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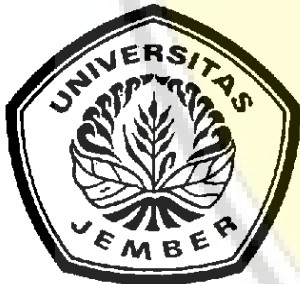
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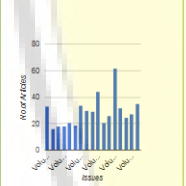
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Hookworm infection and the risk factors among plantation workers in Jember, Indonesia

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Abstract:

Hookworm infection is one of the neglected tropical disease (WHO) and 428 200 000 people are infected with hookworm in worldwide. Hookworm's larvae requires soil media in plantation area. Contamination of plantation soil with hookworm's larvae can occur through unhygienic defecation habit. Hence, workers in plantation areas have a high risk of being infected with hookworms. This study was conducted to determine the prevalence hookworm infection in plantation workers in five plantation areas of Jember, Indonesia District and the risk factors for hookworm infection. This study was observational analytic study with cross-sectional design. The prevalence of hookworm infection in plantation workers was determined based on qualitative methods (Sedimentation and Flootation Method). The defecation habits of the workers were known through questionnaires by interview method. The characteristics of workers and the risk factors were analyzed by the Chi-square test or Fisher's exact test. The results of the study showed that the prevalence of hookworm infection in five plantation areas was 21.30 %. Based on the results of the questionnaire, most plantation workers (80.5 %) did not defecate in the toilet but at the river or the plantation area. This risk factor had significant association with the prevalence of hookworm infection ($p < 0.05$). The results of this study indicated that unhygienic defecation patterns cause hookworm contamination in the soil of the plantation area. Therefore, the plantation workers could be always expose by the infective larvae as long as they don't change their defecation habit.

Keywords: *Ascaris lumbricoides*, contamination, defecation, *Trichuris trichiura*.

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Introduction

Hookworm infection is one of the soil transmitted helminth (STH) infections that are included in neglected tropical disease according to the World Health Organization (WHO) ⁽¹⁾. Hookworm larvae can develop well on moist, loose soil and contain lots of compost such as soil in plantation areas ⁽²⁾. A previous study has shown the presence of soil contamination by hookworm larvae in the vegetable and fruits plantation area ⁽³⁾. Therefore, people who work at the plantation areas have a high risk of being infected with hookworms. The risk is even greater if workers have unhealthy defecation habit and working without using footwear and gloves. Jember is a district in province of East Java, Indonesia which plantation area is quite extensive. In this study, we determined the prevalence and risk factors of significance associated with hookworm infection among plantation workers of Jember, Indonesia.

Materials and methods

Study area and population:

The research protocol was reviewed and approved by the Ethics Committee No.1 174/H25.1.11/KE/2018 of the Faculty of Medicine, University of Jember, Indonesia. Informed consent was obtained from the enrolled participants from the five plantation area in Jember District. The study was conducted at five plantation area including Gunung Pasang (Panti sub-district), Kaliputih (Ledokombo Sub-district), Widodaren (Bangsalsari and Tanggul Sub-district), Sumberwadung and Garahan Kidul (Silo Sub-district). Those plantation areas are planted with coffee, cocoa and rubber. All plantation area were located in Jember District, Province of East Java, Indonesia. The total number of workers in the five plantations was 637, but the total number of enrolled participants was 277 who took interviews and collected feces. First all of participants were interviewed face-to-

face, after that faecal pots were handed to each participant with their code number and collected next morning. The faecal samples were examined to identify hookworm infection by qualitative method (sedimentation and floatation). After study, the plantation workers who were positive infected with hookworm, received anthelmintic drug, *i.e* Albendazole 400 mg as single dose.

Questionnaires:

Standardized questionnaires were used to determine the risk factors for hookworm infection. Enrolled participants were interviewed face-to-face covering demographic data, personal hygiene, *i.e* hand washing, toilet use, nail hygiene, eating habit, and environment sanitation, *i.e* type of house floor, drinking water source, also use of personal protective equipment such as shoes and gloves.

Parasitological techniques:

Faecal samples were examined for hookworm infection using a standard sedimentation in water technique followed by centrifugal floatation in saturated sodium chloride and microscopy.

Statistic analysis:

Statistical analyses were performed using SPSS version 20.0 for Windows. The Chi-square test or Fisher’s exact test served for the comparison of qualitative variables. To assess the factors impacting the hookworm infection, odds ratio with 95 % confidence interval (CI) was used with logistic regression analyses. All variables with $p < 0.25$ in univariable analyses were introduced in the multivariable model. The level of statistical significance was set at p value < 0.05 .

Results

There were 637 workers in five plantation areas (Gunung Pasang, Kaliputih, Widodaren, Sumber wadung, and Garahan Kidul) of Jember District, Indonesia, but only 277 workers participated in this study by collecting feces and willing to be interviewed. The overall prevalence of Hookworm infection was 21.3 % (59 of 277). The sedimentation and floatation technique could identify not only hookworm, but also another species of STH, *i.e* *Ascaris lumbricoides* [Linnaeus, 1758] in 32 workers (11.5 %) and *Trichuris trichiura* [Linnaeus, 1771] in one worker (0.36 %).

The characteristics of the enrolled subjects are shown in table 1. No significant difference was found among gender, age group, educational level, workers status and length of work. The interesting thing about the characteristics of participants was that most workers that infected with hookworms have a low level of education, the status of workers as freelance and the longest working period.

Table 1: Characteristics of the enrolled participants and prevalence of hookworm infection

Characteristics	Enrolled subjects	No. of hookworm-infected person(%)	p value
Gender			
Male	92	20 (7.22)	0.900
Famale	185	39 (14.08)	
Age group			
≤ 35 yr	38	6(2.16)	0.372
> 35 yr	239	53(19.13)	
Educational level			
No education	87	22(7.94)	0.278
Elementary school	148	31(11.19)	
Junior High	32	3(1.08)	
Senior High	7	3(1.08)	
Diploma	1	0	
Bachelor	2	0	

(Continued on next page)

Table 1: Continued

Characteristics	Enrolled Subjects	No. of hookworm–infected person(%)	p value
Worker status			
Freelancer	241	54(19.49)	0.244
Permanent worker	36	5(1.81)	
Length of work			
< 5 yr	44	7 (2.53)	0.455
5 yr to 10 yr	25	4(1.44)	
> 10 yr	208	48(17.33)	

The results of bivariate statistics (chi–square and Fisher’s test) analysis for the risk factors of acquiring hookworm infection are shown in table 2 (risk factors of personal hygiene) and table 3 (risk factors of sanitation). From personal hygiene factors, there was no association between the habit of hand washing, site defecation at home and nail hygiene, but site defecation when at work has significant association with hookworm infection ($p < 0.05$). Use of footwear and gloves have no association, but the logistic regression analysis showed that contact with the ground while working was the predictor of hookworm infection in this study (OR 3.48; 95 % CI 1.196 to 10.112, $p = 0.016$). No association was found between risk factors of sanitation and hookworm infection.

Table 2: Risk factors of personal hygiene associated with hookworm infection (p value of < 0.05)

Risk Factors of Personal Hygiene	No. of hookworm–infected person (%)				p value	OR	95 % CI	
	Negative		Positive				lower	Upper
	n	%	n	%				
Washing hand before eat								
No	13	4.69	4	1.44	0.765*	0.872	0.273	2.78
Yes	205	74	55	19.85				
How to wash hands								
Using water only	99	35.74	21	7.58	0.177	1.505	0.83	2.732
Using water and soap	119	42.96	38	13.71				
Washing hand after work								
No	62	22.38	20	7.22	0.979	0.99	0.485	2.025
Yes	156	56.31	39	14.07				
Wash hands After touching the ground								
No	62	22.38	20	7.22	0.415	0.775	0.419	1.432
Yes	156	56.31	39	14.07				
Wash hands after defecation								
No	6	2.16	5	1.8	0.60*	0.306	0.09	1.039
Yes	212	76.53	54	19.49				
How to wash hands after defecation								
Using water only	64	23.1	12	4.33	0.168	1.628	0.81	3.27
Using water and soap	154	55.59	47	16.96				
Defecation when at home								
Plantation area	28	10.1	5	1.8	0.302			
River	81	29.24	36	12.99				
Toilet	109	39.35	18	6.49				

(Continued on next page)

Table 2: Continued

Risk Factors of Personal Hygiene	No. of hookworm–infected person (%)				p value	OR	95 % CI	
	Negative		Positive				lower	Upper
	n	%	n	%				
Defecation when at work								
Plantation area	32	11.55	18	6.49	0.020**			
River	142	51.26	31	11.19				
Use of footwear while working								
No	3	1.08	2	0.72	0.289*	0.398	0.65	2.437
Yes	215	77.61	57	20.57				
contact with the ground while working								
No	44	15.88	4	1.44	0.016*	3.48	1.196	10.112
Yes	174	62.81	55	19.85				
Use of gloves when working								
No	109	39.35	31	11.19	0.72	0.903	0.508	1.607
Yes	109	39.35	28	10.1				
Always keep your nails clean								
No	90	32.49	28	10.1	0.39	0.778	0.437	1.387
Yes	128	46.2	31	11.19				

Table 3: Risk factors of sanitation associated with hookworm infection having a p value of < 0.05

Risk Factors of Sanitation	No. of hookworm–infected person (%)				p value	OR	95 % CI	
	Negative		Positive				lower	Upper
	n	%	n	%				
Type of house floor								
Soil	14	5.05	5	1.8	0.567*	0.741	0.256	2.148
cement/ceramic	204	73.64	54	19.49				
Bathroom floor type								
Soil	67	24.18	11	3.97	0.067	1.936	0.947	3.96
Cement / ceramic	151	54.51	48	17.32				
Origin of drinking water used								
River water	61	22.02	18	6.49	0.703	0.885	0.472	1.659
Well water / PAM	157	56.67	41	14.80				
The physical quality of drinking water used								
Not eligible	2	0.72	1	0.36	0.514*	0.537	0.048	6.027
Qualify	216	77.97	58	20.93				

*expected count less than five, p value using fisher exact test

Discussion

The life cycle of hookworms involves soil for the development of their larvae as an infective form. Several studies have proven agricultural and plantation areas to be endemic areas ⁽²⁾. Our research on coffee and rubber plantations in Jember district also proved the same thing that hookworm infection still dominated in plantation areas with a higher prevalence (21.3 %) than national prevalence (6.46 %). This study was consistent with previous finding showing that hookworm infection was the predominant infection among tea pluckers in Bangladesh ⁽⁴⁾. The condition of the soil in the plantation area that is shady, moist and loose is an ideal place for the development of hookworm eggs into larvae three stage as an infective form that can live for several weeks. Hookworm eggs exit

the body in feces. Therefore, the presence of hookworm larvae in the soil is related to the habit of defecation which contaminate the soil in the plantation area as one of the risk factors studied.

Hookworm infection has been linked to low socio-economic status, age and low education ^(2,4-6). This study, most of the enrolled participants (87 %) were freelance workers that accept salary cheaper than permanent workers and the most infected were also from this group (19.49 %), although the result of statistical analysis showed no significance ($p>0.05$). This result was consistent with the previous study in Keroram Regency, Papua Province, Indonesia that showed the socioeconomic factors were not significant ($p>0.05$) related to hookworm infections ⁽⁷⁾. Hookworm can infect children and adult, but some studies of population in Southeast Asia suggest that peak prevalence is observed among the middle aged or even individuals over the age of 60 yr ⁽²⁾. This study most of workers that infected with hookworm were the middle aged (19.3 %), although there was no association with the infection. This can also be explained by the age distribution of workers who are mostly in middle age because the youth who live around the plantation area are more interested in other types of work. This study found that as level education increased the likelihood of infection with hookworm declined significantly. This findings was consistent with previous studies in Thailand and in India that most of infected people had lower educational level ^(5,8).

Even though some studies has reported that males are more commonly infected with hookworm than females, the findings in this study showed opposite result that more females (14.08 %) were infected than males (7.22 %) ^(2,7). This finding is in accordance with some previous studies that reported high hookworm infection among females ^(5,8,9). The skewed prevalence rate towards female could result from the bias in this study since the number of female workers were more than males. Another characteristic of enrolled participants that were interested in this study was the length of work. Most of infected workers had a long working period, which is more than 10 yr. This could be caused by chronic infection and continuous exposure to hookworm larvae in the soil, resulting in frequent reinfection. Hookworms in the intestines of humans can live for 1 yr to 3 yr for *A.duodenale* and 3 yr to 10 yr for *N.americanus*, with a maximum life span of 18 yr ⁽²⁾. Limitations in this study we did not identify hookworm species and ask the medical history of anthelmintic drugs.

The site of defecation and soil contact behavior at work were risk factors associated ($p<0.05$) with hookworm infection in plantation workers. Most workers infected with hookworms have a defecation habit not in the toilet when they were at home (14.79 %) and when working in the fields (17.68 %), but they defecate mainly in rivers and in the garden. This finding is consistent with previous study among tea-growing communities in India that female tea-pickers usually defecate in the furrows between the tea trees ⁽⁵⁾. This defecation habit could contaminate the soil in plantation area. The environment of sandy soil, shaded by coffee or rubber tree, provides a perfect environment for development of hookworm larvae and would constitute a significant source of infection and re-infection. The soil type can influence the transmission of hookworm because infective larvae thrive in areas with sandy soil than clay soil. The sandy soil contain small particle size and well-aerated texture, so the larvae are able to rapidly migrate in the soil and during the rains are able to migrate vertically as moisture permits ⁽²⁾.

The plantation workers that contact with soil that has been contaminated with hookworm larvae could be infected or re-infected. This condition was also supported by lack of self protection to prevent direct contact with soil. Although almost all of the workers have used footwear while working, but the number of infected workers who were not wearing gloves when working was greater (11.19 %). This condition still allows for the transmission of hookworms due to cutaneous penetration of larvae as an infection source. Footwear has been associated with hookworm infection as significant risk factor because walking in barefoot have high chance of hookworm infection ^(7,8,10). This study, this risk factor didn't have association with hookworm infection because almost all of the workers have used footwear while working. Therefore, the workers got infection from another way by their hands that were in contact with the soil without using gloves.

Sanitation should be a risk factor that has an important role in hookworm infection, where poor sanitation increases the risk of this infection ⁽¹¹⁾. This study did not show an association between sanitation and hookworm infection ($p>0.05$). This result agrees with the study conducted by Sandyet *al.*, where the environmental sanitation found no significant association with hookworm infection ⁽⁷⁾. This results could be biased because we only conducted interviews without observing the conditions of sanitation of the house.

In conclusion, this study showed that habitual defecation and soil contact were significant risk factors associated with hookworm infection. This risk factors could be related to the educational level and the socioeconomic

conditions of the workers. Therefore, improvement of sanitation facilities by the construction of public toilets around houses and plantation areas, the use of gloves when in contact with the soil and changes in defecation behavior through education would lead to improved living and working condition for this community. The strategy to control hookworm infection would be effective by including giving chemotherapy and supported by health education, improved sanitation, and socioeconomic status.

Conclusion

The results of this study indicated that unhygienic defecation patterns cause hookworm contamination in the soil of the plantation area. Therefore, the plantation workers could be always expose by the infective larvae as long as they don't change their defecation habit.

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References

1. World Health Organization. Helminth control in school-age children: A guide for managers of control programmes. [Online]. 2011 [cited 2018 December 27]. Available from: https://www.who.int/neglected_diseases/resources/9789241548267/en/.
2. Brooker S, Bethony J, and Hotez PJ. Human hookworm infection in the 21st century. *Advances in Parasitology*. 2004;58: p.197–288.
3. Nwoke EU, Ibiama GA, Odikamnoroo OO, Umah OV, Ariom OT, and Orji I. Examination of soil samples for the incidence of geohelminth parasites in Ebonyi north-central area of Ebonyi State, south-east of Nigeria. *Archives of Applied Science Research*. 2013;5(6): p.41–48.
4. Gilgen DD, Mascie-Taylor GN, and Rosetta LL. Intestinal helminth infections, anaemia and labour productivity of female tea pluckers in Bangladesh. *Tropical Medicine and International Health*. 2001;6(6): p.449–457.
5. Traub RJ, Robertson ID, Irwin P, Mencke N, and Thompson RCA. The prevalence, intensities and risk factors associated with geohelminth infection in tea-growing communities of Assam, India. *Tropical Medicine and International Health*. 2004;9(6): p.688–701.
6. Brooker S, Jardim-Botelho A, Quinhell RJ, Geiger SM, Caldas IR, Fleming F, *et al.* Age-related changes in hookworm infection, anaemia and iron deficiency in an area of high *Necator americanus* hookworm transmission in south-eastern Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2007;101(2):p.146–154.
7. Sandy S, Sumarni S, and Soeyoko. Footwear as a risk factor of hookworm infection in elementary school students. *Universa Medicina* 2014;33(2): p.133–140.
8. Jiraanankul V, Aphijirawat W, Mungthin M, Khositnithikul R, Rangsin R, Traub RJ, *et al.* Incidence and risk factors of hookworm infection in a rural community of central Thailand. *The American Journal of Tropical Medicine and Hygiene*. 2011;84(4): p.594–598.
9. Walana W, Aidoo ENK, and Tay SCK. Prevalence of hookworm infection: A retrospective study in Kumasi. *Asian Pacific Journal of Tropical Biomedicine*. 2014;4(1): p.S158–S161.
10. Hossain M, and Bhuiyan JU. Hookworm infection: A neglected tropical disease of mankind. *Journal of Advanced Veterinary and Animal Research*. 2016;3(4): p.297–320.
11. Ziegelbauer K, Speich B, Mäusezahl D, Bos R, Keiser J, and Utzinger J. Effect of sanitation on soiltransmitted helminth infection: Systematic review and meta-analysis. *Journal Public Library of Science Medicine*. 2012;9(1) e1001162: p.1–17.