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Environmental Risk Factors and Hookworm Infection among Schoolchildren in rural areas of Indonesia

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	Background: The prevalence of hookworm infection is a serious public health concern globally. Java Island and Kalimantan Island have differential environmental risk factors of hookworm infection especially in rural areas of Indonesia have high-risk environmental factors of the prevalence of hookworm infection.
	Aim: In this study showed the infection rates, correlation analysis between environmental risk factors, and prevalence of hookworm infection.
Abstract:	Method: We performed a cross-sectional study among 226 schoolchildren from rural East Java p province, Central Java Province, and East Kalimantan Province, Indonesia. This study used two diagnostic methods: Kato Katz and Koga agar plate culture/KAP culture for diagnosing hookworm infections. Pearson chi-square analysis was used to study the correlation between environmental factors and hookworm infection.
	Results: Hookworm, Strongyloides sp, and Ascaris sp infections were found in this study; 137(60.63%), 25(11.1%), and 124(9.84%) respectively. Environmental risk factors such as; rainy season, quality of soil, and infection hookworm in pets have a significant correlation (p-value < 0.05) with hookworm infection among schoolchildren in a rural area in Indonesia.
	Conclusion: The prevalence of hookworm infection correlates with environmental factors, and the finding in this research could be contributed to decreasing the program of hookworm infection especially among schoolchildren in rural areas.



Environmental Risk Factors and Hookworm Infection among Schoolchildren in rural areas of Indonesia

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Conclusion: The prevalence of hookworm infection correlates with environmental factors, and the finding in this research could be contributed to decreasing the program of hookworm infection especially among schoolchildren in rural areas.

Introduction

The prevalence of hookworm infection is of serious public health concern globally. Hookworm infection is prevalent in poor rural communities tropical and subtropical areas in many developing countries.1 They are transmitted through protected contact with soil are endemic in tropical and temperate regions. The prevalence of hookworm infection 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. 2,3,4

Hookworm infection is transmitted through protected contact with soil is endemic in tropical and temperate regions. Humans acquire hookworm infection 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 the small intestine. 5

Generally, hookworm infection is found among poor people with poor environmental sanitation and where the climate is warm and humid.6,7 Factors affecting the difference in the distribution of hookworm infection may include good hygiene practices among the population, availability of sewerage systems, and the length of the rainy season. Environmental factors have contributed to the transmission of diseases as well as the growth and development of worms. 8,9

Environmental factors especially the long rainy season may affect the decrease in the prevalence of strongyloidiasis but not for hookworm infection. The prevalence of strongyloidiasis in south Thailand is lower than in other parts of the country, in contrast, the prevalence of hookworm infection is still high in the south. It is possible because of 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.10 The study in Cambodia reported a lower prevalence of strongyloidiasis in an area with heavy rainfall than in low rainfall areas. Moreover, a high amount of soil organic carbon content affects the lower prevalence of strongyloidiasis.11. Epidemiology study of hookworm infection and strongyloidiasis in Southern Laos showed 56.1% and 41% respectively where has heavy rainfall and poor sanitation. In this study, Baerman and Kato-Katz techniques were used for detecting them.12

Indonesia has environmental risk factors of prevalence of hookworm infection and strongyloidiasis especially for school children in rural areas where have differential characteristics with other southeast Asia country.13. Rural East Java Province, Central Java Province, and East Kalimantan province have differential environmental risk factors of hookworm infection. The study is important to exploration association both of prevalence of hookworm infection, and environmental risk factors. We performed a cross-sectional study in school children in Sumberwringin district, Bondowoso regency, East Java Province, Pakis district, Magelang Regency, Central Java Province and Muara Kaman district and Marangkayu

district, East Kalimantan province to the analysis of geography, texture of the soil, humidity, hookworm, and Strongyloides in pet, vegetation, elevation, volume rain, number day and month of rain yearly, temperature and quality of soil as clay content, organic carbon of soil and pH of soil then was correlated with the prevalence of hookworm infection14.

Materials and methods Ethical consideration

Official permission and ethical clearance for the collection of human fecal samples were obtained from the local provincial government of East Java, Central Java, and East Kalimantan. The study protocol was approved by the Ethical Clearance Committee on human rights related to research involving human subjects, Walailak University HE: number WUEc-18-034-01.

Study Setting and Population

The study was carried out in the rural area of East Java Province, Central Java Province, and East Kalimantan province especially among schoolchildren, Indonesia. This research is schoolchildren-based, was conducted from July 2018- July 2019. The total of number participants is 226 schoolchildren who were joined and sent stool samples. The sample population is counted These areas were selected based on the potential risk of hookworm infection and strongyloidiasis, consist rural area, poor sanitation and hygiene, agriculture activity and surrounding forest and have not yet data study of hookworm and S. stercoralis infection from both areas. The sample size was determined using the single population formula by Stanly Lemeshow technique sampling. It was calculated using a prevalence rate (p) of 37% as detail in previous study.15. with 95% confidence interval (z=1.96) and a 10% margin error (d=0.1). The calculated sample size was 90 participants per province of the school area. We assumed that the final sample size would end up being reduced by around 10% due to subjects being unable to pass stool on the study date and we aimed for a sample size of 100 schoolchildren. A simple random sampling method was used to select the population from the district of the school area. Inclusion criteria were participants who were ≥ 7 years old and studied in both school areas. We collected 226 schoolchildren participants who have completed questionnaires and stools.

Field Procedures

For collecting stool samples, the first day was requested to head of the master of school children and the parents of school children for requesting stool sample, second day in the morning would start to collect stool samples, were brought to parasitology laboratory of B2P2RV Salatiga, Ministry of Health Republic Indonesia for samples from East Java Province and Central Java Province and samples from East Kalimantan were brought to Parasitology Mulawarman University for diagnosis samples. On other days was done observed environmental conditions of houses surrounding the village.

Laboratory Procedures

Agar plate culture and Kato Katz technique

Agar plate culture will be done as described by Koga et al., 1991.16 Briefly, a few grams of stool will be placed at the center of nutrient agar and kept at room temperature for five days. Tracks from larva crawling and larvae or adult worms will be observed. If positive, 10 ml of 10% formalin will be added to the agar surface for 5-10 minutes and transferred to centrifuged tube. Centrifugation at 2,500 rpm for 5 minutes and supernatant will be discarded. The sediment will be examined for hookworm larvae and S. stercoralis larvae or adult worm, Kato-Katz thick

smear, 50 mg of stool will be placed on the slide and covered with a cellophane paper soaked in glycerin solution for 24 hours. The stool will be spread out using a rubber stick. After 30 minutes will be examined and counted for eggs.17,18.

Environmental data

Environmental data were collected consist such as vegetation, the elevation of soil, kind of pets, kind of soil around houses, length of the rainy season, humidity, and temperature per year. Quality of soil as organic carbon content, clay content, and pH were diagnosed by soil laboratory Mulawarman University. Vegetation and kind of soil around houses will be collected by observation form, kind of pet will be collected by questioner and observation, and length of the rainy season, humidity and temperature per year will be collected from Central Bureau of Statistics (https://www.bps.go.id) and Central Bureau of meteorology, climatology, and Geophysical of Indonesia (https://www.bmkg.go.id).

Data Analysis

The prevalence of hookworm and *Strongyloides* infection was stratified according to environmental data and reported by the descriptive statistic. Statistical analysis was performed by Chi-square and regression using SPSS verse 21. The correlation analysis was analyzed by Pearson Chi-square to evaluate the association of hookworm and *Strongyloides* infections with environmental risk factors and the level of significance was considered as P < 0.05.19.

Results and Discussion

Study Sample

A total of 226 schoolchildren participated in this study. We collected data from five schools such as three schools from East Kalimantan, one school from East Java and one school from Central Java, with detail three provinces are Central Java Provinces, East Java Provinces and East Kalimantan Province, all of areas research were conducted in rural and agriculture area with The areas have differences characteristic of environmental risk factors.

Parasitological Findings

Prevalence hookworm and *Strongyloides* infection/strongyloidiasis were diagnosed by Kato Katz technique and APC method showed of 226 tested samples from children have 137(60.63%) cases found positive with hookworm infection and 25(11.1%) cases found positive with *Strongyloides* infection and addition finding of *Ascaris lumbricoides* as 124(9.84%). Detailed data on the prevalence of hookworm and Strongyloides infections were explained in table 1.

The prevalence of hookworm infection 137(60.63%) is highest than other infections and the Prevalence of strongyloidiasis only was found in East Kalimantan Province with 25(11.1%) cases, and have not been found in East Java Province and Central Java Province.

Characteristic participant and Hookworm Infection

The highest prevalence of hookworm infection was found in East Java is 69(97.2%) from total of 71 children, therefore the lowest prevalence of hookworm infection was found in Central Java province is 20(41.7%) among 48 children, Percentage of the prevalence of hookworm infection in East Kalimantan 48(44.9%) is higher than in Central Java.

Correlation between the prevalence of hookworm infection and province of school children area was showed by *Pearson X2* that hookworm infections were significantly correlated with the province (p < 0,0001). East Java province has deferential characteristics with central Java and East Kalimantan Province such as environmental risk factors including as quality of soil, kind of vegetation, number of days rain yearly, humidity, and temperature those are determinant the highest percentage of hookworm infection than other provinces.

Hookworm infection in females was known higher than the male with 72(63.2%) and 65(58.0%) respectively from 114 female participants and 112 male participants. Gender has not significantly correlated with hookworm infections (p = 0.431). Hookworm infection was found dominant among school children class level 1(100%) and school children class level 2

(88,9%). The present showed a significant correlation between the level of study with hookworm infection is negative (p = 0.223).

Sensitivity of diagnostic method for hookworm and Strongyloides infections

By KAP culture technique showed 33(14.6%) of the prevalence of hookworm infection is higher than Kato Katz technique 137(60.6%) KAP culture method is higher of sensitivity than the Kato-Katz technique on the diagnosis of hookworm infection among schoolchildren in Indonesia.

As far as we know, the most sensitive method for S. stercoralis diagnosis is KAP culture.20. We used Kato-Katz thick smear and KAP culture on a double stool sample from school children, in total found 14.6% infected of hookworm infection diagnosed by Kato Katz. KAP culture has sensitivity more than Kato Katz in this research with the founding of prevalence of hookworm infection was 60.6%. This technique can explain the detail of growing up each step development of filariform larvae particularly for detecting of filariform larvae of hookworm

Environmental Factors and Hookworm Infection

Statistical analysis of hookworm and *Strongyloides* infection between environmental factors such as geography, the texture of the soil, infection status of pet, humidity, vegetation, elevation, amount day of rain, the volume of rain, temperature, pH, clay content of the soil, organic carbon of soil, explained detail in table 2.

Sandy soil with 89(74.8%) hookworm infection is the highest level of the prevalence of hookworm infection than other types of soil; organic carbon content in area 1 (1.50%)

with 69(97.2%) hookworm infection is the highest level of the prevalence of hookworm infection than others cluster; clay content in Area 3 (3.0%) with 97.2% hookworm infection is the highest level of the prevalence of hookworm infection than others cluster and pH of the soil in cluster Area 5 (7.79) with hookworm infection 97.2% is the highest level of the prevalence of hookworm infection than others cluster. Texture, organic carbon content, clay content, and pH of soil have significantly correlated with hookworm infection (p < 0.0001).

Climatology and Hookworm Infection

Correlation climatology and hookworm infection detail was explained in table 2. The table showed that temperature in area 2 (22.00C) with hookworm infection 97.2% is highest than other areas. Humidity in area 4 (90) with hookworm infection 97.2% is highest than other areas. Temperature and humidity have significantly correlated with hookworm infection (p = 0.000).

Prevalence of hookworm infection stratified by several day rain yearly Station 1 (122 days) is highest hookworm infection with prevalence was 97.2%. Area 1 (7 months) where has 7 months of the long rainy season is the highest prevalence of hookworm infection 97.2%.

Station 1 (2937mm) of the volume of rain yearly was found hookworm infection 97.2% is highest. The number of days, months, and volume of rain yearly have correlated with hookworm infection (p < 0.0001).

Vegetation, Location, Pet, and Hookworm Infection

Detailed distribution and correlation of vegetation, location with hookworm infection are explained in table 3. The table explained that the highest percentage for the prevalence of hookworm infection by stratified vegetation is palm plantation, which 55.2% hookworm infection. Prevalence of hookworm infection that showed by geography area is highest in the buffer of the river (59.9%), where location village of the participant is a surrounding river. The elevation where was found highest of prevalence hookworm infection in station 3 (50m) with hookworm infection 69.8%.Vegetation, geographic area, and elevation are all of the environmental risk factors that have a significant correlation with hookworm infection (p <0.0001).

Participants were infected by hookworm with infected cat by hookworm 48(44.9%) while they whose non-infected cat by hookworm 89(74.8%), participants whose Participants were infected dog by hookworm 117(65.7%), analysis pearsonX2 hookworm in human has significantly correlated with infected cat and dog by hookworm with p-value 0.000 and 0,002 respectively

The result of statistical analysis showed that environmental factors have an association with the prevalence of hookworm infection, the environmental factors, including the geography of an area, hookworm in a dog, humidity, vegetation, elevation. number day of rain yearly, several months of rain yearly, the volume of rain and quality of soil as texture, organic carbon of soil, clay content of the soil, and pH of soil have significance (P value<0.05) with the prevalence of hookworm infection in Indonesia.

Discussion

The prevalence of hookworm infection 137(60.63%) is highest than other infections and the Prevalence of strongyloidiasis only was found in East Kalimantan Province with 25(11.1%) cases, and have not been found in East Java Province and Central Java Province. The correlation of Prevalence of hookworm infection and strongyloidiasis is significant (p = 0.000). A similar study in Manufahi District, Timor Leste where is a rural area with a prevalence of hookworm infection was 62.8%.21 Hookworm infection and strongyloidiasis are both neglected tropical diseases.22 In poor countries with a tropical climate, conditions favorable for transmission of these parasites have a higher prevalence of hookworm infection and strongyloidiasis (Jongwutiwes s et al, 1999).23 Furthermore, low socioeconomic status and low hygiene living conditions of the rural population are strongly associated with hookworm infection and strongyloidiasis. In southeast Asia, recent work in Cambodia reported a very high infection rate of Takeo Province .11.

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Environmental factors of hookworm and strongyloidiasis in East Kalimantan has similar with south Thailand including long rainy season, temperature, and several geography areas, then the prevalence of hookworm infection in East Kalimantan Province (44.1%) is higher than in south Thailand but equal for Strongyloidiasis, that condition was caused other environmental risk factors like quality of soil such as organic carbon of soil, clay content and pH. 10

The prevalence of hookworm infection in East Kalimantan has similar to a study in southern Laos and Cambodia where hookworm is still high but more than the prevalence of strongyloidiasis. The study in Cambodia reported a lower prevalence of strongyloidiasis in an area with heavy rainfall than in low rainfall areas. Moreover, a high amount of soil organic carbon content affects the lower prevalence of strongyloidiasis .11 Epidemiology study of hookworm infection and strongyloidiasis in Southern Laos showed 56.1% and 41% respectively where were heavy rainfall and poor sanitation. In this study, Baerman and Kato-Katz techniques were used for detecting them.12

Schoolchildren were infected by hookworm with infected cat by hookworm 48(44.9%) while they whose non-infected cat by hookworm 89(74.8%), participants whose Participants were infected dog by hookworm 117(65.7%), analysis pearsonX2 hookworm in human has significant correlated with infected cat and dog by hookworm with p-value < 0.0001 and 0,002 respectively. This research has a similar condition with Cambodian research that dogs in rural Cambodian villages are largely kept as guard dogs and allowed to roam freely, especially during

the day. The dogs are also allowed inside the house and around rice and vegetable fields and ponds. At night-time, the dogs then often stay in or around the house. Dogs, therefore, pose a serious zoonotic risk as they have the potential to transmit zoonotic parasites through their close association with household members as well as through heavy contamination of the environment, including soil, fresh produce, and waterways, with eggs or larvae hookworm, in our observation sew behavior of cats almost all day and night stayed around houses and rare contact with ponds and did not stay around rice and vegetable fields, the behavior of the dog is a higher potential zoonotic risk for hookworm infection than the behavior of the cat. 25,26 Behavior of cat defecation makes the un-save survive of the egg or larvae of hookworm infection. Defecation of the cat was not risk of hookworm infection. usually, the cat closed the feces after defecation with dry soil and the cat has defecated around houses that did not spread other places.27

The result of statistical analysis showed that environmental factors have an association with the prevalence of hookworm infection, the environmental factors, including the geography of an area, hookworm in dogs, humidity, vegetation, elevation. number day of rain yearly, several months of rain yearly, the volume of rain and quality of soil as texture, organic carbon of soil, clay content of the soil, and pH of soil have significance (P value<0.05) with the prevalence of hookworm infection in Indonesia. The transmission of hookworm infection and strongyloidiasis was caused by the tropical climate and the environmental condition that favorable for the survival of hookworm infection.23

Environmental factors of hookworm infection and strongyloidiasis among schoolchildren in Indonesia have significance with high of prevalence of hookworm infection and strongyloidiasis such as geography, vegetation, humidity, volume and amount day of rain organic carbon of soil and clay content of the soil, the environmental factors make survive of infective larvae of hookworm28 Explained with Garcia (2007) that a significant increase in the prevalence of hookworm infection with environmental conditions.29. Changing environmental conditions, specifically deforestation and subsequent silting of the local river, have caused periodic flooding with deposition on a layer of sandy loam topsoil and increased soil moisture24. These conditions, all of which are conducive to hookworm transmissions, have allowed hookworm to reemerge as an important human pathogen in this area.30

Conclusions

The prevalence of hookworm infection among schoolchildren in Indonesia correlates with environmental factors. The result of the study analysis can make a strong contribution to preventing programs by ecological root. Preventing program of reduced prevalence of hookworm infection by treatment of environmental risk factors is an effective program for decreasing hookworm infection among schoolchildren in Indonesia.

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Tables and Figures Legends

Table 1. Prevalence of hookworm among Schoolchildren in Indonesia (226 Participants)

Infections	East J	(%)	Central	l Java (%)	East Kali	mantan (%)	Overall
	Positive	Negative	Positive	Negative	Positive	Negative	Positive
Hookworm	69(97.2)	2(2.8)	20(41.7)	28(58.3)	48(44.9)	59(55.1)	137(60.63)
S. stercoralis	0	71(100)	0	48(100)	25(23.4)	82(76.6)	25(11.1)
Ascaris sp	3(4.2)	68(95.8)	2(4.2)	46(95.8)	11(10.3%)	96(89.7)	124(9.84)

Quality of quality of soil and climatology and Hookworm Infection

Table 2. Correlation Quality of Soil and Hookworm infection

Quality of Soil	Status of D	Piagnosis Participants	- D volu
Quality of Soli	Positive	Negative	<i>r-</i> value
Texture of soil			< 0.000
Sandy clay	17(70.8%)	7(29.2%)	
Loamy sand	13(38.2%)	21(61.8%)	
Sand	89(74.8%)	30(25.2%)	
Clay	18(36.7%)	31(63.3%)	
Organic Carbon content (5 School			< 0.000
children areas)			
Area 1 (1.50%)	69(97.2%)	2(2.8%)	
Area 2 (1.83%)	17(70.8%)	7(29.2%)	
Area 3 (2.77%)	20(41.7%)	28(58.3%)	
Area 4 (3.13%)	18(36.7%)	31(63.3%)	
Area 5 (7.22%)	13(38.2%)	21(61.8%)	
Clay content (5 areas)			< 0.000
Area 1 (1.2%)	20(41.7%)	28(58.3%)	
Area 2 (1.9%)	13(38.2%)	21(61.8%)	
Area 3 (3.0%)	69(97.2%)	2(2.8%)	
Area 4 (34.7%)	18(36.7%)	31(63.3%)	
Area 5 (38.6%)	17(70.8%)	7(29.2%)	
pH of soil (5 areas)			< 0.000
Area 1 (4.26)	17(70.8%)	7(29.2%)	
Area 2 (6.60)	20(41.7%)	28(58.3%)	
Area 3 (7.22)	18(36.7%)	31(63.3%)	
Area 4 (7.40)	13(38.2%)	21(61.8%)	
Area 5 (7.79)	69(97.2%)	2(2.8%)	
Temperature			< 0.000
Area 1 (20.5°C)	20(41.7%)	28(58.3%)	
Area 2 (22.0°C)	69(97.2%)	2(2.8%)	
Area 3 (28 °C)	13(38.2%)	21(61.8%)	
Area 4 (29.5 °C)	35(47.9%)	38(52.1%)	
Humidity	· /	× /	< 0.000

Area 1 (65%)	13(38.2%)	21(61.8%)	
Area 2 (66%)	35(47.9%)	38(52.1%)	
Area 3 (82)	20(41.7%)	28(58.3%)	
Area 4 (90)	69(97.2%)	2(2.8%)	
Number day of rain yearly			< 0.0001
Station 1 (122 days)	69(97.2%)	2(2.8%)	
Station 2 (139 days)	20(41.7%)	28(58.3%)	
Station 3(152 days)	35(47.9%)	38(52.1%)	
Station 4(174 days)	13(38.2%)	21(61.8%)	
Volume of rain yearly			< 0.0001
Station 1 (2937mm)	69(97.2%)	2(2.8%)	
Station 2 (2990mm)	35(47.9%)	38(52.1%)	
Station 3 (3689mm)	20(41.7%)	28(58.3%)	
Station 4 (4000mm)	13(38.2%)	21(61.8%)	

Table 3. Vegetation, Location, Pet, and Hookworm Infection

Environmental viels for stores	Status of Dia	gnosis Participants	Dualua
Environmental risk factors	Positive	Negative	<i>P</i> -value
Vegetation			< 0.0001
Coffee plantation	69(97.2%)	2(2.8%)	
Vegetable plantation	20(41.7%)	28(58.3%)	
Palm and rubber plantation	48(44.9%)	59(55.1%)	
Geography of villages area			< 0.0001
Buffer of sea/coastal area	13(38.2%)	21(61.8%)	
Buffer of river	17(70.8%)	7(29.2%)	
Hill	18(36.7%)	31(63.3%)	
Mountain area	89(74.8%)	30(25,2%)	
Elevation from above of sea surface			< 0.0001
(m)			
Station 1 (32m)	35(47.9%)	38(52.1%)	
Station 2 (50m)	13(638.2%)	21(61.8%)	
Station 4 (700m)	69(97.2%)	2(2.8%)	
Station 5 (841m)	20(41.7%)	28(58.3%)	
Hookworm in cat			< 0.0001
Infected cat by hookworm	48(44.9%)	59(55.1%)	
Non infected cat by hookworm	89(74.8%)	30(25.2%)	
Hookworm in dog			0.002
Infected dog by hookworm	117(65.7%)	61(34.3%)	
Non infected hookworm by dog	20(41.7%)	28(58.3%)	

Tables and Legends

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