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ORIGINAL PAPER

Cryptosporidium and *Giardia* detection in environmental waters of southwest coastal areas of Thailand

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Abstract The aim of this study was to investigate water samples collected in coastal areas of Southern Thailand in the years of 2005 and 2008 for their contamination by the protozoan parasites *Cryptosporidium* and *Giardia*. One hundred eighteen water samples of different origin were collected from six Tsunami affected southern provinces of Thailand in early 2005, and they have been analyzed using standardized methodology. Fifteen out of 118 samples (12.7%) were positive for *Cryptosporidium* spp. and nine (7.6%) positive for *Giardia* spp. Additional 42 samples from two same areas were examined 3 years later, in the early 2008. Five out of 42 (11.9%) samples were positive for *Cryptosporidium* spp., and three out of 42 (7.1%) were positive for *Giardia* spp.. Both protozoans were found in reservoir, river/canal, and pond waters. It appears no significant differences ($p<0.05$) between *Cryptosporidium* and *Giardia* (oo)cysts levels during the two monitoring periods; however, the number of the investigated areas and samples in the second period was significantly less than in the first period. This is the first description on *Cryptosporidium* and *Giardia* (oo)cysts in water sources of Thailand, and it suggests the need for water quality control in the interest of public health safety.

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Introduction

Cryptosporidium and *Giardia* are waterborne protozoa of human and animal fecal origin that causes widespread gastroenteritis in humans that is normally self-limiting but potentially life-threatening in the immune compromised persons. The transmissive stages, oocysts and cysts, are ubiquitous in aquatic environment, resistant to environmental stresses, and cannot be completely removed by conventional water treatment practices (Wolfe 1992; Graczyk et al. 1997; Karanis et al. 1996a, 1998, 2007). *Cryptosporidium* and *Giardia* (oo)cysts are transmitted by the fecal–oral route, not only interpersonally but also by food and water (Karanis et al. 2007). Drinking water and recreational waters particularly in water resources inhabited by domestic and wild animals can be significant sources of human exposure to these organisms (Bednarska et al. 1998; Cox et al. 2005; Heitman et al. 2002; Karanis et al. 1996b; Mead et al. 1999; Robertson and Gjerde 2001). Waterborne outbreaks of cryptosporidiosis and giardiasis have been reported worldwide associated with water from various sources including the contributions of farm animals, principally dairy cattle and sheep (Barwick et al. 2000; Craun et al. 1998; Karanis et al. 2007; Gray 1998; Lemmon et al. 1996; Ribeiro and Palmer 1986; Shield et al. 1990).

Although *Cryptosporidium* and *Giardia* have been reported as common enteric protozoa in many countries, details of their transmission to humans via drinking water remain unclear. In Southeast Asia, investigations on the distribution of *Cryptosporidium* and *Giardia* in surface waters and sources of water supply have not been reported. No previous data are available describing the occurrence of these organisms in water supplies in Thailand. After the tsunami in Asia on Sunday the 26th December 2004, the threat of waterborne disease was more significant than before. The heavily affected provinces in Southern Thailand are main tourism districts of Phang Nga, Ranong, Trang, Phuket, and Krabi where more than 5,000 people, including many foreigners, have been killed. More than 100,000 of the local population were displaced and accommodated in relocation camps having initially only with rudimentary sanitation facilities for up to a year or more. Contamination of drinking water sources by pathogenic bacteria, viruses, and protozoa represented a major human health hazard. Water supplies and sanitation facilities in affected areas were heavily damaged. For example, in Phang-nga, 1,000 households were without water or sewerage. Over 200 wells, 11 tap water supplies, eight surface water sources were unusable. Similar conditions occurred in the coastal portions of Krabi and somewhat less in similar area of the other provinces. Marine environments, including shallow coastal areas and river estuaries, were contaminated by untreated sewage introducing fecal contaminants. Coastal as well as inland surface waters were polluted by human and livestock fecal discharges including those from human corpses and animal remains following the disaster. These waters represent a potential risk for disease transmission via drinking and recreational water or through incidental contact while fishing or swimming.

The present study was conducted to determine the presence of the protozoan parasites *Cryptosporidium* and *Giardia* in environmental waters collected in areas of Southern Thailand and to provide information on (oo)cysts distribution in the investigated waters. Information on sources' contamination of public water supplies would provide a basis for assessing their contribution to local public health risk.

Materials and methods

Locations and water sample collection

Sampling was conducted in tsunami-affected areas including the six southern provinces of Krabi, Phang-nga, Phuket, Ranong, Satun, and Trang (Fig. 1). Water samples were collected in two periods: the first set in the months immediately following the tsunami, between January 20th to May 10th 2005; the second just 3 years later, between February to April 2008. Samples were collected from a range of water sources including near-shore coastal, river and estuary, reservoir, ditch, and a functioning tap sites. Sites were selected to include potential sources of waterborne pathogen contamination. Overall in 2005, 118 samples were collected from various water sources included: 11 tap water, 42 well water, 12 estuary and river water, four reservoir water, six ditch water, 25 pond water, and 18 marine water. Additional of 42 samples have been collected from the same locations in Phuket and in Phang-Nga in the 2008 period. They included: two tap water, 10 well water, 12 estuaries and river water, four reservoir water, 12 pond water, and two marine water. The physical and chemical quality of each water sample has been examined used in situ using portable instruments. Water samples have been subsequently transported on ice to the laboratory in the Bangkok University facilities for further processing and examination.

Isolation and concentration of (oo)cysts from water

For both *Cryptosporidium* and *Giardia* (oo)cysts analysis, one of two protocols below was followed according to the turbidity levels of the water sample. For water samples with turbidity above of five NTU, a 10- to 20-l sample was filtered in the field through a Gelman Envirocheck filter (Pall Gelman Sciences, Inc; Ann Arbor, Mich) at 1 to 2 l/min according to manufacturer's recommendation. Filters were transported to the laboratory on ice, and samples were eluted according to manufacturer's recommendations within 36 h of sample collection. Eluted solids from the capsules were resuspended in 10 ml of laboratory grade water for each 0.5 ml of solids, stored at 4°C, then further processed for (oo)cysts isolation and concentration. For water samples with a turbidity less than five NTU, an each 20- to 30-l sample was collected in a clean container, and (oo)cysts were concentrated by filtration through a membrane filter (1.2 to 3 μm pore size, 47 mm diameter) using a vacuum pump. To keep moisture, the filters were replaced to a sterile plastic universal bottle containing 10 ml of sterile distilled water. Each bottle was then vortexed at low speed for 5 min to recover the (oo)cysts from the surface of the filters. Following centrifugation at 15,000×*g* for 10 min, the supernatant was carefully decanted and the pellet was resuspended in 1 ml of sterile distilled water and stored at 4°C until further processing. (Oo)cysts were further concentrated by sucrose flotation via density gradient centrifugation and, if the suspension after sucrose flotation contained debris, by immunomagnetic separation (IMS) with the Aureon CG kit (Aureon Biosystems GmbH, Vienna, Austria). Briefly, 100 μl of anti-*Cryptosporidium* and anti-*Giardia* beads were added

Fig. 1 Map of Thailand demonstrating the location and sampling areas during the present study. The study settings are along the Tsunami-affected areas in six provinces of southwest coastal Thailand, Krabi, Phang-nga, Phuket, Ranong, Satun, and Trang (*blue circle*)

to a 5-ml concentrate obtained after centrifugation or membrane filtration of the eluate. The sample was then processed according to the recommendations of the manufacturer (Dynal), using Dynal equipment for IMS processing. After dissociation from magnetic beads, (oo) cysts were transferred to a new microcentrifuge tube and treated with 5 μl of 1 N NaOH to neutralize the pH. The resultant suspension for screening was clean and approximately 50 μl total volume. This resulted final sample was divided into two portions, and one portion was examined by the immunofluorescent assay (IFA) to detect *Cryptosporidium* oocysts and *Giardia* cysts.

Detection and identification of *Cryptosporidium* and *Giardia* (oo)cysts by IFA and by DIC

The *Cryptosporidium* oocysts and *Giardia* cysts were stained with FITC conjugated anti-*Cryptosporidium*/anti-*Giardia* monoclonal antibodies (MERIFLUOR® C/G, UK), processed according to the recommendations of the manufacturer, and then were enumerated using fluorescence microscopy (OLYMPUS; Model BX 51/BH-2) and differential interference contrast (DIC) microscopy following the standard methodology as it is recommended by the Method 1623 (USEPA 2001). All samples which were found positive by IFA have been examined with DIC by ×100 magnification to confirm internal structures inside of the (oo)cysts.

Statistical data analysis

Statistical analyses were performed on logarithmically transformed data of *Cryptosporidium* oocysts and *Giardia* cysts numbers and then process using Pearson correlation analysis (SPSS for Windows software: version 11.00). The result were considered to be significant at $\alpha=0.05$.

Results

Among the 118 water samples of various quality and origin from the 2005 investigation period (11 tap water, 42 well water, 12 estuary and river water, four reservoir water, six ditch water, 25 pond water, and 18 marine water), 15 out of 118 samples (12.7%) were positive for *Cryptosporidium* spp., and nine (7.6%) were positive for *Giardia* spp. The highest detection rates were in samples from sources in

Table 1 Summarized results on the detection of *Cryptosporidium* and *Giardia* in coastal waters of Southwest Thailand in the years 2005 and 2008

Investigations in the year 2005

Water types (total)	Phuket		Phang-Nga		Ranong		Krabi		Trang		Satun	
	No. of water samples tested	IFA (C/G) Pos (%)	No. of water samples tested	IFA (C/G) Pos (%)	No. of water samples tested	IFA (C/G) Pos (%)	No. of water samples tested	IFA (C/G) Pos (%)	No. of water samples tested	IFA (C/G) Pos (%)	No. of water samples tested	IFA (C/G) Pos (%)
Tap water (11)	2	0 (0)	4	0 (0)	1	0 (0)	2	0 (0)	1	0 (0)	1	0 (0)
Well (42)	4	0 (0)	26	3 (11)	2	0 (0)	6	0 (0)	2	0 (0)	2	0 (0)
Canal/River (12)	2	0 (0)	8	2[a] (25)	1	0 (0)	–	–	1	0 (0)	–	–
Water reservoir (4)	1	0 (0)	3	1+1[a] (66)	–	–	–	–	–	–	–	–
Ditch water (6)	–	–	2	1 (50)	–	–	4	1 (25)	–	–	–	–
Ponds (25)	3	1[a] (33)	22	5 [a] (22)	–	–	–	–	–	–	–	–
Marine water (18)	3	0 (0)	13	0 (0)	–	–	2	0 (0)	–	–	–	–
Total samples (118)	15	1	78	13	4	0	14	1	4	0	3	0

Investigations in the year 2008

Water types (total)	Phuket		Phang-Nga	
	No. of water samples tested	IFA (C/G) positive (%)	No. of water samples tested	IFA (C/G) positive (%)
Tap water (2)	1	0 (0)	1	0 (0)
Well (10)	4	0 (0)	6	0 (0)
Canal/River (12)	5	0 (0)	7	1[a] (8.33)
Water reservoir (4)	1	0 (0)	3	1 (25)
Ditch water (0)	–	–	–	–
Ponds (12)	4	1 (8.33)	8	2[a] (16.66)
Marine water (2)	1	0 (0)	1	0 (0)
Total samples (42)	16	1	26	13

Number of positive samples; in parentheses the total number of the investigates samples

[a] Samples positive for both *Cryptosporidium* and *Giardia* parasites identified by IFA

Phang-Nga. *Cryptosporidium* spp. oocysts have been found in three of 42 (7.1%) well samples, two of 12 (16.7%) estuarine and river samples, two of four (50%) reservoir samples, two of six (33.3%) ditch samples, and six of 25 (24%) pond samples. *Giardia* spp. cysts have been detected only in estuarine and river water, reservoir, and pond water samples at 16.7%, 25%, and 24%, respectively (Tables 1 and 2). Whereas in the 2008 investigation period samples, five of 42 samples (11.9%) were positive for *Cryptosporidium*, and three of 42 (7.1%) were positive for *Giardia* (Tables 1 and 2).

For samples in which *Cryptosporidium* and/or *Giardia* (oo)cysts were detected, the geometric mean of *Cryptosporidium* oocysts was 5.16 oocysts per liter, ranging from 0.73 to 24.25 oocysts per liter (Table 3). For *Giardia* cysts, the geometric mean was 3.26 cysts per liter, ranging from 0.00 to 13.12 cysts per liter (Table 3). On the average, *Cryptosporidium* oocysts were presented 1.6 times more numerous than *Giardia* cysts (Fig. 2). *Cryptosporidium* occurrence was significantly correlated (r=0.717, P<0.01) to the *Giardia* occurrence. *Cryptosporidium* oocysts numbers were also significantly related to sample turbidity (r= 0.614, P<0.05; Fig. 3) and *Giardia* cysts numbers similarly correlated to turbidity (r=0.465, P>0.05; Fig. 4), but neither organism numbers were significantly correlated to either total nor fecal coliform levels (data not shown here). No *Cryptosporidium* oocysts or *Giardia* cysts were found in any of the samples from Ranong, Trang or Satun (Table 1).

Dicussion

Although infections with *Cryptosporidium* spp. in HIV-infected patients, in cattle, and in food (green mussels) have been already reported from Thailand (Uga et al. 2000; Gatei et al. 2002; Saksirisampant et al. 2002; Tiangtip and Jongwutiwes 2002; Nuchjangreed et al. 2008; Srisuphanunt et al. 2009), no outbreaks of waterborne cryptosporidiosis or giardiasis have been reported in the country, and they could not be included in the systematic review covering all reports worldwide of waterborne protozoan outbreaks by Karanis et al. (2007). No data exist regarding protozoan parasite concentrations in wastewaters or in reuse and recycling of wastewaters for agriculture and industry and in backwash water from drinking water treatment plants in Thailand.

The present study aimed to provide information on the occurrence and distribution of *Cryptosporidium* and *Giardia* spp. (oo)cysts in environmental waters of Southern Thailand. The areas which have been affected by the tsunami at December 2004 have been investigated in the early and in late post-tsunami periods almost 3 years later in the year 2008. Water environments including fresh, estuarine, and marine areas were heavily affected during and after the tsunami period. Physical damage and inundation occurred in drinking water supplies, waste systems, estuaries and fisheries, and recreational areas. The microbiological quality of water and associated risks of diseases have been additionally affected from fecal contamination following these extreme events of the tsunami in which human and animal wastes were distributed indiscriminately with wastes from toilets, latrines, underground drain fields, sewage lagoons, and treatment facilities. Due to the study design, it will not be possible to give concrete comparative information on the (oo)cysts numbers before and/or after the tsunami events. The prevalence of the *Cryptosporidium* oocysts and *Giardia* cysts in both periods (early and late post-tsunami period) were similar (12.5% versus 11.9% for

Table 2 Number of positive samples (and in %) for *Cryptosporidium* and *Giardia* (oo)cysts in coastal waters of southwest Thailand in the years 2005 and 2008

Water type	No. sample test by year		Positive samples/investigated samples (%)			
			Cryptosporidium		*Giardia*	
	2005	2008	2005	2008	2005	2008
Tap water	11	2	0/11	0/2	0	0
Well	42	10	3 /42 (7.14)	0/10	0	0
Estuary and river	12	12	2/12 (16.66)	1/12 (8.33)	2 (16.66)	1/12 (8.33)
Water reservoir	4	4	2/4 (50)	1/4 (25)	1/4 (25)	0/4
Ditch water	6	0	2/6 (33.33)	NI	0/6	NI
Pond	25	12	6/25 (24)	3/12 (25)	6/25 (24)	2/12 (16.66)
Marine water	18	2	0/18	0/2	0/18	0/2
Total	118	42	15/118 (12.71)	5/42 (11.9)	9/118 (7.63)	3/42 (7.1)

NI not investigated

Table 3 Turbidity of water samples and numbers of *Cryptosporidium* oocysts and *Giardia* cysts found in the investigated water sources in 2005 and 2008

Sampling date	Site code (water type)	Location (province)	Turbidity (NTU)	No. of parasites/liter	
				Cryptosporidium oocysts	*Giardia* cysts
Jan. 2005	KL4 (pond)	Phang-Nga	28.52	5.35	2.25
	KH2 (pond)	Phang-Nga	37.21	8.25	13.12
	PK 1(pond)	Phuket	32.64	16.9	5.95
Feb. 2005	NK15 (pond)	Phang-Nga	44.6	24.25	9.8
	KK3 (pond)	Phang-Nga	36.3	15.9	6.75
	WR2(reservoir)	Phang-Nga	19.8	2.33	0
	BH6 (well)	Phang-Nga	16.7	0.73	0
	BK2 (bore)	Phang-Nga	29.4	2.75	0
Mar. 2005	PT4 (well)	Phang-Nga	46.42	3.45	0
	PT13 (well)	Phang-Nga	42.0	9.3	0
	KK7 (pond)	Phang-Nga	38.2	11.75	4.5
	RV3(reservoir)	Phang-Nga	33.73	4.17	1.27
	PT1 (river)	Phang-Nga	12.38	2.67	0.2
	PT10 (canal)	Phang-Nga	27.77	6.50	3.17
April 2005	PP2 (bore)	Krabi	16.5	1.35	0
Total no. of positive/sample tests				15/118	9/118
Feb. 2008	NK15 (pond)	Phang-Nga	39.54	18.54	7.43
	RV3(reservoir)	Phang-Nga	28.90	2.90	0
Mar. 2008	PK1 (pond)	Phuket	34.72	23.78	9.25
	PT10 (canal)	Phang-Nga	22.85	3.2	0
April 2008	KK9 (pond)	Phang-Nga	33.45	12.64	5.9
Total no. of positive /sample tests				5/42	3/42

Cryptosporidium; 7.6% versus 7.1% for *Giardia*); however, the number of the investigated areas and samples differ remarkable (118 samples and six areas investigated in 2005 versus 42 samples and two areas investigated in 2008). These are reasons for indirect comparative figures of the degree of contamination of the investigated water supplies with the investigated protozoan. Furthermore, the different periods of investigations rely to the different

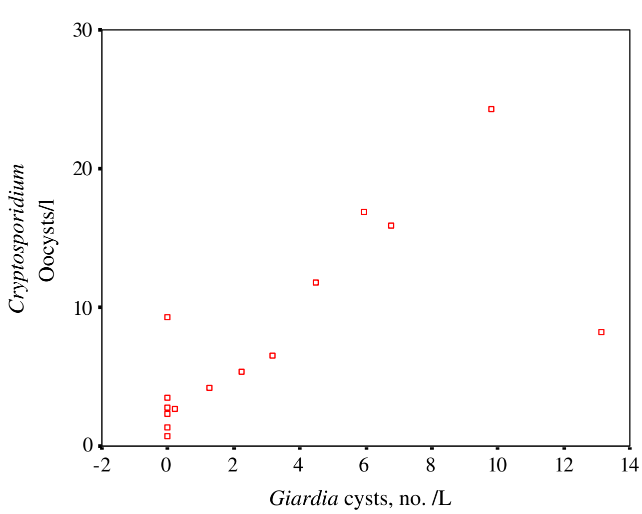

Fig. 2 Relationship between densities of *Cryptosporidium* oocysts and *Giardia* cysts in water samples (r=0.717, P<0.01)

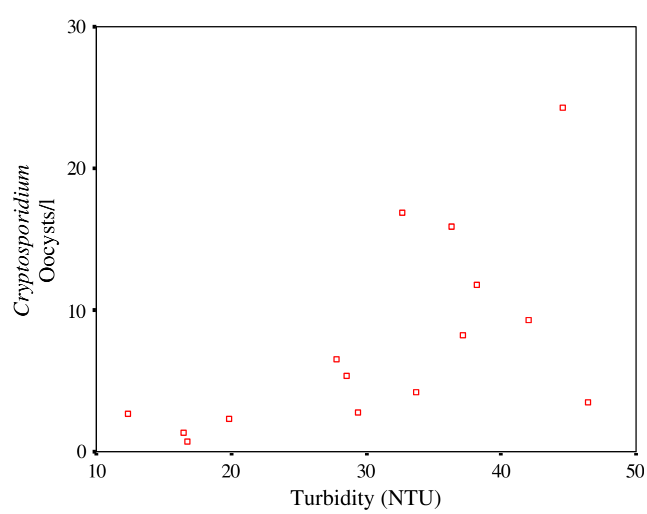

Fig. 3 Relationship between turbidity and *Cryptosporidium* oocysts concentration (r=0.614, P<0.05)

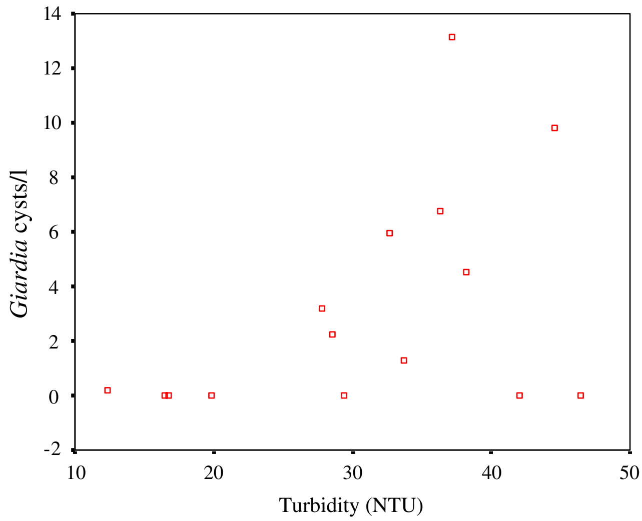

Fig. 4 Relationship between turbidity and *Giardia* cysts concentration (r=0.465, P>0.05)

contamination levels of the water sources, and this is valid also for periods independent of the tsunami or other physical phenomena. This will be difficult for direct comparisons in such studies because the concentration of both protozoans in water supplies is considerably affected by seasonal factors and other mechanisms of transportation and due to the differences in dry seasons or washed out in the rainy seasons. From the methodological point of view, the methods applied here for the detection leads to the underestimation of the parasites' numbers. Nevertheless, it is obvious from the data presented that in both periods, the occurrence of *Cryptosporidium* and *Giardia* (oo)cysts in the samples correlated with the turbidity in the samples. In our investigations, we used membrane filtration with IFA as described by USEPA method 1623 that is widely used for the detection of *Cryptosporidium* and *Giardia* (oo)cysts in water. Recovery of *Cryptosporidium* and *Giardia* (oo)cysts using Method 1623 have been shown to vary significantly, depending on water quality (Ferguson et al. 2004; Lee et al. 2002; Quintero-Betancourt et al. 2003; Weintraub 2006). USEPA Method 1622/23 provides an estimation of the total oocyst count. Data produced by this method include essentially many species of *Cryptosporidium* and *Giardia*, having some ability to discriminate between live and dead organisms but virtually no ability to discriminate between genotypes. In assessing water quality in relation to public health risk, the first priority is the information on the presence or absence of a pathogen. This may be of overriding importance during or after natural disasters.

It has been suggested in the literature that some genotypes of both *Cryptosporidium* and *Giardia* are more strongly associated with human illness than others, and some genotypes are apparently related to specific symptomatology in human giardiasis and cryptosporidiosis cases, and methodologies have been described for the genetic differentiation of the pathogens (Ryan et al. 2005; Plutzer et al. 2008). Accordingly, more detailed information on pathogen occurrence including genotype, particularly for human-adapted *Giardia* species and *Cryptosporidium* species, would support to make risk estimation more precise in the future. However, it cannot be used directly for risk assessment, as it does not determine the fraction of infectious oocysts.

Thailand is a frequently visited country by tourists, and the number of travelers among the different districts is increasing every year. Hence, safe drinking water should be always a general concern to the Thai government. However, monitoring for *Cryptosporidium* and *Giardia* in water supplies in Thailand is not practiced, and epidemiological studies have been hindered by the lack of funding for systematic investigations. Systematic research studies are needed to assess the distribution of parasites in catchments areas of surface and groundwater resources (WHO 2005).

The present work is the first report on contamination with *Giardia* and *Cryptosporidium* spp. in natural and drinking water resources in Thailand, and it provides information of the contamination *Cryptosporidium* and *Giardia* spp. in investigated areas. In most cases, *Cryptosporidium* oocysts' presence was at higher levels than that of *Giardia* cysts. In Southeast Asia, published reports on the occurrence and distribution of *Cryptosporidium* and *Giardia* in water supplies are sparse, and no data have previously described on detection of these organisms in the water environments. It is obvious that additional work to identify waterborne contaminants like *Cryptosporidium* and *Giardia* and in relation to the incidence of human illness in Asian countries towards the efforts for the protection of public health is needed.

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