PROTOZOAL CONTAMINATION OF WATER USED IN THAI FROZEN FOOD INDUSTRY

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Abstract. This study evaluated the prevalence of contamination of water that was used for food preparation. Since protozoal cysts can be found in small numbers in water, 1,000 liters of either untreated or treated water were filtered through activated carbon block filters (1 μm nominal porosity). Identification of protozoa was performed using specific monoclonal antibodies against *Giardia* and *Cryptosporidium* parasites followed by fluorescence microscopy. Twelve of 20 untreated water samples (60%) were found to be contaminated by *Giardia* cysts, with an average of 53.33cysts/1,000 liters (geometric mean 39.43), whilst 7 samples (35%) were contaminated by *Cryptosporidium* oocysts, with an average of 28.57 oocysts/1,000 liters (geometric mean 26.92). Three samples of untreated water (15%) were positive for both organisms. In contrast, none of the treated water samples were contaminated.

INTRODUCTION

Due to worldwide free trade, Thailand encounters an intense competition in the frozen food export market. For this reason, it is a must for Thai frozen food operators to expeditiously improve their capabilities in production and product development with better quality and better price compared with those of their rivals.

Water is used in many steps of frozen food production. Contaminated water can affect food products. Apart form chemical, virus, and bacterial contaminants, protozoa such as *Giardia* and *Cryptosporidium* are possible agents that could contaminate water and have a significant impact on human health.

Giardia spp and *Cryptosporidium* spp have presented a challenge to water suppliers (Gordon,1996) in low concentration. Both parasites are resistant to adverse environmental conditions because they can survive months under sub-optimal conditions and are resistant to minor exposure to disinfectants. The nature of these protozoa means that *Giardia* spp and *Cryptosporidium* spp are of serious concern to the water industry (Ferrari *et al*, 1999).

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Due to low concentrations, detection of protozoal contamination in water requires a specific method and collection of a large volume of water (100-1,000 liters). So far, there is no information about protozoal contamination of water in the food industry in Thailand. We, therefore, determined the prevalence of *Giardia* spp and *Cryptosporidium* spp in water that is used in Thai frozen food industries.

MATERIALS AND METHODS

Water samples collection

Both treated and untreated water were collected by filtration technique through a 1 μm nominal porosity activated carbon block filter (Siam Cast Nylon, Thailand). The collection rate was 4-5 liters/minute for 5-6 hours. Therefore, at least 1,000 liters of each water sample were passed through the filter. For each filter, the date, time, and place of their collection were recorded. The filter was then stored in a cool box and returned to the laboratory of Department of Protozoology, Faculty of Tropical Medicine.

Elution and concentration

Elution. The filters were cut lengthways, separated from the plastic core and divided into three sections. Each section was teased apart and 750 ml of 0.1% Tween 80 solution was added. The mixture (filters + Tween 80) was hand agitated for 5 minutes and repeated thrice. The washing solution was centrifuged at 1,500*g* for 10 minutes. The supernatant was discarded without disturbing the pellet. The pellet was re-suspended and pooled in a volume of 200 ml. The washing solution was centrifuged at 1,500*g* for 10 minutes. The sample was reduced to

a volume of 20 ml. The 10 ml of sample was added to the 10 ml of 2% Tween 80 solution and vortexed for 30 seconds and then centrifuged at 1,500*g* for 10 minutes. The supernatant was carefully aspirated and left behind about 10 ml of fluid above the pellet.

Concentration. Ten ml of cool sucrose solution (1.18S.G.) was underlaid into the suspension by inserting the tip of the cannula into the bottom of centrifuged tube and slowly releasing the sucrose.The solution was centrifuged at 1,000*g* for 5 ml (two times). All the fluid, including the interface, was recovered without disturbing the pellet. About 15 ml of the fluid was recovered. Sufficient distilled water was added to fill the centrifuge tube to remove traces of sucrose then centrifuged at 1,500*g* for 10 minutes (two times). The washing volume was reduced to 1 ml by further centrifugation.

Identification. Three 25 µl replicates were dispensed in 3 spots on each slide. The air-dry samples were covered with acetone or methanol. The air-dried slides were applied with 25 µl monoclonal antibody of *Giardia*, *Cryptosporidium* and *Giardia*/ *Cryptosporidium*, respectively. The slides were incubated in a humidified chamber in the dark at 37°C for 30 minutes. The slides were then immersed in a jar containing PBS to rinse each slide individually with a gentle stream of PBS to remove the residual monoclonal antibody. The slides were dried and examined by fluorescence microscopy.

RESULTS

By using monoclonal antibodies under fluorescence microscopy to detect protozoa in the water, the positive parasites showed bright green fluorescence according to their specific characteristics, such as size and shape. *Giardia* cysts were elliptical in shape, 2-4 µm × 8-12 µm in size; *Cryptosporidium* oocysts were round in shape, 4-6 µm in size (Fig 1A and 1B). Mixed contamination of both parasites was distinguished by the difference in their size and shape (Fig 2). We collected untreated and treated water samples from 20 frozen food industries in Samut Sakhon Province. Untreated water samples were found to be contaminated with *Giardia* cysts in 12 industries (60%) with a mean of 53.33 cysts/1,000 liters (geometric mean 39.43) (Fig 3) and *Cryptosporidium* oocysts in 7 industries (35%) with a mean of 28.57 oocysts/1,000 liters (geometric mean 26.92) (Fig 4). Both parasites were found in 3 factories (15%). None of the treated water samples were contaminated with protozoa. Table 1 showed the result of all frozen food industries.

DISCUSSION

In this study, about 1,000 liters of water samples were collected in order to detect low numbers of parasites similar to a reported study (Lechevallier and Norton, 1995). After filtration, the final concentration of parasites was performed as described previously (Skerret and Holland, 2000). The use of a sucrose flotation facilitated the visualization of the *Giardia* and *Cryptosporidium*. Some researchers, however, suggested using the immunomagnetic electro-cheluminescence method to detect protozoa in water samples, a technique which they found to be more sensitive (Kuczynska *et al*, 2003).

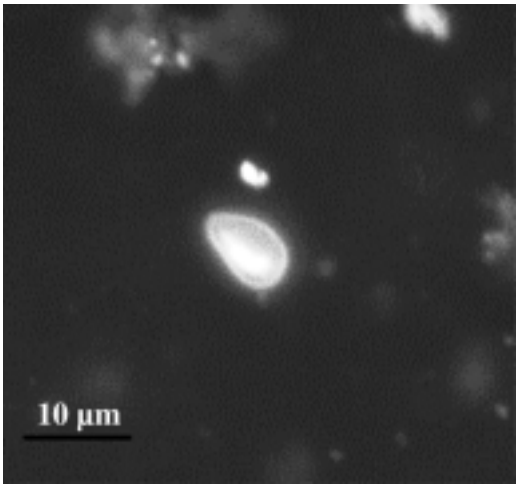

Fig 1A- Positive control of *Giardia* cyst with elliptical shape, 2-4 µ, and 8-12 µ, 1,000×.

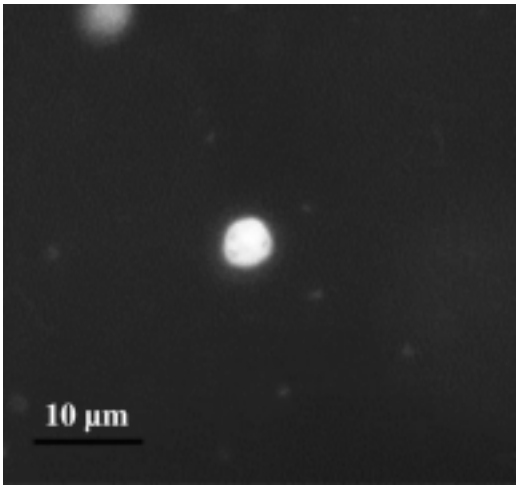

Fig 1B- Positive control of *Cryptosporidium* oocyst with oval shape, 4-6 µ diameter, 1,000×.

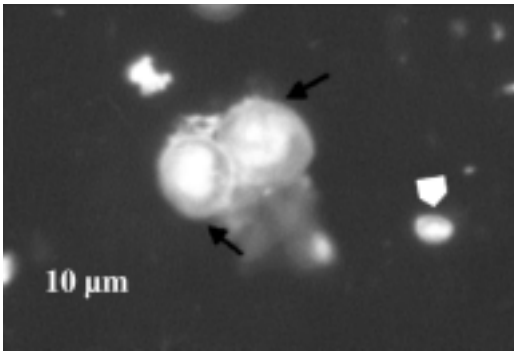

Fig 2- Positive control of mixed infection of *Giardia* cyst (arrows) and *Cryptosporidium* oocyst (arrow head), 1,000×.

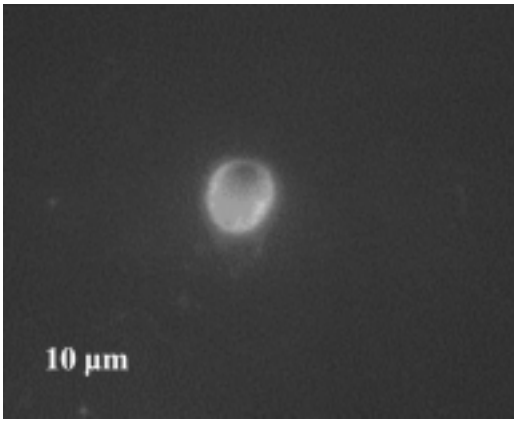

Fig 3- Positive untreated water sample of *Giardia* cyst, 1,000×.

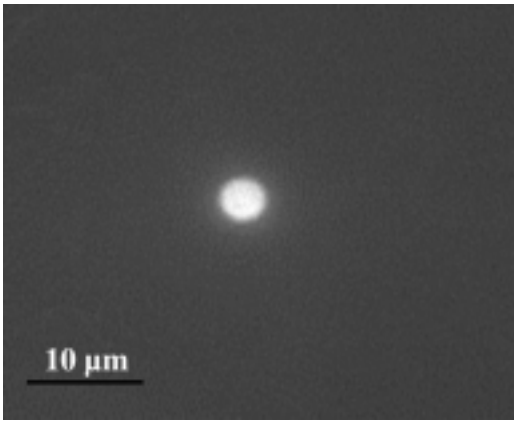

Fig 4- Positive untreated water sample of *Cryptosporidium* oocyst, 1,000×.

Unlike *Giardia* or *Cryptosporidium* in feces, waterborne cysts or oocysts may have been in the aquatic environment for a long time and therefore their morphology and potential for taking up conventional stains such as Giemsa, modified Ziehl-Neelsen, safranin, methylene blue and phenol-auramine can vary (Smith and Rose, 1989). Thus, the use of monoclonal antibodies may be more specific and sensitive under such conditions. The immunofluorescent antibody detection method was successfully applied for the sensitive detection of protozoa in environmental water (Ongerth and Stibbs, 1987). The use of immunofluorescent dyes has also been shown to be superior to conventional staining methods (Arrowcod and Sterling, 1989). However, a drawback of using immunofluorescent monoclonal antibodies is that antigen-antibody complex stability is maintained under a variety of environmental conditions. If the target epitope slips off from the organism by some kind of environmental perturbation, no reaction will occur and a false negative result will be obtained (Rose, 1990). The identification of *Giardia* and *Cryptosporidium* can also be misleading because of particles that have an inherited autofluorescence (Vesey *et al*, 1997). Identification by molecular-based technique could be an alternative method.

In the present study, 60% and 35% of untreated water samples were contaminated with *Giardia* cysts and *Cryptosporidium* oocysts, respectively. The result is in agreement with different studies from other parts of the world. For example, in Germany, *Giardia* cysts were found in 14%, *Cryptosporidium* oocysts in 29.8% and both together in 38.3% of the investigated water supplies (Karanisa *et al*, 1998). Further, in San Pedro Sula, Honduras, between 380-2,100 *Giardia* cysts/100 liters of water and between 58-260 oocysts /100 liters of *Cryptosporidium* were found (Solo-Gabriele *et al*, 1998). In a similar study, Hsu *et al* (2002) reported an average of 66.6 *Giardia* cysts and an average of 59.2 *Cryptosporidium* oocysts per 100 liters of investigated water. All of these studies utilized the immunofluorescence antibody method.

In contrast, all treated water samples in this study were free from both protozoal parasites similar to recent reports from Ottawa and Quebec City, Canada (Chauret *et al*, 1995). However, there were a few studies that reported contamination of swimming pool water with *Giardia* and *Cryptosporidium* parasites (Fournier *et al*, 2002). In Japan, filtered water samples were also contaminated by *Cryptosporidium* oocysts and *Giardia* cysts (Hashimoto *et al*, 2001).

Table 1
Number of *Giardia* cysts and *Cryptosporidium* oocysts in untreated and treated water from twenty frozen food factories.

Name of factories	Untreated water		Treated water	
	Giardia cysts/ 1,000 liters	*Cryptosporidium* oocysts/1,000 liters	*Giardia* cysts/ 1,000 liters	*Cryptosporidium* oocysts/1,000 liters
1. MFP	60	None detectable	None detectable	None detectable
2. GO	240	None detectable	None detectable	None detectable
3. KH	40	None detectable	None detectable	None detectable
4. MMP	60	None detectable	None detectable	None detectable
5. DO	None detectable	40	None detectable	None detectable
6. TSCS	40	None detectable	None detectable	None detectable
7. TF	40	40	None detectable	None detectable
8. SE	20	None detectable	None detectable	None detectable
9. NS	None detectable	20	None detectable	None detectable
10. CC	20	None detectable	None detectable	None detectable
11. TUM	None detectable	None detectable	None detectable	None detectable
12. TPF	20	None detectable	None detectable	None detectable
13. SRCS	None detectable	20	None detectable	None detectable
14. BSAFP	None detectable	20	None detectable	None detectable
15. APFF	40	None detectable	None detectable	None detectable
16. TKSF	40	20	None detectable	None detectable
17. ANF	None detectable	None detectable	None detectable	None detectable
18. SF	None detectable	None detectable	None detectable	None detectable
19. LSP	20	40	None detectable	None detectable
20. UP	None detectable	None detectable	None detectable	None detectable

Clearly the underground water in Thailand used in the production of frozen food is contaminated with *Giardia* and *Cryptosporidium*. Even though, in most cases, underground water is treated by sand filtration and chlorination, the effect of chlorine on *Giardia* cysts can be very limited (Jadwiga and Ewert, 1998). Moreover, *Cryptosporidium* oocysts are nearly insensitive to normal disinfectants used in hospitals and laboratories (Tzipori, 1983) and they are insensitive to the concentration of chlorine routinely used for treating water (Robertson and Gjerde, 2001). A previous study provides evidence that ultraviolet light can be an effective barrier against *Giardia* spp in the treatment of drinking water supplies (Hayes *et al*, 2003).

It must be stated that although clean treated water is essential for the production of frozen food, the untreated water can also contaminate food products and result in a lowered food product quality. Because untreated water is often used for cleaning floors and other equipment, accidental contamination of ingredients for food could occur. Accordingly, frozen food industries should seriously consider either the use of treated water in each step of food production or introduce ultraviolet light to untreated water before use.

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