HUMAN STRONGYLIODIASIS AND PREVENTION AND CONTROL MODEL

WANCHAI MALEEWONG AND COLLEQUES

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ACKNOWLEDGEMENTS

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PREFACE

Strongyloidiasis a global disease, infection caused by *Strongyloides stercoralis*, a nematode parasite, is well known as a potentially fatal soil transmitted helminth explained as a unique and complex human parasite, is endemic in tropical and sub-tropical regions. The parasite infects human hosts mainly through skin contact with contaminated soil and poses infected person at risk of fatal cases from hyper-infection (in cases of immunosuppression due to medical conditions, immunosuppressant therapies, or both). The diagnosis and effective therapy are essential in order to eradicate the infection and the lifelong risk involved

Then, this study performed the impact of health education and preventive equipment package (HEPEP) on prevention of *Strongyloides stercoralis* infection among rural communities in northeast, Thailand. The HEPEP was the first effective model to control *S. stercoralis* transmission among a rural community in northeast, Thailand. The results should encourage policy makers and public health personnel to improve control programs for parasites as well as health promotion.

CONTENTS

Name of Contributors

Wanchai Maleewong

Department of Parasitology, Faculty of Medicine, and Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University, Khon Kaen, Thailand

Pewpan Maleewong Intapan

Department of Parasitology, Faculty of Medicine, and Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University, Khon Kaen, Thailand

Pokkamol Laoraksawong

Department of Public Health Administration, Health Promotion, Nutrition, Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand

Oranuch Sanpool

Department of Parasitology, Faculty of Medicine, and Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University, Khon Kaen, Thailand

Tongjit Thanchomnang

Faculty of Medicine, Mahasarakham University, Mahasarakham, Thailand

Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University, Khon Kaen, Thailand

Wanida Kanarkard

Department of Computer Engineering, Faculty of Engineering, Khon Kaen University, Khon Kaen, Thailand

Ratthaphol Kraiklang

Department of Public Health Administration, Health Promotion, Nutrition, Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand

Rutchanee Rodpai

Department of Parasitology, Faculty of Medicine, and Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University, Khon Kaen, Thailand

Chapter 1

Strongyloides of man and medical importance

Tongjit Thanchomnang[1],[2,] Rutchanee Rodpai[2,3], Pewpan M. Intapan[2,3] and Wanchai Maleewong[2],[3]

[1]Faculty of Medicine, Mahasarakham University, Mahasarakham, Thailand
[2]Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University, Khon Kaen, Thailand
[3]Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

I. Introduction

Roundworms in the genus *Strongyloides* have been found infecting the small intestine of varieties of animals including mammals, reptiles, birds and man (dos Santos et al., 2010). One species of medical importance is *Strongyloides stercoralis* (Bavay, 1876) Stiles and Hassal, 1902 which is cosmopolitan in distribution and the infection cause signs and symptoms ranging from asymptomatic to severe disease like disseminated strongyloidiasis which cause morbidity and mortality. The worm long existence in the world is unique in that it has free-living generation in soil environment and autoinfection feature. Another less important species is *S. fülleborni* von Linstow, 1905 which is primarily monkey's parasite and human infection is limited to certain area.

II. Classification and phylogeny

Strongyloides nematodes has phasmids, a paired chemoreceptors situated posterior to anus, and is therefore placed in Class Secernentia of Phylum Nematoda as follows:

Kingdom Animalia

Phylum Nematoda

Class Secrenentia

Subclass Rhabditida

Suborder Rhabditina

Superfamily Rhabditoidea

Family Strongyloididea

Genus Strongyloides

Genus *Strongyloides* is originally claim as a member of family Strongyloididae Chitwood and McIntosh, 1934, superfamily RHABDIASOIDEA (Little, 1966a). The characteristic of the genus is as follows- RHABDIASOIDEA: *Free=living generation with oral opening guarded by two lateral cephalic lobes. Esophagus with corpus, isthmus, and valved bulb. Female with two divergent uteri, ovaries reflexed; vulva near middle of body. Male with 1 testis, equl spicules. A gubernaculum, and pattern genital papillae; caudal alae absent. Parasitic generation pathenogenetic or dioecious, filariform. Stoma cup-shaped or greatly reduced. Esophagus greatly elongate. Reproductive systems in females and males (when present) similar to free-living generation. Live in gastronintestinal tract of most vertebrates. The family has two genera, i.e., Strongyloides and Parastrongyloides.* They differ in that the parasitic form of the latter is dioeceous and has a cup-shaped, thick-wall stoma (Little, 1966a). Genus *Strongyloides* comprises of over 50 species (Table 1). Morphological detail of each species are described in relation to feature of genus including adult worms, the development stages or stage passed in feces of the host (Little, 1966a; Speare, 1989; Sato et al., 2008).

Molecular phylogenetics of Strongyloides

Their phylogenetic relationship has been studied by molecular methods. Analysis of 10 species from a snake, bovid, rodents, primates and humans using small subunit ribosomal RNA gene (SSU rDNA) sequences revealed very similar sequences which made phylogenetic separation quite difficult (Figure 1). Nevertheless, the findings suggest existence of two clades within the genus (Dorris et al., 2002).

Strongyloides fülleborni collected from apes and monkeys of Africa and Japan, and *S. stercoralis* from humans, apes and dogs were analyzed using the hyper variable region IV (HVR-IV) of 18S ribosomal DNA and partial mitochondrial cytochrome c oxidase subunit 1 gene (*cox*1). The results can place isolates of *S. fülleborni* into three groups, which corresponded to geographical localities but not to host species (Hasegawa et al., 2010). In the opposite, isolates of *S. stercoralis* were grouped into dog parasitic and primate parasitic clades, and not to geographical regions, which then suggested a much shorter period for diversification of *S. stercoralis* than that of *S. fülleborni*. The analysis lead to a proposal that worldwide dispersal of *S. stercoralis* may occur more recently than that of *S. fülleborni*, possibly with the migration of modern humans. This may also be applied with the canine strain of *S. stercoralis* as dogs accompany human migration generally (Hasegawa et al., 2010). More studies are needed to elucidated phylogenetics of *Strongyloides* species of man and animal.

Dorris et al (2002) analyzed the molecular phylogenetic of the genus *Strongyloides*, using small subunit ribosomal RNA gene (SSU rDNA) sequences, that result are ten species of *Strongyloides* were sampled from a representative wide host range, including a snake, bovid, rodents, primates and recognised parasites of humans. The *Strongyloides* SSU sequences were all very similar which made the resolution of their phylogeny problematic with distance and

likelihood methods, many branch lengths are inferred to be very short (Figure 1). In addition, molecular phylogenetic analysis of *Strongyloides* has suggested the existence of two clades within the genus which support by neighbour joining bootstrap employing maximum likelihood parameters based on the maximum likelihood tree (Dorris et al., 2002). Hasegawa et al (2010) analyzed and compared the *S. fuelleborni* collected from apes and monkeys of Africa and Japan, and *S. stercoralis* from humans, apes and dogs used the hyper variable region IV (HVR-IV) of 18S ribosomal DNA and *cox*1. Phylogenetic analysis with the maximum-likelihood method based on DNA sequences of *cox*1 (Figure 2) largely divided isolates of *S. fuelleborni* into three groups, which corresponded to geographical localities but not to host species. While, isolates of *S. stercoralis* were grouped by the phylogenetic analysis into dog parasitic and primate parasitic clades, and not to geographical regions, that suggested a much shorter period for diversification of *S. stercoralis* than that of *S. fuelleborni*. It is thus surmised that worldwide dispersal of *S. stercoralis* seems to have occurred more recently than that of *S. fuelleborni*, possibly with the migration of modern humans. As dispersal of dogs has occurred with human migration and activities generally, it is plausible that the canine strain of *S. stercoralis* has also extended its distribution rather recently (Hasegawa et al., 2010).

Table 1 Lists of *Strongyloides* species

Species	Host	Area	References
S. papillosus Wedl, 1856	cattle, sheep	USA, India, Germany	(Eberhardt et al., 2007)
S. stercoralis Bavay, 1876	human, primates, dogs, cats	worldwide	(Schar et al., 2013)
S. fuelleborni Von Linstow 1905	monkeys and human	Africa, Southeast-Asia	(Labes et al., 2011)
S. cebus Darling, 1911	new world monkeys	Brazil	(Mati et al., 2013)

Species	Host	Area	References
S. westeri Ihle, 1917	horses, donkeys, zebra, pigs	USA	(Lyons and Tolliver, 2014)
S. vituli Brumpt, 1921	cattle	Mali	(Kulkarni et al., 2013)
S. ratti Sandground, 1925	rodents	Worldwide	(Little, 1966a)
S. felis Chandler, 1925	cat	India, Australia	(Speare and Tinsley, 1987)
S. ophidiae Pereira, 1929	reptiles	Brazil	(dos Santos et al., 2010)
S. avium Cram, 1929	poultry	Japan	(Sakamoto and Sarashina, 1968)
S. ophidiae Pereira, 1929	snake	Brazil	(Mati and Melo, 2014)
S. ransomi Schwartz and Alicata 1930	swine	Burkina Faso	(Tamboura et al., 2006)
S. myopotami Artigas and Pacheco, 1933	nutria	USA, Korea	(Choe et al., 2014; Little, 1966a)
S. mustelorum Cameron and Parnell 1933	mustelids	Scotland France	(Little, 1966b; Torres et al., 2008)
S. venezuelensis Brumpt, 1934	rodents	worldwide	(Hino et al., 2014; Little, 1966a)
S. putorii Morosov, 1939	polecat	no data	(Grove, 1996)
S. vulpis Petrov, 1940	red fox	Belarus	(Grove, 1996; Shimalov and Shimalov, 2003)
S. martis Petrov, 1940	mustelids	Russia, Japan	(Little, 1966b; Sato et al., 2006)
S. tumefaciens Price and Dikmans 1941	cat	USA	(Malone et al., 1977)

Species	Host	Area	References
S. rostombekowi Gamzemlidse, 1941	hedgehog	no data	(Grove, 1996)
S. robustus Chandler 1942,	sciurid	North- America	(Bartlett, 1995)
S. amphibiophilus, Perez Vigueras 1942	toad	Cuba	(Little, 1966b)
S. planiceps Rogers, 1943	cat, raccoon dog	Malaya, Japan	(Sato et al., 2006; Sato et al., 2008)
S. turkmenicus Kurtieva, 1953	birds	Czech republic	(Okulewicz and Koubek, 1994)
S. mirzai Singh, 1954	Snakes	India	(Singh, 1954)
S. bufonis Rao & Singh, 1954	Malayan toad	no data	(Grove, 1996)
S. lutrae Little, 1966	otter	USA	(Little, 1966b)
S. dasypodis Little, 1966	armadillo	USA	(Little, 1966b)
S. ardeae Little, 1966	birds	USA	(Little, 1966b)
S. physali Little, 1966	toad	USA	(Little, 1966b)
S. serpentis Little, 1966	snake	USA	(Little, 1966b)
S. gulae Little, 1966	snake	USA	(Little, 1966b)
S. procyonis Little, 1966	raccoon	USA	(Little, 1966b)
S. akbari Mirza and Narayan, 1935	shrew	India, Japan	(Shimabukuro et al., 1995)
S. cruzi Rodrigues, 1968	lizards	Brazil	(Mati et al., 2013)
S. darevskyi Sharpilo, 1976	skink	no data	(Grove, 1996)
S. spiralis Grabda-Kazubsak, 1978	edible frog	Poland	(Grabda-Kazubska, 1978; Grove, 1996)
S. ophiusensis Roca & Hornero, 1992	insular lizard	Balearic islands	(Roca and Hornero, 1992)
S. natricis Navarro & Lluch, 1993	reptile	no data	(Mati and Melo, 2014)
S. callosciureus Sato et al. 2007	Asian sciurids	Japan	(Sato et al., 2007)

III. Species infecting man

It is not known if animal *Strongyloides* can infect human at all. But at least two species of *Strongyloides* successfully establish and maintain life cycle in human- *S. stercoralis* and *S. fülleborni*. The *S. stercoralis* has a cosmopolitan distribution mainly in tropical and subtropical zones where about three million to one hundred million individuals are estimated to be infected worldwide (Schar et al., 2013). Dogs, cats and primate are also natural host and could be a reason for widespread infection. *S. fuelleborni,* however, is very limited in distribution. It is primarily the parasite of non-human primates in Africa (Viney and Lok, 2007). Sporadic human cases have been reported from Africa and Papua New Guinea (Dorris et al., 2002; Hasegawa et al., 2010). *Strongyliodes fuelleborni* then is of minor importance in terms of health and distribution. In Thailand, the results using molecular approaches, demonstrated that transmission of *S. stercoralis* and *S. fuelleborni* between humans and animals may occur frequently in the same area (Thanchomanag et al., 2017). The mitochondrial cytochrome c oxidase subunit 1 gene revealed that the parasites recovered from humans were related to *S. fuelleborni* recovered from the closely contacted primate.

Strongyloides stercoralis is an unusual parasitic nematode in several respects, it can multiply within the host, it has a free living life cycle in addition to its parasitic one, and only parthenogenic females are found in the host. It is an intestinal helminth that infects humans through contact with soil containing the larvae with *S. stercoralis* (Schar et al., 2013).

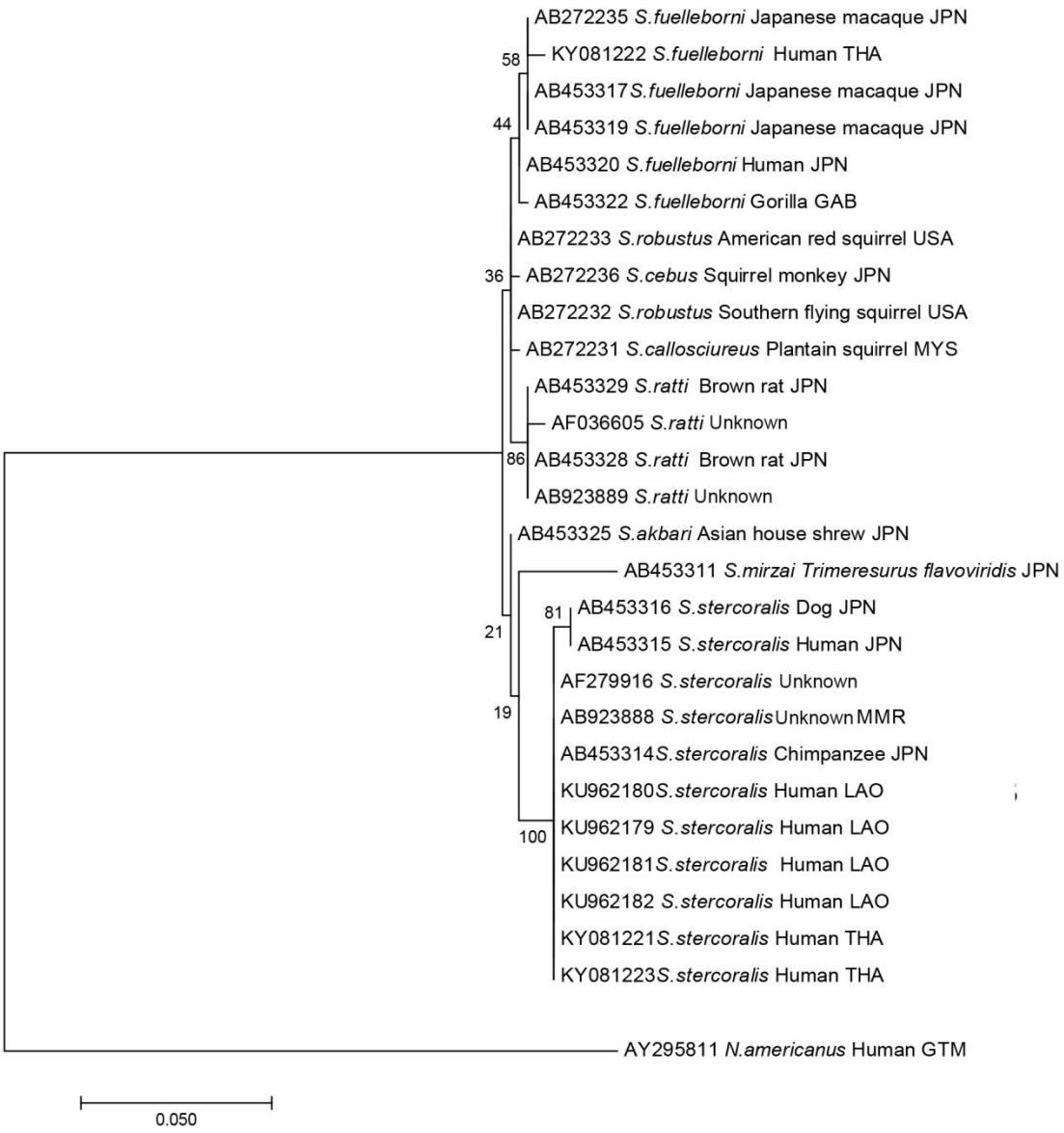


Figure 1 Maximum-likelihood reconstruction of phylogeny based on 494 nucleotides in the 18S rRNA gene of *Strongyloides* species. Bootstrap scores (percentages of 1000 replications) are presented for each node. The sequences of *Strongyloides* species obtained from GenBank database are indicated with their accession number, species name, hosts, and country code. (LAO Lao People's Democratic Republic, THA Thailand, JPN Japan, USA United States of America, GAB Gabon, MYS Malaysia, MMR Myanmar, GTM Guatemala) (Original)

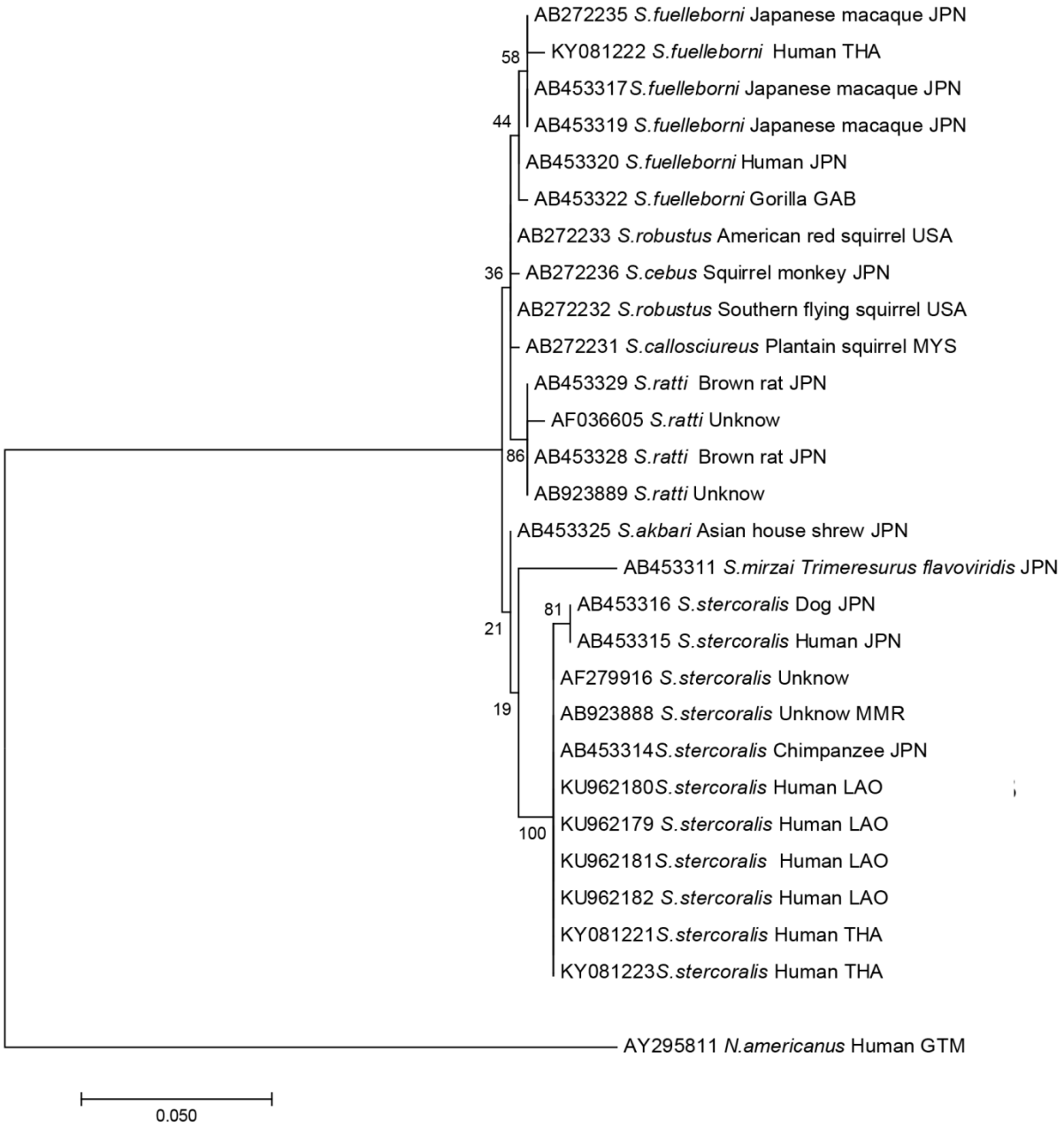


Figure 2 Maximum-likelihood reconstruction of phylogeny based on 710 nucleotides in the cox1 gene of *S. fuelleborni* (A) and *Strongyloides stercoralis* (B). Bootstrap scores (percentages of 1000 replications) are presented for each node. The sequences of *Strongyloides* species obtained from GenBank database are indicated with their accession number, species name, hosts, and country code. (LAO Lao People's Democratic Republic, THA Thailand, JPN Japan, USA United States of America, CAF Central African Republic, GAB Gabon, TZA Tanzania, CHN China) (Original)

IV. Morphology and life cycle

Strongyloides is the only facultative nematode of human. It can reproduce within human (parasitic phase), and in soil environment (free-living phase) (Figure 3). Determinants of route of developments are host, parasite and environmental factors (Viney, 1999).

S. stercoralis

Parasitic cycle (Homogonic development):

Adult parasitic females live in duodenum and jejunum of man and animal hosts. The filariform worm is 2.1-2.7 mm. in length and 30-40 µm in width (Figure 4). Stoma is hexagonal in shape and ovaries are always straight. Eggs in short uteri are few, usually no more than six. Eggs (Figure 5) usually hatch in crypts of Liberkühn of the intestinal mucosa and release first-stage larvae, 180 to 240 µm long by 14 to 15 µm wide with rhabditiform esophagus 80 to 90 µm long before discharge in feces. Larvae appear in feces grow but remain their first-stage, 325 to 380 µm long by 17 to 20 µm wide with esophagus 89 to 94 µm long. Molting occurs within 2 hours after passage and they become second-stage larvae (Figure 6). They have conspicuous mass of 9-cell genital primordium in the mid-ventral body which push the intestinal wall inward (Lopez et al., 2000). This feature is used for differentiating from hookworm larva. Upon suitable environmental conditions, larvae molt and develop into infective filariform larvae (Figure 7) within 3 days. They have filariform type of esophagus and notched tail (Little, 1966a). The body size of infective third-stage larva is about 630 long and 16 µm wide (Georgi, 1982)

The filariform larvae in contaminated soil penetrate the human or animal skin, and are transported via blood circulation to the heart and reach the lungs where larvae disrupt capillaries and enter the alveolar spaces. From there they are carried through the bronchial tree to

the pharynx, swallowed and then reach the small intestine. Other migratory routes in viscera are possible from study in dogs (Mansfield et al., 1995). In the small intestine, they molt twice and become adult female worms. The females live threaded in the epithelium of the small intestine and by produce eggs without the presence of male by process termed parthenogenesis, of which the detail is discussed elsewhere (Streit, 2017).

Free-living cycle (Heterogonic development):

In certain environmental conditions, larvae in feces undergo 4 molts to become free-living adult males and females. The free-living female is shorter and broader than parasitic female, being 0.92-1.7 mm. in length and 52-85 µm in width (Figure 8). The body is slightly constricted behind vulva. The uterus contains up to 28 eggs, not in a single row. The free-living male is 0.81-1.00 mm. in length and 40-50 µm in width with a pair of slightly bow copulatory spicules (Figure 9). Their tails bend anteriorly and give a look of "J" letter.

Worms mate and eggs are produced by female worms. Eggs are ellipsoidal with very thin wall, about 40 x 70 µm (Grove, 1996). Eggs laid into fecal environment are mostly in early cleavage stage. Eggs later hatch into rhabditiform larvae, molt twice and develop into infective filariform larvae. The limited ability to repeat free-living cycle of *S. stercoralis* to one cycle differs from other *Strongyloides* species, e.g., *S. planiceps* has 9 generation of free-living cycle (Yamada et al, 1991).

S. fülleborni

Adult parasitic females live in duodenum and jejunum of African and Asian primates, e.g., chimpanzee, baboon, macaque. The worm is slightly longer than *S. stercoralis*, being 2.9-4.2 mm. in length and 43-55 µm in width. Stoma appears in modified X-shape. Ovaries spiral

situate around intestine, anterior with 3 and posterior with 1/3 spirals. Lips of vulva are prominent. Eggs in uteri are 10-15 in number. Oval, thin-shell eggs are 43-58 µm in length and 34-38 µm in width (Hira and Patel, 1977). Eggs usually do not hatch in the intestine, but rather in early cleavage when pass into feces. Posterior end shows abrupt narrowing behind anus. Tail is truncate and tapering to finger-like projection. Free-living females is shorter, being 1.2-1.3 mm. in length and 60-70 µm in width, with 40 or more eggs in uteri. Free-living male is 0.85-1.1 mm. in length and 38-52 µm in width with long tail (Little, 1966a). Heterogonic development occurs more than one generation (Hansen et al., 1969).

S. fülleborni kellyi

Worm morphology is indistinguishable from *S. fülleborni* under the microscope except the characteristic of the peri-vulval cuticle of the parasitic female and the position of the phasmidial pore of the free-living male (Viney et al., 1991).

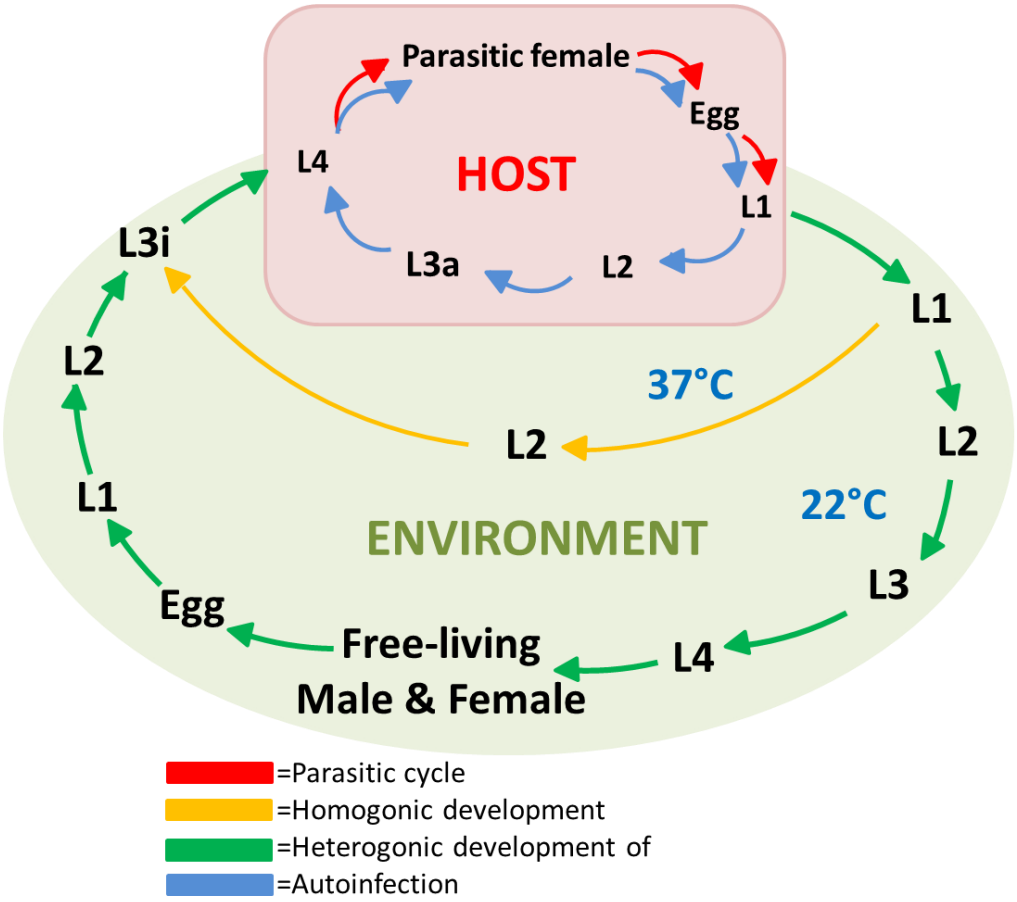
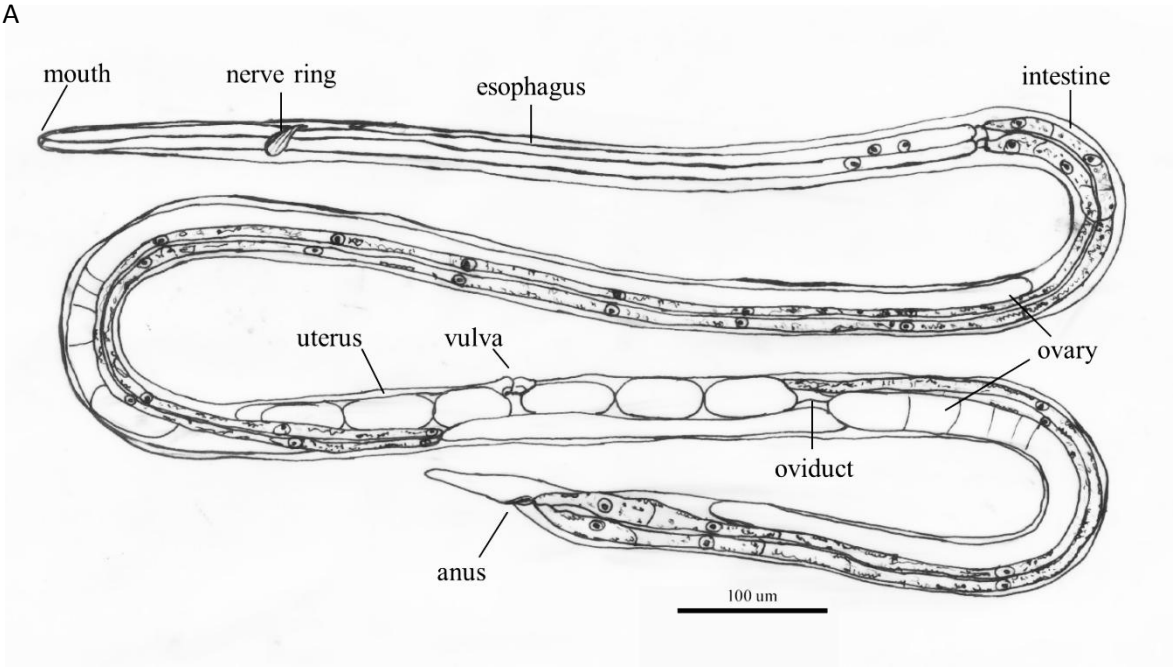


Figure 3. The life cycle of *S. stercoralis*. L1-L4 indicates each developmental larval stage of *S. stercoralis*; L3i indicate the infectious third-stage larva; L3a indicate the autoinfective third-stage larva. (Original)

A



B

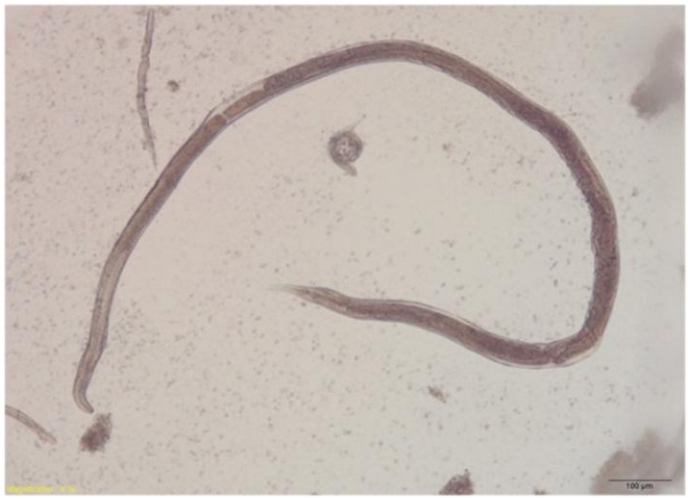


Figure 4. Major morphological features of parasitic female of *Strongyloides stercoralis*. A, Drawing of whole worm. B, Whole worm body collected from infected patient stool, was fixed in formalin. (Original)

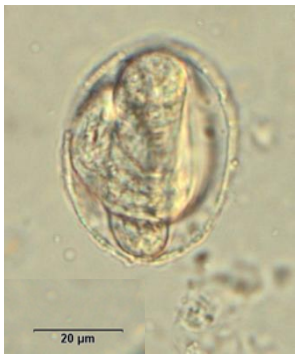


Figure 5. Egg of *Strongyloides stercoralis*. (Original)

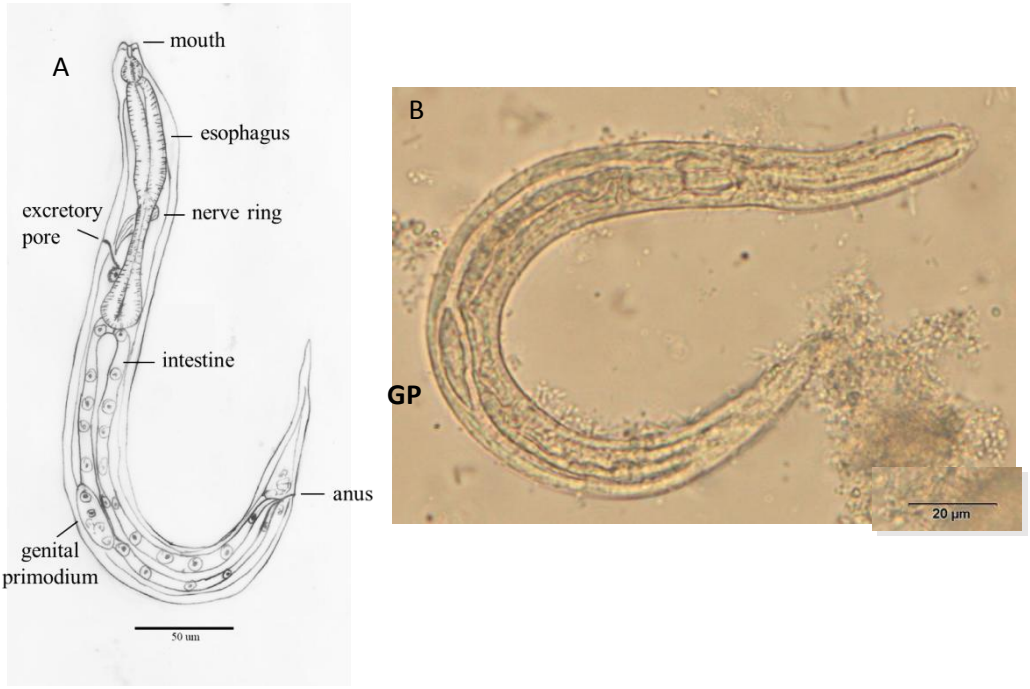


Figure 6. Major morphological features of rhabditiform larva of *Strongyloides stercoralis*. A, Drawing of whole body. B, The rhabditiform larva collected from infected patient stool, was fixed in formalin. (Original). GP, genital primodium

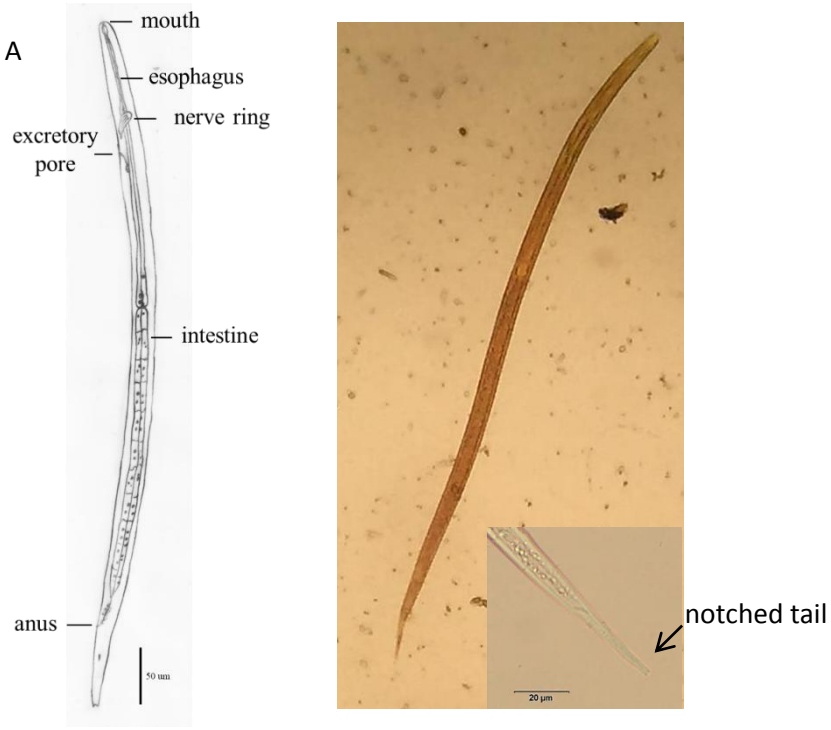


Figure 7. Major morphological features of filariform larva of *Strongyloides stercoralis*. A, Drawing of filariform larva. B, The filariform larva collected from agar plate culture method, stained with 1% iodine. The arrow indicated a notched tail character. (Original)

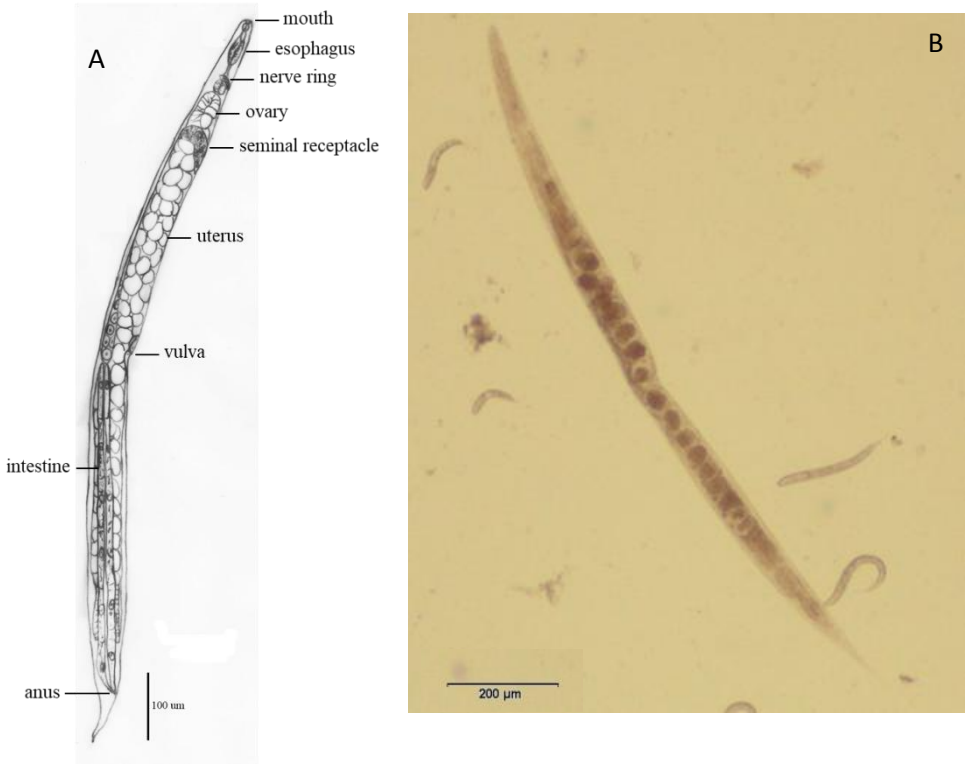


Figure 8. Major morphological features of free-living female of *Strongyloides stercoralis*. A, Drawing of whole worm in lateral view. B, Whole worm body collected from agar plate culture method, was fixed in formalin. (Original)

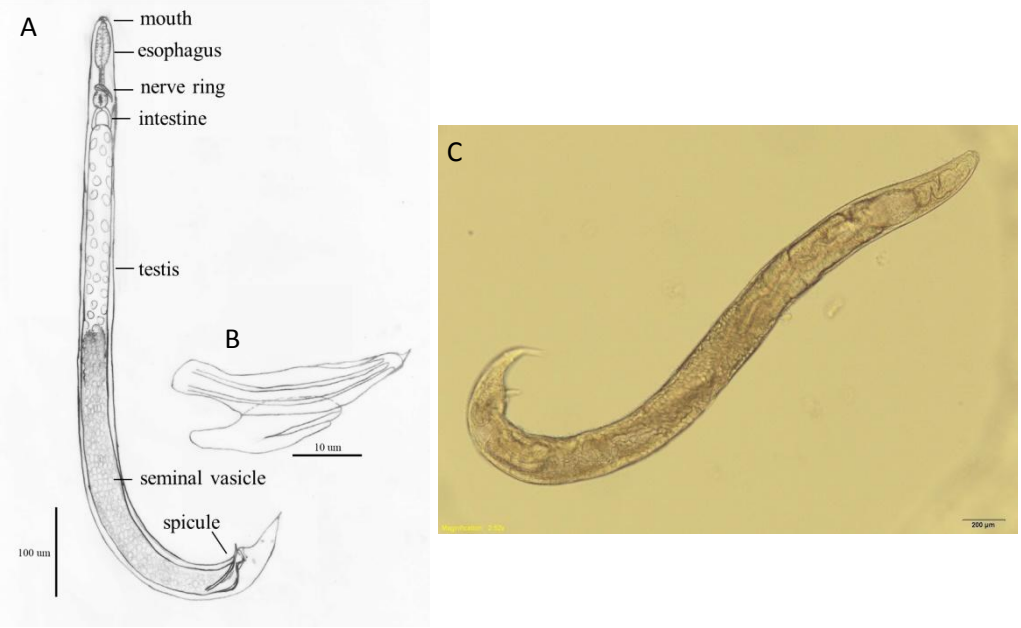


Figure 9. Major morphological features of free-living male of *Strongyloides stercoralis*. A, Drawing of whole worm in lateral view. B, Drawing of spicule and gubernaculum. C, Whole worm body collected from agar plate culture method, was fixed in formalin. (Original)

V. Epidemiology

Geographic distribution and prevalence

Strongyloides of man are distributed widely in tropical and subtropical zones as warm temperature promotes worm development. It is estimated that *Strongyloides* infects 30—100 million people worldwide (Bethony et al., 2006). The figure could be underestimated of the true prevalence as studies used different detection methods of varying sensitivities. Schär et al. (2013) collected data from articles on *S. stercoralis* in the PubMed database published between January 1989 and October 2011, and used a Bayesian meta-analysis that included the diagnostic-test sensitivity to obtain country-specific prevalence estimates. Overall estimated prevalence was between 10-40% of the population in tropical and subtropical countries. As other neglected tropical diseases, population of poor socio-economy have higher prevalence rate of up to 60%. Moreover, prevalence rates of up to 75% is also found in refugee and immigrants in developed countries. Similar figure of prevalence reported during 1992-2011 is demonstrated by Puthiyakunnon et al. (2016) that global prevalence rates are as high as 50%. In Latin America, countries with prevalence 20% or more include Argentina, Ecuador, Venezuela, Peru and Brazil (Buonfrate et al., 2015). Prevalence increases by age, inclines from children below 6 years old to peak around middle age, then declines (Forrer et al., 2018) or remains so (Becker et al., 2011; Sithithaworn et al., 2003). Men appear to have higher prevalence than women (Wongsaroj et al., 2008; Forrer et al., 2018; Jongsuksuntigul et al., 2003).

In Southeast Asian countries, strongyloidiasis can be of high prevalence in remote areas. Using highly sensitive agar-plate culture technique, surveys of northeastern Thailand population were showed prevalence rate of 23.5% and 28.9%, respectively (Jongsuksuntigul et al., 2003; Sithithaworn et al., 2003). The same technique revealed prevalence rate of 20.6% in the South

(Wongsaroj et al., 2008). Prevalence among villagers of Preah Vihear Province, Cambodia was 48.6% (Forrer et al., 2018), in three provinces in Lao PDR (Luang Prabang in the north, Khammouane in the center, and Champasack in the south) (Laymanivong et al., 2016) was 41.% and the villages of Kenethao district, Xayaburi Province, Lao PDR was 44.2% (Senephansiri et al., 2017). In Lao PDR, phylogenetic analyses revealed parasite specimens from community cross-sectional surveys sequenced belonged to *S. stercoralis* (Bavay, 1876) Stiles and Hassall, 1902. The *cox*1 sequences revealed high diversity (24 haplotypes) (Laymanivong et al., 2016). In Thailand, sequenced a portion of the 18S ribosomal RNA gene (rRNA) and of the *cox*1 gene of *Strongyloides* from humans in Thailand were *S. stercoralis* and *S. fülleborni* (Thanchomnang et al., 2017). Up to date, the median-joining network showed that the *S. stercoralis cox*1 sequences fell into 43 known distinct haplotypes (Thanchomnang et al., 2017).

In comparison to *S. stercoralis*, *S. fülleborni* has a more limited in distribution. Human infection was found in Zambia, Central African Republic, Cameroon and Ethiopia (Kelly et al., 1976; Hira and Patel, 1977). There are case reports of human infection who are in close contact with monkeys- one was American soldier in the Philippines who had pet monkey (Wallace et al., 1948) and one was a villager in Thailand who lived in vicinity of close contact with monkeys (Thanchomnang et al., 2017). Thus strongylidiasis from *S. fülleborni* is zoonotic in nature despite the fact that animal host of the latter not yet found.

Strongyloides fülleborni-like nematodes were found in feces of several habitants in Papua New Guinea (Kelly and Voge, 1973; Kelly et al., 1976). The worm was well-described and later found to cause swollen belly sickness in infants of the Kamea people in Papua New Guinea which can be fatal (Ashford et al., 1978; Vince et al., 1979). The worm was given name *S. fülleborni kellyi* in honor to the author (Viney et al., 1991; Ashford et al., 1992). A survey in

children under 5 years of age revealed 27% of them were infected, with varying intensity as demonstrated by fecal egg count (King and Mascie-Taylor, 2004). Animal host is still unknown as there are no non-human primate on the island of New Guinea (Viney et al, 1991).

Transmission

Like other soil-transmitted nematodes such as *Ascaris lumbricoides*, hookworms, and *Trichuris trichiura*, *Strongyloides* are spread to human by soil contact. In endemic area, infected person defecates on ground where homogonic and heterogonic development take place and results in infective filariform larvae in a week. Rainfall helps spreading infective larvae. Heavy rainfall resulting in flood, however, is detrimental to larval development because they are deprived of oxygen which is essential for growth and development (Anamnart et al., 2013). Occupation is one of predisposing factor of infection. Gardeners and people walking bare feet are then susceptible to skin penetration by the larva. Farming activities and walking barefoot were shown to be important risk factors (Senephansiri et al., 2017). Other significant risk factors are HIV infection, HTLV-1 infection and alcoholism (Schär et al., 2013).

Transplacental transmission did not occur, but transmammary transmission was evident from experiments in dogs (Shoop et al., 2002).

Another important mode of infection is autoinfection. For an external autoinfection, rhabditiform larva in fecal remnant around perianal area may develop into filariform larva quickly and penetrate the skin and complete the life cycle in the same person. For internal autoinfection, experiments in immunological naïve puppy showed that autoinfective larvae developed in the intestine. They are shorter and wider than free-living filariform larva (Schad et al., 1993). In human case, rhabditiform larva in the intestine molt in rapid fashion due to certain

stimuli, develop into filariform larva and penetrate the intestinal wall. Autoinfection may be used to explain the persistence of worm in human and chronic infection (Gill et al., 2004). The internal autoinfection feature of the worm contributes to massive infection or hyperinfection syndrome in patients administering corticosteroids (Keiser and Nutman, 2004).

Although it is widely accepted that infected human is important source of infection, dogs may play some role in natural maintenance of the worm. Two distinct genetically different populations of *S. stercoralis* were found in dogs. One of them is indistinguishable from worm of human (Jaleta, et al., 2017).

VI. Clinical features of human strongyloidiasis

As life cycle of *Strongyloides stercoralis* begins from skin penetration and migration through lungs by larva, then finally developed adults embed in the crypt of intestinal mucosa of the small intestine. Signs and symptoms may appear relating to these organs.

1. Skin manifestation

Cutaneous reaction to the larvae migration can result in serpiginous or urticarial tracts with severe pruritus lasting for several days due to inflammatory response. The condition is termed “larva currens” which differs from “larva migrans” caused by other nematode larvae (Iwamoto et al., 1998). The rash may be difficult to distinguish from cutaneous larva migrans, a condition caused by animal species of hookworm that penetrate human skin but are unable to migrate further than the epidermis. Lesion may appear from exposed area such as lower limbs and subsequently trunk (Corte et al., 2013) or buttocks (Rao and Rao, 2006). Path of migration advance rapidly about 2-10 centimeters in one hour and persist up to many days before waning

(Amer et al., 1984; Page and Speare, 2016). Signs could be detected in both acute and chronically infected cases even infection was initiated several decades earlier (Showler and Boggild, 2012; Bailey et al., 2015). This chronic feature is evident from a report showing 70% prevalence of larva currens in former British World War II Far East prisoners of war (Gill et al., 2004). Horses *Strongyloides* have been reported to cause similar skin involvement (Roeckel and Lyons, 1977).

2. Pulmonary manifestation

As the larvae migrate through the lungs they can produce respiratory symptoms, such as a dry cough or wheeze. Loeffler's syndrome, characterized by fever, dyspnea, wheeze, pulmonary infiltrates on chest radiographs, and accompanying blood eosinophilia could be seen.

3. Gastrointestinal manifestation

Infected person is often asymptomatic or experience mild gastrointestinal disturbance and often pass unrecognized. Symptomatic cases may experience nonspecific signs and symptoms which described elsewhere (Greaves et al., 2013; Grove, 1996; Siddiqui and Berk, 2001) including bloating, diarrhea, anorexia, vomiting, indigestion, cramping lower abdominal pains, intermittent or persistent diarrhea, pruritus ani, and sometimes weight loss, and epigastric pain worsened by eating.

4. Other manifestation

Eosinophilia is presented in about 70% of infected cases (Lim et al., 2004). Signs and symptoms of acute infection is the best illustrated by infected tourists from temperate countries visiting tropical countries. Two Italian tourists returning from Thailand, Malaysia, and Singapore suffered from a diffuse urticarial rash, itching, high fever, cough, and fatigue and being

diagnosed of having strongyloidiasis. Incubation period was estimated ranging from 11 to 14 days (Angheben et al., 2011). Chronic strongyloidiasis, however, is usually asymptomatic or causing mild gastrointestinal disturbance which can hardly differentiate from other cause especially in tropics where food safety is poor.

Severe complicated strongyloidiasis

While clinical importance of strongyloidiasis is under-recognized due to mild or asymptomatic nature of uncomplicated strongyloidiasis, cases with severe disease described as disseminated strongyloidiasis, overwhelming strongyloidiasis, hyperinfection or massive strongyloidiasis with worldwide distribution attract attention of scientists (Grove, 1996). The condiction is the result of heavy infections due to enhancing autoinfective cycle of the worm. The term “hyperinfection” is often used in autoinfection, a phenomenon in which the number of worms increases tremendously and the worms are detectable in extraintestinal regions while the term “disseminated” is usually restricted to infections in which worms are found in ectopic sites (Siddiqui and Berk, 2001). Major risk factors for development of hyperinfection syndrome are immunosuppressive therapy especially using corticosteroids, transplantation, hematologica malignancies and human T-lymphotropic virus-1 infection. A systematic review showed that administration of steroids accounted for 67% of hyperinfection and disseminated strongyloidiasis cases (Buonfrate et al., 2013) Additional risk factors include diabetes mellitus, chronic renal failure, chronic alcoholic consumption, organ transplantation, chronic obstructive pulmonary disease (COPD) (Lim et al., 2004). HIV infection, acquired immunoglobulinopathies and immune deficiencies do not usually predispose to hyperinfection or dissemination (Bollela et al., 2013; Khuroo, 2014). The hyperinfection phenomenon is explained by the increase of

ecdysteroid like substances in host tissues as a result of corticosteroid administration. These substances send strong molting signals to rhabditoid larva which subsequently undergo molting intraluminally into filariform larva which in turn penetrate intestinal wall before passing out into feces as usual. Likewise, chronic alcohol consumption results in increase endogenous cortisol which mimics worm hormone ecdysone (Marcia et al., 2016). Repeated autoinfection then increases adult females in the small intestine rapidly and consequently hyperinfection and disseminated strongyloidiasis develop (Genta, 1992).

Cases with *Strongyloides* hyperinfection syndrome have intensified signs and symptoms of uncomplicated strongyloidiasis due to a massive larval migration and increasing number of worms interacting with intestinal mucosa. Purpuric patches were described all over the body (MacDonald and Moore, 2017). Acute pulmonary symptoms are often associated with wheezing, shortness of breath, and pleuritic chest pain (Grove, 1996; Newberry et al., 2005). Acute respiratory failure was a common indicator for pulmonary manifestation (Nabeya et al., 2017). Other pulmonary complications include asthma or exacerbation of preexisting obstructive pulmonary disease, pneumonitis, respiratory failure, acute respiratory distress syndrome (ASDS), alveolar hemorrhage, pleural effusion, granulomatous lung disease. A chest radiograph may reveal pulmonary infiltrates, which can represent a combination of oedema, haemorrhage, and pneumonitis.

Along gastrointestinal tract there may be disruption of mucosa and progress to paralytic ileus (Greaves et al., 2013; Siddiqui and Berk, 2001) or bleeding (Yee et al., 2015; Zaghlool et al., 2016). The inflammatory factors might contribute to mucosal disruption and massive gastrointestinal (GI) bleeding (Csermely et al., 2006). Histologically, worm eggs and/or larva were found to distribute in mucosa and submucosa of GI tract including stomach, duodenum,

jejunum and descending colon (Khuroo, 2014; Yee et al., 2015; Mohamed et al., 2017). Bacterial and fungal infections often occur in cases of hyperinfection because of the leakage of gut flora from a bowel damaged by moving larvae (Grove, 1996; Siddiqui and Berk, 2001). Gram negative bacteria are the most common organisms involved (Greaves et al., 2013; Newberry et al., 2005). Systemic sepsis is then a common complication (Greaves et al., 2013). Other presentations include arterial mesenteric occlusion, small bowel infarction, papillary stenosis with biliary obstruction, ulceration of the colon (Grove, 1996). Mortality rate is high, ranging from 70 to 85 percent (Montes et al., 2010). Eosinophilia is not reliable indicative marker in hyperinfection cases (Marcos et al., 2008).

Dissemination of larva to central nervous system (CNS) occurs, although rarely, in patients with *Strongyloides* hyperinfection. The most common manifestations of CNS involvement is alteration in mental status and meningismus. Larval penetration of vessel walls can cause mycotic aneurysm and intracranial hemorrhage, even vasculitis (Walker and Zunt, 2009). Secondary bacterial infection causes meningitis, such as *Escherichia coli* meningitis (Newberry et al., 2005). A retrospective study of 77 patients diagnosed of strongyloidiasis and meningitis at Japanese Academic Medical Centers revealed causative bacteria namely *E. coli*, *Klebsiella pneumoniae*, *Streptococcus gallolyticus* (Mukaigawara et al., 2016). Other enterobacteria like *Streptococcus faecalis* also contributed to meningitis (Sukhwani et al., 2017). Brain abscess, caused by *E. coli* in about 30% of cases, may produce focal neurological symptoms, e.g., fever, headache, nausea, vomiting, neck stiffness, or convulsions or coma (Grove, 1996; Walker and Zunt, 2009). Lumbar puncture may reveal evidence of bacterial meningitis with increased neutrophils and protein concentration but a reduced glucose level in the cerebrospinal fluid (Grove, 1996).

VII. Diagnosis of human strongyloidiasis

Like other intestinal helminth infection, definite diagnosis relies on direct stool examination for eggs or larva. Typically, rhabditiform larvae of *Strongyloides stercoralis*, when detected, are easy to identify under the microscope. The problem, however, is low number of larvae in feces in asymptomatic chronically infected cases. Moreover, larvae appearance in stool is fluctuated and several samples are then required to confirm the infection (Dreyer et al, 1996; Uparanukraw et al, 1999; Requena-Mendez et al., 2013). Albendazole was found to stimulate the secretion of *S. stercoralis* larvae and can increase sensitivity of detection by stool examination (Anamnart et al., 2010), but it is not practical. Molecular detection of larva in stool has been developed to increase sensitivity of detection. Other indirect methods for diagnosis of infection may be used in an epidemiological survey as well as for diagnosis of strongyloidiasis. Methods include detection of antibodies or worm antigen in serum or other samples, but they await standardization and mass production to make available to community hospitals and health centers worldwide. Sensitivity and specificity of both direct and indirect methods has been reviewed and summarized by Requena-Méndez et al (2013) and Buonfrate et al (2015).

Stool examination

Conventional stool examination techniques among health centers or hospitals include direct simple smear, cellophane thick smear and formalin-ether concentration method (FECT) of which *Strongyloides* eggs (*S. fülleborni*) or larvae (*S. stercoralis*) are detected and identified microscopically (WHO, 1991). Simple fecal smear in saline is a simple and inexpensive routine procedure, but it has low sensitivity because only two to three milligrams of feces are examined. Cellophane thick smear, originally designed for hookworms survey, uses up to 40 milligrams of feces. Difficulties are placed on inexperienced microscopists where *Strongyloides* larvae are

colorless and poorly visible due to glycerol penetration. Formalin-ether concentration technique uses up to one gram of feces and designed for detection of gastrointestinal protozoa, helminth eggs and larva. *Strongyloides* larvae, however, may be trapped in fat/debris layer of the process. Use of above mentioned techniques results in a wide range of reported prevalence of strongyloidiasis in epidemiological surveys.

Special stool examination techniques are designed to detect hookworms or *Strongyloides* in stool based on development of filariform larva in soil environment and their crawling ability. In addition, free-living generation feature of *Strongyloides* helps to amplify its products. These consideration yields increased sensitivity of detection methods of strongyloidiasis. Special techniques are the Baermann method, Harada-Mori filter paper method, water-emergence method, charcoal culture method, and agar-plate culture (APC).

The Baermann method is a cheap and simple technique. Fecal mass is put on gauze in a funnel filled with water. A rubber hose connect funnel to a test tube immersed in warm water. After two hours, the content of test tube is centrifuged and sediment is examined for rhabditiform larva (Lima and Delgado, 1961). The technique may not be suitable for a large survey. Modified Baermann in various forms have been reported. One study showed that it was superior to direct smear and have equal efficiency with APC (Hernández-Chavarría and Avendaño, 2001). Another larger study involving 427 stool samples, however, showed that a modified Baermann was 3.6 times more efficient than the direct smear. But it was still 0.8 times less efficient than that of APC (de Kaminsky, 1991). Comparing to FECT, the modified Baermann showed four times more efficient (Assefa et al., 1991).

In the Harada-Mori technique, a mass of feces is pasted on a filter paper, then place in a tube containing water at the bottom, and left at room temperature for 10 days in which filariform

larva develop and migrate into the water. The content in the bottom of the tube is then examined for *Strongyloides* filariform larvae. The efficiency is proved consistently to be inferior to the APC (Koga et al., 1990; Sato et al., 1995; Jongwutiwes et al., 1999). In one study, the Harada-Mori positivity rate was 24% compared to 69.7% by APC, 48.5% by the Baermann and FECT (Blatt and Cantos, 2003). Despite the disadvantage of delayed diagnosis, it becomes useful in some cases (Martín-Rabadán et al., 1999).

Water-emergence method was employed in only one study which was a survey of human *Oesophagostomum bifurcum*, hookworm and *S. stercoralis* infections in Ghana. A central depression is made in fresh stool specimen and filled with warm water (about 37°C). It was incubated at 37 C for one hour. Rhabditiform larvae migrate into the water and can be easily detected. The sensitivity was almost twice that of direct smear and FECT (Yelifari et al., 2005).

Charcoal culture is performed by mixing two grams of feces with an equal quantity of vermiculite or coarsely ground charcoal, put on a filter paper which is mounted on a culture plate and incubated for seven days. The sediment of the centrifuged water is examined for the presence of filariform larvae (Yelifari et al., 2005).

The most interesting technique is APC which was first developed by Arakaki et al. (1990) and later widely used for survey and diagnosis of strongyloidiasis. It has consistently been found to be 1.6 to 6.0 times more effective than the traditional methods such direct fecal smear, filter paper culture, FECT or Baermann method. (Ines Ede et al., 2011; Intapan et al., 2005; Koga et al., 1990; Sato et al., 1995). Still, in chronic infections, the sensitivity of these methods might not be pleasurable. The study by Sato et al (1995) the detection rate of APC was still less than 60% if only one sample was tested (Sato et al., 1995). Thus, it is essential that stool examinations must

be repeated to increase the diagnostic sensitivity of stool examination technique including APC (Requena-Mendez et al., 2013).

Other diagnostic techniques

One technique is detection of larva in duodenal contents, a string test. A nylon yarn coiled inside a lined gelatin capsule is swallowed and the capsule is delivered to the stomach and duodenum. Then the line is pulled back with adhered bile stained duodenal mucus. Although it has higher sensitivity than stool examination, this invasive method should perhaps be recommended only in selected cases, example in an of immunosuppressed patient to maximize the chance of detecting larvae when a prompt diagnosis is essential (Goka et al., 1990; Requena-Mendez et al., 2013).

Endoscopy is another diagnostic method which may give clue to strongyloidiasis. The most common endoscopic appearances, including loss of vascular pattern, serpiginous ulcerations, duodenal spasm, mucosal edema, thickened duodenal folds, or brown discoloration of the mucosa, erythema, aphthous ulcers, erosions, serpiginous ulcerations, xanthoma-like lesions, yellowish-white nodules and friable mucosa (Minematsu et al., 2011; Requena-Mendez et al., 2013). Colonoscopic evaluation and biopsies are very useful to diagnose strongyloidiasis (Rios et al., 2015). Yellowish-white nodules may be a characteristic finding of colonic lesions in strongyloidiasis and can be a cautious marker to prevent fatal disseminated strongyloidiasis in endemic regions (Minematsu et al., 2011). The histological examinations can confirm the diagnosis showing sections of larvae, eggs and some adult forms, predominantly in the gastric or duodenal crypts with eosinophilic infiltration in the lamina propia directly correlated with the intensity of infection (Minematsu et al., 2011; Requena-Mendez et al., 2013; Khuroo, 2014; Yee et al, 2015).

Sputum or bronchial lavage examination may reveal *Strongyloides* larva in disseminated strongyloidiasis (Mokhlesi et al., 2004; Buresch et al., 2015; Kinjo et al., 2015). Adult worm can also be found but rarely (Bava et al., 2013).

Immunological techniques

These are methods which provide indirect evidence of strongyloidiasis, i.e., worms are not directly demonstrated.

Intradermal skin test

The skin test using somatic and excretory-secretory antigens of filariform larva demonstrated 82-100% positivity among infected people. Cross-reactions with other nematodes infections especially filarial have frequently occurred and the persistence of a positive skin test reaction after treatment is also plausible (Neva et al., 2001). Lower positivity rate was associated with human T-Cell Lymphocytotropic virus type 1 infection (HTLV-1,). The test is not a realistic option for routine diagnosis or epidemiological survey of strongyloidiasis (Requena-Mendez et al., 2013).

Serological tests for antibodies

Several serum antibody detection using a variety of antigens have been already tested over many years, including immunofluorescence antibody test (IFAT), gelatin particle agglutination (GPAT) test, enzyme-linked immunosorbent assays (ELISAs), immunoblot analysis (Western blot; WB) and luciferase immunoprecipitation system (LIPS) (Levenhagen and Costa-Cruz, 2014). Their diagnostic sensitivity and specificity have been reviewed by Requena-Méndez et al. (2013) and Levenhagen and Costa-Cruz (2014) as follows;

(1) Immunofluorescence antibody test (IFAT)

The technique employs filariform larva as antigen and serum antibodies bind to surface or internal organs are visualized by fluorochrome-labelled anti-human immunoglobulin. Binding is visualized under the fluorescent microscope. Serum antibody can be quantitated by serum dilution and presented as antibody titer. This technique has demonstrated high sensitivity and specificity, with minimal cross-reactivity with sera from patients that were positive for other helminthic infections. The titer ≥ 20 is best used for screening of an infection.

(2) Enzyme-linked immunosorbent assay (ELISA)

The ELISA technique is extremely useful and comprises one of the main methods used in the diagnosis of infectious and parasitic diseases. The technique used enzyme instead of fluorochrome and reaction is measured by colorimetry which is a result from substrate-enzyme interaction. Quantitation of serum antibody level is reflected by optical density measured by commercially available machine. This technique is considered to be superior to other serological tests regarding its practicality, automation and the availability of reagents. However, despite the high level of sensitivity, one of the greatest difficulties faced in developing these tests is the possibility of cross-reactivity with other helminth infections. Nevertheless ELISA has been reported to have up to 98% sensitivity and 100% specificity. Since antigen preparation and availability is of great obstacle for sustainable use of the assay, a 31 kDa recombinant antigen (named NIE) was developed from an *S. stercoralis* L3 cDNA library that demonstrated positive and negative predictive values of 88% and 99% to detect IgG, and 100% and 64% to detect IgG4 (Ramanathan et al., 2008). Alternatively, ELISA using *S. ratti* as antigen gave ELISA sensitivity of 76.6 % compared with 75.7 % of ELISA using *S. stercoralis* antigen (Eamudomkarn et al., 2015). Fractionated soluble antigens from *S. venezulensis* female adult

worms up to 85% sensitivity and 95.8% specificity (Corral et al., 2015). Antigen of *S. venezulensis* larvae has been reported to give better sensitivity (96.7%) and specificity (100%) in serum IgG-ELISA (Bosqui et al., 2015). Synthetic peptides have been proposed for use in serodiagnosis with 93.3% efficiency (Feliciano et al., 2016). The ELISA method has been useful in seroepidemiological surveys in different populations, including the detection of infection in immigrants, refugees and travelers in developed countries, due to the increase in the number of cases in this group of people (Ramanathan et al., 2008). Moreover, the method could be applied as a measure for assessment of treatment and outcome of intervention program (Vargas et al., 2017). In one study, seroprevalence fallen from 21% to 5% six months after mass drug administration (Kearns et al., 2017). Commercially available ELISA kits showed 70% sensitivity and 100% specificity (Bisoffi et al., 2014).

(3) Immunoblotting

Immunoblotting assays are also useful in the immunodiagnosis of human strongyloidiasis as a complementary method demonstrating high levels of sensitivity and specificity, such as ELISA. For this assay, it is necessary to apply an antigen or recombinant protein, such as surface antigens or excretion/secretory products from infective larvae of *S. stercoralis*, in an SDS-PAGE gel and subsequently transfer the bands to a nitrocellulose membrane using a transfer vessel. Early experiments revealed 29-kDa and 33 kDa protein bands reacting to strongyloidiasis sera (Sudré et al, 2007). Subsequent study showed that two polypeptide bands of approximate molecular mass of 26 and 29-kDa were potential markers. A sensitivity of 90 and 80 %, and a specificity of 76.5 % and 92.2 % were observed with the 26-kDa and 29-kDa band, respectively (Rodpai et al, 2016).

(4) Luciferase immunoprecipitation system (LIPS)

The LIPS technique uses recombinant antigens of 31 kDa (NIE Ag) obtained from a cDNA library of infective *S. stercoralis* larvae and/or employing the immunoreactive antigen of *S. stercoralis*. LIPS is a technology that can directly identify antibodies in serum, specific to antigens, and for generating a quantitative profile of the antibody response. LIPS-IgG assay gave 97% sensitivity and 100% specificity (Ramanathan et al., 2008).

(5) Dipstick assay

Van Doorn et al (2007) developed a dipstick technique for the diagnosis of this parasitosis, which showed high diagnostic accuracy with 91% sensitivity and 97.7% specificity. The assay has advantages over others in terms of practicality, simplicity and the use of small amounts of antigen. Use of filariform larva extract as antigen poses limitation to application of dipstick for serodiagnosis and serological survey.

(6) Gelatin particle agglutination test (GPAT)

Sato et al (1991) developed a gelatin particle agglutination (GPAT) test for mass examination for strongyloidiasis. The test was performed in 1,199 individuals in Sashiki Town, Okinawa Island. Among those who were seropositive, 41.7% had larva in feces. The test was simple to perform and not complicated procedures. A survey in communities of northeastern Thailand along with ELISA and APC showed sensitivity was 81% by GPAT, while that by ELISA was 73%. But, the specificity of GPAT was 74%, which slightly lower than that ELISA (86%) (Sithithaworn et al., 2005).

Coproantigen detection

Ideally, parasite antigens in stool, coproantigen, should be present in fecal samples of individuals having intestinal parasitic infections. Detection of coproantigen can

provide diagnosis as well as response to treatment. Nageswaran et al. (1994) demonstrated in experimental S. ratti/rat model that capture ELISA using rabbit antibodies against adult and larva somatic antigens could detect coproantigen. The positivity coincided with patency of infection and cross reaction was not detected with hookworm infection. Sykes and McCarthy (2011) raised antibodies against *S. ratti* excretory/secretory (E/S) antigen and used in coproantigen ELISA. The assay was positive with three diluted formalin extract of stool samples from three strongyloidiasis patients. El-Badry et al. (2009) prepared a rabbit hyperimmune serum against adult *S. stercoralis* excretory/secretory (ES) antigen applied and used in sandwich ELISA to capture *S. stercoralis* coproantigen from infected patients. The assay was without cross-reactions with the *Capillaria philipinensis* or with the *Schistosoma mansoni* and *Fasciola gigantic*a. The method could provide an easy and inexpensive technique, although more studies are needed on its performance for the diagnosis of strongyloidiasis.

Molecular diagnostic techniques

Molecular technique for detection of parasite in biological samples has a widespread use nowadays particularly the polymerase chain reaction (PCR). An epidemiological survey in north-western Ethiopia school-aged children showed that strongyloidiasis was detected 13.4% by PCR, 12.1% by the Baermann and 3.5% by FECT technique (Amor et al., 2016). In another study, PCR was positive in 29.9% of first stool specimen of individuals which was higher than 27.4% positivity obtained from conventional coproparasitological techniques (Repetto et al., 2016). Use of PCR for follow up after ivermectin treatment showed that patients with negative stool examination by conventional techniques were still positive by PCR (Repetto et al., 2018). PCR in many cases, however, did not achieve 100% sensitivity when applied to parasitologically proven specimens (Sitta et al., 2014; Paula et al., 2015). Interestingly, PCR can detect worm

DNA in urine specimens. A survey revealed 28% of stools of individuals of northern Argentina positive by fecal examination techniques excluding APC. Urine-PCR, however, gave 44.8% positive rate. Among stool positive individuals, 4.6% were urine-PCR negative. Conversely, half of individuals with urine-PCR positive were positive by stool examination (Lodh et al., 2016).

Real-time PCR assay was developed targeting a small subunit rRNA gene for DNA detection from *S. stercoralis* in stool samples (18S rRNA and 28S rRNA) (Kramme et al., 2011; Verweij et al., 2009). Janwan et al. (2011) have designed duplex real-time PCR to detect different intestinal parasites which have resulted in high specificity and with a higher sensitivity than conventional parasitological methods (Janwan et al., 2011). In a survey, sensitivity and specificity of real-time PCR were 88.9% and 92.7% as compared to the combination of the Baermann and APC (Schär et al., 2013). Superiority of real-time PCR in terms of sensitivity comparing to other conventional stool examination techniques was also evident in laboratory specimens. The sensitivity as compared to a combination of all diagnostic techniques was 21.4%, 37.5% and 76.8% for APC, the Baermann and real-time PCR, respectively (Becker et al., 2015). Real time PCR due to its high sensitivity could decreases the number of serial stool samples necessary to confirm a diagnosis (Dacal et al., 2018). Nested PCR has been developed and reported by Sharifdini et al. (2015) to have 100% sensitivity. In their study 12.7% and 18.2% of stool samples were found positive for *S. stercoralis* by FECT and APC, while 18.9% and 25.1% were positive by real-time PCR and nested PCR, respectively.

Molecular techniques not only provide detection of *Strongyloides* in specimens, but also allow accurate identification of *Strongyloides* species, regardless of the development stage. Larva in sputum could be identified as *S. stercoralis* using PCR on *cox*1 gene and 18S rRNA sequence followed by DNA sequencing (Wang et al., 2017).

VIII. Treatment of human strongyloidiasis

The aim of treatment in most worm infections is to reduce the number of worms to the point where the infection is unlikely to cause disease. Since the parasites are often difficult to detect in the first place, the problem is compounded frequently by uncertainty in determining whether or not they have all been eradicated. *Strongyloides stercoralis* is relatively resistant to anthelmintics and most attention has focused on benzimidazole agents and ivermectin (Grove, 1996).

For, benzimidazole agents belonging to this class of anthelmintics appear to act by binding totubulin and disrupting the assembly of microtubules, and by altering transmembrane proton discharge (Grove, 1996). These benzimidazoles not only kill adult gut dwelling stages of the parasite but also sterilize the larvae and eggs to some extent (Puthiyakunnon et al., 2014). Drugs in this group include, thiabendazole, mebendazole and albendazole, they are used for the treatment of acute and chronic strongyloidiasis but showed varied results in many drug trials (Puthiyakunnon et al., 2014). Albendazole has a high affinity binding capacity to free beta-tubulin in parasite cells, thereby inhibiting tubulin polymerization. This eventually results in loss of cytoplasmic microtubules and thus decreases adenosine triphosphate (ATP) production in worms, ultimately leading to energy depletion, immobilization, and death. Mebendazole inhibits microtubule formation and causes worm glucose depletion but shows variable efficacy against strongyloides. Thiabendazole was a therapeutic option for strongyloidiasis for quite a long time but has been discontinued due to its unfavorable side effects (Gann et al., 1994).

Ivermectin is the drug of choice for acute and chronic strongyloidiasis in intestinal stages, hyperinfection syndrome, and disseminated strongyloidiasis. This drug is a semi-synthetic

derivative of the macrolide mold product avermectin (Gann et al., 1994; Grove, 1996), which binds selectively to glutamate gated chloride ion channels of invertebrate nerve and muscle cells, thereby increasing the cell membrane permeability with hyperpolarization and causing paralysis and cell death. The drug is commonly administered as an oral preparation. Underlying reviewed of Greaves et al. (2013), that four studies have compared the efficacy of a single oral dose of 200 µg/kg of ivermectin with two oral doses of 200 µg/kg given either on consecutive days or two weeks apart (Greaves et al., 2013). Only one study showed a greater efficacy of two doses (100% cure) over a single dose (77% cure) whereas the other three showed comparative efficacy (>93%) for both regimens. Additionally, ivermectin is generally well tolerated with few side effects (Greaves et al., 2013; Puthiyakunnon et al., 2014).

IX. Prevention and Control

Strongyliodes stercoralis infects human by skin penetration mostly in soil transmitted parasites endemic area. Activity must be done to avoid contact with infective soil, fecal materials or contaminated surface water. All proven infective case should be treated and repeatedly treatments in order to protect internal or perianal autoinfection before treatment with immunosuppresstive drugs i.e. steroid treatment. Control of strongyliodiasis is distinctly dependent with the improving economic cases with implementation of good human waste disposal systems and reliable water supplies. Infection is likely to disappear from a community with improving socio economic status (Grove 1996). Mass chemotherapy is not the good method for community control, however, the targeted therapy directed at people that at risk of infection is suitable (Conway 1995). Direct treatment of water supply for agriculture possible be helpful (Grove 1996). (See more information in Chapter 2)

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Chapter 2

Prevention control model of *Strongyloides stercoralis* infection among rural communities in northeast, Thailand

Pokkamol Laoraksawong[1], Oranuch Sanpool[2,3], Rutchanee Rodpai[2,3], Tongjit Thanchomnang[3,4], Wanida Kanarkard[5], Wanchai Maleewong[2,3], Ratthaphol Kraiklang[1], and Pewpan M. Intapan[2,3]

[1]Department of Public Health Administration, Health Promotion, Nutrition, Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand

[2]Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

[3]Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University, Khon Kaen, Thailand

[4]Faculty of Medicine, Mahasarakham University, Mahasarakham, Thailand

[5]Department of Computer Engineering, Faculty of Engineering, Khon Kaen University, Khon Kaen, Thailand

Abstract

Background: Strongyloidiasis is prevalent in the Thailand, including northeast region. This study aimed to evaluate the impact of health education and preventive equipment package (HEPEP) on prevention of *Strongyloides stercoralis* infection among rural communities in northeast, Thailand.

Methods: This study was an intervention trial was conducted in populations of 12 villages (6 interventions and 6 controls) in rural communities in northeast, Thailand, during March 2016 to September 2017. Single stool sample was collected from each participant and examined by agar plate culture technique (APC). Each participant was interviewed with a pre-tested questionnaire. All participants were then treated with single dose ivermectin (200 µg/Kg) and allocated into intervention and control group. The intervention group was provided 1) "The practice to prevent strongyloidiasis" poster, *S. stercoralis* and strongyloidiasis advertising vinyl boards, and lectured *S. stercoralis'* life cycle via poster before treated with ivermectin; 2) the protective equipment package; and 3) the participants was reminded about health educations and using equipment monthly by village health volunteers (VHVs), while the control group was provided only lecture of strongyloidiasis about five minutes. Assessment for new infection was conducted three months later, including 327 and 318 participants in intervention and control groups, respectively).

Results: The HEPEP had 59% of efficacy in preventing *S. stercoralis* infection in intervention group more than the control group (aOR= 0.59, *P-value* = 0.005). The intervention group had knowledge scored significantly higher on all aspects of a test of *S. stercoralis* knowledge compared with control group (mean dif. = 7.19, *P-value* = <0.001).

Conclusions: The HEPEP was the first effective model to control *S. stercoralis* transmission among a rural community in northeast, Thailand. The results should encourage policy makers and public health personnel to improve control programs for parasites as well as health promotion.

Keywords: *Strongyloides stercoralis,* Health education and preventive equipment package, Thailand

Background

Human strongyloidiasis caused by infection with a nematode parasite in genus *Strongyloides* is a one important public health problem in the world, especially tropical and sub-tropical countries (Grove, 1996; Prasongdee et al., 2017). Currently, an estimate of 100 million people is infected with *Strongyloides stercoralis* worldwide (Jourdan et al., 2017; WHO 2015). *Strongyloides stercoralis* has a unique life cycle including free-living life cycle, parasitic life cycle and autoinfection (Jourdan et al., 2017; Olsen et al., 2009; Puthiyakunnon et al., 2014). Autoinfection life cycle together with asymptomatic nature of chronically infected persons enable the parasite to persist in human and environment (Toledo et al., 2015).

Thailand is a country in tropical region which has a suitable environment for soil-transmitted helminths including strongyloidiasis. In northeastern region, the prevalence of *S. stercoralis* infection from community surveys ranged from 2.5% to 33.3% (Jongsuksuntigul et al., 2003; Jongwutiwes et al., 2014; Nontasut et al., 2005; Prasongdee et al., 2017; Sithithaworn et al., 2005; Sithithaworn et al., 2003;Wongsaroj et al.,2014). Moreover, an eleven-year retrospective hospital-based study showed prevalence of infection range from 11.0% to 24.3%

(Prasongdee et al., 2017). Accordingly, strongyloidiasis is among helminthiases of public health importance in the Thailand, and integrated approach in prevention and control should be developed and implemented including screening, mass treatment, and health education (Prasongdee et al., 2017). It was recommended that developed strategies should incorporate delivery of multiple interventions to maximize sustainability of control programs (WHO, 2012). This paper aimed to evaluate the impact of health education and preventive equipment package (HEPEP) on prevention of *S. stercoralis* infection among rural communities in northeast, Thailand.

Methods

Study design

This study was an open-label controlled trial which aimed to evaluate the impact of health education and preventive equipment package on prevention and control of *S. stercoralis* infection among communities in northeast, Thailand, during March 2016 to September 2017. Participants from one area serve as experimental while those from nearby area serve as control group.

Study area and study population

This study was carried out in two areas of Kalasin province, northeastern Thailand (1) Nong Bua sub-district, Nong Kung Si district (Intervention group) and (2) Phu Din sub-district, Mueang Kalasin district (Control group),. Both areas are located near Lam Pao dam. Nong Bua sub-district is located at 16.716733° latitude and 103.383900° longitude and Phu Din sub-district is located at 16.643328° latitude and 103.517948° longitude (Figure 1). People in both areas are

agriculturists, i.e., rice field and cassava, sugarcane, and Para rubber farms (Office of Agriculture Economics, 2016). The two areas were selected purposively based on data from previous studies showing that the province had a high prevalence of strongyloidiasis (Jongsuksuntigul et al., 2003; Yahom et al., 2013). The sample size was determined using STATA Version 10.1 (College Station, Texas: StataCorp LLC) by command "clustersampsi, binomial sample size". It was calculated from the prevalence rate (p_1) of 23.0% from a previous study (Jongsuksuntigul et al., 2003), the prevalence rate after added intervention (p_2) of 10.0% with a 95% confidence interval ($Z^2_{\alpha/2} = 1.96$), 80% confidence interval ($Z_\beta = 0.84$), and design effect = 2. The calculated sample size was 300 per area. We assumed that the final sample size would be reduced by around 20% due to unavailability of stool on the day of collection and thus the sample size was adjusted to 360 per area. The simple random sampling method was used to select subjects from each sub-district. They were given plastic containers for stool collection with instructions. Subject inclusion criteria were 1) the residents of Nong Bua or Phu Din sub-district; 2) age $\geq$ 20 years old. Exclusion criteria were 1) recently migrating from other areas 2) drop out from the study. Eventually, a total of 689 populations returned stool specimens, which consisted of 349 from 6 villages in intervention group and 340 from 6 villages in control group (Figure 1).

Baseline Data collection and empirical methods

The baseline data collection included screening eligibility and diagnosis for *S. stercoralis* infection, and measuring knowledge and behaviors regarding *S. stercoralis* infection using questionnaires. The collection of data surrounding demographic, socioeconomic and environmental factors was conducted between January and May 2017. The intervention study for

evaluating the package was conducted later between June and September 2017-a 3 month assessment and follow-up.

Questionnaire survey

After giving written consent, research participants were interviewed face-to-face in their homes using 2-part questionnaires consisting of 15 questions. The first part consisted of demographic, socioeconomic and environmental data, habits and health status, and the second part revolved around the knowledge regarding *S. stercoralis*. The questions in the second part were designed to test the understanding of respondents on the subject of *S. stercoralis* (biology, transmission, symptom, prevention and control). The knowledge scores was translated following Bloom et al., (1971); high knowledge: 13-15 scores or >80.00%, medium knowledge: 10-12 scores or 60.01 - 79.99%, and low knowledge: 0-9 scores or 0.00 to 60.00%.

Stool examination

Stool samples were collected at baseline, 21-28 days after treatment and 3 months. Clean plastic containers labeled with the participants' name and code number was distributed to all participants by VHVs in each villages. On the following day the full container was returned to the field staffs that performed agar-plate culture (APC) as described by Koga et al. (1991) for detection of *S. stercoralis* infection. The plates were transported to Parasitology Laboratory, Faculty of Medicine, Khon Kaen University and observed under a stereo microscope by qualified parasitologist. Negative result was record when *S. stercoralis* was not detected within 5 days of culture.

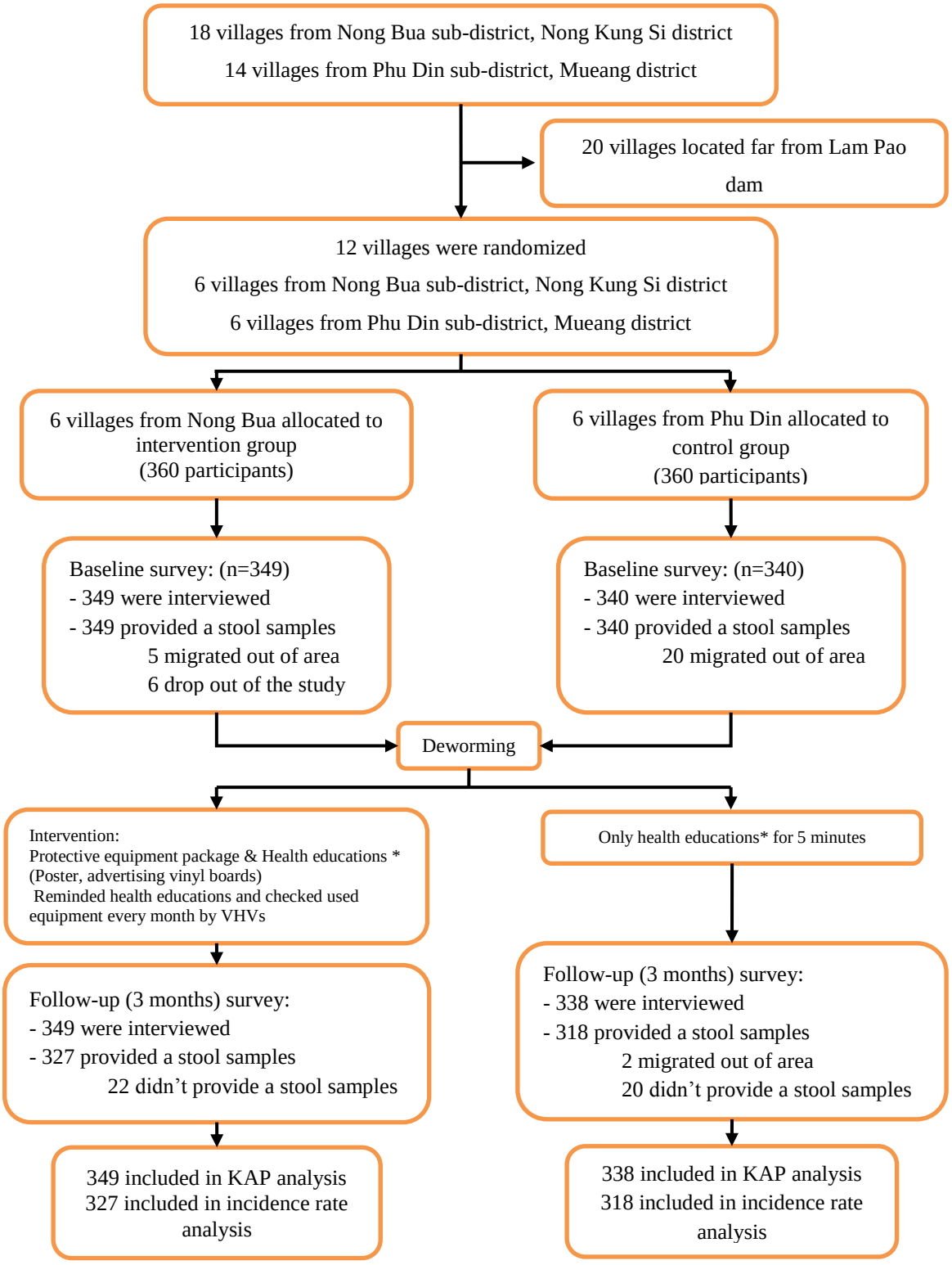

Figure 1. Flow chart of the study's activities and follow up; *Health education program different between intervention group and control group. KAP: A Knowledge, Attitude and Practices; VHVs: Village health volunteers.

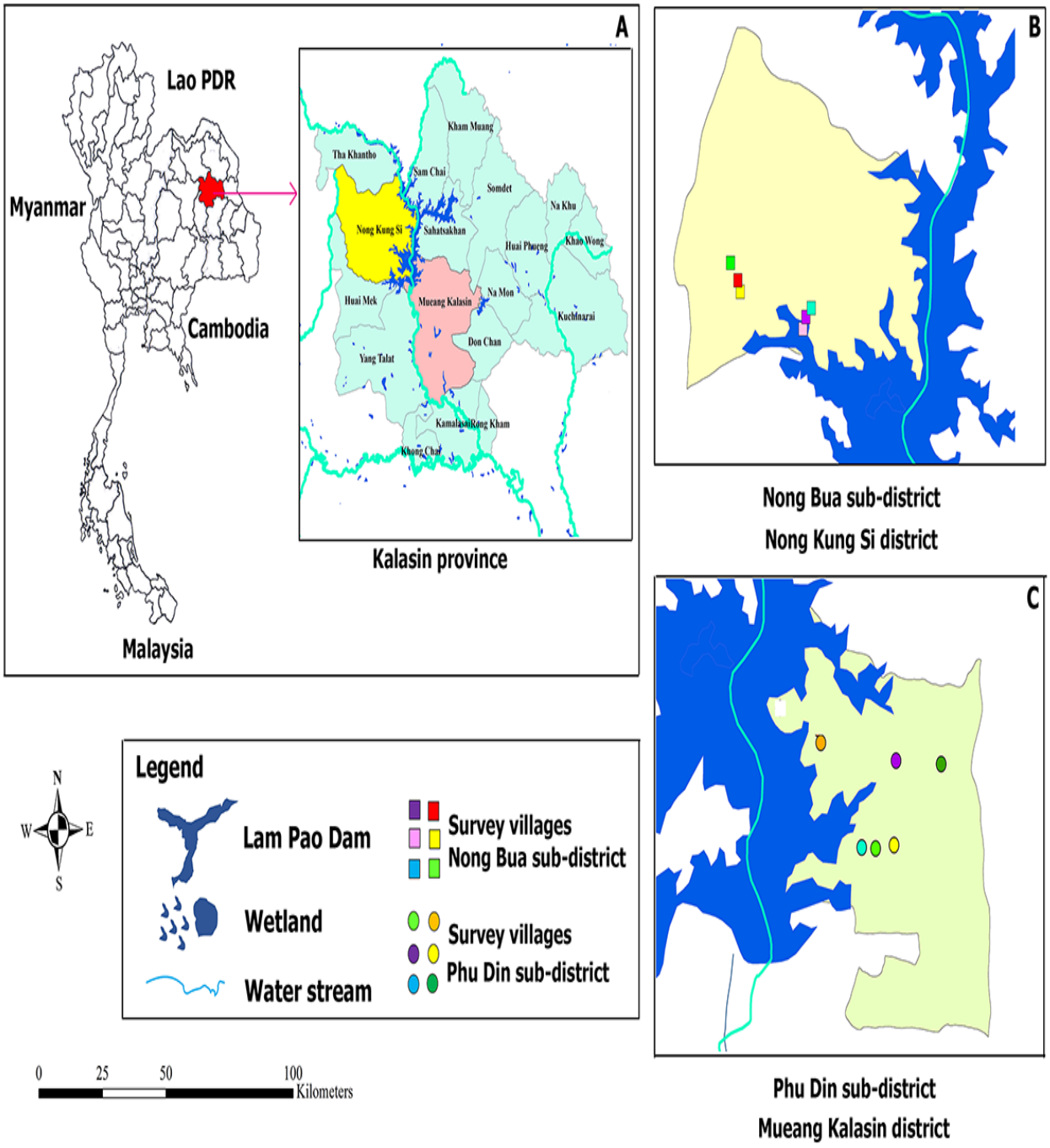

Figure 2. A geographic map showing Kalasin Province northeast Thailand and location of the selected villages in Nong bua and Phu Din sub-districts.

Deworming

Research participants who were infected with *S. stercoralis* as demonstrated by APC received a single dose of 200 µg/kg ivermectin tablets (Atlantic Laboratories Corporation Ltd., Samut Prakan, Thailand).

Preventive equipment package and follow up

The preventive equipment package was then only provided to participants in the intervention group but health education was provided to participants in both groups. Intervention group was provided the full health education of *S. stercoralis* to participants consisted of "The practice to prevent strongyloidiasis" poster (size 29 X 40 cm) to promote in participants' house (supplement figure 1), "*S. stercoralis* and strongyloidiasis advertising vinyl boards" (size 2 x 3 m) to promote in each village of this group (supplement figure 2), and "*S. stercoralis* life cycle poster" (90x120 cm) (supplement figures 3 and 4) with lecture given to participants about 20 minutes in this group. Subsequently, intervention group was reminded health education every month and checked used equipment every month by village health volunteers (VHVs). The control group was provided with health education of *S. stercoralis* infection including only a lecture of human strongyloidiasis about 5 minutes. The participants from both areas being recalled for follow up on *S. stercoralis* examinations and interviewed over the next 3 months (Figure 1). The follow up of HEPEP activities were performed regularly by visiting at the farm and villages monthly by researchers and VHVs and observed the participants' practice of wearing shoes and gloves while working in theirs farms (supplement file 1). For health education, the VHVs reminded the

participants about *S. stercoralis* transmission via broadcast tower in each village every month (Figure 2).

Statistical analysis

The frequency, percentage, mean, and standard deviation (SD) were used described a demographic characteristics. The prevalence of *S. stercoralis* infection used proportions and 95% CI for description. To investigate the impact of health education and protective equipment package on *S. stercoralis* infection, the prevalences of *S. stercoralis* infection were compared between the intervention group and control group by using logistic regression and generalized estimating equation (GEE). To investigate the impact of health education and protective equipment package on knowledge score in each group, the knowledge score was compared between the baseline and 3 months by using pair *t*-test. To investigate the impact of health education and protective equipment package on behavior in each group, the behavior was compared between the baseline and 3 months assessment by using pair McNemar's test. Additionally, the knowledge score was compared between intervention and control group by using *t*-test. A *P-value* less than 0.05 were considered statistically significant. The statistical analysis was done by the STATA package version 10.1. (College Station, Texas: StataCorp LLC).

Ethical consideration

This study was approved by the Khon Kaen University Ethics Committee for Human Research (HE601048). All participants were informed of the study methods, risks, and benefits of the

process. Written and signed or thumb-printed informed consent was obtained to conduct the study from participants before starting the study.

Results

Demographic characteristic

A total of 689 study participants, comprising 349 from Nong Bua sub-district (intervention group) and 340 from Phu Din sub-district (control group), were enrolled in the study. Of participants 323 (46.88%) were male and 366 (53.12%) were female, mean of age (±SD) was 51.19 (±12.04) year-olds (range = 20–78 years). 467 of participants (67.77%) graduated from primary school and 472 (68.51%) were agriculturists. Most of participants 370 (53.70%) had normal body mass index (BMI) (18.5 to 24.9) and had household income lower than $454.54 per month (Exchange Rates as of 1 Nov 2017) 633 (91.87%), average household income (±SD) was $167.54 (±214.15) (range = 0-1757.58$). Most of participants 495 (71.84%) were healthy and 618 (89.70%) married. Fourteen participants (2.03%) observed larva currens symptom appeared on their skin. For residential environment, the participants reported a damp soil around their houses in 441 (64.01%) and had a pet in 494 (71.70%). Most of participants 688 (99.85%) used cesspool and professional toilet personnel for feces management (Table 1). For knowledge levels, 278 (40.35%) participants had a good knowledge and average knowledge scores was 71.69 ± (14.48) (range = 0-100 scores). The participants in the intervention group had an average knowledge scores of 73.81 (±11.11) (range = 40-100 scores). The control group had an average knowledge scores of 69.51 (±17.03) (range = 0-93.33 scores) (Table 1). For behaviors, most of participants 644 (93.46%) directly contacted soil and 423 (65.68%) contacted soil in their farm area. Additionally, 540 participants (78.37%) used animal dung as fertilizer and 155 (22.5%) had

used steroid drug. Importantly 508 participants (73.73%) defecate in environment (Table 1). Differences in age, occupations, underlying diseases, had a pet at house, area of direct contact with soil, and using animal dung fertilizers were significant between the intervention area and the control area (Table 1).

Prevalence of S. stercoralis infection at baseline

Overall 226 (32.80%) of participants were found to be positive for *S. stercoralis* infection by APC (Figure 3). Positive rate was higher in male, (21.92%), than in female, (10.88%). Peak infection rate was at 40-59 year-olds age group 19.30% (Figure 3). The baseline prevalence of *S. stercoralis* infections in intervention group and control group were comparable, being 31.23% and 34.41%, respectively, with no statistically significant difference (Figure 3).

Impact of health education and preventive equipment package on the prevalence of S. stercoralis at 3 months assessment

After treatment, all participants were examined for the presence of *S. stercoralis* infection in third month by APC. The prevalence of *S. stercoralis* infections in intervention group and control group were 2.75% and 6.60%, respectively (Figure 4). There was statistically significant difference in the prevalence of *S. stercoralis* infection between intervention group and control group. The health education and protective equipment package can reduce 40% of *S. stercoralis* infection in the intervention group (cOR= 0.40, 95%CI: 0.18 to 0.89, *P-value* = 0.02). For multivariate analysis, the health education and protective equipment package can reduce 59% of *S. stercoralis* infection in the intervention group (aOR= 0.59, 95%CI: 0.41 to 0.85, *P-value* =0.005) (Figure 4, Table 2).

Table 1 Baseline characteristic of participants in the intervention (HEPEP) and control group

Variables	Intervention (n= 349)	Control (n= 340)	Total (n= 689)	P-value for tests of between-group differences
	Number (%)	Number (%)	Number (%)	
Individual characteristic				
Gender				0.058
Male	176 (50.43)	147 (43.24)	323 (46.88)	
Female	173 (49.57)	193 (56.76)	366 (53.12)	
Age				0.0001
Maen ±SD (Min:Max)	49.40±11.81 (20:78)	53.03±12.01 (20:87)	51.19±12.04 (20:87)	
Education levels				0.177
Graduated or higher	14 (4.01)	17 (5.00)	31 (4.50)	
Diploma	7 (2.01)	8 (2.35)	15 (2.18)	
Grade 10-12	56 (16.04)	37 (10.88)	93 (13.50)	
Grade 7-9	36 (10.32)	32 (9.41)	68 (9.87)	
Primary school	232 (66.47)	235 (69.12)	467 (67.77)	
unlearned	4 (1.15)	11 (3.24)	15 (2.18)	
Occupations				<0.0001
Trade/owner business	28 (8.02)	103 (30.29)	131 (19.01)	
Government/private officer	13 (3.72)	21 (6.18)	34 (4.93)	
Students	1 (0.29)	1 (0.29)	2 (0.29)	
Agriculture	298 (85.39)	174 (51.18)	472 (68.51)	
other (Elder/House wife)	9 (2.58)	41 (12.06)	50 (7.26)	
BMI				0.089
<18.50	19 (5.44)	31 (9.12)	50 (7.26)	
18.50 to 24.99	199 (57.02)	171 (50.29)	370 (53.7)	
25.00 to 29.99	108 (30.95)	121 (35.59)	229 (33.24)	
≥30.00	23 (6.59)	17 (5.00)	40 (5.81)	
Mean ±SD (Min : Max)	24.10±3.81 (15.06 : 36.72)	23.94±4.07 (13.12 : 44.82)	24.02±3.94 (13.12 : 44.82)	0.6081
Household income ($)				0.703
<454.54 $	322 (92.26)	311 (91.47)	633 (91.87)	
≥454.54 $	27 (7.74)	29 (8.53)	56 (8.13)	
Mean ±SD (Min:Max)	160.42±199.15 (0 : 1696.97)	174.86±228.58 (0 : 1757.58)	167.54±214.15 (0 : 1757.58)	0.3767
Marital status				0.71
Single	14 (4.01)	16 (4.71)	30 (4.35)	
Married	312 (89.40)	306 (90.00)	618 (89.70)	
Devoted	23 (6.59)	18 (5.29)	41 (5.95)	
Underlying diseases				0.006
No	267 (76.50)	228 (67.06)	495 (71.84)	
Yes	82 (23.50)	112 (32.94)	194 (28.16)	
Larvae currens				0.961
No	342 (97.99)	333 (97.94)	675 (97.97)	
Yes	7 (2.01)	7 (2.06)	14 (2.03)	

Table 1 Baseline characteristic of participants in the intervention (HEPEP) and control group (Conts.)

Variables	Intervention (n= 349)	Control (n= 340)	Total (n= 689)	*P*-value for tests of between-Group differences
	Number (%)	Number (%)	Number (%)	
Residential environment				
Has damp soil around house area				0.372
No	120 (34.38)	128 (37.65)	248 (35.99)	
Yes	229 (65.62)	212 (62.35)	441 (64.01)	
Ever flooding in area				0.834
No	341 (97.71)	333 (97.94)	674 (97.82)	
Yes	8 (2.29)	7 (2.06)	15 (2.18)	
Have a pet in house				<0.0001
No	125 (35.82)	70 (20.59)	195 (28.30)	
Yes	224 (64.18)	270 (79.41)	494 (71.70)	
Type of toilet				0.311
Cesspool	349 (100.00)	339 (99.71)	688 (99.85)	
Pit latrines	0 (0.00)	1 (0.29)	1 (0.15)	
Feces management				0.311
Professional toilet personnel	349 (100.00)	339 (99.71)	688 (99.85)	
fertilizer	0 (0.00)	1 (0.29)	1 (0.15)	
Knowledge scores*				
Bad (0.00 to 60.00)	59 (16.91)	85 (25.00)	144 (20.90)	0.007
Medium (60.01 to 79.99)	132 (37.82)	135 (39.71)	267 (38.75)	
Good (80.00 to 100.00)	158 (45.27)	120 (35.29)	278 (40.35)	
Mean ± SD (min:max)	73.81±11.11 (40.00:100.00)	69.51±17.03 (0:93.33)*	71.69±14.48 (0.00:100.00)*	<0.0001
Behaviors				
Directly contact soil*				0.099
No	17 (4.87)	28 (8.24)	45 (6.53)	
Yes	332 (95.13)	312 (91.76)	644 (93.46)	
Area of touch soil or barefoot walking*	n = 332	n = 312	n = 644	<0.0001
Owner house area	63 (18.98)	122 (39.10)	185 (28.73)	
Owner farm area	255 (76.81)	168 (53.85)	423 (65.68)	
Other person's farm	14 (4.22)	22 (7.05)	36 (5.59)	
Animal fertilize using				0.007
No	61 (17.48)	88 (25.88)	149 (21.63)	
Yes	288 (82.52)	252 (74.12)	540 (78.37)	
Steroid using				0.014
No	284 (81.38)	250 (73.53)	534 (77.50)	
Yes	65 (18.62)	90 (26.47)	155 (22.50)	
Excrete to environment				<0.0001
No	57 (16.33)	124 (36.47)	181 (26.27)	
Yes	292 (83.67)	216 (63.53)	508 (73.73)	

*Number of participants follows by the participants who directly contacted soil.

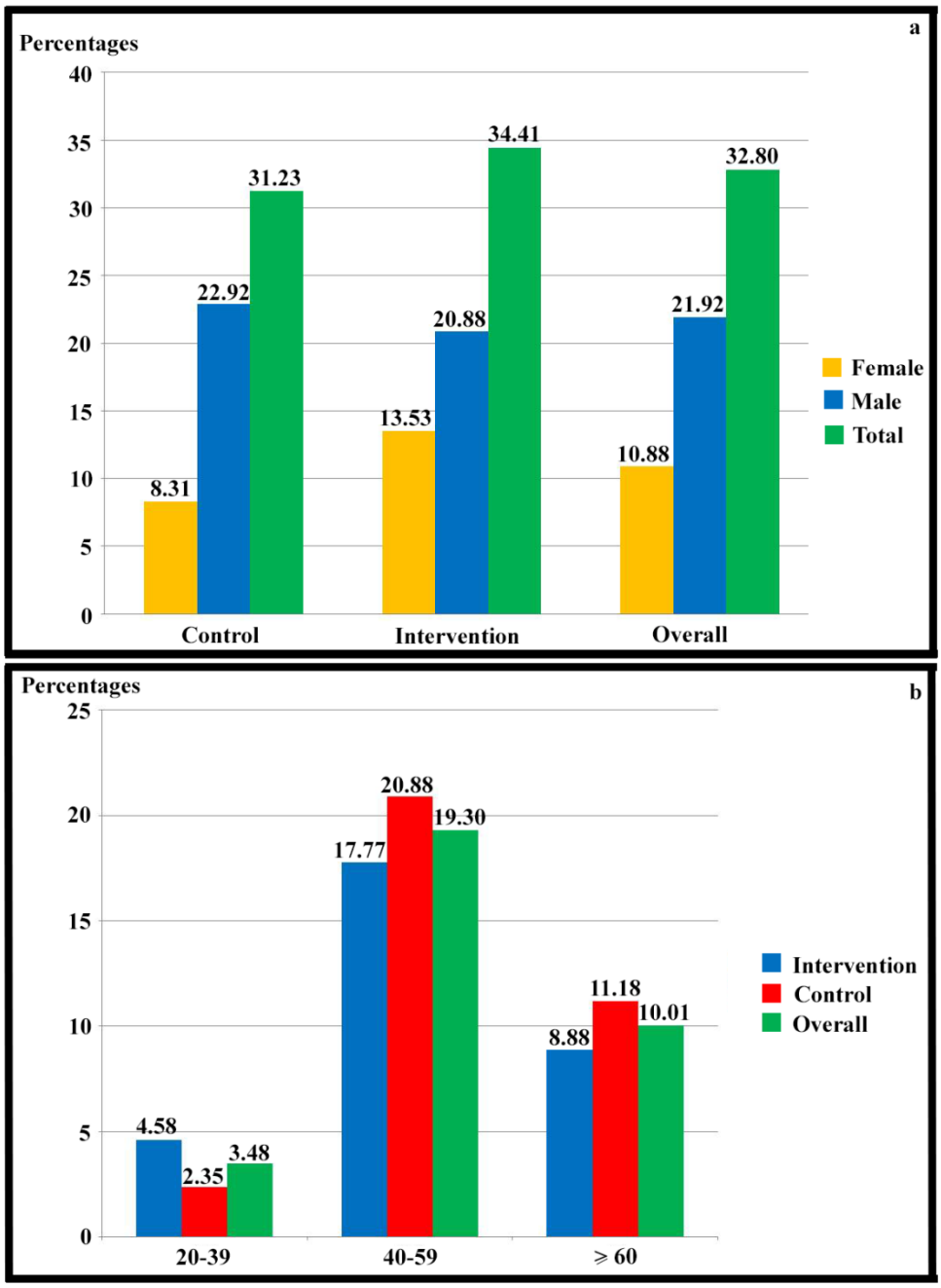

Figure 3 Prevalence of *S. stercoralis* infection at the baseline assessment a: prevalence of *S. stercoralis* infection at the baseline assessment classified by gender, b: prevalence of *S. stercoralis* infection at the baseline assessment classified by age groups

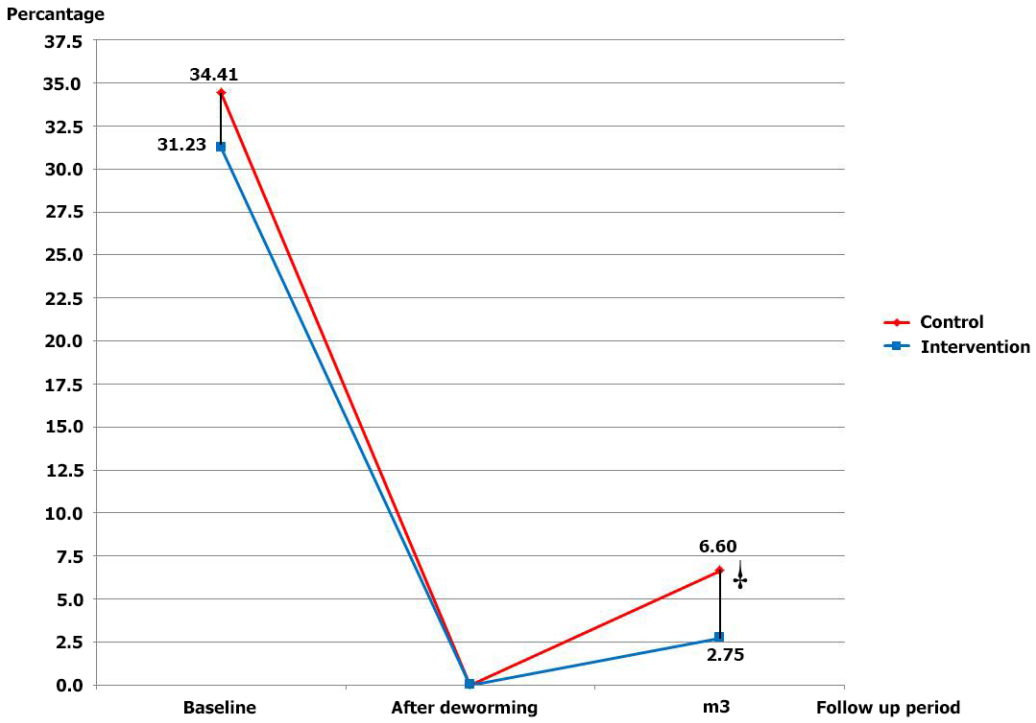

Figure 4 Prevalence and incidence of *S. stercoralis* infection among the intervention and control groups.

Statistically significant difference *P*-value <0.05.

Table 2 Effect of the health education and protective equipment package on prevalence of *S. stercoralis* infection at 3 months assessment in generalized estimating equation (GEE)

Outcome variable	Unadjusted	Adjusted
	cOR (95% CI)	aOR* (95% CI)
S. stercoralis prevalence	0.40 (0.18 to 0.89) **	0.59 (0.41 to 0.85) **

** Statistically significant difference *P*-value <0.05

Remark: Odds ratios adjusted for gender, age, education levels, occupations, household income ($), underlying diseases, ever appear larva currens, has a pet in house, ever directly contact soil, animal fertilizer, and baseline *S. stercoralis* prevalence.

Gender, age, and education were compared among two groups at 3 months. Statistically significant difference is as follows. Males were 2.74 times more likely to be infected than females (aOR 2.74; 95%CI: 1.94 to 3.88, *P-value* <0.001). For every one year of age increase in

the participants, there were about a two persons infected with *S. stercoralis* (aOR 1.02; 95%CI: 1.001 to 1.04, *P-value* 0.036). Participants with a primary school/no formal education were 4.14 times more likely, and those who had completed grades 7-12 were 4.22 times more likely to be infected than those with a diploma, bachelor degree, or higher to be infected *S. stercoralis* (aOR 4.14; 95%CI: 1.35 to 12.70, aOR 4.22; 95%CI: 1.37 to 12.97, *P-value* = 0.012, respectively) (data not shown).

Impact of health education and preventive equipment package on the knowledge of the participants in both groups

The knowledge of the participants from both groups was assessed at baseline and 3 months after starting the intervention study. The knowledge score in the intervention group at baseline and 3 months were 73.81 (±11.11) scores and 83.82 (±10.35) scores, respectively. There was significantly difference in the knowledge score between baseline and 3 months assessment [mean difference (mean dif.) = -10.01, 95%CI: -11.44 to -8.58, *P-value* = <0.0001] (Table 3). For control group, the knowledge score at baseline and 3 months were 69.51 (±17.03) scores and 76.63 (±13.02) scores, respectively. There was significantly difference in the knowledge score between baseline and 3 months assessment (mean dif. = -7.12, 95%CI: -9.12 to -5.12 *P-value* = <0.0001) (Table 4). For knowledge scores comparison between intervention and control groups, the intervention group has a knowledge score 83.82 (±10.35) higher than knowledge score 76.63 (±13.02) in control group. There was a significantly difference in the knowledge score between baseline and 3 months assessment (mean dif. = 7.19, 95%CI: 5.43 to 8.95, *P-value* = <0.0001) (Table 4).

Impact of health education and preventive equipment package on the behavior of participants at 3 months post-intervention

After starting intervention, the increased knowledge score in the participants in intervention group translated into behavioral change with regard to directly contact soil, use of animal dung fertilizer, use of steroid drug, and defecate into the environment (Table 3). In the intervention group, the participants were less likely to directly contact with soil (mean dif. = 8.88; 95%CI: 4.70 to 13.07), using animal dung fertilizer (mean dif. = 51.86; 95%CI: 45.76 to 57.96), use of steroid drug (mean dif. = 7.45; 95%CI: 2.26 to 12.63), and defecate into surrounding environment (mean dif. = 27.51; 95%CI: 21.56 to 33.45) which were significant statically compared with a baseline assessment (Table 3). In control group, the increased knowledge score in the participants after starting only health education translated into behavioral change with regard to directly contact soil, using animal dung fertilizer, steroid drug use, and defecate into the

Table 3 Behaviors factors at 3 months assessment after starting full health educations in intervention group and only lectured health education in control group

Behaviour	Intervention			Control			OR 95%CI Compare between intervention and control group at 3 months
	Baseline (n=349)	3 month (n=349)	difference between proportions	Baseline (n=338)	3 month (n=338)	difference between proportions	
	n (%)	n (%)	difference (95%CI)[a]	n (%)	n (%)	difference (95%CI)[b]	
Directly contacted soil							
Yes	332 (95.13)	301 (86.25)	8.88 (4.70 to 13.07) *	310 (91.71)	289 (85.50)	6.21 (1.75 to 10.68) *	0.94 (0.61 to 1.44)
Animal fertilizer using							
Yes	288 (82.52)	107 (30.66)	51.86 (45.76 to 57.96) *	250 (73.96)	124 (36.69)	37.27 (31.04 to 43.52)*	1.31 (0.95 to 1.78)
Steroid drug using							
Yes	65 (18.62)	39 (11.17)	7.45 (2.26 to 12.63) **	88 (26.04)	60 (17.75)	8.29 (2.68 to 13.88) **	1.71 (1.11 to 2.65) **
Excreted to environment							
Yes	292 (83.67)	196 (56.16)	27.51 (21.56 to 33.45) *	214 (63.31)	109 (32.25)	31.06 (24.79 to 37.34) *	0.37 (0.27 to 0.51) *

[a]Mean difference in intervention group between baseline and 3 months assessment after deworming using pair McNemar's test

[b]Mean difference in control group between baseline and 3 months assessment after deworming using pair McNemar's test

[c] Compare between intervention and control group at 3 months using chi-squared test.

* Statistically significant difference *P*-value <0.0001

Table 4 *Strongyloides stercoralis* knowledge scores at baseline and follow-up (3 months assessment post-deworming)

Variables	Intervention (n=349)	control (n=338)	Mean difference between group differences[a]
	Mean (SD)	Mean (SD)	Mean (95%CI)
Baseline assessment			
Knowledge scores	73.81 (11.11)	69.51 (17.03)	4.24 (2.15 to 6.45)*
3 months assessment			
Knowledge scores	83.82 (10.35)	76.63 (13.02)	7.19 (5.43 to 8.95)*
Mean difference between baseline and 3 months differences[b]	-10.01 (-11.44 to -8.58)*	-7.12 (-9.12 to -5.12)*	

* Statistically significant difference *P*-value <0.0001

[a] Compare knowledge scores between intervention group and control group using *t*-test

[b] Compare knowledge scores between baseline and 3 month assessment within intervention group and control group using Pair *t*-test

environment (Table 3). At follow-up in the control group, significant differences were detected in that participants were less likely to directly contact soil (mean dif. = 6.21; 95%CI: 1.75 to 10.68), animal dung fertilizer using (mean dif. =37.27; 95%CI: 31.04 to 43.52), steroid drug use (mean dif. = 8.29; 95%CI: 2.68 to 13.88), and defecate into the environment (mean dif. =31.06; 95%CI: 24.79 to 37.34) compared with a baseline assessment which were significantly statistic (Table 3). Additionally, steroid drug use and defecate into the environment differed significantly when compared behavior change between intervention group and control group at 3 month post-intervention (Table 3).

Discussion

This study evaluated the health education and preventive equipment package (HEPEP) on the interruption of transmission of *S. stercoralis* infection among a rural community in northeast, Thailand. At baseline, 32.80% of the participants were found to have *S. stercoralis* infection,

which was higher than in previous studies (Intapan et al., 2005; Nontasut et al., 2005; Sithithaworn, et al. 2005; Sithithaworn et al. 2003; Wongsaroj et al., 2014; Boonjaraspinyo et al., 2013; Kitvatanachai, Boonslip and Watanasatitarpa, 2008; Wongsaroj et al., 2008). The difference were contributed by variations in examination technique, environmental sanitation, socioeconomic factors, and education level of participants (Hotez et al., 2008; Hotez et al., 2006; Punsawad et al., 2917). People aged 40-59 year-olds had a 19.30% prevalence of *S. stercoralis* infection which was higher than other groups. Older adults are at major risk for *S. stercoralis* infection because they are continually exposed to sources of infection (Prasongdee et al., 2017; Wongsaroj et al., 2008).

At the three months assessment, the prevalence of *S. stercoralis* infection was decreased from 31.23% to 2.75% in the intervention group. In addition, the prevalence of *S. stercoralis* infection in control group (was provided only health education for 5 minutes) was decreased from 34.41% to 6.60%. For evaluating the efficacy of HEPEP in the intervention group and control group (only health education), it was found that the HEPEP had 59% efficacy in preventing *S. stercoralis* re-infection more than the control group. This result was similar to other studies in control of soil-transmitted in children (Al-Delaimy et al., 2014, Bieri et al., 2013, and Gyorkos et al., 2013). This study represented that the HEPEP was effective to decreasing the *S. stercoralis* infection. Additionally, this is the first effective model to control *S. stercoralis* in adults among a rural community in Thailand.

Notewithstandingly, participants in both groups had a flush latrine (cesspool) in their house (99.85%) but the prevalence of *S. stercoralis* infection was still high. Thus, sanitary improvement only appears not enough for reducing the prevalence of *S. stercoralis* infection because they are not always using a latrine, (Arfaa et al., 1977; Asaolu & Ofoezie, 2003). Most

of participants were agriculturist and they defecate into surrounding environment while working in their farm. In rural community as reported previously in Vietnam, Lao PDR, the presence of latrine alone is not sufficient to decrease the prevalence of helminthiasis if fresh feces are used as fertilizer (Yajima et al., 2009). Furthermore, the lack of knowledge regarding *S. stercoralis* transmission is an important factor promoting to *S. stercoralis* transmission among participants. This study showed that the knowledge of participants in intervention group (received HEPEP) at the 3 months assessment was higher than the knowledge of participants in control group 7.19 scores. Furthermore, the knowledge score was associated with the decreasing in the prevalence of *S. stercoralis* infection and changed their behaviors resulting in decreased infection, which was similar to the previous study (Al-Delaimy et al., 2014; Gyorkos et al., 2013).

The health education and preventing equipment package (HEPEP) was developed and distributed to rural communities in Kalasin province, northeast Thailand as the first heath education program to control *S. stercoralis* infection in this region. The HEPEP proved effective among these people, especially in terms of decreasing the prevalence of *S. stercoralis* infection. The HEPEP may also be useful model in controlling other intestinal parasites, especially hookworm infection in southern Thailand.

Despite the implementation of an intensive national parasite control program in rural areas of northeast Thailand decades ago, strongyloidiasis is still highly prevalent and was sympatric with opisthorchiasis (Boonjaraspinyo et al., 2013). The result of this study supports an urgent need to start an integrated and effective *S. stercoralis* control program using developed HEPEP and follow up in a long term

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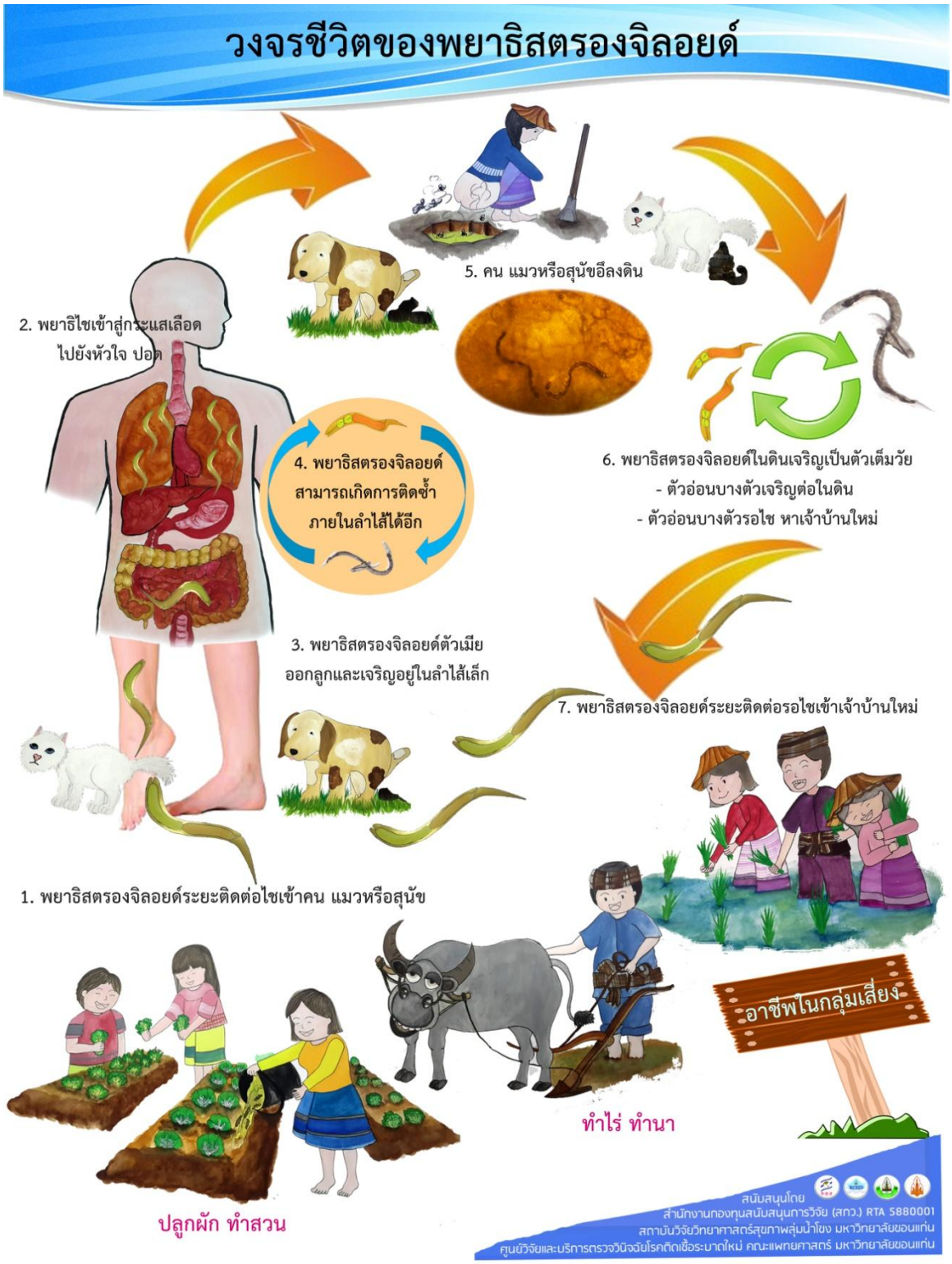
Supplement figure 1 a: equipment package (groves and boots), b: The practice to prevent strongyloidiasis" poster, c: lecturing of human strongyloidiasis by using *Strongyloides stercoralis* life cycle poster, d: *S. stercoralis and* strongyloidiasis advertising vinyl boards to promote in each village of this group, e and f: checked equipment using every month by VHVs and researchers.

Supplement figure 2 the practice to prevent strongyloidiasis" poster.

Supplement figure 3 *Strongyloides stercoralis* and strongyloidiasis advertising vinyl boards to promote in each village of this group.



Supplement figure 4 *Strongyloides stercoralis* life cycle poster