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INTESTINAL PARASITIC INFECTIONS AMONG PRE-SCHOOL CHILDREN IN SANGKHLABURI, THAILAND

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Abstract. This study was conducted to investigate the presence of intestinal parasites among pre-school children (aged 3 months to 5 years) in Sangkhlaburi, a rural district in the west of Thailand along the Thai-Myanmar border. Stool specimens were collected from October 2001 through October 2002. A total of 472 pre-school children, 233 males and 239 females, 236 children with diarrhea and 236 asymptomatic children were recruited for the study. Each specimen was processed and examined by direct wet smear, modified acid fast stain, formalin-ethylacetate sedimentation concentration technique, and trichrome stain. In detecting *Giardia lamblia* and *Cryptosporidium* species ProSpecT Microplate assays (Alexon-Trend, Lenexa, KS) were performed. There were 107 individuals (22.7%), 41 diarrheal and 66 asymptomatic children, infected with intestinal parasites. The most frequent parasites identified in cases and controls were *G. lamblia* and *Cryptosporidium* spp. Eighteen specimens (3.8%) showed mixed parasite infections. Highest proportion of intestinal parasites occurred during the rainy season (June–October).

INTRODUCTION

Three factors that separate the developing world from the developed world are access to safe drinking water, sanitation, and nutrition.[1] The most prevalent nutritional deficiency worldwide, and hence a cause of morbidity, is iron-deficiency anemia.[2] In a helminth endemic region, a rice-farming village in the Philippines, 507 children, 18 years old and younger, were studied to determine that low-intensity infections, increased severity of polyparasitism infections, and increased number of parasites causing infection all resulted in a greater risk of developing anemia.[3] In another rural, helminth endemic region, Côte d'Ivoire, West Africa, researchers identified 500 age diverse study participants to learn the extent of polyparasitism and determine the significance between polyparasitism and self-reported morbidity indicators. Only 10% of this single village population were not infected with any of the intestinal parasites (*Schistosoma mansoni*, soil-transmitted helminths, and intestinal protozoa—probably underreported because only single formol-ether processed stool samples were done), 10% had mono-infections (infants and young children mostly), and 75% harbored three or more parasite species concurrently. The authors speculate that polyparasitism is likely the norm rather than the exception in other parts of the developing world also, and proved that increasing numbers of parasites harbored in any one person results in an increase in morbidity indicators.[4]

Since intestinal parasitic infections are associated with poor socioeconomic class and unsanitary environments, people living in such conditions in rural Thailand are at risk for developing parasitism. Previous intestinal parasite studies undertaken in Thailand have focused on school-age children and older or pre-school orphans living in crowded conditions. Hence, we sought to assess and quantify the extent of parasitic infections and polyparasitism in a symptomatic and asymptomatic pre-school child population. Additionally, we evaluate seasonal prevalence over a year's time.

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MATERIALS AND METHODS

Study area. Sangkhlaburi, a district in Kanchanaburi province, is about 450 km northwest of Bangkok. Bordering Thailand and Myanmar, Sangkhlaburi averages an annual rainfall of 2359 mm. Most precipitation occurs during the rainy season (June–October). Temperatures range from daily averages of 29°C during the hot season (March–May) to 24°C during the cool months (November–February). During this study period the district's population was 26,484. The major nationalities living in Sangkhlaburi from highest population to lowest are Karen, Thai, Mon, and Burmese.[5]

Study site. This investigation was conducted from October 2001 through October 2002. The center for the study was AFRIMS-Kwai River Christian Clinical Center (AKCC) housed in the Kwai River Christian Hospital (KRCH). The hospital is in Sangkhlaburi district, Nongloo subdistrict, and services six villages. The villages are located within a 9-km radius of AKCC and in addition to the hospital, share a market, three health care centers, and schools.

Enrollment and specimen collection. A diarrheal stool specimen was collected from 236 children, aged 3 months–5 years, who came to the hospital or one of three nearby health care centers with complaints of diarrhea. All children who met the age criteria and had diarrhea were enrolled as cases. Control specimens matched by the patients' age (± 3 months) were collected from children who came to the hospital or health care centers for reasons other than diarrhea on the same day or not more than one week after the age-matched case was enrolled. Controls had not experienced diarrhea, vomiting, food poisoning, or gastrointestinal disease for at least 2 weeks and reported no use of antiprotozoal or antihelminth medications. A total of 472 fresh stool specimens were collected and examined for intestinal parasitic pathogens. With each specimen a consent form was completed by a parent or guardian. Demographics gathered included age, sex, nationality (278 Karen, 113 Thai, 60 Mon, 14 Burmese, 4 Lao, and 3 other), and village name.

Specimen processing and examination. Stool specimens were processed by mixing one portion stool with three portions preservatives, both 10% buffered formalin and Zinc PVA.[6] Saline and iodine wet mount smears of fresh stool were prepared, fixed in absolute methanol for 10 minutes, and air dried before performing DMSO-mAFB staining as described by Bronsdon for the detection of *Cryptosporidium* and *Cyclospora* oocysts.[7] Iodine wet mounts and DMSO-mAFB smears were prepared from 345 formalin-preserved specimens after performing a formalin-ethylacetate sedimentation concentration; not all 472 specimens were of a quantity sufficient to concentrate. Trichrome stains were performed on stool specimens preserved in Zinc PVA.[8] Microscopic examination was performed by a seasoned medical parasitologist with 10 years experience. Two hundred to three hundred oil immersion fields were examined for the presence of ameba, protozoan cysts and trophozoites of flagellates (to include *G. lamblia*, *B. hominis*), and soil-transmitted helminth eggs and larvae (to include *Ascaris lumbricoides* and *Trichuris trichiura*—both round worms). A portion of 434 stool specimens was kept in a cryogenic vial at −20°C before weekly shipment on dry ice to AFRIMS; not all 472 specimens were available for freezing and further testing due to insufficient quantity. At AFRIMS the 434 samples were thawed and tested for the presence of *G. lamblia* and *Cryptosporidium* specific antigens by ProSpecT *Giardia*/*Cryptosporidium* Microplate assay, an enzyme immunoassay. All positive samples were then tested by single antigen assays, ProSpecT *Giardia* Microplate and ProSpecT *Cryptosporidium* Microplate assays (Alexon-Trend, now Remel, Inc., Lenexa, KS). The previously reported sensitivities of ProSpecT *Giardia*/*Cryptosporidium*, *Giardia*, and *Cryptosporidium* Microplate assays were 95.5%, 94.9%, and 100%, respectively.[9]

Statistical methods. Statistical analysis was performed by using a two-tailed χ^2 test. A P value of < 0.05 was considered significant.

RESULTS

From October 2001 through October 2002, stool specimens from a total of 472 pre-school children, 233 males and 239 females, 236 children with diarrhea (cases) and 236 asymptomatic children (controls), were examined for the presence of intestinal parasites by the described methods. There were 107 individuals, 41 cases and 66 controls, infected with intestinal parasites. A statistically significant ($P < 0.01$) higher proportion was found in control children (27.9%) compared with case children (17.4%). The proportion between males (18.5% and 25.7%) and females (16.1% and 29.9%) was similar in both cases and controls (p = NS), respectively. Children between 2 and 3 years of age had the highest proportion of intestinal parasites compared with the other four age groups in cases; children between 4 and 5 years of age led the control group in highest percentage of intestinal parasites. The youngest age group included in this study, children between 3 months and 1 year of age, showed a statistically significant lower parasitism compared with the other four age groups. The Karen, Thai, Mon, and Burmese children had fractions of 22.5% and 32.2%, 11.9% and 15.2%, 12.1% and 29.6%, and 0 and 25%, in cases and controls, respectively (Table 1). Karen people had a statistically significant higher proportion of intestinal parasites compared with Thai preschoolers ($P < 0.05$) in controls, but not in cases. Amongst the other nationalities, significant differences in parasitism were not observed in controls or cases.

TABLE 1

Number of intestinal parasites for cases and controls grouped by sex, age, and nationality in Sangkhlaburi

Group	No. infected/No. investigated	
	Cases	Controls
Sex		
Male	23/124	28/109
Female	18/112	38/127
Age (years)		
3 months–1	4/84	9/99
1–2	15/92	33/82
2–3	14/33	16/31
3–4	4/14	2/13
4–5	4/13	6/11
Nationality		
Karen	29/129	48/149
Thai	8/67	7/46
Mon	4/33	8/27
Burmese	0/2	3/12
Lao	0/2	0/2
Other	0/3	0/0
Total	41/236 (17.4%)	66/236 (27.9%)

Further analysis of the parasites revealed two species of round worm and four species of protozoan. The parasite detected most frequently in cases and controls was *G. lamblia*, followed by *Cryptosporidium* spp. In cases, *Cyclospora* spp. was the next most frequently identified parasite followed by *Ascaris lumbricoides* and *Blastocystis hominis*, whereas in controls, *Ascaris lumbricoides* identification was followed by *Cyclospora* spp., *Blastocystis hominis*, and *Trichuris trichiura* (Table 2). Eighteen specimens (3.8%) showed mixed parasitic infections (Table 3). Fraction of infection in cases and controls was lowest in the hot season (8.3% and 20%, respectively) and peaked during the rainy season (30.5% and 32.2%, respectively) (Figure 1).

The number of both *G. lamblia* and *Cryptosporidium* spp. detected increased significantly with the addition of an enzyme immunoassay technique (three ProSpecT Microplate assays). Of 434 stool specimens screened using ProSpecT *Giardia*/*Cryptosporidium* Microplate assay, 99 were positive and hence tested further using the single antigen assays for *Giardia* and *Cryptosporidium*. Of 73 tests positive for *G. lamblia*, 34 were simple smear positive, 40 were microscopy positive after concentration, and 71 (97.3%) were positive by ProSpecT *Giardia* Microplate assay. Of 16 tests positive for *Cryptosporidium* spp., 4 were simple smear positive (DMSO-

TABLE 2

Proportion of intestinal parasites among 472 pre-school children in Sangkhlaburi

Intestinal parasites	No. infected	
	Cases (N = 236)	Controls (N = 236)
G. lamblia	32 (13.6%)	55 (23.3%)
B. hominis	2	1
Cryptosporidium	4 (0.8%)	12 (2.5%)
Cyclospora	3	5
A. lumbricoides	2	11
T. trichiura	0	1
Total	43	85

TABLE 3
Occurrence of mixed intestinal parasite infections in 18 pre-school children in Sangkhlaburi

Intestinal parasites detected	No. of infections from cases and controls
G. lamblia + B. hominus	2
G. lamblia + Cyclospora	1
G. lamblia + Cryptosporidium	9
G. lamblia + A. lumbricoides	5
G. lamblia + A. lumbricoides + T. trichiura	1
	18 (3.8%)

mAFB stain), 5 were microscopy positive after concentration, and 16 (100%) were positive by ProSpecT *Cryptosporidium* Microplate assay (Table 4). The EIA tests incorporated in this study were easy to use, improved sensitivity of detecting both *G. lamblia* and *Cryptosporidium* spp. compared with traditional microscopy, showed no signs of cross-reactivity with other parasites, and reduced labor and capital expenditure costs.

DISCUSSION

Other intestinal parasite studies have been conducted in at least these seven provinces in Thailand–Bangkok, Nonthaburi, Pathum Thani, Phichit, Nan, Khon Kaen, and Chiang Mai—all north of Bangkok (Figure 2 and Table 5). We are unaware of another seasonal study of intestinal parasites taking place in Kanchanaburi province, northwest of Bangkok. The limitation in comparing studies lies in differing study designs, number of stool samples examined, and ova and parasite examination techniques, but factors influencing the prevalence of intestinal parasites are constant. Climate, food and water supplies, personal and community hygiene, sanitation, proximity to domestic and wild animals, and socioeconomic class all play into the likelihood of exposure to intestinal parasites.[1]

In this study of pre-school children living in Sangkhlaburi, Thailand, we found that 27.9% of asymptomatic controls and 17.4% of symptomatic cases had intestinal parasites (Table 1). We were unable to find another study of asymptomatic and symptomatic age-matched pre-school children living in rural communities in Thailand to compare our overall intestinal

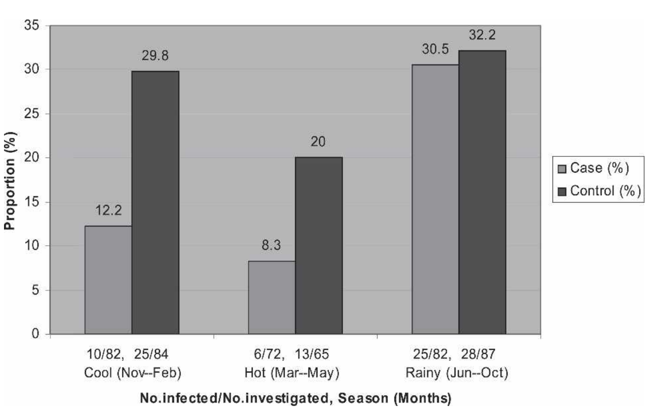

FIGURE 1. Seasonal proportion of parasites in cases and controls in Sangkhlaburi during study period of October 2001–October 2002.

TABLE 4
Detection of *G. lamblia* and *Cryptosporidium* spp. by simple smear, concentration technique, and EIA Microplate assays (n = 345)

	Simple smear	Concentration technique	EIA Microplate assays	Total detected
G. lamblia	34	40	71	73
Cryptosporidium	4	5	16	16

parasites detected. Janoff and colleagues found that 17% of asymptomatic pre-school orphans living in an urban Bangkok home had intestinal parasites compared with 15% of symptomatic cases.[10] Other studies in Thailand of only institutionalized, asymptomatic children show prevalence of intestinal parasites ranging from 30.8% in an urban setting up to 81.1% in a rural setting.[11–13] The possibility of a dilutional effect masking the number or presence of parasites detected in children with diarrhea in our study is worthy of consideration.

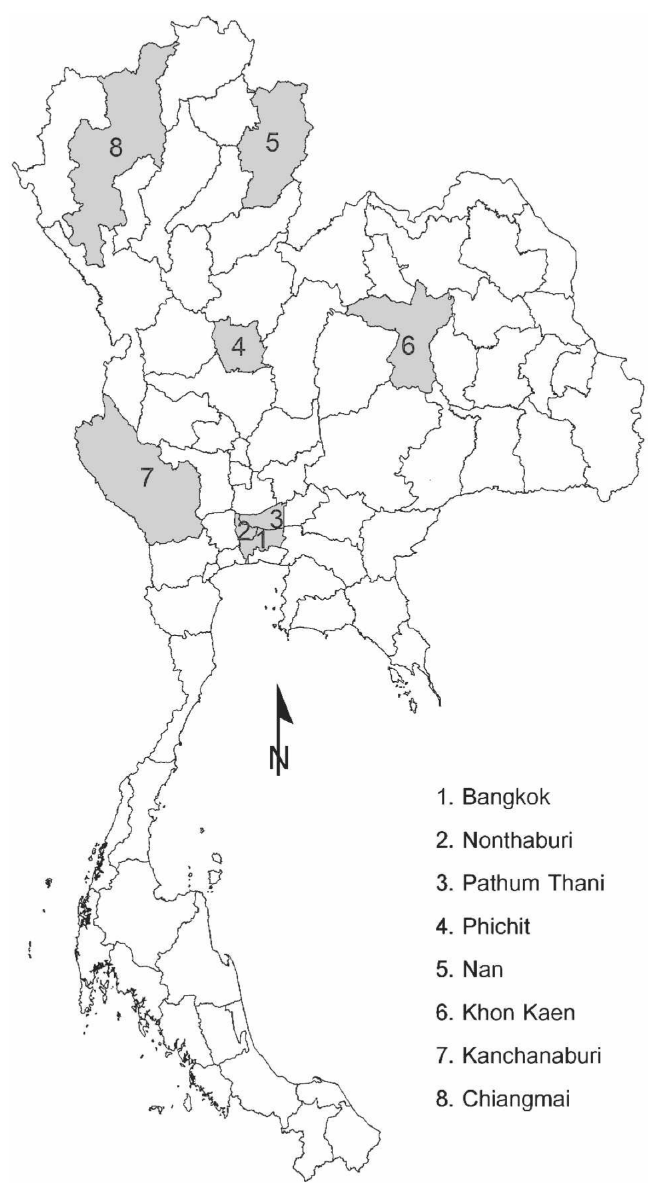

FIGURE 2. Provinces of Thailand relevant to the discussion and Table 5.

TABLE 5
Summary of intestinal parasite studies in Thailand

Study	Province	Population		*G. lamblia* detected	*Cryptosporidium* detected
Chavalittamrong et al., 1978[15]	Bangkok/urban	Children in low socioeconomics	Asymptomatic	18.18%	N/A
			Symptomatic	18.58%	
Chavalittamrong and Jirapinyo, 1984[19]	Bangkok/urban	Children in low socioeconomics	Symptomatic	6.1%	N/A
Saksirisampant et al., 2003[13]	Pathum Thani/rural	Pre-school orphans	Asymptomatic	37.7%	None
Mungthin et al., 2001[12]	Bangkok/urban	Children/orphans Phayathai BH	Asymptomatic	None	7.8%
		Childcare workers	Asymptomatic	None	None
Termmathurapoj et al., 2000[11]	Bangkok/urban	Pre-school children/orphans Phayathai BH	Asymptomatic	None	12%
		Childcare workers	Asymptomatic	None	None
Janoff et al. 1990[10]	Bangkok/urban	Pre-school children/orphans	Asymptomatic	18%	6%
			Symptomatic	2%	2%
Jongwitiwes et al., 1990[18]	Nonthaburi/urban	Children/orphans	Asymptomatic	6.4% overall	None
			Symptomatic		7.3%
Bunnag et al., 1982[21]	Phichit/rural	Village dwellers		7%	N/A
	Bangkok/urban	Slum dwellers		10%	N/A
Sornmani et al., 1973[22]	Khon Kaen/rural	Village dwellers on periphery of a lake		7.2%	N/A
Waikagul et al., 2002[25]	Nan/rural	School children		5.3%	N/A
Kasuya et al., 1989[24]	Chiang Mai/rural	Primary school children		7.7%	N/A
Echeverria et al., 1989[14]	Bangkok/urban	Pre-school children	Asymptomatic	1.3%	0.3%
			Symptomatic	2%	1.8%
Boonchai et al., this study	Kanchanaburi/rural	Pre-school children	Asymptomatic	23.3%	2.5%
			Symptomatic	13.6%	0.8%

G. Giardia; E. Entamoeba; B. Blastocystis; BH Babies' Home; N/A not applicable.

In the 472 pre-school children studied in our investigation, *G. lamblia* was the most frequent parasite detected in 87 of 472 (18.4%) cases and controls with significantly greater proportion in asymptomatic controls compared with symptomatic cases, 55 of 236 (23.3%) versus 32 of 236 (13.6%) ($P < 0.01$) (Table 2). This finding is consistent with results from a study of urban, institutionalized pre-schoolers in which *G. lamblia* was detected in 18% of asymptomatic controls, and only 2% of symptomatic cases (Table 5).[10] By contrast, two other studies conducted in Bangkok found little difference in detection of *G. lamblia* between children with diarrhea and those without gastrointestinal symptoms. *G. lamblia* was detected in 2% of symptomatic pre-schoolers and 1.3% of age-matched asymptomatic controls, whereas in another study, *G. lamblia* was detected in 18.6% of symptomatic children and 18.2% of asymptomatic children.[14,15] It is hypothesized that chronic exposure to *G. lamblia* in places with an unreliable water supply protects persons against parasite-associated symptoms.[16] However, evidence suggests that children do not acquire immunity to re-colonization with *G. lamblia* in highly endemic areas.[17]

The second most frequently identified intestinal parasite amongst our study population was *Cryptosporidium*. *Cryptosporidium* oocysts were detected in 12 asymptomatic controls (2.5%) yet only in four symptomatic children (0.8%). Janoff and colleagues found a similar division between institutionalized, asymptomatic pre-school children with *Cryptosporidium* (6%) versus symptomatic pre-school children with *Cryptosporidium* living in the same institution (2%).[10] Frequent exposure may have resulted from both water and fecal–oral transmission, and was intensified by crowded living quarters.[10] Two groups of investigators in Thailand have reported the reverse—a statistically significant higher percentage of symptomatic children with *Cryptosporidium* (1.8% and 7.3%, respectively) versus age-matched, asymptomatic children with *Cryptosporidium* detected in stools (0.3% and none, respectively).[14,18]

In our study population of children less than 5 years of age, we found the proportion of *G. lamblia* to be highest in 2–3 year olds in cases and controls. Amongst cases, *Cryptosporidium* was detected only in 1–2 year olds, whereas in controls the proportion was highest in 4–5 year olds. Other studies designed to detect proportion of intestinal parasites and group participants by age in Thailand have also shown that children are typically infected with *G. lamblia* and/or *Cryptosporidium* at 1 year of age and older.[13–15,19] Although Termmathurapoj and colleagues agree that *G. lamblia* is more often detected in children older than 1 year of age, they found that the risk of acquiring *Cryptosporidium* in children living in an orphanage was 2.4 times higher for those younger than 1 year.[11] Janoff and colleagues found both *Cryptosporidium* and *G. lamblia* were significantly more common in children 2 years or older; the children were living in close quarters.[10] While exposure to *G. lamblia* and *Cryptosporidium* begins early in life in these communities in Thailand, there are subtle differences in age of infectivity.

No association has been found between positive antibody titers to *G. lamblia* and *Cryptosporidium* and positive stool samples, infection, or symptomatic disease in areas where exposure is elevated compared with more developed countries.[10,20] Studies in *G. lamblia* and *Cryptosporidium* endemic areas, Bangladesh and Thailand, describe serum antibody titers to *G. lamblia* and *Cryptosporidium* being acquired at early ages and remaining high across all age groups, but not reliable in determining disease.[10,20]

Two parasitological studies of age diverse populations in rural Thailand report high percentages of parasites detected, 63% and 69.1%, respectively. Helminths were detected more often than protozoa with *Opisthorchis viverrini* and hookworm most frequently identified.[21,22] In a 2001 national survey of parasitic infections in children and adults conducted by the Department of Communicable Disease Control, Ministry of Public Health/Thailand, helminths ranked 22.3% in prevalence. Hookworm, the most common helminth detected, accounted for 11.3% of the parasitic infections.[23] Investigators in northern Thailand reported hookworm as the most frequent parasite detected (26.3%) in a study of primary school children in which the overall percentage of parasites detected was 48.7%.[24] We did not identify hookworm in any of the 472 stool samples analyzed, and suspect that decreased exposure in pre-school age children is one explanation.

Mixed parasitic infections are not uncommon amongst children of developing countries. In our study population, *G. lamblia* was mixed with another parasite in 18 pre-school children, 3.8% of cases and controls. In Nan province where the rate of parasitic infections in school children exceeded the national average, mixed parasitic infections accounted for 33% of all parasitic infections.[25] Additionally, Chavalittamrong and colleagues concluded that parasitic infections may increase one's susceptibility to infection with other intestinal pathogens, bacterial and viral.[15,19]

We have shown that high temperatures in combination with high rainfall are conducive to acquiring intestinal parasites. The percentage of infected cases and controls peaked during the rainy season, 30.5% and 32.5%, respectively. Jongwutiwes and colleagues made the same observation of *Cryptosporidium* infection.[18] In our study, the lowest percentage of infected cases and controls occurred during the hot season, 8.3% and 20% respectively.

The effects of an intervention can be seen in Cambodia, a neighboring country. As a result of clinical investigations, prevalence plotting, and a committed Ministry of Health, the need to treat Cambodian school-age children against intestinal parasites was identified. The World Health Organization reports that as recent as 5 years ago in 1999, 70% of Cambodian children were infected with intestinal helminths. The impact of these infections could be seen on school attendance, children's performance in reasoning, reading comprehension, memory, and general health. Improvement was seen in all of these areas when 75% of Cambodian's nearly three million school age children were treated prophylactically with inexpensive, antihelmintic drugs, such as albendazole, between 2001 and 2004.[26] Similarly, a series of parasitologic surveys in Chiang Mai conducted between 1969 and 1989 identified a dramatic decrease in the number of *Ascaris* cases following improvements in sanitary conditions and introduction of elimination projects to include a Helminthiasis Control Project, a public health education campaign, and easy access to antihelmintics.[25]

We have identified an at-risk population who could benefit from health education, promotion of good personal hygiene, improvement in sanitation, provision of safe drinking water, and possibly mass treatment. The study of parasitic infections in different populations is necessary to develop effective prevention and control strategies. There continues to be a pressing need for parasitological studies of protozoan and helminth parasites in endemic areas.

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