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Research Report

Correlation of Nutritional Status with Hookworm and *Strongyloides stercoralis* Infection in Children Under Five Years in Kokar Public Health Center, Alor Regency, East Nusa Tenggara

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ABSTRACT

Malnutrition can reduce immune response particularly in cytokine (IL-4, IL-5, IL-10) production and immune effector (eosinophil, IgE, and mast cell), thus increasing the probability of intestinal nematode infection. Through this study, intestinal nematode infections occurred among children under five years, at different nutrition status, in Kokar Public Health center, Alor Regency, East Nusa Tenggara was captured. Hookworm and *Strongyloides stercoralis* were studied as both of them have devastating impacts compare to other helminthes compare to other helminths. This study is a cross-sectional study with a quote sampling technique. As many as 238 children, aged 12-59 months living in Kokar's Public Health Center area, Alor regency were recruited in this study i.e. 7.7% severely underweight, 19.2% underweight, 70.5% normal and 2.6% overweight. Data were collected in August - October 2016. Hookworm and *S. stercoralis* infection were determined from collected fecal samples of all subjects using either Baermann test, Koga Agar Plate (KAP), or Harada-Mori culture method. The prevalence of hookworm and *S. stercoralis* infection was 8.82%, and 0.42%. Correlation between nutritional status and hookworm infection were analyzed by Mann-Whitney test with p value = 0.54 ($p > 0.05$). Prevalence of hookworm and *S. stercoralis* among children under five years in Kokar were 8.82% and 0.42%. There was no significant correlation between nutritional status with hookworm infection prevalence.

Keywords: Hookworm infection prevalence, nutritional status, Kokar, Alor Regency

ABSTRAK

Malnutrisi dapat menyebabkan penurunan pada sistem imun terutama pada produksi sitokin (IL-4, IL-5, IL-10) dan kinerja sel efektor pada respon imun (eosinofil, IgE, dan sel mast). Gangguan ini dapat berakibat pada meningkatnya risiko infeksi nematoda usus. Selain malnutrisi, faktor perilaku tidak higienis juga dapat meningkatkan risiko infeksi nematode usus. Infeksi nematoda usus pada balita dapat menyebabkan gangguan pertumbuhan dan perkembangan. Nematoda usus yang menyerang anak-anak usia balita umumnya disebabkan oleh soil-transmitted helminth (STH), diantaranya adalah hookworm dan *Strongyloides stercoralis*. Studi ini bertujuan untuk mengetahui hubungan status gizi terhadap prevalensi infeksi pada balita di Puskesmas Kokar, Kabupaten Alor. Rancangan penelitian bersifat cross-sectional, dengan teknik quote sampling. Sampel 238 balita di Puskesmas Kokar, kabupaten Alor. Pengumpulan data pada bulan Agustus - Oktober 2016. Subjek dan orangtua yang memenuhi kriteria penelitian diwawancara menggunakan panduan kuisioner. Sampel feses dikumpulkan dan diperiksa jenis infeksi menggunakan metode uji Baermann, Koga Agar Plate (KAP), dan Harada-Mori. Prevalensi infeksi hookworm dan *S. stercoralis* pada balita di Puskesmas Kokar adalah 8,82% dan 0,42%. Korelasi antara status gizi dan infeksi hookworm dianalisis menggunakan Mann-Whitney test didapatkan nilai $p = 0,54$ ($> 0,05$). Perilaku tidak higienis tidak memiliki korelasi terhadap infeksi hookworm. Prevalensi infeksi hookworm dan *S. stercoralis* pada balita di Kokar adalah 8,82% dan 0,42%. Tidak ada

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hubungan bermakna antara status gizi balita dan prevalensi infeksi hookworm. Tidak ada korelasi antara perilaku tidak higienis dengan prevalensi infeksi hookworm.

Kata kunci: prevalensi infeksi hookworm, status gizi balita, perilaku, Kokar, Alor

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INTRODUCTION

Nutritional status is a health condition of children under five that is measured by age, body weight (BW) and body height (BH)¹. Nutritional status could be influenced by nutrition intake in food, parenting style, children's health services, environmental health, economic factors, sociocultural factors, and parents' educational factors (knowledge). In 2012, the prevalence of severe-weight and underweight children in Indonesia is at 19.60% spread across all levels of the community's economy².

Malnutrition may lead to increasing risk of intestinal nematode infection. The relationship of malnutrition to intestinal nematode infection occurs through two pathways, namely: malnutrition causes an increased risk of infection and helminthiasis is caused malnutrition^{3,4}. Increased risk of infection is probably due to reduced production of cytokines (IL-4, IL-5, IL-10) and effectors cell performance on immune (eosinophils, IgE, and mast cells)⁵⁻⁷. Thus low level of IL-4 is suggested to increase the probability of STH infection due to less immune system to prevent helminth infection. This study focused on hookworm and *S. stercoralis* infection as it may cause anemia and malabsorption syndrome due to haemorrhagic and desquamation of intestinal epithelium respectively⁸. Briefly, both infections may lead to a growth disturbance in children.

Approximately 1.4 billion of the world's population are estimated infected with Soil Transmitted Helminths (STH) with the highest number of cases occurring in developing countries. The prevalence of STH infection in Indonesia is ranging low to high, with the highest prevalence occurs usually in remote or underdeveloped areas. Study reports showed a

high prevalence of STH helminthiasis in children, such as: in Jayapura (50%), Central Mollucas Regency (99.4%), Padang (51.3%), Nangroe Aceh Darussalam (59.2%), East Nusa Tenggara (27.7%), and West Kalimantan (26.2%)^{9,10,11}.

In 2013, the province of East Nusa Tenggara ranked second highest in malnourished children in Indonesia with a percentage of 11.50%². The numbers are escalating in the work area of Kokar's public health center, with a percentage of severe-weight (6.00%), underweight (12.70%), normal weight (77.80%), and overweight (3.50%). Such condition had put Kokar to ranked third highest in percentage of severe-weight and underweight children after Apui and Kabir in Alor Regency. Kokar selected as study area due to complete category of nutritional status and sufficient facilities related to this study (electricity).

This study focused on finding correlation between nutritional status with hookworm and *S. stercoralis* infection. This study also try review about *S. stercoralis* prevalence due to lack of strongyloidiasis data in Indonesia until present time which perhaps due to difficulty in performing *S. stercoralis* detection method such as Baermann and Koga Agar Plate (KAP) tests. Results of this study might be useful for further action was taken by stakeholders and local government.

MATERIALS AND METHODS

A cross-sectional study with nutritional status as an independent variable and hookworm and *S. stercoralis* infection as a dependent variable. The reachable population of this study were children under 5 years for in Kokar totaling 631 people. Through Slovin formula¹², total samples in this study determined as 245 children of 12-59 months old. Samples were selected from population based

on inclusion and exclusion criteria as follows. Inclusion criteria include: (1) children aged 12 to 59 months and (2) children did not consume the anthelmintic drugs for the last 4 months. Exclusion criteria include: (1) feces contaminated by dirt, water, and urine, (2) feces were given for more than 24 hours after defecate, (3) children's parent did not approve inform consent.

Nutritional status data were secondary data based on *Kartu Menuju Sehat/* Children Growth Chart (KMS/CGC) obtained from Kokar Health Center. Nutritional status data based on anthropometric measurements of body weight (kg) for age (month). Subjects recruited in four categories nutritional status, in line with the proportion in the population, i.e. 7.7% severely underweight, 19.2% underweight, 70.5% normal and 2.6% overweight.

Data Collection

Data collection was held in August - October 2016 in the working area of Kokar public health, Alor Barat Laut district, East Nusa Tenggara Province. Children's parent are given an explanation of how to collect and store stool samples e.g. stool sample collected must be fresh (no more than 24 hours) without any contamination from water, soil and urine, and the respondent was not given any anthelmintic treatment in the last four months. Stool samples collected in stool containers.

After accommodated, the sample was submitted The Public Health center staff and researcher will retrieve afterward. Each stool sample were examined using 3 diagnostic methods: Baermann test, Koga Agar Plate (KAP), and Harada-Mori culture methods to identify the presence or absence of infection.

Baermann Method (BM)

In this study, the Baermann Method composed of tea strainer, plastic funnel, gauze bandages, hose and hose clamp. Each tea strainers placed on funnel mouth with gauze bandage on strainer. Warm water used to check whether there is a bubble in the hose. Part of fresh feces (5 gram), wrapped with gauze bandages, then placed on

strainer. A 40W bulb lighted at the bottom of hose for 2-3 hours. The filtrate poured as many as 15 ml, centrifuged at 2,500 rpm for at least 5 minutes. The supernatant discarded fast, the sediment examined under light microscope with a magnification of 100 and 400 times¹³.

Koga Agar Plate (KAP)

KAP Method started with the making of agar medium consist of 15 gram of agar-agar gepulvert, MERCK, Art.1615; 5 gram of bacto-liver, DIFCO, Control 763182; 10 gram of tryptone peptone, DIFCO, 211705, and 5 gram solid NaCl MERCK, 1.06404.1000. All the ingredients were dissolved with 1 liter of hot aquades. Agar poured into petri dish as much as 10 ml. A 5 gram of feces were placed in the center of agar, and given an identification code number. The covered dishes incubated at room temperature for 48 hours. After 48 hours, agar medium cleaned with 10 ml of sodium acetate-acetic-formalin (SAF). The SAF solution retrieved then, poured into a centrifuge tube, and centrifuged at 2,500 rpm for 5 minutes. The supernatant discarded fast, the sediment examined under a light microscope with a magnification of 100 and 400 times^{13, 14}.

Harada-Mori Culture Method (HM)

Feces (0.5-1 gram) smeared in the center of the filter paper. Smeared filter paper entered in a plastic bag filled with ± 5 ml water, with a record the smeared section that is not submerged in the water. Plastic bag attached with paper clips and hung up for 7 days at room temperature, avoid exposure to direct sunlight. Water retrieved from the plastic bags by way of cut and placed in a centrifuge tube. The volume of water added up to 15 ml before centrifuged at a speed of 2,500 rpm for 5 minutes. The supernatant was discarded fast, the sediment examined under a light microscope with a magnification of 100 and 400 times¹⁵.

Data Analysis

Results were analyzed statistically and descriptively. Descriptive analysis was meant to determine the frequency distribution of measurement results independent variables and

the dependent variable. Correlation between hookworm and *S. stercoralis* infection with nutritional status were analyzed by Mann-Whitney's test.

Ethical Considerations

The study was approved by the Ethics Committee for Medical and Health Research, Medical Faculty, Gadjah Mada University with reference number Ref: KE/FK/892/EC/2016.

RESULT AND DISCUSSION

One of the criteria for children to participate in this study did not anthelmintic drugs for the last 4 months due to STH reinfection processes. Reinfection time varies on the helminth species, *A. lumbricoides* takes 2 months, *T. trichiura* takes 3 months, and hookworm takes 35 days to reach sexually mature inside the human body^{8,16}. Another condition is that the feces should not be contaminated by dirt, water, and urine. Contamination by dirt may lead to false positive due to contamination by hookworm rhabditiform larva live in soil. Condition of sample should not be contaminated by water and urine due to fecal sample in this study were used together with Lalangpuling¹⁷ which used Kato-Katz method. Contamination by water and urine can lead feces become inconvenient to be mold in Kato-Katz due to feces low consistency.

Table 1 shows that from total of 238 children under 5 years fecal samples, 21 were found positive in hookworm infection and only 1 subject

Table 1. Identification of fecal samples from children under 5 years in Kokar public health center

	Total Infection (n =22)	
	<i>Strongyloides stercoralis</i> n(%)	Hookworm n(%)
KAP	-	15 (68.18%)
Harada Mori	-	11 (50 %)
Baermann test	1 (4.54%)	-
KAP + Harada Mori	-	21 (95.45%)

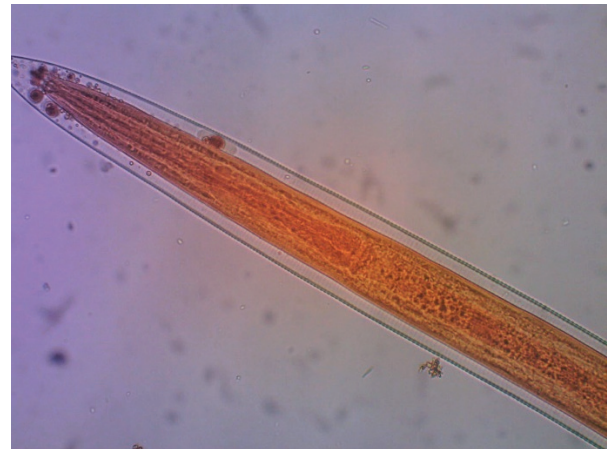


Figure 1. *Necator americanus* filariform larvae, 400 X magnification



Figure 2. Rhabditiform larvae tracks in Koga Agar Plate

found positive in *S. stercoralis* infection. Table 1 also shows that the KAP method has a better sensitivity with success rate (efficacy) 68.18% of detect hookworm infection compares to Harada Mori method with success rate of 50%. This result agrees with Reiss et al¹⁸ which found that KAP were superior to Harada Mori methods in hookworm larvae detection. Baermann test were the only method to reach its goal to detect *S. stercoralis* infection, with only on 1 subject. Examination in multiple diagnostic methods needed to reach a true prevalence due to absence of a gold standard for *S. stercoralis* detection¹⁴. Filariform larvae which found in Harada Mori method identified as *Necator americanus* from its appearance of gap between esophagus and intestine³¹ (see Figure 1).

A study of hookworm claims that KAP is better than Harada-Mori in hookworm detection due to diversity in stool weight requirement in both methods, which also found in this study that Harada-Mori requires a lesser than 5 gram of faeces¹⁸. Other study held in the same period and with the same subjects reveals that there were only a light infection of hookworm (1-1999 epg) in children under 5 years in Kokar¹⁷. A low concentration in a few stools can lead to the decrease in the probability of hookworm eggs to be carried in filter paper of Harada-Mori culture method.

One of KAP method's results is a larva track in agar surface (see Figure 2), though not all positive KAP were found with larva tracks on the agar (21). This occurrence also happen in this study. The tracks might be made by rhabditiform larvae and become obvious due to growing of bacterial colonies that grow along path^{19,20}. In KAP with larva tracks, it is advised to use a dissecting microscope to search for larvae directly. Compare to another study of hookworm and *S. stercoralis* in other regions of Indonesia and from other countries, the prevalence of hookworm and *S. stercoralis* in this study are considered low. Many studies of hookworm infection in many regions in Indonesia found a higher prevalence like in Jayapura (14.3%) and in Maluku Tengah Regency (56.8%)^{9,10}. Prevalence of *S. stercoralis* are found higher in Bali (1.6%)²¹.

Table 2. Distribution of hookworm and *S. stercoralis* cases in children under 5 years old based on age and nutritional status in Kokar public health center, Alor Regency

Characteristic of Respondent	Hookworm n(%)	<i>S. stercoralis</i> n(%)	N
Age (month)			
12-23 months	4 (1.68)	0	84
24-59 months	17 (7.14)	1 (0.42)	154
Nutritional Status			
Severe-weight	1 (0.42)	0	18
Underweight	3 (1.26)	0	46
Normal weight	17 (7.14)	1 (0.42)	168
Overweight	0	0	6
Total	21 (8.82)	1 (0.42)	22 (9.24)

This study found that the prevalence of hookworm and *S. stercoralis* infection obtained in this study is at 8.82% and 0.42% respectively (see Table 2). Hookworm infection occurs in almost all categories of respondents, except in overweight children. The highest score was in children under five years with normal nutritional status (7.14%). *Strongyloides stercoralis* infection only found in 1 child from overall 238 children.

The low prevalence of hookworm might be due to MDA (mass drug administration) with DEC (diethylcarbamazine) and albendazole as an anthelmintic drug for a brugian filariasis and STH infections. The program was held in Alor regency from 2002 to 2007, with a target of Alor citizens from 3-50 years old. In post evaluation, 3 years after the program was held, hookworm (*N. americanus*) prevalence showed a 75% decrease, from 28% become 7%¹². A study by Pion *et al.*²² and Supali *et al.*²³ found that filariasis treatment for 12 months with ivermectin and albendazole could diminish STH infection to 91%. This low prevalence of infection could lead to low risk of transmission from parents to their children.

Children under 5 years with normal status have the highest score, both in percentage and quantity of hookworm infection (see Table 3). The relationship between nutritional status and hookworm infection were analyzed using Mann Whitney test with p-value = 0.54, which shows that nutritional status has no correlation with

Table 3. Correlation between variable of nutritional status and hookworm infection in children under 5 years in Kokar Public Health Center, Alor Regency, August-October 2016

Nutritional Status, n (%)	Hookworm Infection (n=238)			p
	Negative (n=217)	Positive (n=21)	Total (n=238)	
Severe weight	17 (7.14)	1 (0.42)	18 (7.56)	0.54
Underweight	43 (18.07)	3 (1.26)	46 (19.33)	
Normal	151 (63.44)	17 (7.14)	168 (70.58)	
Overweight	6 (2.52)	0 (0)	6 (2.52)	
Total	217	21	238	

hookworm infection. This study was in line with another study of elementary school children in Purus, Padang¹¹.

The absence of correlation might be due to the adequacy of protein requirements. This condition leads to body capable in normal production of IL-4, which is the main cytokine in IgE production. IgE antibody is an adaptive immunity response against helminth infection^{5,24}. Other reason might be due to other confounding variables such as children's behavior. A study by Alemu *et al.*²⁵ shows that hookworm infection has an association with children's habit of shoe wearing. Outdoor activities are the main cause of hookworm infection due to the practice of open defecation in society^{14,26-28}. Therefore playing with soil and walking barefooted in outdoor activities may lead children to increase the risk probability of filariform infection. In addition to behavior, social-economic status may also have an effect on STH infection. In Kokar most of children's parents are farmer (71,4%) with low monthly household income (95,3%)¹⁷. Some studies reveal that social- economic status such as family income were significant risk factors for STH infection^{23,29}.

Our results do not display a correlation between nutritional status and *S. stercoralis* infection due to quantity of subjects with positive infection were too few, therefore cannot be used as a reference for data processing.

CONCLUSION

Our result found that the prevalence of hookworm and *S. stercoralis* among children under 5 years old in Centre of public health in Kokar, Alor Regency, is 8.82% and 0.42% respectively. There was no correlation between nutritional status and hookworm infection among children under 5 years. This study recommended collaborating KAP and Harada-Mori to detect the presence of hookworm infections as it is able to increase the detection level up to 95.45%.

CONFLICT OF INTEREST

There is no conflict of interest of this study.

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