

Diagnosis and prevalence of hookworm and *Strongyloides stercoralis* infections among schoolchildren in rural southern Thailand

Blego SEDIONOTO^{1,2,*}, Sueptrakool WASESSOMBAT²,
Chuchard PUNSAWAD³ and Witthaya ANAMNART⁴

¹Department of Environmental Health, Faculty of Public Health, Mulawarman University, Samarinda 75123, Indonesia

²Division of Biomedical Sciences, School of Allied Health Sciences, Walailak University, Nakhon Si Thammarat 80161, Thailand

³Division of Parasitology, School of Medicine, Walailak University, Nakhon Si Thammarat 80161, Thailand

⁴Division of Environmental Health, School of Public Health, Walailak University, Nakhon Si Thammarat 80161, Thailand

(* Corresponding author's email: blego_kesling@yahoo.com)

Abstract

The prevalence of hookworm and *Strongyloides stercoralis* infections is serious public health concern globally. In rural southern Thailand, the prevalence of hookworm infection and strongyloidiasis was high and endemic area. In this study would show the infection rates, compared two diagnostic methods and analysis of climate change that has potential impact for reducing of prevalence of hookworm and *Strongyloides stercoralis* infections among school children in southern Thailand. We performed a cross-sectional study among 75 schoolchildren from one primary school at a rural village of Mokhalan sub district, Thasala, Nakhon Si Thammarat province in southern Thailand. In this study used two diagnostic methods: Kato Katz and Koga agar plate culture/KAP culture for diagnosing of hookworm and *Strongyloides stercoralis* infections. Hookworm, *Trichuris trichiura* and *Strongyloides stercoralis* infections were found in this study; 10.8, 5.3, and 2.7% respectively. The prevalence *Strongyloides stercoralis* in the study decreased when was compared with data prevalence in 2009 (28.5%) and 2014 (28%). The prevalence of hookworm and *Strongyloides stercoralis* infections was reduced among schoolchildren even the facilitate sanitation and personal hygiene unchanged. This study showed potential impact of dry season reduced of *Strongyloides stercoralis* infection. The sensitivity of Koga agar plate culture/KAP culture (100%) is higher than Kato Katz technique (0%) on diagnosis of *Strongyloides stercoralis* infection.

Keywords: Diagnosis, prevalence, hookworm and *Strongyloides stercoralis* infections, schoolchildren, rural southern Thailand

Introduction

The prevalence of hookworm infection and strongyloidiasis is of serious public health concern globally. Hookworm infection and strongyloidiasis are prevalent in poor rural communities tropical and subtropical areas in many developing country (Wardell et al. 2017). They are transmitted through in protected contact with soil are endemic in tropical and temperate regions. The prevalence of hookworm infection and strongyloidiasis was estimated in 2010 that 438.9 million people were infected with hookworm and 100 million with *strongyloides*. Almost 70% of these infections occur in Asia. (Pullan et al. 2014; WHO, 2011; Bethony et al. 2006).

Hookworm infection and strongyloidiasis are transmitted through in protected contact with soil are endemic in tropical and temperate regions. Human acquire the hookworm infection and strongyloidiasis through direct skin contact with infective third stage larvae where the soil was contaminated by human feces penetrate the intact human skin and eventually reach small intestine (Forrer et al. 2016).

Generally, hookworm infection and strongyloidiasis are found among poor people with poor environmental sanitation and where the climate is warm and humid (Bannon et al. 1995; Hall et al. 1994). Factors affecting difference in distribution of hookworm infection and strongyloidiasis may include good hygiene practices among population, availability of sewerage system and the length of rainy season. Environmental factors have contributed for transmission of diseases as well as growth and development of the worms (Anamnart et al. 2013; Prasit et al. 2016).

Environmental factors especially long rainy season may affect the decrease in prevalence of strongyloidiasis but not for hookworm infection. Prevalence of strongyloidiasis in south Thailand is lower than other parts of the country, in contrast, prevalence of hookworm infection is still high in the south. It is possibly because the failure in the control of hookworm infection due to 10 months long rainy season in southern Thailand contrasted with 4 months long rainy season in other parts (Anamnart et al. 2015). The study in Cambodia reported the lower prevalence of strongyloidiasis in area with heavy rainfall than in low rainfall area. Moreover, high amount of soil organic carbon content affect to the lower prevalence of strongyloidiasis (Khieu et al. 2014). Epidemiology study of hookworm infection and strongyloidiasis in Southern Laos showed 56.1 and 41% respectively where was heavy rainfall and poor sanitation. In this study Baerman and Kato-Katz techniques were used for detecting them (Vonghachack et al. 2015).

In rural southern Thailand, currently three years has climate changed, long rainy season is shorter than previously period. We perform a cross-sectional study on *S. stercoralis* in school children with compared prevalence in same primary school in 2009 and 2014.

Materials and methods

Ethical consideration

Official permission and ethical clearance for collection human fecal samples was obtained from head master and teacher. The study protocol was approved by the Ethical Clearance committee on human right related to research involving human subjects, walailak University HE: number WUEc-18-034-01.

Study setting and population

The study was carried out in primary school in Mokhalan sub district rural southern Thailand.

This research is a school-based survey was conducted during rainy season January-February 2017. Participants was taken from 4-6 grads of students in Wat Mokhalan School Children.

Field procedures

For collecting stool samples, the first day were requested to head master of School children and teacher and then to students, second day in the morning would start to collect stool samples, were brought to parasitology laboratory Walailak University for diagnosis samples. Others day was done observation environmental condition houses surrounding school children.

Laboratory procedures

Stool samples were first subjected to a KAP culture, then Kato-Katz thick smear. First, KAP culture (Koga et al. 1991) was used for identifying *S. stercoralis* and possibly hookworm larvae. For the purpose, agar plate was prepared one per week and stored at 4°C in humid condition. A hazelnut-sized stool sample was placed in the middle of plate and closed petri dish was incubated in humid chamber for 48 hours at 28°C. Afterwards, the plates were rinsed with sodium acetic acid formalin [SAF] solution. The cluent was centrifuged and the sediment microscopically examined for the presence of *S. stercoralis* and hookworm larvae. The two species were distinguished by characteristic morphology of the larvae [i.e, size of buccal cavity, presence genital primordium [L1], presence of forked tail-end [L3]].

Then, a single Kato-Katz thick smear (Katz et al. 1972) was prepared using the WHO standard template and examined under light microscope for the presence of helminth eggs.

Data analysis

The prevalence of hookworm infection and Strongyloidiasis was stratified according to sociodemographic data and reported by descriptive statistic. Directly, we observed facilitate sanitation and evaluated and compared duration of rainy season and dry season from 2009 to 2014. In this study compared the data of prevalence hookworm infection and strongyloidiasis from 2009-2014 with changed of climate between them.

Results and discussion

Study sample

A total of 75 individuals participated in this study. The age ranged between 10 and 12 years.

Parasitological findings

The observed and estimated prevalence of three different soil transmitted helminth species found from stool sample diagnoses. In total schoolchildren 5.3% were infected with *Trichuris trichiura*, 10.8% were infected with hookworm and 2.7% were infected with *S. stercoralis*. Prevalence STH in previous data (2014) showed of four different: 3.7 were infected with *A. lumbricoides*, 17% were infected with *T. trichiura*, 44.4% were infected with hookworm and 8 were infected with *S. stercoralis*. Prevalence STH in previous data (2014) showed of four different: 5% were infected with *A. lumbricoides*, 19% were infected with *T. trichiura*, 52% were infected with hookworm and 28.5 were infected with *S. stercoralis* (**Table 1**).

Table 1 Prevalence Soil Transmitted Helminths (STH) in school children Mokhalan.

STH	Prevalence STH (%) in year			Notification
	2017	2014	2009	
<i>A. Lumbricoides</i>	0	3.7	5	On 2009 after diagnoses of infected sth, children were brought drugs and 2014 to 2017 without drugs for sth infected children
<i>T. trichiura</i>	5.3	17	19	
Hookworm	10.7	44.4	52	
<i>S. stercoralis</i>	2.7	8	28.5	

Infection finding of hookworm infection and strongyloidiasis

In total, 8 hookworm infected schoolchildren (10.7%) were found in this research, and 2 strongyloidiasis infected were found in this study out of 75 participants (Table 2).

Table 2 Performance of the diagnostic methods.

Soil transmitted helminth infection	Infection finding with Diagnostic technique		
	Direct wet smear	Kato-Katz tick smear	Koga agar plate culture
<i>A. Lumbricoides</i>	0	0	-
<i>T. trichiura</i>	2/2.7%	4/5.3%	-
Hookworm	2/2.7%	1/1.3%	8/10.7%
<i>S. stercoralis</i>	0	0	2/2.7%

KAP technique only was used for hookworm and *S. stercoralis* diagnosis

Sensitivity of diagnostic method for hookworm infection and strongyloidiasis

By KAP culture technique showed 8/100% of prevalence of hookworm infection and 2/100% of prevalence of strongyloidiasis, KAP culture method is highest of sensitivity than direct wet smear and Kato-Katz technique on diagnosis of hookworm infection and strongyloidiasis in schoolchildren diagnoses of soil transmitted helminth infection in Mokhalan school children rural southern Thailand (Table 3).

Table 3 Sensitivity of diagnostic method for hookworm infection and strongyloidiasis.

Soil transmitted helminth infection	Diagnostic technique		
	Direct wet smear	Kato-Katz tick smear	Koga agar plate culture
Hookworm	25%	12.5%	100%
<i>S. stercoralis</i>	0	0	100%

Hookworm infection and strongyloidiasis are both of neglected tropical diseases (Anamnart et al. 2010). In poor countries with tropical climate, condition favorable for transmission of these parasites have higher the prevalence of hookworm infection and strongyloidiasis (Jongwutiwes et al. 1999). Furthermore, low socioeconomic status and low hygiene living conditions of the rural population are strongly associated with hookworm infection and strongyloidiasis. In Southeast Asia, a recent work in Cambodia reported a very high infection rate of Takeo Province (Khieu et al. 2014). In southern Thailand, there have been few studies on both of hookworm infection and strongyloidiasis.

Prevalence of strongyloidiasis in south Thailand is lower than other parts of the country, in contrast, prevalence of hookworm infection is still high in the south. It is possibly because the failure in the control of hookworm infection due to 10 months long rainy season in southern Thailand contrasted with 4 months long rainy season in other parts (Anamnart et al. 2015). The study in Cambodia reported the lower prevalence of strongyloidiasis in area with heavy rainfall than in low rainfall area. Moreover, high amount of soil organic carbon content affect to the lower prevalence of strongyloidiasis (Khieu et al. 2014). Epidemiology study of hookworm infection and strongyloidiasis in Southern Laos showed 56.1 and 41% respectively where was heavy rainfall and poor sanitation. In this study Baerman and Kato-Katz techniques were used for detecting of them (Vonghachack et al. 2015).

As far as we know, the most sensitive method for *S. stercoralis* diagnosis is KAP culture (Peter et al. 2015). We used direct wet smear, Kato-Katz tick smear and KAP culture on single stool sample from each school children, in total found 10.7% infected of hookworm infection and 2.7% infected of strongyloidiasis. KAP culture has sensitivity 100% both of diagnosis hookworm infection and strongyloidiasis on stool samples of schoolchildren Koga agar method has high sensitivity to diagnosis hookworm infection and strongyloidiasis. This technique can explain detail of growing up each step development of filariform larvae particularly for detecting of filariform larvae of *S. stercoralis*. Quoted by Steinmann et al. (2007), *S. stercoralis* was found only by Baermann technique and Koga agar plate method. In diagnosis *S. stercoralis* larvae KAP culture is more sensitivity than Baermann technique that quoted by Anamnart et al. (2015) and Khieu et al. (2015).

The prevalence of hookworm infection and strongyloidiasis in this study decreased when compared with previously data of hookworm infection and strongyloidiasis in 2014 were 44.4 and 8%, respectively. But the data before drugs program by government of Thailand in 2009 showed prevalence of hookworm infection and strongyloidiasis were 52 and 28.5%. Had been seen data in 2009 with 2014 that decreased of hookworm infection and strongyloidiasis maybe be caused by drugs program. But for decreasing prevalence of hookworm infection and strongyloidiasis in schoolchildren with data 2014 to 2017 may be

caused by climate change, have been changed of long duration rainy season and dry season in southern Thailand. The facilitate sanitation and behavior were not changed but naturally the prevalence hookworm and strongyloidiasis is reduced.

Conclusions

The prevalence of hookworm infection and *S. stercoralis* in endemic area in rural mokhalan subdistrict thasala Nakhon si thammarat southern Thailand was shown decreased may be was caused by climate change, but contribution improving sanitation and hygiene are important for continuing control program of hookworm infection and strongyloidiasis in rural southern Thailand.

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References

- Anamnart, W., Pattanawongsa, A., Intapan, P.M., & Maleewong, W. (2010). Albendazole Stimulates the excretion of *Strongyloides stercoralis* larvae in stool specimens and enhances sensitivity for diagnosis of strongyloidiasis. *Journal of Clinical Microbiology* 48, 4216-4220
- Anamnart, W., Pattanawongsa, A., Intapan, P.M., Morakote, N., Janwan, P., & Maleewong, W. (2013). Detrimental effect of water submersion of stools on development of *Strongyloides stercoralis*. *PloS One* 8, e82339.
- Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D., & Hotez, P.J. (2006). Soil-transmitted helminth infections: Ascariasis, trichuriasis, and hookworm. *Lancet* 367, 1521-1532.
- Hall, A., Conway, D.J., Anwar, K.S., & Rahman, M.L. (1994). *Strongyloides stercoralis* in an urban slum community in Bangladesh: Factors independently associated with infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 88, 527-530.
- Jongwutiwes, S., Charoenkorn, M., Sitthichareonchai, P., Akaraborvorn, P., & Putaporntip, C. (1999). Increased sensitivity of routine laboratory detection of *Strongyloides stercoralis* and hookworm by agar-plate culture. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 93, 398-400.
- Katz, N., Chaves, A., & Pellegrino, J. (1972). A simple device for quantitative stool thick-smear technique in *Schistosomiasis mansoni*. *Revista do Instituto de Medicina Tropical de São Paulo* 14, 397-400.
- Khieu, V., Schär, F., Forrer, A., Hattendorf, J., Marti, H., Duong, S., Vounatsou, P., Muth, S., & Odermatt, P. (2014). High prevalence and special distribution of *Strongyloides stercoralis* in rural Cambodia. *PloS Neglected Tropical Diseases* 8, e2854
- Khieu, V., Schär, F., Marti, H., Bless, P.J., Char, M.C., Muth, S., & Odermatt, P. (2014). Prevalence and risk factors of *Strongyloides stercoralis* in Takeo province, Cambodia. *Parasit Vectors* 7, 221.15
- Khieu, V., Schär, F., Marti, H., Sayasone, S., Duong, S., Muth, S., & Odermatt, P. (2013). Diagnosis, treatment and risk factors of *Strongyloides stercoralis* in schoolchildren in Cambodia. *PloS Neglected Tropical Diseases* 7, e2035.
- Kitvatanachai, S., & Pipitgool, V. (1999). Efficacy of three methods in the detection of hookworm and *Strongyloides stercoralis* infections. *Journal of Tropical Medicine and Parasitology* 22, 80-81.
- Koga, K., Kasuya, S., Khamboonruang, C., Sukhavat, K., Ieda, M., Takatsuka, N., Kita, K., & Ohtomo, H. (1991). A modified agar plate method for detection of *Strongyloides stercoralis*. *American Journal of Tropical Medicine and Hygiene* 45(4), 518-521.
- Na-ek, P., Sanpool, O., Jongthawin, J., Anamnart, W., Intapan, P.M., Chamavit, P., & Maleewong, W. (2016). Restoration of hookworm egg development after prolonged storage in stool suspension. *Parasitology Research* 115(7), 2817-2823.
- Olsen, A., Lieshout, L.V., Marti, H., Polderman, T., Polman, K., Steinmann, P., Stothard, R., Thybo, S., Verweij, J.J., & Magnussen, P. (2009). Strongyloidiasis: The most neglected of the neglected tropical diseases? *Transactions of the Royal Society of Tropical Medicine and Hygiene* 103, 967-972.
- Siddiqui, A.A., & Berk, S.L. (2001). Diagnosis of *Strongyloides stercoralis* infection. *Clinical Infectious Diseases* 33(7), 1040-1047.
- Steinmann, P., Yap, P., Utzinger, J., Du, Z.W., Jiang, J.Y., Chen, R., Wu, F.W., Chen, J.X., Zhou, H., & Zhou, X.N. (2015). Control of soil-transmitted helminthiasis in Yunnan province, People's Republic of China: Experiences and lessons from a 5-year multi-intervention trial. *Acta Tropica* 141, 271-280.
- Vonghachack, Y., Sayasone, S., Bouakhasith, D., Taisayavong, K., Akkavong, K., & Odermatt, P. (2015) Epidemiology of *Strongyloides stercoralis* on Mekong Islands in Southern Laos. *Acta Tropica* 141, 289-294.
- Wardell, R., Clements, A.C.A., Lal, A., Summers, D., Llewellyn, S., Campbell, S.J., McCarthy, J., Gray, D.J., & Nery, S.V. (2017). An environmental assessment and risk map of *Ascaris lumbricoides* and *Necator americanus* distributions in Manufahi district, Timor-Leste. *PloS Neglected Tropical Diseases* 11, e0005565.