What is learned from water-related outbreak of cryptosporidiosis
- Sign phenomenon and Oocyst monitoring -

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The WHO's definition of a water- or a foodborne outbreak is when two or more persons experience a similar illness after ingestion of the same type of food or water from the same source and when the epidemiological evidence implicates the food or the water as the source of the illness (Schmidt, 1995). A useful definition of a waterborne outbreak, for the purposes of active surveillance, is when more cases than would be expected are clustered, geographically and in time.

Table 1. Reported outbreak cases and their causal events.

Area		Causal Events	Date
Bradford	**(GB)**	**Amount of tapwater consumed**	**1992.10 - 12**
Milwaukee	**(US)**	**Treatment failure (high turbidity)**	**1993.03 - 04**
Ogose	**(JPN)**	**Amount of tapwater consumed (elevation of temperature)**	**1994.05 - 06**
London	**(GB)**	**Oocyst detected (Active surveillance)**	**1999.04 - 05**
Battlefords	**(Can)**	**Treatment failure (high turbidity)**	**2001.03 - 04**

From this view point, we have re-examined the outbreak reports published elsewhere (Table 1), and tentatively summarized as follows:

1) A small increase in the diarrhea (cryptosporidiosis) cases preceded almost exclusively the large-scale outbreak of the disease in the community (Fig. 1a-c), suggesting that, prior to the disease in outbreak, leakage of a small number of oocysts in tapwater has lasted on the order longer than a month.
2) It is most plausible that leakage is attributable to the increase in the number of oocysts in the source water to a level that can not be removed thoroughly by the treatment process. (This supports the idea that waterborne outbreak of infectious diseases occurs when failure in,

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or accidental defect(s) of, the treatment system happen to occurs under the conditions where there has been relatively heavy contamination of source water with the oocysts.)

3) Sudden temperature shift, and thus elevated intake of unboiled tapwater, can also be another type of variable to cause the outbreak.
4) It was demonstrated that this sign phenomenon (a small number of diarrhea cases associated with a water supply) was not detectable against the general background of infection by the health-based monitoring, and could only be detected by the post-outbreak investigation.

However, there is still hope to detect the sign phenomenon by monitoring oocysts in source water. By doing so, pre-warning system can be established for large-scale outbreak of cryptosporidiosis.

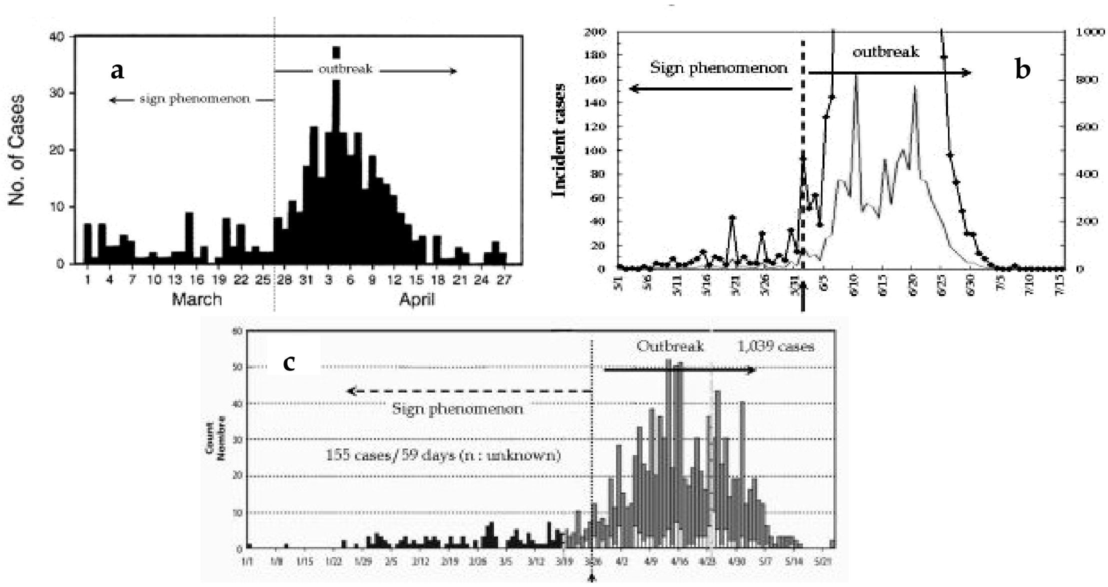

Fig. 1. Sign phenomena of the waterborne outbreak of cryptosporidiosis.
Small increase in the diarrhea (cryptosporidiosis) cases in the community preceded the large-scale waterborne outbreaks. Number of onset of illness within a: Milwaukee, b: Ogose and c: Battlefords health service areas.

The calculated number of oocysts in tapwater during pre-outbreak period was something around ~ 0.02 oocysts/L which is far from the detection limit of the routine test for *Cryptosporidium*. In the source water, however, 10 to 1,000 times of the concentration of the oocysts will be expected depending on the particle removal efficacy of the treatment process (Table 2). Given that a 2.7 log removal (≒1/500) is assured by the overall treatment process, the number of oocyst in source water is ~ 10 oocysts/L. Considering that the new sign phenomenon (or high load of oocysts in source water) is not a transient event but lasts for several weeks as reported in many post-outbreak survey reports, and that isolation of oocyst from 200 ~ 1,000 mL of source water is not time consuming, it is, therefore, feasible to incorporate the *Cryptosporidium* monitoring in source water into the routine monitoring work at intervals of every one or two weeks.

In this presentation, we are going to discuss a new monitoring system, such as using DNA amplification method, for detecting oocyst to prevent large-scale waterborne outbreak of cryptosporidiosis.

Table 2. Number of oocysts estimated in source and tap water during pre-outbreak period.

Outbreak Cases	Amount of tapwater consumed daily	Number of oocyst estimated (/L)			
		in tapwater	in source water with varying removal rate of the treatment plant		
			1-log	2-log	3-log
Milwaukee, US	200 ml	0.1	1.0	10	100
	1,000ml	**0.02**	**0.2**	**2**	**20**
North Battlefords, Canada	200 ml	0.05	0.5	5	50
	1,000ml	**0.01**	**0.1**	**1**	**10**
Ogose, Japan	20ml	0.5	5.0	50	500
	1,000 ml	**0.01**	**0.1**	**1**	**10**

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It is generally said that a useful definition of a waterborne outbreak, for the purposes of active surveillance, is when more cases than would be expected are clustered, geographically and in time.

⇕

A small number of diarrhea cases associated with a water supply is hardly detectable against the general background of infection by the health-based monitoring, and could only be detected by the post-outbreak investigation.

⇩

Need a new sign phenomenon of the disease in outbreak

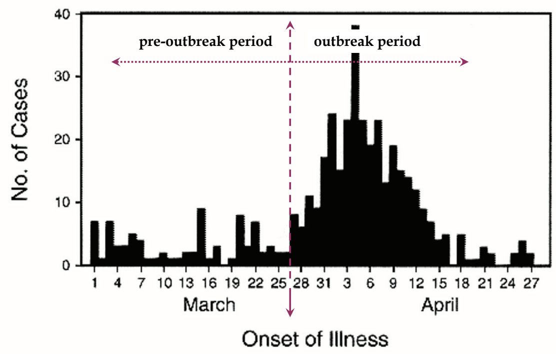
Table. Reported outbreak cases and their causal events.

Area		Causal Event(s)	Date
Bradford	(GB)	Amount of tapwater consumed	1992.10 - 12
Milwaukee	(US)	Treatment failure (high turbidity)	1993.03 - 04
Ogose	(JPN)	Amount of tapwater consumed (elevation of temperature)	1996.05 - 06
Battlefords	(Can)	Treatment failure (high turbidity)	2001.03 - 04

In general, waterborne outbreak of infectious disease occurs when failure in, or inadequate design of, the treatment system happen to occur under the conditions where there is relatively heavy contamination of source water with a pathogen.

Sudden temperature shift, and thus elevated intake of unboiled tapwater, can also be another type of variable to cause the outbreak.

Waterborne Outbreak of cryptosporidiosis in Milwaukee in 1994



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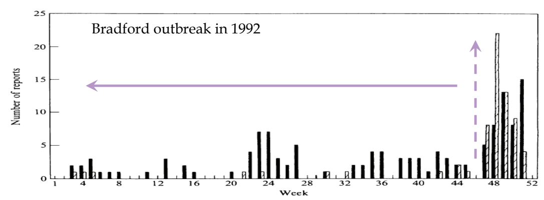

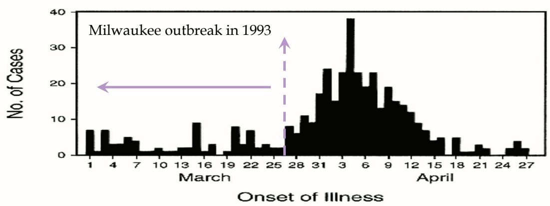

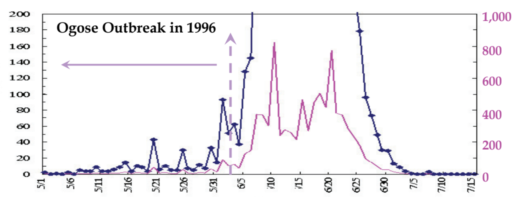

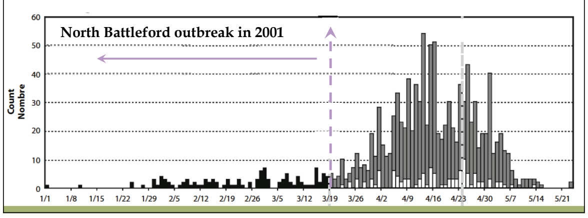

Summary of Ogose Outbreak Case

Among 11,619 responders, 215 reported having had diarrhea during pre-outbreak period from May 1 to May 28, 1996 with average daily infection rate of 0.066%.

215÷11,619÷28=6.6x10^{-4} (=0.066%)

$$P_d = 1 - e^{-rx_d} \Rightarrow x_d = -\frac{\ln(1-P_d)}{r}$$ (Haas et al. 1996)

where P_d is a daily infection rate, x_d is number of infectious oocyst ingested, and r (=0.09) is the probability of infection from ingesting a single infectious oocyst.

The number of oocysts in tapwater was estimated to be 0.012, 0.04 or 0.4 oocysts /L, given the amount of unboiled tapwater consumed daily was 600ml (3 cups of water), 200ml or 20ml, respectively.

Summary of Milwaukee Outbreak Case

Of 1,663 responders, 81 reported having had diarrhea during pre-outbreak period between March 1 and March 26,1994.

The average daily infection rate was 0.0019.

Number of oocyst consumed was estimated to be 0.02 per person per day.

The number of oocysts in the tapwater was estimated to be 0.02 or 0.1 oocysts/L, given the amount of unboiled tapwater consumed daily was 1,000 or 200ml, respectively.

Summary of Battlefords Outbreak Case

A total 155 residents reported having had diarrhea during pre-outbreak period between January 23 and March 25, 2001.

The average daily infection rate was 0.0011.

Number of oocyst consumed was estimated to be 0.012 per person per day.

The number of oocysts in the tapwater was estimated to be 0.01 or 0.05 oocysts/L, given the amount of unboiled tapwater consumed daily was 1,000 or 200ml per day, respectively.

Number of oocysts estimated in source and treated water during pre-outbreak period

Outbreak Cases	Number of oocyst estimated (/L)				
	Amount of tapwater consumed daily	Tapwater	Source water with varying removal rate of the treatment plant		
			1-log	2-log	3-log
Milwaukee, US	1,000 ml	0.02	0.2	2	20
	200ml	0.10	1.0	10	100
North Battleford, Canada	1,000 ml	0.01	0.1	1	10
	200ml	0.05	0.5	5	50
Ogose, Japan	1,000 ml	0.01	0.1	1	10
	20ml	0.4	4.0	40	400

In general, it is said that waterborne outbreak of infectious disease occurs when failure in, or inadequate design of, the treatment system happen to occurs under the conditions where there is relatively heavy contamination of source water with a pathogen.

1. According to the case reports, small increase in the diarrhea (cryptosporidiosis) cases in the community preceded the outbreak of the disease. (⇒ sign phenomenon)
2. The estimated number of oocysts in tapwater during pre-outbreak period was ~ 0.02oocyst/L.
3. Leakage of small number of the oocysts into the finished water, and thus causes cryptosporidiosis, is attributable to a high load of oocysts in the source water.
4. Given that a 2.7 log removal (1 / 500) is assured by the treatment process, the number of oocyst in source water is ~ 10 oocysts/L. (⇒ new sign phenomenon)
5. Leakage of oocyst in tapwater (≑ high load of oocyst in source water) lasts on the order longer than a month.

Oocyst Detection

~ 1,000ml of source water ← sample

↓

1hr: filtration / density gradient centrifugation / immuno-beads separation ← oocyst isolation

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1hr: freeze and thaw / proteinase treatment ← DNA extraction

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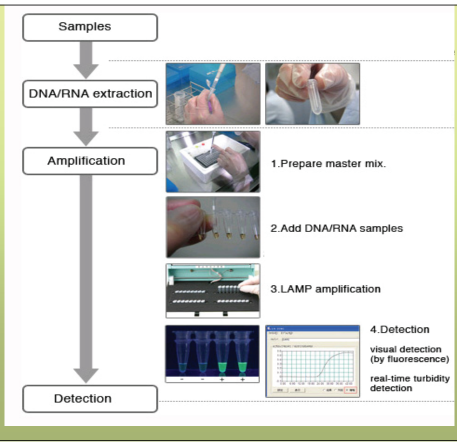
1hr: LAMP-MPN quantification method / 3 dilutions and 3 tubes per dilution ← DNA amplification

↓

outcome



"Oocyst Monitoring System" to be developed

Test Sample	:	Source water
Sample Volume	:	200 ~ 1,000 mL (depending on the removal efficacy)
Oocyst Detection	:	DNA Amplification (LAMP, PCR) Microscopy
Frequency of Testing	:	At least Once a week or Two
Pre-cautions to be made	:	Strengthen treatment processes Boil notice ※ Protection or replacement of source water, etc.

※ Sudden temperature shift, and thus elevated intake of unboiled tapwater, can also be another type of variable to cause the outbreak.

ACKNOWLEDGEMENTS

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