Prevalence of Intestinal Parasitic Infections in Military Personnel and Military Dogs, Thailand

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Objective: To determine the prevalence of intestinal parasitic infections and risk factors among military personnel and military dogs at the Military Dog Center, Veterinary and Remount Department, Royal Thai Army, Thailand.

Material and Method: A cross-sectional study was conducted in January 2006 to examine intestinal parasitic infections using wet preparation and, formalin-ethyl acetate concentration. Modified acid fast and gramchromotrope stains were used to identify Cryptosporidium spp. and microsporidia, respectively. Culture for Blastocystis was performed using Jone's medium. Genotypic characterization of Blastocystis and Giardia duodenalis were also determined using PCR-RFLP. To determine the risk factors and outcomes of intestinal parasitic infections, standardized questionnaires were used in the present study.

Results: Of 317 military personnel, the prevalence of intestinal parasitic infections was 22.4%. Blastocystis was the most predominant intestinal protozoa infection of 14.5 % while G duodenalis was only 1.3 %. The prevalence of other helminthic infections were 4.8% which were Strongyloides stercoralis (2.5%), Hookworm (1.0%), Opisthorchis viverrini (1.0%), and Taenia spp. (0.3%), respectively. Blastocystis subtype 1 was identified in 25 positive culture specimens while all 4 positive of G duodenalis were analyzed as Assemblage B, subgenotype IV. The presented data could not indicate that intestinal parasitic infections and blastocystosis in this army population were significantly linked to risk association among groups with regard to rank, age group, working unit, area of residence, animal contact, source and treatment of drinking water. Of 189 military dogs, the prevalence of intestinal parasitic infections was only 3.7% which was Blastocystis sp. (2.6%), S. stercoralis (0.5%), and Entamoeba coli (0.5%), respectively.

Conclusion: The predominant intestinal parasites found in this population, such as Blastocystis sp. and G. duodenalis transmit to humans via fecal-oral route so that improvement of sanitation and personal hygiene should be emphasized.

Keyword: Intestinal parasitic infection, military personnel, military dog, Thailand

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Parasitic infections in Thai military personnel still remain a public health concern in the army since the high prevalence of 53.9% and 55.7% were observed in the authors surveys in the military bases, central Thailand during 2000 and 2002, respectively^(1,2). The most prevalent protozoa found was *Blastocystis* infection followed by *Giardia duodenalis*. Others were

soil-transmitted helminthes (hookworm, *Strongylodes stercoralis*) and food borne trematode (*Opisthorchis viverrini*). Privates had significantly higher risk of acquiring parasitic infections than noncommissioned officers and officers⁽²⁾. For effective control program of intestinal parasitic infection, base-line data including the prevalence and risk factors of parasitic infections in military personnel in other regions of Thailand are required. The authors aimed to investigate the prevalence of intestinal parasitic infections among military personnel and military dogs at the Military

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Dog Center, Veterinary and Remount Department, Nakornrachsrima province, the frontier of the northeast region of Thailand. Since some of these parasites can transmit to humans from animals, the role of zoonotic transmission in this military base was determined. Associated risk factors were also analyzed using standardized questionnaires.

Material and Method

Study population

A cross-sectional study of intestinal parasitic infections was undertaken in January 2006 among military personnel and military dogs at the Military Dog Center, Veterinary and Remount Department, Royal Thai Army, Nakornrachasrima province, Thailand. Research protocol was approved by the Ethical Committee of Medical Department, the Royal Thai Army (no.138/2005). A total of 317 stool specimens were collected from military personnel including privates, noncommissioned officers, officers, and other employees, who voluntarily enrolled into the present study with the informed consent. Additionally, 189 stool specimens were also collected from dogs, trained for military missions at the Military Dog Center. These dogs were composed of German Shepherd, Labrador Retriever, Rottweiler, Belgian Malinois, Doberman, Dalmatian and others.

Stool collection and examination

Stool specimens were examined for intestinal parasites after the collection by wet smear preparation in normal saline and Lugol's iodine solution. All specimens were then processed for formalin/ethyl-acetate concentration. For genetic characterization of Giardia, sodium nitrate floatation was performed to collect Giardia cysts. For detecting Blastocystis, a short-term in vitro cultivation was performed for each stool sample using Jone's medium supplemented with 5-10% horse serum^(3,4). The cultures were incubated at 37°C for 48-72 hours and then examined at objective 10X and 40X under a light microscope. The composition of Jone's medium previously described⁽³⁾ was prepared as follows; dissolve 1.244 g of Na₂HPO₄ in 131.25 mL distilled water, 0.397 g of KH₂PO₄ in 43.75 mL distilled water, and 7.087 g of NaCl in 787.50 mL distilled water, respectively. The three solutions were mixed together to a final volume of 962.5 mL, then discard 12.5 mL of the mixture. Add 1 g of yeast extract (Oxoid) into the mixture. Autoclave the solution and leave until it is cool. Add 5-10 mL of horse serum to 95-90 ml of the sterile medium. Aliquot 3-5 ml of the medium into a sterile screw cap tube, kept at 4°C until used. The present study also examined for oocysts of *Cryptosporidium* spp. and spores of microsporidia using modified acidfast and gram-chromotrope stains, respectively.

Genotypic characterization

The purified Giardia cysts were washed thrice by phosphate buffered saline (PBS). DNA extraction was performed using FTA filter paper as previously described⁽⁵⁾. Genotypic characterization of G. duodenalis was determined by polymorphic sites using semi-nested PCR of a 432 bp region of the glutamate dehydrogenase (gdh) gene and PCR-RFLP method described by Read et al⁽⁶⁾. Briefly, amplification of gdh gene was performed using primer pairs of GDHeF/GDHiR and GDHiF/GDHiR. A total mixture of 50 ml contained DNA template using a piece of FTA filter paper, 1x PCR buffer, 1.0 U of taq polymerase, 1.5 mM MgCl., 0.2 mM dNTP and 25 pmol of each primer. The PCR condition was as follows; 1 cycle of 94°C for 2 min, 56°C for 1 min and 72°C for 2 min, followed by 55 cycles of 94°C for 30 s, 56°C for 20 s and 72°C for 45 s and a final extension of 72°C for 7 min. RFLP analysis was performed by digesting 10 ml of the PCR product with 5 U of NlaIV in 1X enzyme buffer (New England Biolabs, England) in a final volume of 20 ml for 3 h at 37°C. PCR products and restriction fragments were separated by electrophoresis in 2 % agarose gel, respectively. Gels were stained with ethidium bromide and visualized under UV light and documented on high-density printing paper by using a UV-save gel documentation system I (UVItec, Cambridge, United Kingdom). DNA sequencing of PCR products were also performed to confirm with the sequence of GenBank accession number; L40509 (G. duodenalis assemblage AI), L40510 (G. duodenalis assemblage AII), AF069059 (G. duodenalis assemblage BIII), and L40508 (G. duodenalis assemblage BIV). Multiple alignment and restriction map analysis were performed using program Bioedit version 7.

Positive samples of *Blastocystis* from culture medium were used for subtype identification. Genomic DNA of *Blastocystis* was extracted using FTA filter paper as previously described⁽⁵⁾. A pair of primers described by Clark et al⁽⁷⁾, was used as a primary primer while secondary PCR was performed using a specific pair of primers described by Bohm-Gloning et al⁽⁸⁾. The secondary PCR product produced the expected size of 1100-bp. Genotypic characterization of *Blastocystis* was determined using PCR-RFLP analysis of 1100 bp of partial SSU-rRNA gene. Digestion of PCR products was performed using three restriction enzymes, *Hinf*I, *Rsa*I, and *Alu*I endonucleases (Gibco, BRL, Gaithersburg, Md.), separated by 2% agarose gel electrophoresis and then visualized under UV light and documented on high density printing paper using a UVsave gel documentation system I (UVItec, Cambridge, United Kingdom). To confirm the subtypes, nucleotide sequencing of the SSU rRNA gene of *Blastocystis* was conducted by Bioservice Unit, Bangkok, Thailand. Chromatograms were manually checked and edited using Sequencher version 4.0.5.

Questionnaires

To determine the risk factors and outcomes of intestinal parasitic infections, standardized questionnaires concerning demographic data, sanitary behaviors including cooking and eating habits, source and treatment method of drinking water, pets or animal contact and also history of present gastrointestinal symptoms were used in the present study. All participants were asked to complete the questionnaires when they provided their specimens. Diarrhea was defined as a change in their normal pattern of bowel movements and at least 3 loose stools during a 24-hour period. Dysentery was defined as at least one passage of mucous-bloody stool in 1 day.

Statistical analysis

The association between potential risk factors and intestinal parasitic infections was assessed by the chi-square test with a 95% confidence interval using EpiInfo version 6.04b. Univariate analysis was performed using SPSS for Windows version 11.5 (SPSS, Chicago, IL). Odds ratios with 95% confidence intervals and p-values were calculated to compare the outcome among the study groups. Logistic regression was performed for multivariate analysis to assess the independent association of risk factors and blastocystosis.

Results

Of 317 enrolled studied population, 182 (57.4%) were military personnel, including privates (18, 9.9%), non-commissioned officers (141, 82.4%), and officers (23, 12.6%). Others were civilian personnel (135, 42.6%) who had been hired to work as dog care-takers (49, 36.3%) and service workers (86, 63.7%) at this army base. Approximately 58.4% originated from the North-eastern region, 22.1% from the central part of Thailand, 5.4% from the North and, 14.1% from other regions. Other characteristics of the studied popula-

tion are shown in Table 1. Seventy-two (22.4%) were found positive for intestinal parasitic infections. As shown in Table 2, *Blastocystis* was the most common protozoa found in this population with the prevalence of 14.5%, followed with *S. stercoralis* (8, 2.5%), *E. coli* (7, 2.2%), *G. duodenalis* (4, 1.0%), hookworm (3, 1.0%), *O. viverrini* (3, 1.0%), *Taenia* spp. (1, 0.3%), *Endolimax nana* (2, 0.6%). *Cryptosporidium* spp. and microsporidia were not found. Those who were

 Table 1. Demographic data of military personnel at the Military Dog Center, Veterinary and Remount Department, Royal Thai Army, Thailand

Characteristic	Number (%)	No. infected (%)	p-value
Rank			
Private	18 (5.7)	2 (11.1)	
Commisioned ofiicer		5 (21.7)	
Noncommissioned officer	141 (44.4)	33 (23.4)	
Civilian employee	135 (42.6)	31 (23.0)	
Total	317 (100)	71(22.4)	0.698
Gender			
Male	292 (92.1)	66 (22.6)	
Female	25 (7.9)	5 (20.0)	
Total	317 (100)	71(22.4)	0.842
Unit			
Medical corps	8 (2.8)	1 (12.5)	
Engineer corps	28 (9.9)	6 (21.4)	
Veterinary and dog	109 (38.4)		
Office and others	139 (48.9)		
Total	284 (100)		0.901
Level of education			
Primary school	79 (24.9)	23(29.1)	
Secondary school	138 (43.5)	30 (21.7)	
Diploma or equal	72 (22.7)	15 (20.8)	
Bachelor degree or higher		3 (13.0)	
Total	312 (100)	71(22.8)	0.350
Age (years)			
< 30	19 (6.2)	1 (5.3)	
31-40	47 (14.8)	10 (21.3)	
41-50	108 (34.1)		
51-60	133 (42)		
Total	307 (100)	70 (22.8)	0.404
Drinking water			
Boiled	144 (45.4)	35 (24.3)	
Not boiled	173 (54.6)		
Total	317 (100)		
Current residence	. /	. ,	
In the camp	236 (76.6)	51 (21.6)	
Outside the camp		18 (25.0)	

Table 2. Intestinal parasitic infections among 317 military
personnel and 189 military dogs at the Military
Dog Center, Veterinary and Remount Department,
Royal Thai Army, Thailand

Intestinal parasite	Positive	Percent
Military personnel (n = 317)		
Blastocystis sp.	46	14.5
Strongyloides stercoralis	8	2.5
Giardia duodenalis	4	1.3
Hookworm	3	1.0
Opisthorchis viverrini	3	1.0
Taenia spp.	1	0.3
Entamoeba coli	7	2.2
Endolimax nana	2	0.6
Total	74	22.4
Military dogs ($n = 189$)		
Blastocystis sp.	5	2.6
Strongyloides stercoralis	1	0.5
Entamoeba coli	1	0.5
Total	7	3.7

infected with pathogenic parasitic infection were treated with proper antiparasitic drugs. In addition, instruction to prevent themselves from acquiring intestinal parasitic infections i.e. foodborne parasite, waterborne parasite and soil-transmitted helminthes were provided to the studied population.

The prevalence of intestinal parasitic infections was not significantly different among groups regarding gender, rank, age group, working unit, area of residence, animal contact, source and treatment of drinking water. Using univariate and multivariate analysis, no significant risk factors were identified regarding gender, rank, age group, working unit, area of residence, animal contact, source and treatment of drinking water. Of 46 positive culture of *Blastocystis* sp., only 25 were available for genetic characterization and were identified as subtype 1. In addition, using PCR-RFLP of the *gdh* gene, all 4 positive human cases of *G. duodenalis* were genetic and characterized as assemblage B, subgroup IV.

All dogs enrolled in the present study were 1-7 years old and had their individual dog caretaker. Of 189 dogs, 7 (3.7%) were positive for intestinal parasitic infections which were *Blastocystis* (5, 2.6%), *S. stercoralis* (1, 0.5%), *and E. coli* (1, 0.5%), respectively. The dog, infected with *S. stercoralis*, was treated with the anti-parasitic drug while others had nonpathogenic protozoa infection and required no treatment. Genotypic characterization of 5 positive cultures of *Blastocystis* sp. found in dogs was unsuccessfully identified for subtype due to specimen storage.

Discussion

The populations enrolled into the present study originated from all regions of Thailand, approximately 60% of them were from the Northeast. During the present study, privates were on active duty outside the military base, thus only 18(5.7%) privates were enrolled into the present study. In the present study, the prevalence of intestinal parasitic infections in military personnel was 22.4%. The authors' previous study demonstrated a significantly increased risk of acquiring intestinal parasitic infections among privates aged 21-23 years old, especially those who finished less than secondary school⁽²⁾. Thus, the prevalence of intestinal parasitic infections found in this population could have been higher if there were more enrollment of the private group. In this population, parasite control program using bi-annual mass chemotherapy of mebendazole for military personnel had been administered which could somehow control the prevalence rate of intestinal helminthic infections to be as low as 4.7%. However, effective prevention and control of soil-transmitted helminthes i.e. S. stercoralis and hookworm infection is still needed in this population. Compared to other studies conducted in the Northeast region⁽⁹⁾, 1.3% of other infections were O. viverrini and Taenia spp., which were less prevalent than expected. Thus, a small number of privates who enrolled into the present study could account for the low prevalence of O. viverrini and Taenia spp.

In Thailand, the prevalence of blastocystosis, an intestinal protozoal infection, extend over a wide range which were approximately 0.8-45% depending on the methods of detection and studied populations. A significance of blastocystosis has increasingly gained more attention since the high prevalences have been reported in all age groups. In the present study, blastocystosis, detected by in vitro cultivation using Jone's medium, a more sensitive method compared to formalin-ethyl acetate sedimentation technique, was predominant among military personnel and dogs at the military dog center. This finding agreed with a few studies of intestinal parasitic surveys performed at other army bases in Thailand, which also showed the highest prevalence of blastocystosis among Thai military personnel i.e. 21.9%, 44.4% and 36.9% in the central region of Thailand^(1, 2, 10). Interestingly, the present result was similar to that reported from the study in Hondurus which reflected a common *Blastocystis* infection. U.S. military both servicemen and women who were assigned to do their duty in Hondurus acquired blastocystosis at the highest prevalence of 35.8%⁽¹¹⁾. The high prevalences of blastocystosis was also demonstrated in children i.e. 45.2% in an orphanage, Pathumthani province⁽¹²⁾ and 8.1% in a primary school, central Thailand⁽¹³⁾. In contrast to studies conducted in other groups using formalin-ethyl acetate sedimentation, a less sensitive technique, showed rather lower prevalence of blastocystosis i.e. 0.19% in primary school children in central Thailand⁽¹⁴⁾, 0.8% in school children in Northern Thailand, 4.1% in Thai laborers⁽⁹⁾ and 6.1% in school children, in Central Thailand⁽¹⁵⁾.

Blastocystis sp. has been identified both in humans and a very wide range of animals. Recent studies confirmed zoonotic transmission of blastocystosis⁽¹⁶⁾. Cyst of *Blastocystis* served as the infective stage and transmits via fecal-oral route. Like other protozoa, transmission of blastocystosis can occur through waterborne or food borne. Thus, source of infection of *Blastocystis* could have come either from human or animal excreta, which contaminated into water or food. The presented data could not indicate that blastocystosis in this army population was significantly linked to any risk association among groups with regard to rank, age, working unit, area of residence, animal contact, source and treatment of drinking water.

Genetic characterization of Blastocystis revealed several subtypes including subtypes 1 to $9^{(17)}$. In the present study, genetic characterization of Blastocystis revealed only subtype 1 while other subtypes could not be excluded for the existence since only 25 samples (54.3%) were available for genetic characterization. These military personnel who harbored Blastocystis subtype 1 did not show any clinical symptoms. Blastocystis subtype 1 has been identified both in humans and a very wide range of animals i.e. pigs, horses, monkeys, cattle, rodents, chickens, quails and, pheasants^(18,19). The authors' previous study of Blastocystis isolated from dogs and humans living in a localized endemic community in Thailand provided molecular-based evidence supporting the zoonotic potential of *Blastocystis* in dogs⁽¹⁶⁾. Unfortunately, the authors did not compare the subtype of Blastocystis isolated from military personnel and military dogs since the subtype of *Blastocystis* from dogs could not be determined. Studies of blastocystosis in military personnel have been conducted in Thailand. It was shown that consuming neither filtered nor boiled water was independently associated with blastocystosis⁽¹⁾. Another study also showed that privates who had education lower than the secondary school level had a significantly greater risk of *Blastocystis* carriage⁽²⁾.

Giardiasis, the most commonly notified waterborne disease and causes a wide range of clinical symptoms, has been reported worldwide. The prevalence of giardiasis tends to be more common in young children than adults. In Thailand, the prevalence studies showed 37.7 % in orphans⁽¹²⁾ while 1.4-7.7% among school children^(10,13,14,20-22). The prevalence of giardiasis in Thai military personnel was previously reported with the prevalence of 3.7%⁽²⁾. There are two major genetic assemblages of G. duodenalis found in humans i.e. assemblages A and B. It has been shown that assemblage A and B were isolated from animals, thus these 2 assemblages have a zoonotic potential. Other assemblages C, D, E, F and G, are found only in animals⁽²³⁾. Subgenotypes within each assemblage were also identified. Assemblage A comprises of subgenotypes AI and AII while assemblage B consists of subgenotypes BIII and BIV. A study showed evidence supporting zoonotic transmission of giardiasis⁽²⁴⁾. In the present study, the authors used PCR-RFLP of *ghd* gene to study both assemblage and subgenotype of G. duodenalis. All 4 positive specimens were successfully characterized as Assemblage B, subgenotype IV. It is possible that the infective stage could have come from the same source. However, there was no statistical measurement to support any risk association due to the small number of sample size. This preliminary data of G. duodenalis subgenotype also urge us to investigate their source of transmission in other population.

The present study showed the low prevalence of intestinal parasitic infections in military dogs. The parasite control program using bi-annual mass chemotherapy for military dogs accounted for the findings. In addition, dog health care and well-being had been routinely handled by the veterinarian. Safe food and clean water were regularly inspected and provided to all dogs. As a result, only one military dog was infected with soil-transmitted helminth, S. stercoralis. The infection was acquired principally by skin penetration of infective filariform larvae of S. stercoralis from the soil or playground. The other 6 dogs were infected with non-pathogenic protozoa which included Blastocystis sp. and E. coli. The morphology of Blastocystis sp. obtained from dogs when observed in Jone's medium was similar to those found in humans. Since transmission route of *Blastocystis* sp. to humans was demonstrated by the presence of zoonotic strains⁽²⁵⁾, genetic characterization of *Blastocystis* found in both humans and dogs could help understanding the epidemiology of the organism. Unfortunately, genotype of *Blastocystis* detected in military dogs could not be performed due to long-term specimen storage.

In conclusion, the present study demonstrated that parasitic infection acquired via fecal-oral route is still a significant problem among military personnel. Base-line information of parasitic infection among military personnel in other regions of Thailand need further studies since continuous surveillance programs for enteric parasites are warranted to reduce morbidity. Moreover, health education provided to the affected population could enable them to protect themselves against infection.

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ความชุกของโรคติดเชื้อปรสิตในลำไส้ในทหารและสุนัขทหารในประเทศไทย

เสาวนีย์ ลีละยูวะ, สุรเดช ศิริพัฒนพิพงษ์, ทวี นาคหล่อ, ปานจิต ธรรมศรี, มฑิรุทธ มุ่งถิ่น

วัตถุประสงค์: ศึกษาหาความชุกและปัจจัยเสี่ยงของโรคติดเชื้อปรสิตในลำไส้ในทหารและสุนัขทหารที่ศูนย์สุนัขทหาร กรมการสัตว์ทหารบก ประเทศไทย

วัสดุและวิธีการ: การศึกษาชนิดตัดขวาง โดยดำเนินการเก็บอุจจาระจากกำลังพลทหาร และสุนัขทหาร ในเดือน มกราคม พ.ศ. 2549 และตรวจโดยใช้ทั้งการตรวจธรรมดาและวิธีเข้มข้นชนิด formalin-ether ย้อมสี modified acid fast และ gram-chromotrope เพื่อตรวจหา Cryptosporidium และMicrosporidia ตามลำดับ เพาะเชื้อเพื่อตรวจหา Blastocystis ใน Jone's medium ศึกษา genotype ของ Blastocystis and Giardia duodenalis โดยใช้ PCR-RFLP รวมทั้งหาปัจจัยเสี่ยงของการติดโรคพยาธิในลำไส้โดยการใช้แบบสอบถามมาตรฐาน

ผลการศึกษา: จากอุจจาระที่เก็บได้ทั้งหมด 317 ตัวอย่างจากทหาร พบความชุกของโรคติดเชื้อปรสิตในลำไส้ 22.4% โดยโปรโตซัวมากที่สุดพบคือ Blastocystis 14.5% และรองลงมาคือ G. duodenalis 1.3% ความชุกของหนอนพยาธิ ทั้งหมดมี 4.7% โดยพบ Strongyloides stercoralis (2.5%), Hookworm (1.0%), Opisthorchis viverrini (1.0%), และ Taenia spp. (0.3%), ผลการทำ PCR-RFLP พบ Blastocystis subtype 1 จำนวน 25 ราย G. duodenalis Assemblage B, subgenotype IV จำนวน 4 ราย นอกจากนั้นการตรวจอุจจาระสุนัขทหาร 189 ตัว พบความชุก ของโรคติดเชื้อปรสิตในลำไส้ 3.7% โดยพบ Blastocystis มากที่สุด (2.6%) ตามด้วย S. stercoralis (0.5%) และ Entamoeba coli (0.5%) จากการวิเคราะห์ข้อมูลทางสถิติ ไม่พบบ้าจจัยเสี่ยงของโรคติดเชื้อปรสิตในลำไส้ ในทหารที่สัมพันธ์กับ ยศ กลุ่มอายุ หน่วยที่สังกัด ภูมิลำเนา น้ำดื่ม ประวัติสัมผัสสัตว์

สรุป: การสำรวจโรคติดเชื้อปรสิตในลำไส้ในทหารและสุนัขทหารในประเทศไทย พบความชุกของโปรโตซัวที่ติดต่อทาง fecal-oral คือ Blastocystis sp. และ G. duodenalis ตามลำดับ การป้องกันควรเน้นสุขอนามัยส่วนบุคคล