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

Benaya Y. Onesiforus1, Indra E. Lalangpuling2, Mahardika A.Wijayanti3, E. Elsa Herdiana Murhandarwati4,*,
1 Study Program of Medical Laboratory Technology of Mangunwijaya Catholic Polytechnic, Semarang, 50135, Indonesia
2 Health Polytechnic Ministry of Health of Republic Indonesia of Manado, Manado 55281, Indonesia
3,4 Department of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

Received: 29th March 2019; Revised: 6th April 2020; Accepted: 6th October 2020

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. This study is a cross-sectional study with a quota 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) production and immune effector (eosinophil, IgE, dan mast cell), thus increasing the probability of intestinal nematode infection. Selain malnutrisi, faktor perilaku tidak higienis juga dapat meningkatkan risiko infeksi nematoda 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 quota 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 jenas 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
**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 economy\(^2\).

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 respectively\(^8\). 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 formula\(^12\), total samples in this study determined as 245 children of 12-59 months old. Samples were selected from population based...
on inculsion 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 to 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 bandeage 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 times13, 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 Lalangpuling\(^17\) 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 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 compared to Harada Mori method with success rate of 50%. This result agrees with Reiss et al\(^18\) 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* detection\(^14\). Filariform larvae which found in Harada Mori method identified as *Necator americanus* from its appearance of gap between esophagus and intestinum\(^31\) (see Figure 1).

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

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

\(^8\) Bentinger, S., et al. (2014). \(^16\) Bauch, J., et al. (2018). \(^17\) Lalangpuling, M. (2017). \(^18\) Reiss, J., et al. (2019).
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 faeces. 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 Kokar. 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. 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%). Prevalence of S. stercoralis are found higher in Bali (1.6%).

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%). S. 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 (diethylcarbamizine) 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 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(%) | S. stercoralis 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) |

### 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)     |
| 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               | 6 (2.52)      |
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 infection5,24. Other reason might be due to other confounding variables such as children’s behavior. A study by Alema 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 society14,26-28. 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 infection23,29.

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.

ACKNOWLEDGEMENT

The authors are grateful for cooperation of Head and all staff of Centre of Public Health in Kokar, and to all local authorities that facilitated this study.

REFERENCES

1. Ministry of Health of Republic of Indonesia. Standar Anthropometri Penilaian Status Gizi Anak. Jakarta : Direktorat Jendral Bina Gizi dan Kesehatan Ibu dan Anak; 2011.
2. Riset Kesehatan Dasar (Riskesdas) 2013. Jakarta: Health Ministry of Republic of Indonesia; 2013.
3. Scrimshaw NP and San Giovanni JP. Synergism of Nutrition, Infection, and Immunity: A Overview. Am J Clin Nutr. 1997; 66(2):464S-77S
4. Gutierrez-Jimenez J, Torres-Sanchez MGC, Fajardo-Martinez LP, Schlie-Guzman MA, Luna-Cazares LM, Gonzalez-Esquinca AR, et al. Original Article Malnutrition and The Presence of Intestinal Parasites in Children From The Poorest Municipalities of Mexico. Journal Infect Dev Ctries. 2013; 7(10): 741–747
5. Ing R, Su Z, Scott ME, Koski KG. Suppressed T helper 2 Immunity and Prolonged Survival of a Nematode Parasite in Protein-Malnourished Mice. PNAS. 2000; 97(13):7078-7083
6. Schaible UE and Kaufmann SHE. Malnutrition and Infection: Complex Mechanisms and Global Impact. PloS Med. 2007; 4(5):e115.
7. Franca TGD, Ishikawa LLW, Zorzella-Pezavento SFG, Chiuso-Minicucci F, da Cunha MLRS, Sartori A. 2009. Impact of Malnutrition on Immunity and Infection. J Venom Anim Toxins incl Trop Dis. 2009; 15(3):374-379
8. Paniker CKJ and Ghosh S. Paniker’s textbook of Parasitology. 7th ed. New Delhi : Jaypee Brothers Medical Publishers (P) Ltd; 2013.
9. Martila, Sandy S, Paembonan N. Hubungan Higiene Perorangan dengan Kejadian Kecacingan pada Murid SD Negeri Abe Pantai Jayapura. PLASMA. 2015; 1(Pt 2) : 87-96.
10. Ndona MV. 2015. Hubungan Pengetahuan, Kondisi Lingkungan, dan Sosial Ekonomi Dengan Infeksi Soil Transmitted Helminth (STH) Pada Anak Usia Sekolah Dasar Di Kecamatan Salahutu dan Lehihiu di Kabupaten Maluku Tengah, Provinsi Maluku [Thesis]. Yogyakarta : Univ Gadjah Mada; 2015
11. Renanti R, Rusjdi SR, Elmatris SY. Hubungan Infeksi Soil Transmitted Helminth dengan Status Gizi pada Murid SDN 29 Purus Padang. Jurnal Kesehatan Andalas. 2015; 4(2).

12. Notoatmodjo S. Metodologi Penelitian Kesehatan (Edisi Revisi). Jakarta: Penerbit Rineka Cipta; 2005

13. Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson, D, et al. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. PloS Negl Trop Dis. 2008; 2(11):e331

14. Khieu V, Schar F, Marti H, Sayasone S, Duong S, Muth S, et al. Diagnosis, Treatment and Risk Factors of Strongyloides stercoralis in Schoolchildren in Cambodia. PloS Negl Trop Dis. 2013; 7: e2035.

15. Vaden SL, Knoll JS, Smith Jr FWK, Tilley LP. Blackwell’s Five-Minute Veterinary Consult : Laboratory Test and Diagnostic Procedures : Canine and Feline. Iowa : Wiley & Sons Publishing Ltd; 2009.

16. Chiodini P, Moody AH, Manser DW. Atlas of Medical Helminthology and Protozoologi. 4th Edition. London : Churchill Livingstone, 2001

17. Lalangpuling IE. Hubungan Infeksi STH dengan Status Gizi dan Anemia Pada Balita di Puskesmas Kokar, Kabupaten Alor; 2017 Ma 634-649; Manado : Seminar Nasional Tahun, 2018

18. Reiss D, Harrison LM, Bungiro R, Cappello M. Short Report: An Agar Plate Method for Culturing Hookworm Larvae: Analysis of Growth Kinetics Ana Infectivity Compared With Standard Coproculture Techniques. Am. J. Trop. Med. Hyg. 2007; 77(6):1087–1090

19. Steinmann P, Zhou XN, Du ZW, Jiang JY, Wang LB, Wang XZ, et al. Occurrence of Strongyloides stercoralis in Yunnan Province, China, and Comparison of Diagnostic Methods. PloS Negl Trop Dis 2007; 1: e75

20. Arakaki T, Iwanaga M, Kinjo F, Saito A, Asato R, Ikeshiro T. Efficacy of Agar-plate Culture in Detection of Strongyloides stercoralis Infection. J. Parasitol 76 1990; (3):25-428

21. Widjana DP and Sutisna P. The prevalence of Strongyloides stercoralis Infection in Rural Population of Bali: A preliminary study. Med J Indonesia. 2001; 10(3):174-177.

22. Pion SDS, Chesnais CB, Bopda J, Louya F, Fischer PU, Majewski AC. The Impact of Two Semiannual Treatments With Albendazole Alone on Lymphatic Filariasis and Soil-Transmitted Helminth Infection : A Community-Based Study in the Republic of Congo. Am. J. Trop. Med. Hyg. 2015; 92(5):959-966

23. Supali T, Djuardi Y, Bradley M, Noordin R, Ruckert P, Fischer PU. Impact of Six Rounds of Mass Drug Administration on Brugian Filariasis and Soil-Transmitted Helminth Infections in Eastern Indonesia. PloS Negl Trop Dis. 2013; 7 (12): e2586

24. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology 7th ed. Philadelphia: Elsevier Saunders, 2012

25. Alemu A, Atanafu A, Addis Z, Shiferaw Y, Teklu T, Mathewos B, et al. Soil transmitted Helminths and Schistosoma mansoni infections among school children in Zarima Town, Northwest Ethiopia. BMC Infectious Diseases. 2011; 11:189

26. Walana W, Aidoo ENK, Tay SCK., Prevalence of hookworm infection: a retrospective study in Kumasi. Asian Pac J Trop Biomed. 2014; 4(Suppl 1):158-161.

27. Walmers M, Hall A, Basanez, MG. Individual Predisposition, Household Clustering and Risk Factors for Human Infection with Ascaris lumbricoides: New Epidemiological Insights. PloS Negl Trop Dis. 2011; 5(4): e1047.

28. Forrer A, Vounatsou P, Sayasone S, Vonghachac, Y, Bouakhasith D, Uztinger, et al. Risk Profiling of Hookworm Infection And Intensity in Southern Lao People’s Democratic Republic Using Bayesian Models. PloS Negl Trop Dis. 2015; 9(3):e0003486.

29. Texeira JC and Heller I. Impact of water supply, domiciliary Water reservoirs and sewage on faeco- orally transmitted parasitic disease in children residing in poor area in Juiz de For a, Brazil. Epidemiol. Infect. 2006; 134:694-698

30. Al-Delaimy AK, Al-Mekhlafi HM, Lim YA, Nasr NA., Sady H, Atroosh WA. Developing and Evaluating health education learning package (HELP) to control soil-transmitted helminth

31. Stiles. Necator americanus. In: Chatterjee, K.D. Parasitology : Protozoology & Helminthology In Relation to Clinical Medicine 13th ed. New Delhi : CBS Publishers & Distributors Pvt. Ltd, 2009

Copyright © 2020, IJTID, p-ISSN 2085-1103, e-ISSN 2356-0991