Tropical Biomedicine 39(4): 539-546 (2022)
https://doi.org/10.47665/tb.39.4.009

RESEARCH ARTICLE

Intestinal parasitic infections and risk factors among the population in Cambodia

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ARTICLE HISTORY

Received: 13 July 2022
Revised: 10 November 2022
Accepted: 11 November 2022
Published: 31 December 2022

ABSTRACT

Many species of helminths and protozoa caused intestinal parasitic infections (IPIs). It belongs to neglected tropical diseases (NTDs) and remains a major public health problem in several Southeast Asian countries. The present study aimed to investigate the prevalence of IPIs and associated risk factors among the population in Kratie Province in northeastern Cambodia and Phnom Penh is the capital that locates in southern Cambodia. Fecal specimens (n=366) were collected in 10 villages in Kratie Province and Phnom Penh from 2019 to 2021. They were processed using the formalin ethyl-acetate concentration technique (FECT) to investigate parasites at egg and cyst stages and then examined under a light microscope. The results revealed that the prevalence of IPIs among the population in Kratie Province (n=317) and Phnom Penh (n=49) was 16.12% (n=59); of Kratie Province (n=50, 13.66%) and Phnom Penh (n=9, 2.46%), 12.02% (n=44) were helminths and 4.10% (n=15) were protozoa. The parasitic infection rate was higher in males (9.02%) than in females (7.10%) and more likely to be due to helminths (7.38%) than protozoa (1.64%). Prevalence of *Opisthorchis viverrini* was the highest (5.74%), followed by those of *Entamoeba coli* (4.10%), hookworm (3.83%), *Ascaris lumbricoides* (1.10%), *Hymenolepis nana* (1.09%), *Taenia* spp. (0.54%), *Trichuris trichiura* (0.55%), and *Enterobius vermicularis* (0.27%), respectively. Moreover, *O. viverrini* infection was the most common infection in the >20-year age group in Kratie Province. In addition, the bivariate and multivariate analyses showed that the association between gender. Gender was a significant risk factor positively associated with *O. viverrini* and hookworm infections (OR_{adj}=0.318, 95% CI=0.122-0.8270, P=0.019 and OR_{adj}=0.085, 95% CI=0.017-0.436, P=0.003, respectively). In conclusion, the IPIs were highly prevalent, especially *O. viverrini* and hookworm infections, among the population in Cambodia. These IPIs impact the public health burden but can be prevented by education regarding good sanitary practices in this community.

Keywords: Intestinal parasitic infections (IPIs); formalin ethyl-acetate concentration technique (FECT); Cambodia; prevalence; Kratie Province and Phnom Penh.

INTRODUCTION

Intestinal parasitic infections (IPIs) are a serious public health problem in several countries throughout the world. IPIs are highly prevalent in Southeast Asia (Dunn *et al*., 2016), including Thailand, Cambodia, Lao PDR, and Myanmar (Han *et al*., 2019). Neglected helminth and protozoa infections have affected almost 200 million people who live in poverty in countries belonging to the Association of Southeast Asian Nations (ASEAN) (Hotez *et al*., 2015). The main cause of intestinal parasite transmission is poor hygiene conditions, such as a lack of clean water and sanitary toilets (Ziegelbauer *et al*., 2012; Echazu *et al*., 2015). More than 10 million people suffer from parasitic infections, including infections of liver and intestinal flukes, in Southeast Asia (Furst *et al*., 2012). In Myanmar, the prevalence of intestinal helminth infection is approximately 11.3 million people (Hotez *et al*., 2015; WHO, 2018). The primary socioeconomic factors related to the high prevalence of IPIs include poor food hygiene, unsanitary housing, congested living conditions, agricultural conditions, insufficient water resources, animal living near the reservoirs, and poor healthcare services (Echazu *et al*., 2015; Hotez *et al*., 2015; Dunn *et al*., 2016;). Nevertheless, these parasitic infections are classified as neglected tropical diseases. The most patients are unawareness of symptoms until progression and illness presentation, leading to individual, economic, and society burdens due to chronic infection or reinfection. Consequently, this problem has persisted. Approximately 9.3 million cases of liver fluke infection have been reported in ASEAN countries (Hotez *et al*., 2015). A previous study reported on the prevalence of intestinal helminth infections among villages in Kratie Province, northeastern Cambodia, using fecal specimens and the Kato-Katz thick smear technique. The highest prevalence of *Opisthorchis viverrini* infections occurred in Roka Kandal A (10.4%), followed by Talous (5.9%); in both these

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villages, adult residents presented with a higher prevalence (19.4% and 9.0%, respectively) than schoolchildren (6.4% and 1.4%) (Sohn *et al*., 2021). Moreover, *Strongyloides stercoralis* and hookworm or soil-transmitted helminth were high prevalence in Preah Vihear Province, northeastern Cambodia. Hookworm, *S. stercoralis*, and co-infections were 49.0%, 48.6%, and 43.8%, respectively (Forrer *et al*., 2018). The prevalence of *Ascaris lumbricoides* and hookworm infections in Kratie Province were 49% and 28.9%, respectively (Sinuon *et al*., 2003). IPI cases have a distinct geographic distribution in the Lower Mekong Basin, including regions of Thailand, Lao PDR, Cambodia, Myanmar, and southern Vietnam. Currently, the gold standard technique, the formalin ethyl-acetate concentration technique (FECT), is used to detect the parasite eggs or helminths in fecal specimens (Won *et al*., 2015). FECT enables accurate diagnosis and reduces the costs of examination. Therefore, the present study aimed to determine human IPI cases using this technique from fecal specimen collected in Kratie Province in northeastern Cambodia and Phnom Penh. Moreover, the present study aimed to investigate the prevalence of and risk factors for each parasite infection, especially the geographic distribution and epidemiological status, to reduce the rate of IPIs in Cambodia.

MATERIALS AND METHODS

Study areas

Kratie Province is located in northeastern Cambodia and is approximately 242 kilometers (km) from Phnom Penh in southern Cambodia, where the Mekong River crosses 5 provinces, running from the north to the south. Kratie Province is at 12.86063 N and 105.969988 E (map coordinates: 12°51'38.268" N and 105°58'11.9568" E). It is located in northeastern Cambodia, bordering Stung Treng in the north, Mondul Kiri in the east, Vietnam and Kampong Cham in the south, and Kampong Thom in the west. The coordinates of Phnom Penh are 11.562108 N and 104.888535 E. The capital is the largest city in Cambodia and a key industrial center. Kratie Province is approximately 11,094 square kilometers (km^2) and is divided into six districts with 46 communes (including Sangkat) and 255 villages. The 2019 census estimated a population of 374,755 residing in the province, and 50% females. The population density in 2008 was 29 persons/km^2, while the density of the average population in Cambodia was 74 persons/km^2. The Mekong River stretching across Kratie Province and flowing towards Phnom Penh (Figure 1).

Population, sample size and fecal specimen collection

The sample size was calculated using the equation: $n=[N(Z\alpha/2)2P(1-P)]/[e^2(N-1)+(Z\alpha/2)2P(1-P)]$, where N= the sample size or population, P=prevalence or proportion of infected individuals, $Z\alpha/2$=normal deviation for two-tailed alternative hypothesis at a level of significance of 1.96, and e=precision margin of error (5% or 0.05) (Ngrenngarmlert *et al*., 2012). The necessary sample size of this study was set at 366 individuals. This study used a cross-sectional design to collect fecal specimens and a simple random sampling technique to select the 366 participants from Kratie Province (*n*=317) and Phnom Penh from (*n*=49) 2019 to 2021. The sample size of Kratie Province was provided more than Phnom Penh due to the capital being more urbanized. A total of 366 fecal specimens were collected from the residents (154 men and 212 women; ages in the range of 3-80 years old). The specimens were collected from 10 villages in subdistricts, including Pithnou, Khsirp, and Snuol in Kratie Province and Phnom Penh. The Kratie Province samples were from the Chrab, Chreav, Lamut, Memot, Trapaing Sre, and Phnomprich villages, and the Phnom Penh samples were from Dangkor, Mean Chey, Posen Chey, and Russey Keo. The procedure was approved by the Human Ethics Committee of the health office in Nakhon Ratchasima Province (EC: NRPH013) and the Ministry of Health of Cambodia. Participants provided informed consent for participation. The parents signed as the witness in the informed consent for young participants. On the other hand, young participants used the fingerprint replaced their name if they can't write names by themselves. The specimens were transported to the Parasitic Disease Research Center (PDRC), Institute of Medicine, Suranaree University of Technology (SUT), Thailand within 2-3 days of collection and stored at 4°C until the examination.

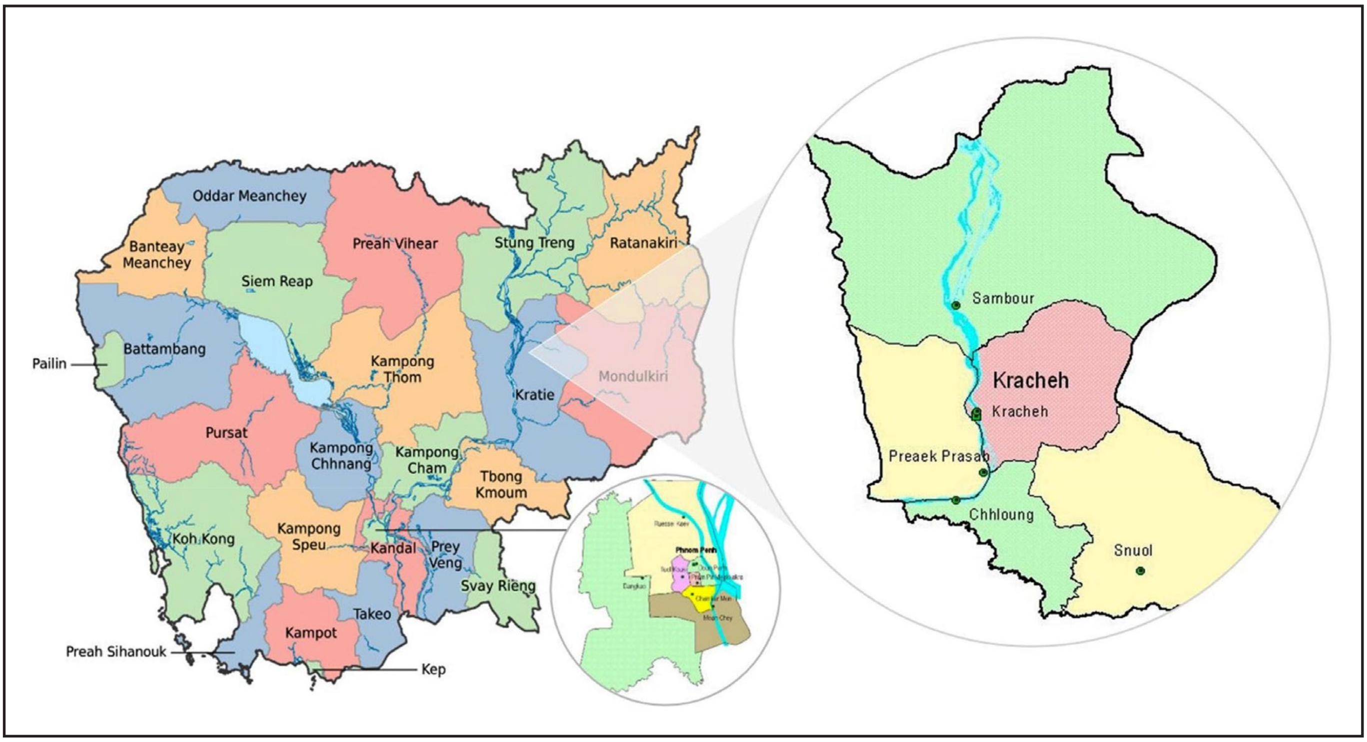

Figure 1. Map of the surveyed area (Snuol district) in Kratie Province and Phnom Penh, Cambodia (modified from a provincial map, 2012).

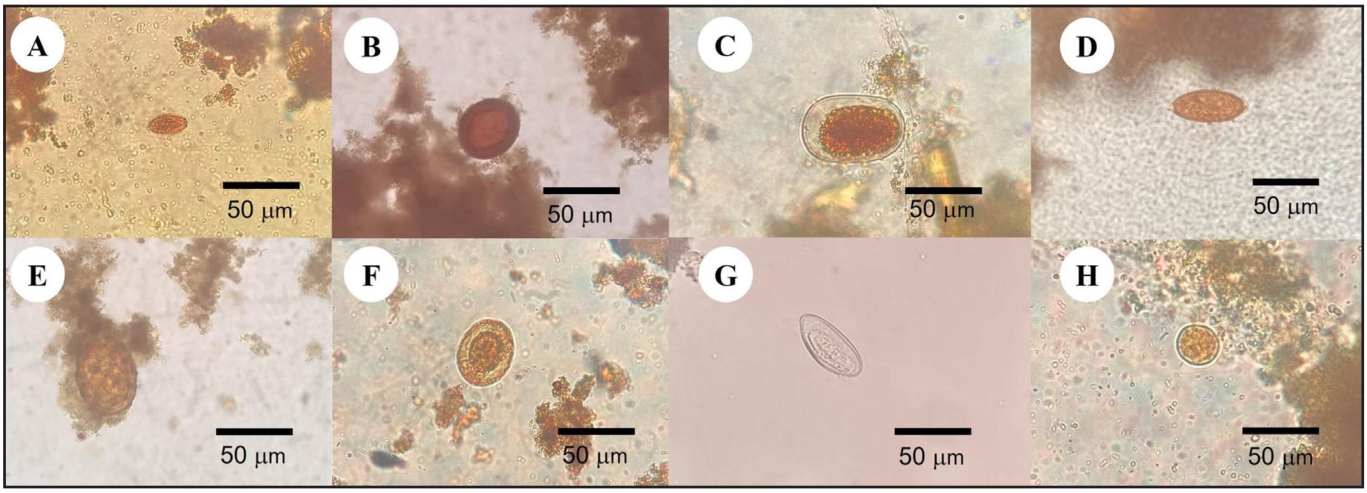

Figure 2. The morphology of intestinal helminth eggs and protozoa cysts in fecal specimens under light microscopy. *Opisthorchis viverrini* (A), *Taenia* spp. (B), hookworm (C), *Trichuris trichiura* (D), *Ascaris lumbricoides* (E), *Hymenolepis nana* (F), *Enterobius vermicularis* (G), and *Entamoeba coli* (H). Scale bar: 50 µm.

Fecal examination

The participants were provided with labeled plastic containers for the fecal specimens. The specimens were preserved with 10% formalin, and intestinal parasite and protozoan infections were detected using FECT. The sample was examined under a light microscope using physiological saline and iodine to observe the parasite eggs and protozoa cysts. Finally, the positive result sample slides were confirmed by parasitologists to confirm the accuracy of detection (Elkins *et al*., 1986; Wararat *et al*., 2021).

Statistical analysis

The statistical analysis was conducted with SPSS version 20.0 for Windows. The prevalence of IPI expression is expressed as the percentage and the 95% confidence interval (95% CI). The associations between IPI prevalence and potential risk factors were examined with bivariate and multivariate analyses (Wararat *et al*., 2021). The difference in IPI prevalence of 6 villages in Kratie Province (Chrab [*n*=63], Chreav [*n*=8], Lamut [*n*=94], Memot [*n*=2], Phnomprich [*n*=50], and Trapaing Sre [*n*=100]) and 4 villages in Phnom Penh (Dangkor [*n*=11], Mean Chey [*n*=17], Posen Chey [*n*=6], and Russey Keo [*n*=15]) were determined. Descriptive statistics are presented as frequencies, crosstabs, and the results of chi-square tests; and *P*<0.05 was considered significant. Moreover, the multivariate model was analyzed using the variable; gender that we selected the variable from a significant risk factor positively associated with each parasite in the bivariate test.

RESULTS

The IPI prevalence among 366 fecal specimens collected in Kratie Province (*n*=317) and Phnom Penh (*n*=49) was 16.12% (*n*=59); of Kratie Province (*n*=50, 13.66%) and Phnom Penh (*n*=9, 2.46%), these IPIs were further classified into helminths (*n*=44, 12.02%) and protozoa (*n*=15, 4.10%). More IPIs were found in males (*n*=33, 9.02%; higher than those in females [*n*=26, 7.10%, crude odds ratio [OR_{cru}]=0.513, 95% CI=0.292-0.900, *P*=0.02 and adjusted odds ratio [OR_{adj}]=0.482, 95% CI=0.272-0.854, *P*=0.012]) and males were also more likely to be infected by helminths (*n*=27, 7.38%, OR_{cru}=0.41, 95% CI=0.215-0.783, *P*=0.007 and OR_{adj}=0.375, 95% CI=0.194-0.726, *P*=0.004) or protozoa (*n*=6, 1.64%, OR_{cru}=1.094, 95% CI=0.381-3.139, *P*=0.868 and OR_{adj}=1.102, 95% CI=0.382-3.183, *P*=0.857), respectively. Most IPIs were found in >51-year-olds (*n*=15, 4.10%, OR_{cru}=1.156, 95% CI=0.95-1.408, *P*=0.149 and OR_{adj}=1.182, 95% CI=0.968-1.442, *P*=0.101); more likely to be infected by helminths (*n*=10, 2.73%, OR_{cru}=1.217, 95% CI=0.973-1.522, *P*=0.085 and OR_{adj}=1.256, 95% CI=1.001-1.576, *P*=0.049) and protozoa (*n*=5, 1.37%, OR_{cru}=0.971, 95% CI=0.673-1.402, *P*=0.875 and OR_{adj}=0.968, 95% CI=0.670-1.400, *P*=0.864), respectively. Kratie Province had

Table 1. The Bivariate and Multivariate analysis of the prevalence of intestinal parasitic infections (IPIs) and participant variables in Kratie Province and Phnom Penh, Cambodia (*n*=366)

Variable		Samples examined (n (%))	IPIs (n (%))	Crude and Adjusted OR (95% CI) and *P* - value			
				Crude OR (95% CI)	*P - value*	Adjusted OR (95% CI)	*P - value*
Sex	Male	154 (42.08)	33 (9.02)	0.513 (0.292-0.9)	0.02	0.482 (0.272-0.854)	0.012
	Female	212 (57.92)	26 (7.10)				
Age	< 20 years	92 (25.14)	13 (3.55)	1.156 (0.95-1.408)	0.149	1.182 (0.968-1.442)	0.101
	21–30 years	77 (21.04)	13 (3.55)				
	31–40 years	72 (19.67)	7 (1.91)				
	41–50 years	66 (18.03)	11 (3.01)				
	>51 years	59 (16.12)	15 (4.10)				
Location	Phnom Penh	49 (13.39)	9 (2.46)	1.201 (0.549-2.631)	0.646	1.295 (0.584-2.869)	0.525
	Kratie Province	317 (86.61)	50 (13.66)				

Significantly different *P*<0.05.

Table 2. The prevalence of intestinal parasitic infections (IPIs) (identified helminthes and protozoa infections) and participant variables in Kratie Province and Phnom Penh, Cambodia

Variable		Samples examined (n (%))	n (%) (95% Confidence interval) and *P*-value								
			Helminthes (%)	Crude OR (95% CI)	*P* - value	Adjusted OR (95% CI)	*P* - value	Protozoa	Crude OR (95% CI)	*P* - value	OR (95% CI)	*P* - value
Sex	Male	154 (42.08)	27 (7.38)	0.41 (0.215-0.783)	0.007	0.375 (0.194-0.726)	0.004	6 (1.64)	1.094 (0.381-3.139)	0.868	1.102 (0.382-3.183)	0.857
	Female	212 (57.92)	17 (4.64)					9 (2.46)				
Age	< 20 years	92 (25.14)	6 (1.64)	1.217 (0.973-1.522)	0.085	1.256 (1.001-1.576)	0.049	7 (1.91)	0.971 (0.673-1.402)	0.875	0.968 (0.670-1.400)	0.864
	21–30 years	77 (21.04)	12 (3.28)					1 (0.27)				
	31–40 years	72 (19.67)	6 (1.64)					1 (0.27)				
	41–50 years	66 (18.03)	10 (2.73)					1 (0.27)				
	>51 years	59 (16.12)	10 (2.73)					5 (1.37)				
Location	Phnom Penh	49 (13.39)	7 (1.91)	1.261 (0.528-3.012)	0.601	1.416 (0.582-3.448)	0.443	2 (0.55)	0.995 (0.218-4.550)	0.995	0.991 (0.216-4.554)	0.99
	Kratie Province	317 (86.61)	37 (10.11)					13 (3.55)				

Significantly different *P*<0.05.

Table 3. Bivariate analysis of the relationship between variables and intestinal parasitic infections (IPIs) in Kratie Province and Phnom Penh, Cambodia

Variable	*Opisthorchis viverrini*			*Taenia* spp.			Hookworm			*Trichuris trichiura*			*Ascaris lumbricoides*			*Hymenolepis nana*			*Enterobious vermicularis*			*Entamoeba coli*		
	n (%), OR (95% CI), and *P* - value																							
Male	14 (3.83)	0.341 (0.134-0.868)	0.024	1 (0.27)	0.725 (0.0451-1.684)	0.821	12 (3.28)	0.113 (0.025-0.511)	0.005	0	1.539 (0.000)	0.996	2 (0.55)	0.724 (0.101-5.196)	0.748	1 (0.27)	2.196 (0.226-21.316)	0.497	1 (0.27)	0.000 (0.000)	0.995	6 (1.64)	1.094 (0.381-3.139)	0.868
Female	7 (1.91)			1 (0.27)			2 (0.55)			2 (0.55)			2 (0.55)			3 (0.82)			0			9 (2.46)		
< 20 years old	4 (1.09)	1.455 (0.477-4.441)	0.510	0	1.007 (0.103-9.806)	0.995	1 (0.27)	4.533 (0.585-35.135)	0.148	0	1.188 (0.000)	0.997	1 (0.27)	1.007 (0.103-9.806)	0.995	0	2.393 (0.000)	0.997	1 (0.27)	0.000 (0.000)	0.995	7 (1.91)	0.365 (0.129-1.037)	0.058
> 20 years old	17 (4.64)			2 (0.55)			13 (3.55)			2 (0.55)			3 (0.82)			4 (1.09)			0			8 (2.19)		
Phnom Penh	4 (1.09)	1.569 (0.505-4.872)	0.436	0	0.000 (0.000)	0.998	0	1.569 (0.505-4.872)	0.436	1 (0.27)	0.000 (0.000)	0.997	0	0.000 (0.000)	0.998	2 (0.55)	6.702 (0.922-48.726)	0.060	0	6.583 (0.405-107.011)	0.185	2 (0.55)	0.995 (0.218-4.550)	0.995
Kratie Province	17 (4.64)			2 (0.55)	.		14 (3.83)			1 (0.27)			4 (1.09)			2 (0.55)			1 (0.27)			13 (3.55)		

Significantly different *P*<0.05.

the most cases (n=50, 13.66%, OR_{cru}=1.201, 95% CI=0.549-2.631, P=0.646 and OR_{adj}=1.295, 95% CI=0.584-2.869, P=0.525) , individuals were more likely to be infected with helminths (n=37, 10.11%, OR_{cru}=1.261, 95% CI=0.528-3.012, P=0.601 and OR_{adj}=1.416, 95% CI=0.582-3.448, P=0.443) and protozoa (n=13, 3.55%, OR_{cru}=0.995, 95% CI=0.218-4.550, P=0.995 and OR_{adj}=0.991, 95% CI=0.216-4.554, P=0.99), respectively (Table 1 and Table 2).

Fecal specimens were investigated using FECT, and the parasites were classified under a light microscope. These methods identified seven species of helminths (*O. viverrini*, *Taenia* spp., hookworm, *Trichuris trichiura*, *A. lumbricoides*, *Hymenolepis nana*, and *Enterobius vermicularis*) and one species of protozoa (*Entamoeba coli*) (Figure 2). The IPIs were classified into helminths such as *O. viverrini*, *Taenia* spp., hookworm, *T. trichiura, A. lumbricoides*, *H. nana*, and *E. vermicularis* or protozoa (*E. coli*). The most IPIs was *O. viverrini* (n=21, 5.74%), which was more common in males (n=14, 3.83%) than in females (n=7, 1.91%) P=0.024, followed by *E. coli* (n=15, 4.10%), which was more common in females (n=9, 2.46%) than in males (n=6, 1.64%) P=0.868. Next came hookworm (n=14, 3.83%), which was more common in males (n=12, 3.28%) than in females (n=2, 0.55%) P=0.005, and *A. lumbricoides* (n=4, 1.10%), which was more common in males (n=2, 0.55%) than in females (n=2, 0.55%) P=0.748. *H. nana* (n=4, 1.09%) was more common in females (n=3, 0.82%) than in males (n=1, 0.27%) P=0.497. *Taenia* spp. (n=2, 0.54%) was equally common in males (n=1, 0.27%) and females (n=1, 0.27%) P=0.821. *T. trichiura* (n=2, 0.55%) was only found in females P=0.996, and *E. vermicularis* (n=1, 0.27%) was only found in males P=0.995. The three most common parasite infections in this investigation were *O. viverrini,* which was most common (n=17, 4.64%) in the >20-year age group, followed by hookworm (n=13, 3.55%) and *E. coli* (n=8, 2.19%), respectively. Moreover, *O. viverrini* infection was the most prevalent in Kratie Province (n=17, 4.64%), followed by hookworm (n=14, 3.83%) and *E. coli* (n=13, 3.55%), respectively (Table 3).

The differences were assessed with the OR at P<0.05. IPIs were compared among sex (male [n=33, 9.02%] or female [n=26, 7.10%]), age group (<20 [n=13, 3.55%] and >20 [n=46, 12.57%] years old) and location (Kratie Province [n=50, 13.66%] and Phnom Penh [n=9, 2.46%]). In addition, co-infection cases were *O. viverrini* and *A. lumbricoides* 2 cases, *Taenia* spp. and hookworm 1 case, and hookworm and *H. nana* 1 case (Table 1 and 3). The bivariate analysis of the association between each parasite and variables (sex, age, and location) among the Cambodian population revealed significant associations with *O. viverrini* and hookworm infections by sex. *O. viverrini* infection was 21 cases (5.74%, OR_{cru}=0.341, 95% CI=0.134-0.868, P=0.024 and hookworm was 14 cases (3.83%, OR_{cru}=0.113, 95% CI=0.025-0.511, P=0.005, respectively (Table 3 and 4). This significant risk factor or sex was positively associated with *O. viverrini* (OR_{adj}=0.318, 95% CI=0.122-0.8270, P=0.019 and hookworm infections (OR_{adj}=0.085, 95% CI=0.017-0.436, P=0.003), respectively (Table 4).

DISCUSSION

The intestinal parasite infection (IPI) rate among the population of Kratie Province in the northeast and Phnom Penh, Cambodia in this study further validated the IPI prevalence along the Mekong River. A previous survey showed the highest prevalence of *O. viverrini* infection in Roka Kandal A (19.4%) and Talous villages (9.0%) in Kratie Province, although these levels were lower than the prevalence reported in three villages in Takeo Province, southern Cambodia (Sohn *et al*., 2012; Yong *et al*., 2014). Helminths are transported in soil, exert a chronic and insidious impact on nutritional status; hookworms are one of the leading causes of protein malnutrition and iron-deficient anemia (Inpankaew *et al*., 2014; de Gier *et al*., 2015;). Hookworm infection (13.5% of individuals) was more prevalent among individuals >12 years old (80.1% of cases). Children in primary school have been reported to be infected with hookworm (6.4%) and *A. lumbricoides* (26.3%) (Lee *et al*., 2002). The odds of hookworm infection increased at a faster rate (0.4%-3.7% faster) in females than in males (Colella *et al*., 2021). In Kratie Province, the prevalence of *A. lumbricoides* and hookworm infections were 49% and 28.9%, respectively (Sinuon *et al*., 2003). The hookworm infected people <15 years old in Peninsular Malaysia at a higher rate than *T. trichiura* (Anuar *et al*., 2014) due to poor sanitation conditions, such as a lack of food and water sanitary, and proper toilets, which posed a serious risk for infection spread (Ziegelbauer *et al*., 2012; Echazu *et al*., 2015; Wararat *et al*., 2021). Therefore, hookworm was the most concerning parasite and hookworm eggs found in 9.6% samples; these infections occurred frequently in adults because the helminth control programs in Cambodia mainly target schoolchildren (WHO, 2004; Schär *et al*., 2014; Schelzig, 2014).

In the present study of IPIs in Cambodia, males were infected with parasites 9.02% more than females, especially with *O. viverrini* and hookworm, which were higher 3.83% and 3.28%, respectively. Sex was associated with the risk of IPIs, and males had a higher risk than females. Differences in gender roles likely explain these differences in the chance of infection with soil-transmitted helminths, because males have worse food hygiene than females, which facilitates helminth infections. Moreover, male workers have a higher risk of *S. stercoralis* infection (OR=1.97, 95% CI=1.45-2.67) than female workers, which was associated with risk factors along the Mekong Islands in southern Laos (Vonghachack *et al*., 2015; Wararat *et al*., 2021). The relationship between risk of IPIs and sex was obvious in rural areas in Kratie Province. Fecal specimens demonstrated the highest infection rate by *O. viverrini*, followed by hookworms in Kratie Province areas. The region of Kratie Province may have differences in geography, temperature, and atmospheric humidity that influence the growth and viability of egg and larval stages of parasites, especially soil-transmitted helminths (Lai *et al*., 2013; Phongluxa *et al*., 2013; Scholte *et al*., 2013). The Mekong River basin subregion includes several Southeast Asian countries,

Table 4. Multivariate analysis of the risk factors for *O. viverrini* and hookworm infections in Kratie Province and Phnom Penh, Cambodia

Risk factor	N (%)	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Opisthorchis viverrini					
Male	14 (3.83)	0.341 (0.134-0.868)	0.024	0.318 (0.122-0.8270)	0.019
Female	7 (1.91)				
Hookworm					
Male	12 (3.28)	0.113 (0.025-0.511)	0.005	0.085 (0.017-0.436)	0.003
Female	2 (0.55)				

Significantly different P<0.05.

especially Cambodia. The maximum prevalence of *O. viverrini* infection in this area 10 years ago was 47.5%, which was noted in Cambodia (Sripa *et al*., 2021). In addition, obvious health inequalities were observed among 80% of the population, as a consequence of an unfair distribution of income, resources, and other determinants of health, including clean water, nutritious food, adequate living conditions, public health services, and adequately trained health personnel (Soeung *et al*., 2012; Schelzig, 2014; Kosal *et al*., 2015). The mentions above might explain the high prevalence of IPIs in Kratie Province. Furthermore, Southeast Asia has several countries with inadequate water and hygiene substructures that have fostered the spread of the parasite. The risk factors for IPIs are specific to personal behaviors, which vary according to the country or culture, such as consuming raw foods that may contain parasites, not washing hands, not trimming fingernails, not wearing shoes, and lack of access to sanitary toilets (Abera *et al*., 2013; Deepthi *et al*., 2014). In contrast, the present study found a lower prevalence of IPIs in Phnom Penh, which is in an urban area; IPIs may therefore be controlled by improving the sanitary system in rural areas.

This investigation observed the highest rate of *O. viverrini* infection in group of >20 year olds, followed by hookworm and *E. coli* infections, respectively. Young individuals (< 20 years old) may have had high rates of *E. coli* infection due to poor personal hygiene habits, such as playing in the dirt, walking without shoes or not washing their hands before meals. *O. viverrini* infection was previously reported in a minor Cambodian province along the Mekong River, with high infection rates in eastern and central Cambodia in 2020 and low infection rates (0.7%) in northwestern Cambodia (Khieu *et al*., 2014). The endemic area of *O. viverrini* infection in Cambodia is likely due to the consumption of raw fish and the expansion of parasites into the Lower Mekong Basin in Cambodia and Vietnam (Thaenkham *et al*., 2010). These results suggest that the prevalence of infection in Cambodia, a country in the Lower Mekong Basin, is estimated at 1 million people (Ting-Ting *et al*., 2021; Zhao *et al*., 2021). Furthermore, *O. viverrini* infections represent 25.62% of those among the ASEAN, such as Thailand (Kaewpitoon *et al*., 2018a). *O. viverrini* infection is widespread along the main Mekong River through Cambodia, especially in the endemic areas of Takeo, Kratie, Stung Treng, and Kampong Cham Provinces (Lee *et al*., 2002; Sinuon *et al*., 2003; Yong *et al*., 2012). Three freshwater fish species are usually consumed of raw by villagers in Kratie Province; thus, they are thought to facilitate *O. viverrini* infections (Sohn *et al*., 2012). The prevalence of *O. viverrini* metacercariae (OvMc) in fish from 3 southern areas in Cambodia along the Mekong River has been reported, including that in Phnom Penh (23.7%), Takeo (38.1%), and Kandal (90.2%) Provinces, with 4, 10, and 6 fish species positive for OvMc, respectively (Sohn *et al*., 2021). Moreover, OvMc is most prevalent in workers and rural inhabitants of Lower Myanmar who consumed raw cyprinoid fish (Aung *et al*., 2017); scientific evidence has revealed that the consumption of raw freshwater fish is a major risk factor for human liver fluke infection (Chavengkun *et al*., 2016; Kaewpitoon *et al*., 2015). A previous study demonstrated that individuals who consumed raw fish had a greater risk of *O. viverrini* infection than those who never consumed raw fish (OR_{adj}=5.22, 95% CI=2.05-13.3, *P*<0.001) (Saiyachak *et al*., 2016).

In the present study, *O. viverrini* and hookworm were highly prevalent among the Cambodian population susceptible to foodborne parasites and soil-transmitted helminths. Southeast Asia and northeastern Thailand were reported as endemic regions and hotspots for *O. viverrini* transmission. The prevalence of *O. viverrini* infection is still high in cultural and ecological complexes such as wet-rice agrarian habitats and centuries-old raw-food culture (Sripa & Echaubard, 2017). Gastrointestinal helminth infections have been reported in Cambodian workers (27.27%), especially *O. viverrini* (25.62%) and hookworm infections (0.83%). Moreover, fecal specimens have presented an *O. viverrini* infection rate of 7.70% (Kaewpitoon *et al*., 2018b). The main factors underlying *O. viverrini* infection include the environment, demographic characteristics, geography, hygiene, health care, and history of *O. viverrini* infection (Pengput & Schwartz, 2020). The *O. viverrini* infection rate among workers in Cambodia was 25.6%. The most infections were in males aged 40 years and who worked as laborers. This infection occurred from the consumption of raw cyprinoid fish, which was associated with an elevated risk of infection (OR_{adj}=2.2, 95% CI=1.2-4.0, *P*=0.014). Moreover, intestinal helminthic eggs were observed among 22.3% of workers from Cambodia (Kaewpitoon *et al*., 2018c). The infection may be associated with certain cultural practices, such as drinking alcohol and consuming raw cyprinoid fish, which are more common in men than women in Cambodia (June *et al*., 2013).

In the previous study, 26.2% of the fecal specimens of children and adults contained intestinal helminth eggs, including hookworms, which comprised most IPIs at 9.6%, followed by *O. viverrini* and/or minute intestinal flukes at 5.7%, *A. lumbricoides* at 4.6%, *T. trichiura* at 4.1%, *E. vermicularis* at 1.1%, *Taenia* spp. at 0.4%, and *Hymenolepis* spp. at 0.2%. Northwestern Cambodia showed a higher prevalence of hookworms (17.4-22.3%) than the other regions, while southwestern Cambodia displayed a high prevalence of *A. lumbricoides* (17.5-19.2%) and *T. trichiura* (6.1-21.0%). Moreover, the prevalence of *O. viverrini* and/or minute intestinal fluke was 23.8-24.0% in the central and southern areas of Takeo and Kampong Cham Provinces, respectively. These regions should receive adding attention to reduce the prevalence of parasitic infection in Cambodia (Yong *et al*., 2014). Additionally, *Taenia saginata* infection in northern Cambodia was investigated with molecular techniques and the Kato-Katz thick smear method. This was the first report of this infection in Cambodia, at 2.4% (Chang *et al*., 2020). Furthermore, a long strobila without scolex of adult tapeworm was revealed in a 27-year-old man after praziquantel treatment (Chang *et al*., 2020). Another study of fecal specimens collected from Koh Kong Province, Cambodia, and the presence of *Taenia* spp. egg was determined. The risk factors for infection with intestinal parasites in this area may be caused by eating the livers of infected cattle (Yong *et al*., 2014). Moreover, the prevalence of *E. vermicularis* infection was found to be 1.1%, despite underestimating prevalence. Finally, hookworm, followed by *A. lumbricoides*, and *T. trichiura* were discovered to be the most prevalent intestinal helminths in Cambodia (Yong *et al*., 2014).

The prevalence of helminth infections in other studies was 2.03%, with *O. viverrini* accounting for the majority (1.31%). No parasite infections exhibited significantly different general characteristics. The infections resulted mainly from foodborne helminths and skin-penetrating nematodes. Therefore, intervention should concentrate on the personal hygiene of the population and improving sanitation to reduce helminth infection in the area (Rattanapitoon *et al*., 2020). Furthermore, the prevalence of *S. stercoralis* infection in Takeo Province, Cambodia, was observed in all age groups; in that study, males had a higher infection rate than females (OR=1.7, 95% CI=1.4-2.0, *P*<0.001), possibly due to working outside and in closer proximity to soil than female workers (Khieu *et al*., 2014). This present research was a primary screening to observe the prevalence of IPIs among the population in Cambodia using the FECT method, a general laboratory method for determining the infection prevalence with high sensitivity and precision (Laoprom *et al*., 2016). Nevertheless, this method has a sensitivity (91.0%) followed by the Kato-Katz technique, fecal parasite concentrator kit (FPCK), and direct simple smear technique, respectively, for examination of *O. viverrini* egg in stool (Charoensuk *et al*., 2019). In these cases, the biomolecule confirmation method or the polymerase chain reaction (PCR) method would be more sensitive and specific for determining parasites in fecal specimens such as *O. viverrini* eggs and confirming the results of the parasitological technique or FECT.

In conclusion, there is a high prevalence of IPIs in Cambodia. Risk factors such as sex, age, and region in Cambodia were associated with higher IPI rates, which may be including personal behaviors, culture, socioeconomic discrimination influencing health, residing in areas with poor sanitation conditions, and epidemiological transition of a rural in Kratie Province of Cambodia when compared with the town area of Phnom Penh, the capital. Therefore, these serious risk factors for IPIs could be ameliorated by health education and recommendations regarding hygiene practices to prevent infections in the targeted regions.

Conflict of interest

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENTS

This work was supported by Suranaree University of Technology, Thailand Science Research and Innovation (TSRI), and National Science, Research, and Innovation Fund (NSRF) (NRIIS number 160366). The authors thank the subjects who participated in this project, Ministry of Health of Cambodia, Kratie Province of Health Department, head of Snuol operational district, head of Snuol sub-district and district, and villages' leaders.

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