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**Brief communication (Original)**

# Intestinal parasitic infections: high prevalence of *Giardia intestinalis* in children living in an orphanage compared with hill-tribe children as detected by microscopy and ELISA

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***Background:*** Data regarding intestinal parasitic infections in preschool-aged children (less than 6 years old) living in an orphanage and remote mountainous areas are very limited.

***Objectives:*** We surveyed infections in orphans and hill-tribe children.

***Materials and Methods:*** They were studied in 2008 by stool examination (simple smear and concentration), Scotch-tape and culture (Boeck and Drbohlav's Lock-Egg-Serum medium) techniques. The *Giardia* copro-antigen ELISA was also performed. The risk correlation between unusual stool types and giardiasis by univariate analysis was tested.

***Results:*** The overall infection rates in 137 orphans and in 145 hill-tribe children were 58.4% and 77.9%, respectively. *Giardia intestinalis* had the highest prevalence in orphans (with microscopy 28.5%, with copro-antigen ELISA 31.4%). Other pathogens included *Blastocystis hominis* (23.4%), *Enterobius vermicularis* (9.5%), and hookworm (0.7%), whereas the nonpathogens were *Trichomonas hominis* (19.0%), *Entamoeba coli* (11.7%), and *Endolimax nana* (2.2%). *Ascaris lumbricoides* had the highest prevalence (62.1%) in hill-tribe children, while *Giardia intestinalis* showed 7.6% with microscopy and 9.0% by ELISA. The other pathogens were *E. vermicularis* (25.5%), *Trichuris trichiura* (10.3%), *B. hominis* (2.8%), hookworm (1.4%), *Sarcocystis hominis* (1.4%) and *E. histolytica* (0.7%), whereas the nonpathogenic organisms were *E. coli* (19.3%), and *E. nana* (0.7%). Giardiasis stools from orphans had significantly greater cyst density than those from the hill-tribe children. The copro-antigen ELISA for giardiasis demonstrated 91.4% specificity, 72.0% sensitivity, 64.3% positive predictive value, and 93.8% negative predictive value, respectively. By univariate analysis, a loose (mushy) stool type was 2.43 times likely to have *Giardia* cysts.

***Conclusion:*** In large-scale epidemiological studies, a *Giardia* ELISA might be a useful aid for diagnosis, because conventional microscopy is time-consuming and relies on the expertise of the microscopist.

***Keywords:*** ELISA, *Giardia intestinalis*, hill-tribe children, intestinal parasite, microscopy, orphanage children, preschool-aged children

*Giardia intestinalis* (also known as *Giardia duodenalis*; previously, *Giardia lamblia*) is a ubiquitous, water-borne diarrhea-causing protozoa, commonly found in children worldwide. Giardiasis is transmitted through the consumption of parasite cysts in contaminated drinking water and less often in food. The parasite can infect both humans and animals, which include birds, reptiles, and mammals [1, 2]. Incidence of human infection worldwide is estimated at 280 million cases per year [3]. Although 60%–80% of infected people may be asymptomatic, those with symptoms may experience mild diarrhea, anorexia,

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flatulence, cramped abdominal pains and/or epigastric tenderness. Giardiasis can significantly affect the child's growth and physical development [1-3]. The prevalence of giardiasis is highly dependent on the individual's educational background, and whether they have high-risk behavior [1, 4]. Chronic and heavy *Giardia* infections can change intestinal villi architecture and lead to the production of an unusual stool consistency [1, 5]. There are generally six stool types (formed, soft, loose, mucous, loose-watery, and watery) as described by WHO [6]. Observations of abnormal stool types with intestinal parasites have been reported. For example, *Entamoeba histolytica* and *Balantidium coli* can produce a mucous bloody stool, while *Dientamoeba fragilis* can be detected in any kind of stool, but mostly in the mushy stool type [7]. Approximately half of *Giardia*-infected stools are of the mushy stool type [8]. *Cryptosporidium* spp. is mostly found with watery stools [9]. HIV/AIDS patients with mucous, loose-watery, or watery stool types were 4.9 times likely to have opportunistic protozoan infections (e.g. *Cryptosporidium* spp., Microspora, *Cyclospora* and *Isospora* spp.) [10]. To date, there is no information regarding the statistical risk correlation between abnormal stool types and giardiasis.

The data for *Giardia* infection rates reported in preschool children from orphanages and from low socioeconomic communities of various foreign countries are varied. The rates in Iraq, Pakistan, and Brazil are reported to be between 16.7%–48.1%. [4, 11, 12]. European preschool children attending day care centers have a prevalence of 1.3%–1.7% [13-15]. In Thailand, there are substantial numbers of parasitic infections in school aged-children both from orphanage and from rural areas [16-24]. However, available data from children under the age of 6 are very limited.

Here, preschool children aged 1–6 years old from an orphanage in Pathum Thani province and from mountainous area of Pang Hin Fon subdistrict, Mae Chaem district, Chiang Mai province, Thailand, were investigated for intestinal parasitic infections by using stool examinations (simple smear and concentration techniques), Scotch-tape and/or cultures. The copro-antigen ELISA for *Giardia* was also performed in this study [25]. Univariate analysis of risk correlation between unusual stool types and giardiasis was conducted.

## Materials and methods

### *Study areas and participants*

During February to May 2008, a cross-sectional descriptive survey of the intestinal parasitic infections was performed in two groups of 1 to 6-year-old children.

Group I. A survey of 137 children in an orphanage located in Pathum Thani province, Thailand was conducted. This center is responsible for orphans and is under the control of the Bureau of Woman and Child protection and Welfare, Department of Social Development and Welfare, Ministry of Social Development and Human Security of Thailand. The children were from impoverished families and abandoned at public places. Informed consent was obtained from the superintendent of the center before the study. The result of the parasitic infections was reported to the doctors of the center who directly treated the children.

Group II. A survey of 145 hill-tribe children from 4 different villages (Mae Fah Luang Community Learning Centre; Murdlong, Maehanai, Pikey, and Sedosa) in Pang Hin Fon subdistrict, Mae Chaem district, Chiang Mai province was conducted. The numbers of the participants in each center were 32, 32, 34, and 47 children respectively. The participants were Karen (86.8%), Hmong (7.5%), Larva (4.1%), and Lesor (1.3%). Informed consent was obtained from the parents or the guardians of the children. The study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University. Communication between the study participants and research staffs was mediated by a translator. After the completion of the study, medication was given to all the infected children by public health personnel. Lectures, educational leaflets, and posters on intestinal parasitic infections were provided to the children and their communities.

### *Examination of intestinal parasites*

The Scotch-tape technique was used to collect the parasites from the perianal area as previously described [26]. The collected stools, together with the Scotch-tape prepared slides, were carried to the laboratory of the Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, for examination. Each stool specimen was examined by simple smear and formalin-ether concentration techniques. Using sediment of the stool concentration technique, the density of *Giardia* cysts was obtained by averaging the results from ten fields under high power magnification. To detect certain protozoa (e.g. *E. histolytica*, *B. hominis* and *T. hominis*), cultures were made by using Boeck and Drbohlav's Lock-Egg-Serum medium (LES). Cultures were grown at 36.5°C for 72 hours as previously described [27]. The types of the collected stools were categorized according to the definitions provided by World Health Organization (WHO): formed, soft, loose, mucous, loose-watery, and watery [6].

### *Detection of Giardia intestinalis copro-antigen*

Each stool sample was tested for the presence of *G. intestinalis* copro-antigen. The antigen was detected using a commercially available enzyme-linked immunosorbent assay (*Giardia* Antigen ELISA, Ref. 6005, Generic Assay, 15827 Dahlewitz, Germany), following the manufacturer's instructions.

### *Data analysis*

Statistical differences of the data were analyzed using a Chi-square test or Fisher's exact test. $P < 0.05$ was considered statistically significant. Univariate analysis was used to analyze the risk factors and association between unusual stool type and giardiasis.

## Results

In 137 orphans (81 boys and 56 girls), the average incidence of intestinal parasitic infections was 58.4%. There were no significant differences in infection rates between boys and girls. The highest prevalence of intestinal parasitic infection was *Giardia intestinalis* (28.5%) followed by *B. hominis* (23.4%), *T. hominis* (19.0%), *E. coli* (11.7%), and *E. nana* (2.2%) as shown in **Table 1**. In orphans between the ages of 5–6 years old, the infection rate tended to be higher than any other age group. The prevalence of *B. hominis* and *T. hominis* infections detected by culture were higher when compared with simple smear and concentration techniques.

In the 145 hill-tribe children (70 boys and 75 girls) from the Pang Hin Fon subdistrict, the average rate of intestinal parasitic infections was 77.9%. There were no significant differences in infection rates between boys and girls. Children between 3–4 and 5–6 years old had a higher prevalence of intestinal parasitic infections than the younger group (**Table 2**). Four species of roundworms were detected: *A. lumbricoides* (62.1%), *E. vermicularis* (25.5%), *T. trichiura* (10.3%), and hookworms (1.4%). The most common species of protozoan detected was *E. coli* (19.3%). Four pathogenic protozoa detected: *G. intestinalis* (11/145, 7.6%), *E. histolytica* (0.7%), *B. hominis* (2.8%), and *S. hominis* (1.4%).

**Table 1.** Prevalence of intestinal parasitic infections among 137 orphans from Pathum Thani province detected by stool simple smear, concentration, Scotch-tape techniques, and cultures and presented by the different age groups

| Age (years) / Parasites | No. infected (%) 1–2 | 3–4 | 5–6 | Total |
|---|---|---|---|---|
| **Helminthes** | | | | |
| *E. vermicularis* | 0 (0.0%) | 5 (3.7%) | 8 (5.8%) | 13 (9.5%) |
| Hookworm | 0 (0.0%) | 1 (0.7%) | 0 (0.0%) | 1 (0.7%) |
| **Pathogenic protozoa** | | | | |
| *G. intestinalis* | 1 (0.7%) | 16 (11.7%) | 22 (16.1%) | 39 (28.5%) |
| *B. hominis* | 2 (1.5%) | 12 (8.8%) | 18 (13.1%) | 32 (23.4%) |
| **Nonpathogenic protozoa** | | | | |
| *E. coli* | 1 (0.7%) | 8 (5.8%) | 7 (5.1%) | 16 (11.7%) |
| *E. nana* | 0 (0.0%) | 2 (1.5%) | 1 (0.7%) | 3 (2.2%) |
| *T. hominis* | 3 (2.2%) | 13 (9.5%) | 10 (7.3%) | 26 (19.0%) |
| **No. mixed infection** | 3 | 25 | 11 | |
| **Total no. infected** | 3 (2.2%) | 45 (32.9%) | 32 (23.4%) | 80 (58.4%) |
| **Total no. investigated** | 4 | 73 | 60 | 137 |

**Table 2.** Prevalence of intestinal parasitic infections among 145 children from the Pang Hin Fon subdistrict detected by stool simple smear, concentration, cultures, and Scotch-tape techniques, presented by the different age groups

| Parasites \ Age (years) | No. infected (%) 1–2 | 3–4 | 5–6 | Total |
|---|---|---|---|---|
| **Helminth** | | | | |
| *A. lumbricoides* | 16 (11.0%) | 37 (25.5%) | 37 (25.5%) | 90 (62.1%) |
| *E. vermicularis* | 7 (4.8%) | 14 (9.7%) | 16 (11.0%) | 37 (25.5%) |
| *T. trichiura* | 1 (0.7%) | 5 (3.5%) | 9 (6.2%) | 15 (10.3%) |
| Hookworm | 0 (0.0%) | 1 (0.7%) | 1 (0.7%) | 2 (1.4%) |
| **Pathogenic protozoa** | | | | |
| *G. intestinalis* | 4 (2.8%) | 2 (1.4%) | 5 (3.5%) | 11 (7.6%) |
| *E. histolytica* | 0 (0.0%) | 0 (0.0%) | 1 (0.7%) | 1 (0.7%) |
| *B. hominis* | 1 (0.7%) | 2 (1.4%) | 1 (0.7%) | 4 (2.8%) |
| *S. hominis* | 0 (0.0%) | 2 (1.4%) | 0 (0.0%) | 2 (1.4%) |
| **Non-pathogenic protozoa** | | | | |
| *E. coli* | 3 (2.1%) | 13 (9.0%) | 12 (8.3%) | 28 (19.3%) |
| *E. nana* | 0 (0.0%) | 0 (0.0%) | 1 (0.7%) | 1 (0.7%) |
| **Mixed infection** | 10 | 22 | 25 | |
| **Total infected** | 21 (14.5%) | 44 (30.3%) | 48 (33.1%) | 113 (77.9%) |
| **Total investigated** | 32 | 51 | 62 | 145 |

Giardiasis was found with higher prevalence in the orphans compared with age-matched children from the hill-tribes (28.5% versus 7.6% by microscopy). The infection was more prevalent in orphaned boys than girls, but the difference was not significant. Stool samples from the orphans, 31.4% (41/137) were positive for *Giardia* copro-antigen. By stool examinations and/or ELISA, *G. intestinalis* showed a higher incidence of infection in orphans (55/137, 40.2%) than either technique alone. In addition, there was 9.0% (13/145) *Giardia* infection rate when copro-antigen ELISA was tested with the stools from hill-tribe children. When combining the data from both techniques, we found a higher rate of giardiasis in these children (15/145) 10.3% (**Figure 1**).

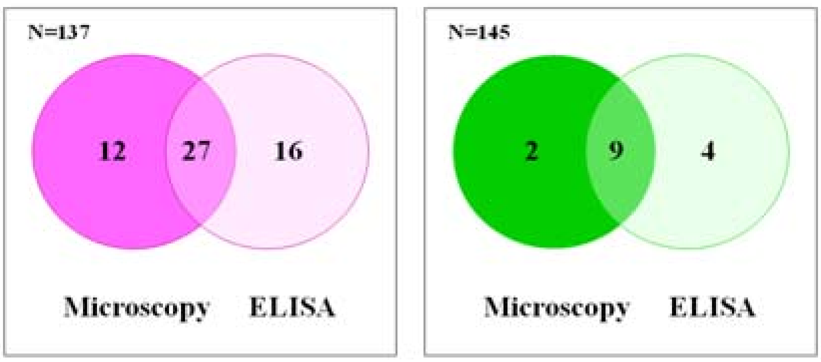


**Figure 1.** Venn's diagrams showing increased positivity for Giardia diagnosis of by copro-antigen ELISA, when compared with microscopy techniques

Of all the 282 stool specimens in our study, the incidence of infection was higher by stool examinations and/or ELISA techniques. The copro-antigen ELISA in this study demonstrated 91.4% specificity, 72.0% sensitivity, 64.3% positive predictive value, and 93.8% negative predictive value, respectively.

The amount of cyst shedding was graded as either1+, 2+, 3+, or 4+. Among the 39 orphans who were positive for shedding cysts, there were 25.7%, 59.0%, 12.8%, and 2.6% with cyst shedding of 1+, 2+, 3+, and 4+, respectively. By contrast, 11 hill-tribe children had only 1+ shedding (81.8%), whereas 18.8% had grading of 2+.

The orphans and the hill-tribe children produced all six types of stools. Upon examination of the samples that had *Giardia* cysts, we found that most were in the loose (mushy) stool type. In the univariate analysis, the loose (mushy) type of stool was 2.43 times more likely to have *Giardia* cysts ($p = 0.014$) as shown in **Table 3**.

## Discussion

The incidence of parasite infection can generally vary from one area to another depending on the climate, community sanitation, and degree of personal hygiene [4]. It is believed that children living in an orphanage or remote rural areas are at increased risk for acquiring parasitic infections and succumb to health and physical development problems [28]. For giardiasis, children are three times more frequently affected than adults [29]. After ingestion of infective *Giardia* cysts with as few as 10 to 25 cysts, 5%–15% pass cysts without symptoms. When the parasite causes symptoms, they may last for 5 to 7 days. These include diarrhea (89%), malaise (84%), flatulence (74%), abdominal cramps (70%), bloating (69%), nausea (68%), anorexia (64%), and weight loss (64%). About 25%–50% develop acute self-limiting diarrhea. Chronic infection may produce steatorrhea (copious light-colored, fatty stools) and full-blown malabsorption syndrome [1, 2, 30]. Unfortunately, we had no clinical records of these children. At the time of stool collection, 48.6% (137/282) abnormal stool consistency (loose (mushy), mucous, loose-watery/watery) were observed. However, the prevalence of giardiasis from previous reports in asymptomatic children of all age groups in Thai-orphanages ranged from 14.3% to 37.7% [16-18]. In this study, we detected a significantly higher prevalence of this protozoal infection in orphans (by using both microscopy and ELISA) when compared with the hill-tribe children. This may reflect persistent problems of sanitary conditions and healthcare. Crowding and behavioral patterns may contribute greatly to the spread of the parasite. Other parasite and host variables could contribute to the outcome of the infection. The immune status of the young age-group orphans may also explain their greater susceptibility to the infection than that of the hill-tribe children. It is known that living in an orphanage can affect the health and development of children [28]. Our results showed a markedly low incidence of helminth infections in our orphans because they were mass treated prophylactically with an anthelminthic drug yearly under the policy of The Bureau of Woman and Child protection and Welfare, Department of Social Development and Welfare, Ministry of Social Development and Human Security of Thailand. By contrast, when we looked at data from western countries, we noticed huge discrepancies. For

**Table 3.** Risk factors for *G. intestinalis* infection by type of stools according to the univariate analysis

| Characteristics | Pathum Thani orphanage | | Pang Hin Fon subdistrict (Hill-tribe) | | Total | | Crude Odds ratio (95%CI) | *P* |
|---|---|---|---|---|---|---|---|---|
| | Investigated (%) | Giardiasis (%) | Investigated (%) | Giardiasis (%) | Investigated (%) | Giardiasis (%) | | |
| **Stool consistency** | | | | | | | | |
| Form/ Soft | 50 (36.5) | 10 (20.0) | 95 (65.5) | 7 (7.4) | 145 (51.4) | 17 (11.7) | 1 | |
| Loose (mushy) | 45 (32.9) | 17 (37.8) | 41 (28.3) | 4 (9.8) | 86 (30.5) | 21 (24.4) | 2.43 (1.20–4.93) | 0.014 |
| Mucous | 34 (24.8) | 9 (26.5) | 6 (4.1) | 0 (0.0) | 40 (14.2) | 9 (22.5) | 2.19 (0.89–5.37) | 0.088 |
| Loose-watery/ Watery | 8 (5.8) | 3 (37.5) | 3 (2.1) | 0 (0.0) | 11 (3.9) | 3 (27.3) | 2.82 (0.68–11.68) | 0.152 |

instance, infection rates of giardiasis in preschool-aged children attending day cares centers in Europe is 1.3%–1.7% [13-15]. Outbreaks of giardiasis reported in the day-care centers in USA and United Kingdom showed a high prevalence of 17%–47%, which was considered associated with poor hygiene and inadequate toilet training of newly registered children [31, 32]. In addition, in this study, we observed that giardiasis tended to be more common in orphaned boys. Our data are supported by a study conducted in an outbreak in mentally retarded children from Manitoba, Canada, which found the prevalence was higher in boys (46.6%) than girls (11.0%) [33]. Along the same line, kindergarten children from Cambodia were also reported to have a higher infection incidence of giardiasis in boys than girls (4.4% versus 1.1%) [34]. There was no previous information of the prevalence of giardiasis in our preschool hill-tribe children aged 1 to 6 years old. However, in schoolchildren in other rural areas, the rates with microscopy were similar to this study (7.6%). Giardiasis in children attending primary schools in Chiang Mai and Nan provinces located north of Thailand, were 7.7% and 5.5%, respectively [19, 20]. Likewise, in the central part of Thailand, the *Giardia* infection incidence from two studies in schoolchildren were 1.3% and 6.2% [23, 24]. The incidence in schoolchildren in other rural areas of the northeast (Surin province) and subrural parts (Samut Sakhon province) were 2.2% and 6.5%, respectively [22]. However, in rural areas at the Thai–Myanmar border (Kanchanaburi province) there were higher rates of asymptomatic cases in preschool-aged children (23.0%–23.3%) than those with acute diarrhea (13.6%–15.0%), respectively [35, 36]. The reasons for this were not demonstrated. The infection incidence of *Giardia intestinalis* in this study could show more positive than microscopy following the introduction of a sensitive enzyme immunoassay diagnosis. Because, even though the protozoan parasite was not evident, antigen may still be a reliable test. Antigen of this protozoan could be detected despite the destruction of the parasite [25]. The false-negative copro-antigen ELISA might the result of the low cyst density in the specimens, as the *Giardia* ELISA used in this study could detect at least at the level of $5 \times 10^3$ cysts/g stool [25]. Conventional microscopy is time-consuming and relies on the expertise of the microscopist. In large-scale epidemiological studies, the ELISA for *Giardia* may be recommended. Recent studies have found ELISA to be a sensitive, cost-effective, and rapid method for the detection of *Giardia* in stools [25]. Higher prevalence could be obtained when more sensitive techniques such as ELISA or PCR had been used [25, 37]. We found a greater incidence of positive stool by ELISA 19.9% (56/282) than microscopy 17.7% (50/282).

Increasing risk for this infection was not only via ingestion of contaminated water, but also via contacting to infected animals [2]. This organism is now globally detected in many vertebrates both domestic and wild life animals. It is a concern by the World Health Organization’s “Neglected Diseases Initiative” [38]. We observed dogs and cats in the orphanage. In addition, there were dogs, swine and cattle in the hill-tribe regions. A recent molecular epidemiological study showed that there is an association between species and assemblages, for example, assemblages A and B are specific to human and primates and assemblages C and D that are specific to dogs whereas, assemblage E infects hoof stock, assemblage F infects cats, and assemblage G infects rodents [2]. Of the 11 cyst positive stools from hill-tribe children, 8 were successfully amplified for genetic characterization. Sequence analysis of the SSU-rRNA gene revealed that 5 (62.5%) and 3 (37.5%) samples were from assemblage A and assemblage B, respectively. Subgenotypes of these isolates was also identified using PCR-RFLP of the *gdh* gene. Of these 8 samples, only 6 (75.0%) were successfully characterized by PCR-RFLP of the *gdh* gene which 1 (16.6%), 3 (50.0%), and 2 (33.3%) were subgenotypes AI, AII, and BIV, respectively (unpublished data). The zoonotic transmission of assemblages A-I or B from dogs and cats has been reported [39]. Genetic characterization of the fecal specimens from animals in the areas of both orphanage and hill-tribe are now being conducted to gain the evidence of zoonotic transmission. A previous report showed the association of the assemblage B and high number of cyst shedding [40]. The parasite dose that can transmit infection was estimated at 10 cysts or less [41]. The infected stools of our orphans had higher numbers of cyst shedding than those of the hill-tribes. The association of the amount of cyst shedding and the assemblage should be further evaluated. These may support a major site of giardiasis and transmission among orphans.

The stool types from normal bowel movement can be hard or soft. The other types of loose stool and/or mucous and/or water may relate to poor absorption of water from the bowel, malabsorption syndromes, rapid movement through the gut, and the presence of pathogenic organisms [42]. However, by univariate analysis, the children with loose (mushy) stools were 2.43-fold likely to have *Giardia* cysts compared with children with normal (form and/or soft) stools (with a 95% confidence interval for the crude odds ratio of 1.20–4.93; $p = 0.014$). Of note, the incidence of parasite infection in our hill-tribe children (aged 1–6 years old) were mainly of soil-transmitted helminthes and nonpathogenic protozoal infections. In addition, unlike the orphans, there were a few infections of *B. hominis* in our hill-tribe children. *In vitro* culture studies in these remote areas were unfortunately rarely performed. The unavailability of fresh fecal samples may be the reason *T. hominis* was not detected [43]. However, this can confirm that an appropriate method is required to provide reliable data about the prevalence of parasitic infection. Stool concentration techniques are not applicable for *Enterobius vermicularis* eggs, which are generally found scattered perianally [44]. In addition, we showed an increase in sensitivity for protozoa detection especially *B. hominis* and *T. hominis* by means of a LES culture technique that can confirm our introduction of this medium for *B. hominis* and *T. hominis* detection [27].

## Conclusion

We have presented the different types of intestinal parasites in stools obtained from orphans and hill-tribe children between the ages of 1 and 6 years old by using simple smear, concentration, Scotch-tape and/or culture techniques. A high prevalence of *G. intestinalis* was detected in orphans. The copro-antigen ELISA for giardiasis in this study demonstrated 91.4% specificity and 72.0% sensitivity. In large-scale epidemiological studies, *Giardia* ELISA may be an aid for diagnosis. Drug treatment of children with positive cases as detected by microscopy and/or ELISA is recommended.

The infected stools of the orphans had greater cyst density than that from hill-tribe children. This study is the first of its kind to report the statistical association of giardiasis and *Giardia* cysts with loose (mushy) stools. A genetic characterization of fecal specimens from animals in our study areas is now being conducted to gain evidence of zoonotic transmission.

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