Prevalence and risk factors of Ascaris lumbricoides infection in children of Manusak Village, Kupang District, East Nusa Tenggara Province, Indonesia

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Research Article

Prevalence and risk factors of *Ascaris lumbricoides* infection in children of Manusak Village, Kupang District, East Nusa Tenggara Province, Indonesia

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ABSTRACT

Worm infection is one of the health problems in all tropical countries, including Indonesia. The most frequent helminthiasis is the infection caused by the Soil-Transmitted Helminth. Primary school-age children are an age group that is susceptible to helminthiasis. Worm infections can cause a serious impact if left untreated with no clean and healthy lifestyle. This study was conducted to determine the prevalence and risk factors of STH in children of Manusak Village, East Kupang Subdistrict, Kupang District, East Nusa Tenggara Province, followed by the characterization of subjects. The analytic observational design with the cross-sectional study was applied to this research. Research subjects were 130 children aged 6-12 years old. The infection was diagnosed microscopically by the Katokatz method. *Ascaris lumbricoides* were the only species found infecting a total of 50 children (38.4%.). The distribution of ascariasis was higher in girls (30 or 23.08%) than in boys (20 or 15.38%), in children whose parents have no formal education (41 or 31.54%), and who their professions are a farmer. Poor hygiene and sanitation such as no washing hands with soap (27 or 20.77%) as well as the habit of not wearing footwear (47 or 36.2%) and bowel habit on the open areas (35 or 26.92%) are the risks factors causing the high incidence of *A. lumbricoides* in the studied area. Enlightenment to the parents regarding good personal and environmental hygiene and sanitation along with chemotherapy from the government and related agencies is needed to achieve a durable reduction of the burden of ascariasis and other STH infection.

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INTRODUCTION

Ascaris lumbricoides is one of the soil-transmitted helminths (STH). STH are intestinal nematode worms that infect humans who ingest their eggs via the oral-fecal route. This worm consists of *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenale* and *Strongyloides stercoralis*. The STH infections are widely distributed in tropical and subtropical areas, with the most incredible numbers occurring in Sub-sahara Africa, America, China, and East Asia. More than 1.5 billion people, or 24% of the world's population, are infected with STH. The high-risk group includes preschool children, school-age children, women of childbearing age (including pregnant women), and adults in certain high-risk occupations such as tea pickers or miners (Sastry & Bhat, 2019) or farmers (Ensink, Hoek, Mukhtar, Tahir, & Amerasinghe, 2005). The current comprehensive prevalence rate of STH in Indonesia is difficult to be ascertained. The prevalence rate in children is higher than in adults due to poor personal hygiene (Lee & Ryu, 2019). No report of a certain number of cases has been published.

Ascaris lumbricoides is the largest nematode of the gastrointestinal tract of humans. This nematode is cosmopolitan in distribution, where the typical habitat of the adult worm is the jejunum. The infection is acquired by the ingestion of the embryonated eggs, and the larvae pass through a pulmonary migration phase for maturation (Khuroo, Zargar, & Mahajan, 1990). The infection is transmitted through the fecal-oral route, from soiled hands and food contaminated with *A .lumbricoides*-infected human feces or unprocessed sludge or biosolids taken from sewage treatment systems where the ova accumulate (Asaolu & Ofoezie, 2018). Intestinal nematode infection is most prevalent among rural communities in warm and humid equatorial regions and where sanitation facilities are inadequate. Infection also occurs in urban areas. Even within areas of low prevalence, small localized regions of high prevalence can exist. Only cold or sweltering, arid climates are free of infection (Brooker, 2010). The number of infections is estimated that in 2010 about 818 million (771.7-891.6 million) people were infected with *A. lumbricoides* (Pullan *et al*, 2014).

Hygiene and sanitation are the factors are closely related to STH infection. The habit of washing hands with soap, wearing footwear, the cutting nails are the best way to prevent the transmission of helminthiasis. Sanitation is one of the risk factors for the disease, such as the use of improper latrines, which will cause contamination of the soil with feces around the yard and clean water availability (Kartini, Kurniati, Jayati, & Sumitra, 2017). The infection of STH is one of the neglected tropical diseases—lack of maximum effort and monitoring by health workers to prevent the disease. The STH-infected persons usually do not realize that they have been infected. The infection can be diagnosed by finding eggs and adult worms in the feces (Juhairiyah, Annida, & Indriyati, 2015). The incidence of helminthiasis in the Province of East Nusa Tenggara (NTT) is at the third position in Indonesia with a percentage of 28% (2018 NTT Health Service) after Banten Province, Which was 60.7%, and Nanggroe Aceh Darussalam Province (NAD) which was 59.2% (Zulhifah, 2016).

This study was conducted in Manusak Village, where poor sanitation and hygiene in this village may develop STH infection. Most of the population of Manusak Village works as farmers. The majority of the population were refugees from Timor Leste during the referendum of free Timor Leste from Indonesia in 2009 who did not return to the country of Timor Leste. The people in this area have a low economic condition, low environmental sanitation, not using footwear during play in the yard, some

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people do not use toilets for defecation and free pets outside the cage. This study aims to find out the prevalence risk factors of STH infection in primary school-age children in the refugee area of Manusak Village, East Kupang Subdistrict, Kupang District, East Nusa Tenggara Province, Indonesia.

METHODS

Ethical clearance

Ethical clearance was obtained from the Health Research Ethical Clearance Commission (HRECC) Faculty of Dental Medicine, Universitas Airlangga, Surabaya as described on the certificate number 053/HRECC.FODM/II/2020.

Location

The research was conducted in the Manusak Village, East Kupang Subdistrict, Kupang District, East Nusa Tenggara (NTT) Province. Kupang District is situated in Timor island of Indonesia (Figure 1).

Geographically, Kupang District has a land area of 7,178.26 Km^2 and is the largest area in East Nusa Tenggara (ENT) Province. Kupang District covers 15.16 % of the total land area of ENT Province. Geographically, Kupang District is located at 123º16'10.66"-124º13'42.15" east longitude and 9º15'11.78"-10º.22 '14.25"south latitude.The territory is bordered by the Savu Sea and Ombai Strait to the north, the Indian Ocean and the Timor Sea to the south, District of South Central Timor and the District Oecusi of the country of Timor Leste to the south, and Kupang City, Rote Ndao District, Sabu Raijua District and Sawu Sea to the west (Pemerintah Daerah Kabupaten Kupang, 2013). Manusak Village is one of 8 villages in East Kupang Subdistrict, which consists of 3,800 population and contains 520 families. This village is a rural and slum area with low social and economic level, limited education level, limited hygienic criteria in the availability of latrines, that resulting in the disposal of sewage in any place such as in the bushes around the place of residence (BPK RI Perwakilan Prop NTT, 2019).

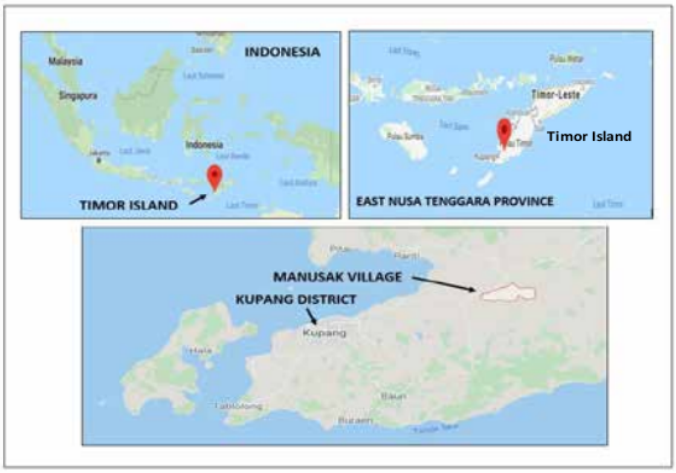

Figure 1. Location of Kupang District and Manusak Village. Kupang District, East Nusa Tenggara Province is located in Timor Island as same as the country of Timor Leste, and Manusak Village is a part of Kupang District.

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Research design

The analytic observational design with the cross-sectional study was applied to this research. Samples collections were done during January-February 2020. Stool samples were collected using stool pots which were distributed on a day before sample collection, followed by an explanation on how to collect stool samples, and the subjects who agree to give the sample were asked to sign the informed concern. The parents of the children are ethically represented to sign the informed concerns. The sampling must meet inclusion criteria that the subjects who had signed the inform concern and gave their stool samples, the children who did not take the anthelminthic medication within the last 6 months when the sampling was ongoing, and the samples which contained STH on microscopy examination. The exclusion criteria were the children who did not give the samples during the data collection and who had taken anthelminthic medication within the last six months when the sampling was ongoing. Stool pots contained stool samples were then labeled with the identity of the subjects. The collected samples were then transported to the Laboratory of Parasitology, Study Program on Medical Technology, Institute of Polytechnic of Ministry of Health, Kupang District prior to microscopy examination.

Kato Katz Method for stool sample examination

Kato Katz's method was done based on the technique explained by WHO in (WHO, 2019) modified based on the materials available in the Laboratory of Parasitology in the Study Program on Medical Technology in the institute of Polytechnic of Ministry of Health, Kupang District. A gram of fecal sample was placed on filter paper, and then a wire mesh was put on the feces. A piece of cardboard was prepared, a hole was made on it then the cardboard was placed on sliding glass. The wire mesh was placed on fecal sample examination top of the feces; then, the cardboard holes were filled with feces, and cardboard was removed. Feces on object glass was then covered with cellophane that has been soaked in the Kato solution. Cellophane tape was pressed with other glass objects to flatten the stool, and it was left for 20-30 minutes at room temperature. Objects glass was then examined under a light microscope using a 10x objective lens to identify the species of STH. The eggs per gram of feces were counted and calculated to determine the intensity of infection based on WHO (WHO, 1994).

RESULTS

Characteristics of subjects

Research subjects were 130 children aged 6-12 years old, consisted of 76 females (58.5%) and 54 males (41.5%). Primary data on the characteristic of subjects were obtained by direct interviews using a questionnaire. The results were presented in Table 1.

Kato Katz stool sample examination

The results of microscopy examination of stool samples showed that *Ascaris lumbricoides* was the only species found in 50 (38.4%) samples out of total of 130 samples. Therefore 80

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Table 1. Characteristic of subjects and their parents

Variable	Number (%)
Age (years)	
6-12	130(100)
Gender	
Female	76(58.5)
Male	54(41.5)
Level of education of parents	
No School	41(31.54)
Primary school	30(23.08)
Junior high school	25(19.23)
Senior high school	20(15.38)
College	14(10.77)
Profession of parents	
Farmer	107(82.31)
State officer	18(13.84)
Entrepreneur	5(3.85)

(61.6%) were negative. Other species of STH were not found in the collected samples. The distribution of positive samples based on the characteristic of subjects and their parents is presented in Table 2.

Table 2 above shows that among 50 positive samples, the distribution of ascariasis was higher in girls than in boys, in children whose parents have no formal education and whose professions are a farmer. Farmer is the kind

Table 2. Distribution of *A. lumbricoides* infection based on the characteristic of subjects and their parents

Variable	Number of the sample (%)			p
	Positive	Negative	Total	
Age (years)				
6-12	50 (38.46)	80(61.54)	130(100)	
Gender				
Female	30(23.08)	46(35.38)	76(58.5)	
Male	20(15.38)	34(26.2)	54(41.5)	
Level of education of parents				0.001
No School	27(20.77)	14(10.77)	41(31.54)	
Primary school	10(7.69)	20(15.38)	30(23.08)	
Junior high school	7(5.38)	18(13.85)	25(19.23)	
Senior high school	5(3.85)	15(11.54)	25(19.23)	
College	1(0.77)	13(10)	14(10.77)	
Occupation of parents				0.021
Farmer	47(36.2)	60(46.2)	107(82.31)	
State Officer	2(1.4)	16(12.31)	18(13.84)	
Entrepreneur	1(0.77)	4(3.08)	5(3.85)	

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of profession which dominated among the parents, and their children have also dominated the positivity of ascariasis case in the studied village. Interestingly, the number of positive samples was decreased along with the increase in the level of education. Chi-square analysis showed a strong significant correlation between the level of education of the parents (p=0.001) and their occupation (p=0.021) with the positivity of ascariasis.

Intensity of infection

The intensity of ascariasis in children of Manusak Village based on the number of EPF of feces is presented in Table 3. The intensity of infection among the positive samples (50) was found to be mild and moderate (WHO, 2017).

The number of samples with a mild intensity of infection contained each male and female were 5 (10%) each of children, while moderate infection contained 15 (75%) male and 35 (83.33%) female of children.

Risk factors of ascariasis in Manusak Village

Based on interviews and direct observations on the behavior of clipping, the nails showed dirty and clean nails; however no correlation of this behavior to the positivity of ascariasis (p=0.868). Handwashing habits before eating and after defecating (p=0.000), the habit of wearing footwear when playing and accompanied their parents to the garden (p=0.019), as well as bowel habits (p=0.035) are the risk factors in children to have *A. lumbricoides* infection (Table 3).

Tabel 3. Intensity of infection based on the number of EPG of feces in children of Manusak Village, Kupang District

A. Lumbricoides infection	Intensity of infection	
	Mild	Moderate
Number of sample (%)	10 (20)	40(80)
Number of EPG of feces	1,000-4,000	6,000-9,600
Mean of EPG of feces	2,420	7,390

DISCUSSION

The STH infection in children has received more attention than in farmers, as shown by the implementation of deworming and providing albendazole treatment to school children in Indonesia (Sungkar, Ridwan, & Kusumowidagdo, 2017).

Children are more susceptible to parasitic infections than adults because of a lower immune response, poor hygiene and sanitation, and favorable environmental conditions for the development of parasites that eventually infect the host. The transmission of ascariasis in Manusak village was caused by poor sanitation and hygiene of the children, especially in defecating behavior. Although 100% of subjects own the family latrine, 26.92% out of them prefer defecating on the ground or garden or house yard (Table 3), and although defecating in the family toilet (11.54%), they are positively infected by *A. lumbricoides.*

Based on the results of microscopy examination of 130 samples, only species of *A. lumbricoides*

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Table 4. Risk factors of ascariasis and distribution of *A. lumbricoides* infection based on behavior of subjects of Manusak Village, Kupang District

Behavior	Number of the sample (%)			
	Positive	Negative	Total	p
Clipping fingernails behavior				0.868
Yes	23(17.7)	38(29.2)	61(46.92)	
No	27(20.77)	42(32.3)	69(53.08)	
Handwashing Habits				0.000
Washing	8(6.2)	39(30)	47(36.15)	
Not washing	42(32.3)	41(31.5)	83(63.85)	
Wearing footwear behavior				0.019
Wear	3(2.3)	17(13.1)	20(15.38)	
Do not wear	47(36.2)	63(48.5)	110(84.62)	
Bowel habits				0.035
Toilet	15(11.54)	39(30)	54(41.54)	
On the ground of garden and house yard	35(26.92)	41(31.54)	76(58.46)	

Table 5. Environmental sanitation and distribution of *A. lumbricoides* infection in children of Manusak Village

Variable	Number of the sample (%)			
	Positive	Negative	Total	p
Latrine Ownership				*
Yes	50(38.46)	80(61.54)	130(00)	
No	0	0	0	
Clean Water Source				0.148
River	47(36.2)	60(46.2)	107(82.31)	
Well	2(1.4)	16(12.31)	18(13.84)	
Tank	1(0.77)	4(3.08)	5(3.85)	

*Statistical analysis cannot be performed because latrine ownership is constant, all subjects have a family latrine.

(100%) with a positive number of 50 children (38.4%) were found. The species of *A. lumbricoides* is the most prevalent STH in the region of South Asia and South East Asia, but there was considerable geographic variation (Silver *et al*., 2018).

The focal nature of *A. lumbricoides* was associated with pH and the soil texture, those are factors that influence STH infection distribution. Furthermore, *A. lumbricoides* were stated to prefer acidic soil. Clay and loam soil are more favorable for eggs of *A. lumbricoides* development than sandy loam soil, which favorable for hookworm (Wardell *et al*., 2017). Those types of soil were found in Kupang District (Pemerintah Daerah Kabupaten Kupang, 2013).

The species of *A. lumbricoides* was the only species found by the Kato Katz method in this study. Other species might present in the samples but could not be detected by this method. The direct method has been applied to diagnose the samples. However, the same results were obtained. Kato Katz is a method

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which has great epidemiological relevance because its simplicity and low cost (by using this method not only detecting the parasite but also the parasite load quantification of the infected patients by calculating the number of egg per gram (EPG) of feces (Speich, *et al*, 2015).

However, the Kato-Katz technique has limitations in terms of sensitivity, especially when the small amount of stool was analyzed (usually 41.7 mg) and whenever the parasites present at low frequency (Taye, 2014).

The sensitivity of the Kato-Katz technique is increased by analyzing multiple thick smears from a single or, ideally, from multiple stool samples (Speich *et al*., 2015).

Hookworm might be present in the community but not detected by the Kato Katz method. A sufficient distance from the sampling area to the laboratory to transport the samples for processing showed that time delay between excretion and laboratory processing of stool samples significantly decreases the fecal egg count, especially hookworm eggs (Krauth *et al*, 2012).

When the sample was too little, and the intensity was too low, the existing hookworm egg could not be detected by Kato Katz method. A more sensitive method should be applied to solve this problem.

Alternatively, a molecular detection technique can be applied to detect and identify the parasite's species of the low frequency of STH (Lamberton and Jourdan, 2015).

Molecular diagnostic tools are highly sensitive and specific, such as multiplex PCR and real-time PCR (qPCR). Multiplex PCR enables the detection of multiple parasite species in a single reaction and can simplify diagnostics by replacing several individual tests with one molecular test. Multiplex PCR showed promising results for *A. lumbricoides*, *T. trichiura* and *N. americanus* (Phuphisut *et al*, 2014)

The qPCR, unlike conventional PCR, which can only indicate the presence of infection, qPCR enables quantification of parasites and associated infection intensity. The qPCR is more sensitive than Kato-Katz and the floatation technique (FS7) for detection of *A. lumbricoides* infections and co-infections. Multiplex qPCR assays have successfully detected *A. lumbricoides* infection alongside multiple intestinal parasites (Basumi *et al*, 2012; Mejia *et al*, 2013).

High incidence of ascariasis in Manusak Village compared with other districts in Indonesia was higher than that of ascariasis in 2-9 years old children in Batakte Village of West Kupang Subdistrict, Kupang District, where 12% out of 59 children was positive (Susilawati & Smaut, 2017), in Northwest Sumba District, where only 31% children infected with *A. lumbricoides* (Olin, Paun, & Rindu, 2019), while in Sorong District, Papua Province was only 3.4% (Yuwono, Husada, & Basuki, 2019), in Karang Asem District, Bali Province was only 0.8% (Bayu, Pradinata, Sudarmaja, & Ariwati, 2019), however in Banjarmasin City, South Kalimantan Province no ascariasis was found (Juhairiyah *et al.*, 2015).

Ascariasis in Manusak village was lower compared with that in Raja Basa District of Lampung Province, where ascariasis cases in children were 88.2% (Wintoko, 2014).

The high incidence of ascariasis might relate to the anthelminthic medication program from the local health center, which was not implemented properly by the children in Manusak village. In Indonesia, according to Decree Minister of Health, No. 424 / Menkes / SK / VI / 2006 regarding Guidelines for Worms Control mass treatment of intestinal worms, is if the prevalence of an area above 30% is carried out mass treatment two times a

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year. For a prevalence below 30%, treatment is carried out selectively for subjects who are positively found worm's eggs in their stools and carried out at the current health facility come for treatment. However, according to WHO 2006, for areas with high infection rates, the medication can be implemented every three times a year (Kementerian Kesehatan Republik Indonesia, 2006).

The direct information regarding the anthelmintic medication obtained from the subjects was that they received albendazole in August, but none of them took medicine because it was too big to swallow. A recommendation given to a community to crush the anthelminthic drug to be easy to swallow might overcome this problem (Vitamine angels, 2020).

However, no published report on the information on the distribution of anthelmintic as well as the use of albendazole and its resistance in Kupang District. Likewise, in the Health Profile of Kupang District 2018, there was no description of albendazole distribution. Only the distribution of vitamin A and iron-containing tablets were explained clearly (Dinas Kesehatan Kupang, 2018).

The intensity of infection was found to be mild and moderate. This finding was different from the study among elementary school children of Suka Village, North Sumatra, where the severe intensity was found in addition to mild and moderate intensities. Mild ascariasis is often asymptomatic. However, individuals with mild and moderate infection intensity are the source of transmission in the community (Bethony *et al*, 2006). Malabsorption of vitamin A and reduction of lactose digestion often occur in *A. lumbricoides*-infected children due to the obstruction of the small intestine by the worm, which leads to growth retardation, undernutrition, impaired cognitive function, and low educational achievements in children (Galgamuwa and Dharmaratne, 2018).

The *A. lumbricoides* infections and undernutrition have a negative impact on the growth and development of the infected person (Quihui-Cota *et al*, 2004).

Characteristics of parents of subjects included education and occupation, have been analyzed. The level of education and knowledge of parents is very influential on the growth, development, and formation of children's behavior (Nokali, Bachman, & Votruba-Drzal, 2010).

The level of education correlated significantly with *A. lumbricoides* infection with p=0.001. Interestingly, the number of positive cases increased oppositely with the level of education (Table 2). This is a fact that the low education of parents risky to cause ascariasis in their children. The low education may have the low knowledge on hygiene and sanitation as well as on worm infection, including preventive care and transmission. This low level of knowledge of being infected. Therefore there is a need for proportion education on STH transmission (Oyebamiji, Ebisike, Egede, & Hassan, 2018).

Farmer is the occupation of parents with the highest number of ascariasis cases (36.2%) of their children. Occupation of parents correlated significantly with the infection with p=0.021 (Table 2). Farmer is one type of occupation that is in direct contact with the soil, which is possible for them to be infected with STH if the soil is contaminated with eggs of STH (Munawaroh, Arwati, & Wardhani, 2020). Risk factors associated with farmers are the use of wastewater for land irrigation, manure fertilizers as plant fertilizers, and the use of personal protective equipment during work, and handwashing behavior after work (Ensink *et al*., 2005).

oStatistically, there was no correlation between the habit of clipping fingernails with infection (p=0.868). The same result was shown by a report from Kulon Progro District in the province of Specific Region of Yogyakarta

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were no correlation of trimming fingernails and the STH infection with (p=0.085); however, fingernail biting increased the risk of STH infection (p=0.019) (Sofiana, Sumarni, & Ipa, 2011). A different result was reported that the habit of fingernails clipping of children in Raja Basa District of Lampung Province was 16,5% affected the contamination of nails with STH eggs (Wintoko, 2014).

Poor hygiene and sanitation in washing hands with soap before eating or after defecating (p=0.000), as well as the habit of wearing footwear (p=0.019) and bowel habit (p=0.035), are the risk factors causing the high *A. lumbricoides* incidence in the studied area. The number of children who did not wash hand with soap (32,4%) more than the number of children who did (6.2%) as well as the number of children who did not wear footwear (36.2%) than who did (2.3%) coupled with the habit of defecating on the open plantation (26.92%) more than on the toilet (11.54%). The association between handwashing and *A. lumbricoides* infection depended largely upon access to a water source (Garn *et al*., 2015).

Shoes are effective for blocking the penetration of STH to the skin. Unfortunately, shoe-wearing is uncommon in many areas where STHs are prevalent because the local populations are unaware of the health benefits of wearing shoes (Paige *et al*., 2017).

Although family toilets were available in all subject's houses, however, they prefer defecating in the open. The habit of defecation on the open plantation and bush was also found in Taabo, Co ˆte d'Ivoire, where 43.4% of participants were due to a deeply rooted tradition of open defecation (Schmidlin *et al*., 2013). The unavailability of clean water was thought to be a problem for the defecation in the toilet. In fact, clean water was available for their daily activities in all houses of the subjects. Apparently, the problem was the observance in implementing good personal hygiene and sanitation.

The interesting results of this study were that the strong correlation between the level of education of the parents play an important role in educating the children's behaviors of personal and environmental hygiene and sanitation as the number of positive cases increased oppositely with the level of education.

The limitation of this study was that other species of STH were not detected by the Kato Katz method. The finding of single species was an important thing to observe whether there was indeed only one species or some other species that were not detected by the Kato Katz method. Since the habit of not wearing footwear has a correlation with *A. lumbricoides* infection, the hookworm infection might exist in the community. The low sensitivity of the Kato Katz method for the detection of hookworm infection may be related to the rapid degeneration of delicate hookworm eggs with time. The PCR method might be used simultaneously with the microscopy examination so that the samples could be preserved in the field to maintain the parasite's DNA prior to further laboratory processing.

CONCLUSION

A high number of ascariasis cases in the studied area was due to the observance of the children in implementing good personal hygiene and sanitation. The level of education of their parents, which correlated with the infection, influenced the habit of their children. Therefore, the interference from the government and related agencies to provide enlightenment to the parents and children in different ways regarding good personal and environmental hygiene and sanitation along with chemotherapy is needed to achieve a durable reduction of the burden of ascariasis and other STH infection.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

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PAGE 10

PAGE 11

PAGE 12

PAGE 13

PAGE 14