FREE-LIVING AMEBA CONTAMINATION IN NATURAL HOT SPRINGS IN THAILAND

Amorn Lekkla[1], Chantira Sutthikornchai[1], Somchai Bovornkitti[2] and Yaowalark Sukthana[1]

[1]Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, Bangkok; [2]The Academy of Science, the Royal Institute, Bangkok, Thailand

Abstract. Thermo tolerant free-living ameba, *Naegleria* spp and *Acanthamoeba* spp contamination in natural hot springs in Thailand were carried out from 13 provinces. The temperature of hot springs water varied from 28° - 65° C and pH from 6-8. We found that 38.2 % (26/68) of water samples were positive, *Acanthamoeba* was 13.2% (9/68) whilst *Naegleria* was 35.3% (24/68). Contamination by free-living ameba in natural hot springs may pose a significant health risk to people who use such water for recreation activities.

INTRODUCTION

Free-living Ameba, *Naegleria* and *Acanthamoeba* are found worldwide in the environment, swampy soil, natural pools or swimming pools. They are thermophilic organisms who live at temperatures between 37° - 45° C and feed on bacteria and detritus (De Jonckheere, 2002). Free-living amebae have been found in many parts of the world such as natural hot springs in Yellowstone and Grand Teton National Parks in USA (Kathy *et al*, 2003). Other reports were isolation of *Naegleria* species in thermal water in Japan (Izumiyama *et al,* 2003) and the identification of free-living amebae in natural springs from Bulgaria (Tsvetkova *et al,* 2004). In Thailand, the prevalence of *Acanthamoeba* and *Naegleria* in aquatic habitats of human environments were 36.7% and 28.6%, respectively (Nacapunchai *et al,* 2001), whilst 10% of pathogenic *Naegleria fowleri* was found in stagnant water around an industrial area (Tiewcharoen and Junnu, 2001).

The first report on free-living amebae in natural hot springs in Thailand was carried out in Lop Buri Province, in the central part of the country (Sukthana *et al,* 2004). We extended our studies to determine the contamination of free-living amebae in natural hot springs in other provinces in Thailand.

MATERIALS AND METHODS

From April to August 2004, 42 natural hot springs in 13 provinces from the central and southern part of Thailand were enrolled in this study (Table 1). Sixty-eight water samples were collected from natural hot springs, daughter pools, adjoining man-made bathing facilities and domestic supplies (Figs 1-3). Temperature and pH were measured in each sampling site, then 500 ml of water, as well as sediments, algae mats and scrapings of biofilm on rock surfaces were collected in sterile bottles. Water filtering and centrifugation followed by morphological identification of free-living amebae using fresh direct examination and trichrome stain under light microscopy were performed in the Department of Protozoology, Faculty of Tropical Medicine, Mahidol University.

RESULTS

There were 28 out of 69 (37.68%) samples positive for free-living amebae. *Acanthamoeba* was found in 13.0% (9/69) whilst *Naegleria* was 34.8% positive (24/69). The temperature ranged from 28°-65° C and pH from 6-8 (Table 1). Figs 4-5 showed acanthopodia and broad pseudopodia of *Acanthamoeba* and *Naegleria* under fresh smear, respectively. Trichrome stain showed one nucleus containing a large central karyosome surrounded by a halo, without peripheral nuclear chromatin, characteristic of free-living amebae (Figs 6-7).

DISCUSSION

Natural hot springs water has been used worldwide for bathing and health purposes for many thousand years. At present, Thailand is famous for health spas and natural hot springs among local people and tourists. There may be possible risks of exposure to harmful microorganisms such as free-living amebae. Both *Acanthamoeba* and *Naegleria,* are recognized causes of lethal infections in both immunocompetent and

Correspondence: Yaowalark Sukthana, Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand.
Tel: +66 (0) 2354-9100 ext 1830,
Fax: +66 (0) 2643-5601
E-mail: tmymv@mahidol.a.c.th

Table 1
Locations and description of contaminated water with free-living amoeba

Site	pH	Temp (°C)	*Naegleria*	*Acanthamoeba*
Krabi				
Klong Bo Nam Ron	6.7	37.5	Not found	Not found
Baan Huay Yoong Tog	7.0	38	Positive	Not found
Baan Nam Ron Kem	6.7	49	Positive	Not found
Sra Namgnoen	7.0	30	Not found	Not found
Sra Morakot	7.0	28	Positive	Not found
Phatthalung				
Baan Rawang Kuan	7	30	Not found	Not found
Baan Na Tung Poh	7	46	Not found	Not found
Baan Lo Jungkra	7.5	44	Not found	Not found
Khou Chaison	7.5	45	Not found	Not found
Bathing pool	7.2	44	Not found	Not found
Trang				
Baan Kuan Sra	7.7	34	Not found	Not found
Baan Kuang Kang	7.2	50	Positive	Not found
Bathing pool	7.2	42	Positive	Not found
Nakhon Si Thammarat				
Bo Krod	7	50	Positive	Not found
Baan Numphuron	7	49.5	Positive	Not found
Surat Thani				
Wat Tharnnamron	7	57	Not found	Not found
Baan Namphuron	7	49.5	Not found	Not found
Bathing pool	7	28	Not found	Not found
Baan Kau Namron Nai	7	43	Positive	Positive
Bathing pool	7	39	Positive	Not found
Children pool	7	39	Positive	Not found
Baan Wanghin	7	37.5	Not found	Not found
Bathing pool	7	39	Not found	Not found
Bann Kauplu	7	52.5	Positive	Not found
Baan Bo Namron	7	40	Positive	Not found
Bathing pool	7	39	Not found	Not found
Baan Kau Noi	7	52	Not found	Not found
Annex pool	7	48	Not found	Not found
Ratanakosy	7	66	Positive	Not found
Baan Ta Hin (Bo Mae)	6	54	Not found	Not found
Bo Oon	8	37	Not found	Not found
Bathing pool	7	40	Not found	Not found
Phang-gna				
Baan Bo Darn	7	45	Not found	Not found
Annex pool	7	40	Not found	Not found
Annex pool	7	39.5	Not found	Not found
Baan Pakphu	7	61.5	Not found	Positive
Adjoining stream	7	52	Not found	Positive
Baan Romany	7	57	Not found	Positive
Adjoining stream	7	47	Not found	Not found

Site	pH	Temp(^{o}C)	*Naegleria*	*Acanthamoeba*
Ranong				
Hauy Nam Sai	7	45	Not found	Not found
Bo 1	7	53	Not found	Not found
Bathroom	7	34	Not found	Not found
Baan Porn Rung	7	53	Not found	Not found
Bathing pool	7	43.5	Not found	Not found
Baan Tung Yo	7	40	Positive	Not found
Raksawarin	7	65.5	Positive	Not found
Chantsom Thara	7	28	Not found	Not found
Bathroom	8	48	Not found	Not found
Bathing pool	7	38	Not found	Not found
Chumphon				
Pool 1	7	45	Not found	Not found
Pool 2	7	42	Not found	Not found
Satun				
Kuan Kalong	7	49	Positive	Not found
Lop Buri				
Nong Yai Toh	7.5	60	Positive	Positive
Nai Tiang's Farm	7.8	36	Positive	Positive
Phetchabun				
Baan Puteuay	7	30	Not found	Positive
Other pool	7	30	Not found	Not found
Baan Pukham	8	49	Not found	Not found
Baan Wangkham	8	48	Not found	Positive
Annex pool 1	7	32	Not found	Not found
Annex pool 2	7	37	Not found	Not found
Baan Nam Ron	7	50	Not found	Not found
Kamphaeg Phet				
Phra Ruang	7	47	Not found	Not found
Bathing pool	7	37	Not found	Not found
Annex pool	7	53	Not found	Not found
Baan Pong Namron	7	38	Positive	Not found
Mae Wong Nation Forest	7	43	Not found	Not found
Bo Rae	7	32	Positive	Positive
Utai Thani				
Baan Samotong	8	64	Positive	Not found

Fig 1- Natural hot spring.

Fig 2- Daughter pool.

Fig 3- Man-made bathing.

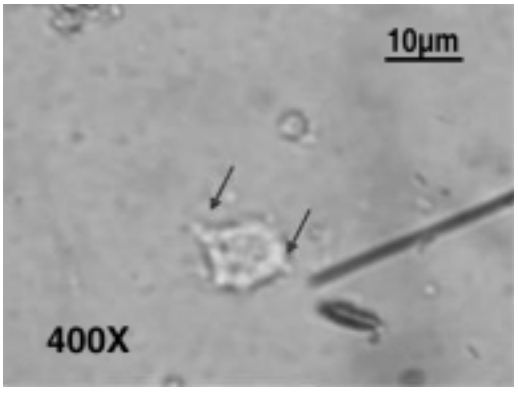

Fig 4- Fresh smear of *Acanthamoeba* spp, note spiky pseudopodia (arrows), 400×.

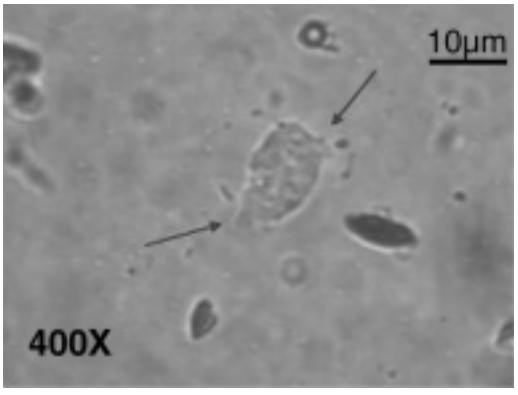

Fig 5- Fresh smear of *Naegleria* spp, note broad pseudopodia (arrows), 400×.

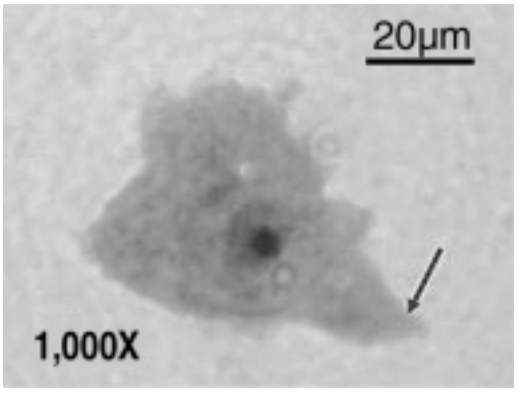

Fig 6- Trichrome stained of *Acanthamoeba* spp, note spiky pseudopodia, 1,000×.

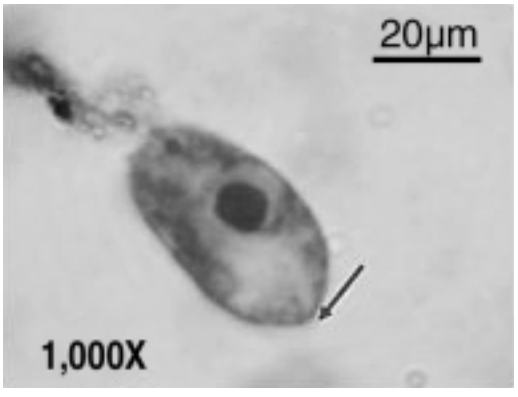

Fig 7- Trichrome stained of *Naegleria* spp, note broad pseudopodia, 1,000×.

immunocompromized persons. Whereas the latter often causes acute fulminant central nervous system (CNS) infection in normal hosts, *Acanthamoeba* causes chronic or subacute granulomatous encephalitis in immunosuppressed persons. Apart from CNS infection, *Acanthamoeba* can cause keratitis, sinusitis, pneumonitis and dermatitis (Carter *et al*, 2004).

We studied the free-living amebae contamination of natural hot springs from 13 provinces in central and southern Thailand. Pathogenic free-living amebae were found in 13.0% (*Acanthamoeba*) (9/69) and 37.8% (*Naegleria*) (24/69), respectively. Our study provided evidence that natural hot springs, a popular tourist attraction, may pose a health risk to people during recreation. However, further studies using diagnostic tools such as molecular technique are needed.

The health authorities concerned should be aware of these possible hazards and provide tactful measures and guidelines to ensure safety without causing undue alarm to foreign and Thai tourists.

ACKNOWLEDGEMENTS

The authors would like to thank all staff of Provincial Health Office, High Way Policemen for their

help. Thanks also to all owners of private hot springs. This study was financially supported by Thanat-Molee Khoman Foundation.

REFERENCES

Carter WW, Gompf SG, Toney JF, Greene JN, Cutolo EP. Disseminated *Acanthamoeba* sinusitis in a patient with AIDS: a possible role for early antiretroviral therapy. *AIDS Read* 2004;14:41-9.

De Jonckheere JF. A century of research on the Amoeboflagellate genus *Naegleria*. *Acta Protozool* 2002;41:309-42.

Kathy BS, Jennifer AF, Michael JF, Joan MH. PCR detection and analysis of the free-living Amoeba *Naegleria* in hot spring in Yellowstone and Grand Teton Nation Parks. *Appl Environ Microbiol* 2003; 5914-8.

Izumiyama S, Yagita K, Furushima-Shimogawara R, Asakura T, Karasudani T, Endo T. Occurrence and distribution of *Naegleria* species in thermal waters in Japan. *J Eukaryot Microbiol* 2003;50(suppl): 514-5.

Nacapunchai D, Kino H, Ruangsitticha C, Sriwichai P, Ishih A, Terada M. A brief survey of free-living amebae in Thailand and Hamamatsu District, Japan. *Southeast Asian J Trop Med Public Health* 2001;32 (suppl 2):179-82.

Sukthana Y, Wootta W, Praveenkittiporn W, *et al*. Study of natural hot springs in Lop Buri Province. *Intern Med J Thai* 2004;20:211-4.

Tiewcharoen S, Junnu V. Distribution of pathogenic *Naegleria* spp in Thailand. *Southeast Asian J Trop Med Public Health* 2001;32 (suppl 2):172-8.

Tsvetkova N, Schild M, Panaiotov S, *et al*. The identification of free-living environmental isolates of amoebae from Bulgaria. *Parasitol Res* 2004;92:405-13.