: overview from a medical perspective. *Acta Trop* 2000; 76: 59-63.

Concha R, Harrington WJR, Rogers AI. Intestinal strongyloidiasis: recognition, management, and determinants of outcome. *J Clin Gastroenterol* 2005; 39: 203-11.

Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nuclic*

Acids Symp Ser 1999; 41: 95-8.

Hasegawa H, Hayashida S, Ikeda Y, Sato H. Hyper-variable regions in 18S rDNA of *Strongyloides* spp. as markers for species-specific diagnosis. *Parasitol Res* 2009; 104: 869-74.

Hasegawa H, Torii H, Fujita S, *et al*. Molecular identification of the causative agent of human strongyloidiasis acquired in Tanzania: Dispersal and diversity of *Strongyloides* spp. and their host. *Parasitol Int* 2010; 59: 407-13.

Junior AF, Gonc alves-Pires MRF, Silva DAO, Gonçalves ALR, Costa-Cruz JM Parasitological and serological diagnosis of *Strongyloides stercoralis* in domesticated dogs from southeastern Brazil. *Vet Parasitol* 2006; 136: 137-45.

Koosha S, Kazemi B, Bonyadi F. The 18S Ribosomal DNA sequence of *Strongyloides stercoralis* in Iran. *Iranian J Parasitol* 2009; 4: 53-8.

McGinnis S, Madden TL. BLAST: at the core of a powerful and diverse set of sequence analysis tools. *Nucleic Acids Res* 2004; 32: 20-5.

Nilforoushan MR, Mirhendi H, Rezaie S, Rezaian M, Meamar AR, Kia EBA. DNA-based identification of *Strongyloides stercoralis* isolates from Iran. *Iranian J Public Health* 2007; 36: 16-20.

Sa-nguankiat S, Maipanich W, Yoonuan T, *et al*. Modification of polyethylene tube culture technique in cultivation of hookworm larvae. *J Trop Med Parasitol* 2011; 34: 30-5.

Tamara K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011; 28: 2731-39.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997; 25: 4876-85.

Vadlamudi RS, Chi DS, Krishnaswamy G. Intestinal strongyloidiasis and hyperinfection syndrome. *Clin Mol Allergy* 2006; 4: 8.