COLD REGIONS ENGINEERING

The Cold Regions Infrastructure:
An International Imperative for the 21st Century

Proceedings of the Eighth International Conference on Cold Regions Engineering

University of Alaska, Fairbanks
Fairbanks, Alaska
August 12-16, 1996

Sponsored by the
Technical Council on Cold Regions Engineering
of the American Society of Civil Engineers

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RECEIVED
OCT 04 2006
DEPT. OF ENVIRONMENTAL
CONSERVATION
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Published by
ASCE American Society of Civil Engineers
345 East 47th Street
New York, New York 10017-2398

Occurrence and Significance of *Cryptosporidium parvum* and *Giardia lamblia* in Surface Waters on Alaska's North Slope

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ABSTRACT

This paper describes the results of a 1995 water quality sampling program in 10 water sources used by Native villages across Alaska's Arctic Coastal Plain, conducted by Northern Testing Laboratories, Inc. (NTL) of Fairbanks, Alaska. The focus is on the parasitic protozoans *Giardia lamblia* and *Cryptosporidium parvum. Giardia* is currently one of the definitive organisms in the Surface Water Treatment Rule, incorporated into the State of Alaska Drinking Water Regulations (18 AAC 80, May, 1994). At least one or both of these microorganisms were detected in each of the ten water sources during one or more rounds of sampling. Overall, eight of the sources had *Giardia lamblia* and eight had *Cryptosporidium* ssp. This is a significant treatment concern for rural Alaskan villages.

INTRODUCTION

Cryptosporidium ssp. has recently emerged as a significant concern in both raw and treated water supplies throughout the world. In 1993, *Cryptosporidium* was responsible for a 400,000 case outbreak which resulted in over 5000 hospitalizations and more than 100 deaths in Milwaukee, Wisconsin (Fox et al. 1993). The organism has since and prior to 1993 been implicated a number of other occurrences of waterborne gastrointestinal illness outbreaks. *Cryptosporidium* is presently emerging as the defining organism in a proposed "Enhanced Surface Water Treatment Rule" being developed by the USEPA, and is also a significant factor in the reauthorization of the Safe Drinking Water Act by Congress.

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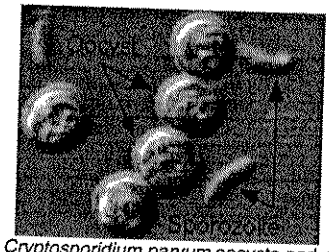


Figure 1: *Cryptosporidium parvum* oocysts and sporozoites

A background on *Giardia* and *Cryptosporidium* and the analytical techniques is discussed, followed by a description of the sampling procedures used by NTL in the 1995 raw water monitoring program. The final section of this paper presents the results of the analyses for these two protozoans and the significance of the occurrence of these organisms on water treatment plant design. This project represents the largest data base for the occurrence of both *Giardia* and *Cryptosporidium* in the Arctic to date.

GIARDIA AND *CRYPTOSPORIDIUM* BACKGROUND

In the United States, protozoans are emerging as the major contaminants of concern for drinking water professionals. Protozoa are eukaryotic protists that occur as single cells or in colonies of cells. There are an estimated 45,000 species of protozoa (Pelczar et al. 1977) They vary widely in size, shape, structure and physiological characteristics. Some are harmless, some are useful, such as those used in wastewater treatment and some are capable of causing disease in humans and animals. Approximately 7,000 species of protozoa are parasitic. *Giardia lamblia* and *Cryptosporidium parvum* belong to this latter category.

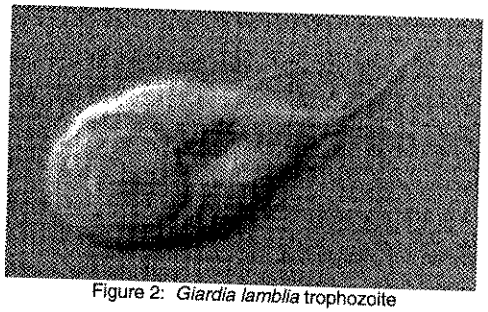


Figure 2: *Giardia lamblia* trophozoite

Giardia and *Cryptosporidium* are pathogenic intestinal protozoa which can occur in domestic and wild animals, as well as in humans. *Cryptosporidium* was first described in 1907 by Ernest Tyzzer. He observed that the organism was causing infection in laboratory mice and

that the mode of transmission was fecal-oral. Infections due to *Cryptosporidium* have been recognized as important causes of morbidity and mortality in farm animals since 1955. *Cryptosporidium parvum* is now recognized as a human pathogen which can cause cryptosporidiosis, a severe diarrheal illness (Hayes et al. 1989). Infections are especially common in neonatal and immunocompromised individuals. At present, there is no effective drug therapy for cryptosporidiosis and infections can be life threatening for the immunocompromised.

Ingestion of drinking water contaminated with viable oocysts is a major mode of transmission for humans. *Cryptosporidium* is introduced to the water supply via animal excreta containing oocysts. Important reservoirs of the oocysts include cattle, sheep and pigs. *Cryptosporidium* is also found in wild animal populations. The organism is most prevalent in ruminants, such as deer, elk and caribou and is found primarily in neonates of those species. Person to person transmission is common, especially in child daycare facilities. Direct contact with infected animals, particularly calves and lambs, can cause illness in exposed persons. Uncooked, contaminated food can also cause infections. The latest studies on infectivity rates indicate that *Cryptosporidium* is potentially infective at very low doses, in some cases as few as 10 oocysts (MMWR 1995). *Cryptosporidium* oocysts are fully sporulated when they leave their host, which renders them immediately infective to susceptible individuals. Sporozoite release occurs in the small intestine after ingestion. Symptoms usually appear within two to ten days after ingestion of the parasite. Symptoms include watery diarrhea, headache, abdominal cramps, nausea, vomiting and low-grade fever. These symptoms may lead to weight loss and dehydration. In otherwise healthy individuals, symptoms usually last from one to two weeks, at which time the immune system is able to stop the infection. However, in the immunocompromised, the infection may continue and become life-threatening.

Cryptosporidium has been implicated in more than five major waterborne disease outbreaks in the United States since 1984, the largest of which occurred in Milwaukee in 1993. That outbreak affected over 400,000 people, with a reported death toll of 104 persons (Fox et al. 1993). *Cryptosporidium* has also been implicated in seven waterborne disease outbreaks in the United Kingdom since 1983. *Cryptosporidium* is widely distributed in both industrialized and developing countries. The organism has been found in every country where surveys have been conducted. In the United States, 87% of surface water sites on the East coast and 77% of surface water sites on the West coast were found to contain oocysts (*"Cryptosporidium"* 1993) Overall, prevalence in industrialized countries is approximately 2.1%. Prevalence rates in developing nations is 8.5%. Prevalence in AIDS patients in the U.S. is about 3% and in AIDS patients in underdeveloped nations is about 40% (*"Cryptosporidium"* 1993). There is some evidence that immunity can be developed against *Cryptosporidium*.

Giardia was first observed by Anton von Leeuwenhoeck in 1681 and taxonomically described by Lambl in 1859. For most of its history, *Giardia* has been considered to be non-pathogenic because of the large number of asymptomatic cases. *Giardia lamblia* has been identified as the major cause of waterborne disease outbreaks in the United States between 1978 and 1991. In 1992, *Giardia* and *Cryptosporidium* were implicated in the same number of outbreaks.

Giardia lamblia is recognized as a human pathogen which causes the disease, giardiasis. *Giardia* cysts are ingested either in water or by the fecal-oral route. *Giardia* cysts are introduced into the water supply by human or animal excreta. There are several animal reservoirs of *Giardia*, including beaver, muskrat, bears and dogs. Excystation takes place inside the small intestine and trophzoites attach themselves to the intestinal wall. This leads to malabsorption of nutrients and liquids which causes diarrhea, cramps, nausea and vomiting. *Giardia* can also cause disease in the bile tract and liver. Immunodeficiency may predispose individuals to chronic and symptomatic giardiasis. There are several drugs available which are effective against *Giardia*. Immunity against *Giardia* can be acquired.

Overall, distribution of human giardiasis is cosmopolitan. Prevalence rates range from 1% in industrial nations to 30% in underdeveloped nations (Lin 1985). Rates of infection are dependent on hygienic standards and prevention depends on proper sanitation and personal hygiene.

The presence of *Giardia* in drinking water supplies is currently regulated under the Surface Water Treatment Rule (SWTR) which requires that all surface water and groundwater under the direct influence of surface water (GUDISW) systems demonstrate a 3-log (99.9%) removal of *Giardia* cysts by filtration and disinfection. Although there are currently no regulations requiring testing for *Cryptosporidium* in drinking water, the US Senate has recently passed the Safe Drinking Water Act Amendments of 1995 (S.1316) which specifically call for EPA to promulgate an Enhanced Surface Water Treatment Rule with standards for *Cryptosporidium*.

GIARDIA AND *CRYPTOSPORIDIUM* ANALYSIS

The approved method for testing water for both *Giardia* and *Cryptosporidium* is the *ICR Protozoan Method for Detecting Giardia Cysts and Cryptosporidium Oocysts in Water*. This is an in-vitro procedure for the simultaneous detection of *Giardia* cysts and *Cryptosporidium* oocysts in environmental samples. In this method, a large volume of water (100L to 3785L) is filtered through a 1 µm nominal porosity, yarn-wound, polypropylene filter. This is accomplished using a sampling apparatus consisting of a filter housing, water meter, inlet and outlet hoses and a flow control valve (See figure 3). The water flow is restricted to 4 gpm and the sample is collected over an appropriate time span. The filter is then returned to the laboratory for analysis.

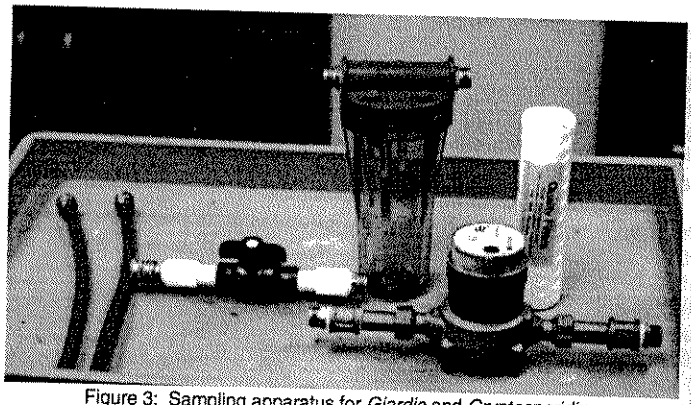
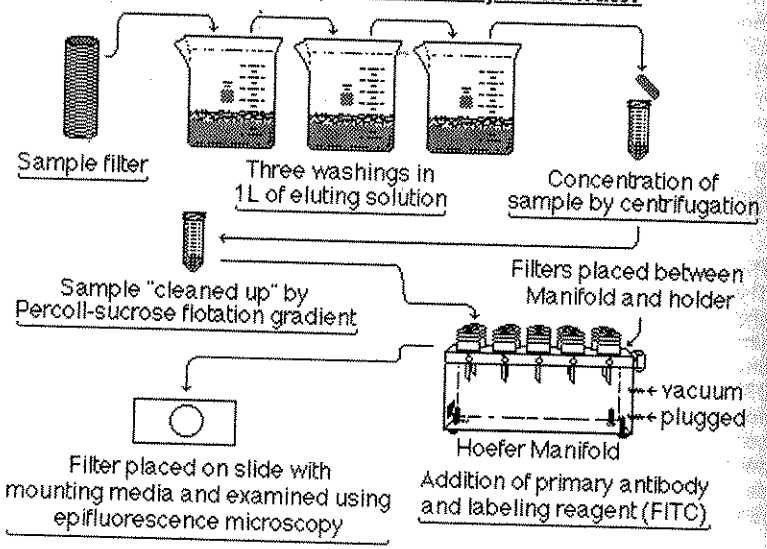


Figure 3: Sampling apparatus for *Giardia* and *Cryptosporidium*.

Upon arrival at the laboratory, the sample is carefully processed (Figure 4). The filter is hand washed in three liters of eluting solution. The three liters of eluate are concentrated by centrifugation. The sample concentrate is "cleaned up" using a Percoll-Sucrose flotation gradient. The cleaned sample is then applied to a filter, conjugated with a monoclonal antibody and labeled with fluorescein isothiocyanate (FITC).

Figure 4: ICR Protozoan Method for Detecting *Giardia* Cysts and *Cryptosporidium* Oocysts in Water



The slide is examined using epifluorescence microscopy generated by a super high pressure mercury lamp (see figure 5). *Giardia* cysts and *Cryptosporidium* oocysts will exhibit characteristic apple-green fluorescence if present on the filter. Cysts and oocysts must also have the correct size and internal morphology to be positively identified as *Giardia* or *Cryptosporidium*.

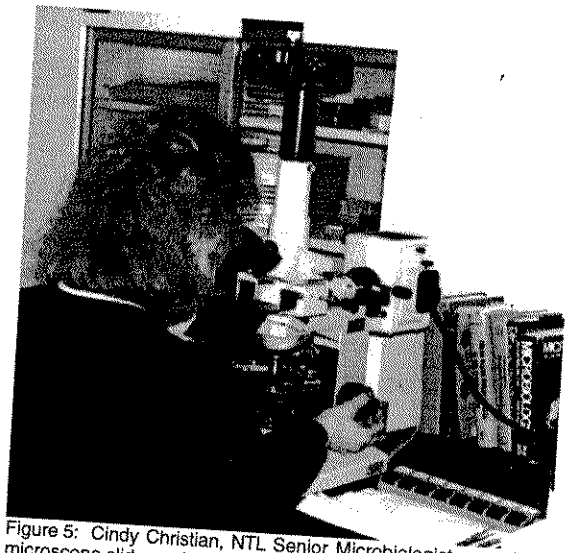


Figure 5: Cindy Christian, NTL Senior Microbiologist, examining microscope slides using epifluorescence microscopy generated by a super high pressure mercury lamp.

This test method provides a quantitative indication of the level of contamination in raw and treated drinking waters with *Giardia* cysts and *Cryptosporidium* oocysts. The method can be useful in determining the source of contamination in water supplies, the occurrence and distribution of the protozoa in water supplies and in evaluating the effectiveness of treatment processes. This method has several limitations, the most severe of which is recovery of cysts and oocysts. Recovery rates ranging from 9% to 69% have been reported by laboratories performing this test (MMWR 1995). The test is very labor intensive and requires a highly trained microscopist to identify the cysts and oocysts, making the test expensive to perform. There are a myriad of organisms in the same size range which can cross react with the primary antibody. This can make positive identification of *Giardia* cysts and *Cryptosporidium* oocysts difficult. The method cannot be used to identify particular species of *Giardia* and *Cryptosporidium* and therefore, host species cannot be identified. Rates of infectivity and viability also cannot be determined by this method. This means that even if cysts and oocysts are observed, it does not indicate

whether or not their presence would lead to waterborne disease outbreaks.

PROJECT SUMMARIES

NTL was directly involved with two separate projects covering 10 surface water sources from Point Hope to Barter Island, Alaska. Nine of the village sources were sampled 4 times throughout the summer. Barrow had a monthly sampling schedule from January through December 1995. Following are descriptions of these two projects.

PROJECT 1: RAW WATER SAMPLING OF NORTH SLOPE BOROUGH VILLAGES

A raw water sampling and analysis program was completed for nine fresh water sources located near the North Slope Borough (NSB) villages of Atqasuk, Kaktovik, Nuiqsut, Point Hope, Point Lay and Wainwright. The purpose of this program was to establish a baseline of raw water quality data for these supplies in support of the NSB water and wastewater improvement effort in these villages. The sampling program was conducted by the Environmental Science staff of Northern Testing Laboratories, Inc. (NTL) of Fairbanks, Alaska with the assistance of the NSB Department of Municipal Services (DMS) water treatment operators in each of these villages under authorization from BTS • LCMF Joint Venture.

The NSBDMS village raw surface water sampling program was accomplished in two phases. In the first phase a "complete analysis" set of time-dependent field tests and laboratory samples were collected by NTL environmental scientists Craig Nordgren and Jonathan Pollen during the week of July 10 to 14, 1995 with the assistance of the NSBDMS water treatment operators. The samples were forwarded to NTL's certified water quality laboratory in Fairbanks for analysis.

All of the villages that were sampled are only accessible by commuter air service. NTL technicians flew from village to village during the first phase of the project. The samples were either taken directly out of the source lakes, the raw water transmission line between the lakes and the treatment plant, or a raw water sampling point inside the water treatment plants. Figure 6 shows the *Giardia* and *Cryptosporidium* sampling apparatus connected to the raw water transmission line in the village of Wainwright. The weather played an important factor in the first phase as both technicians were delayed due to coastal fog. A few samples in the second phase were also delayed due weather conditions, since all the samples were shipped to the laboratory through commuter airlines.

The initial phase sampling effort was also used as a training program for the NSBDMS operators in proper sample collection and shipping procedures. During the second phase of the sampling program, the water plant operators collected three additional sets of samples and shipped

them to the laboratory at approximately two week intervals. The operators also performed field pH and temperature measurements with each of the three follow-on samples.

Figure 6: *Giardia* and *Cryptosporidium* sample being taken from the raw water transmission line in Wainwright, Alaska.

A total of 8 lakes and one river in six villages were sampled as shown in TABLE 4. In one case, the alternate lake at Point Hope, the lake was identified incorrectly by the water plant operator in the field and another lake near the source lake (7 Mile Lake) was sampled instead.

TABLE 4
NSB Village Water Sources Sampled During 1995

Village	Source	Notes
Atqasuk	Meade River	Sampled from shore
Atqasuk	Ikmakrak Lake	Sampled from inside the water plant
Kaktovik	Water Supply Lake	Sampled from inside the water plant
Nuiqsut	Water Supply Lake	Sampled from the shore
Point Hope	7 Mile Lake	Sampled from transmission line tap
Point Hope	Alternate Lake	Sampled near 7 Mile Lake
Point Lay	Water Supply Lake	Sampled from a boat in the lake
Wainwright	North Lake	Sampled from the shore
Wainwright	South Lake	Sampled from transmission line tap

The "complete analysis" parameters included most of the current ADEC regulated drinking water parameters and *Giardia* and *Cryptosporidium*. The parameters selected for the "limited analysis" series were those that we have historically found to be of significance in the village surface water

supplies. This presumption was confirmed from the initial sampling program data. These parameters included microbiological tests for fecal coliform, heterotophic bacteria, *Giardia* and *Cryptosporidium.*

Figure 7: *Giardia* & *Cryptosporidium* Occurances in Nine Village Water Source (Combined)

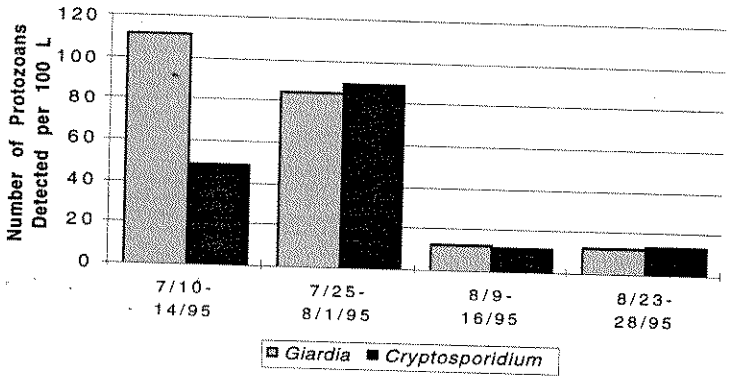


Figure 8: *Giardia* & *Cryptosporidium* Results For Nine North Slope Village Surface Water Supplies

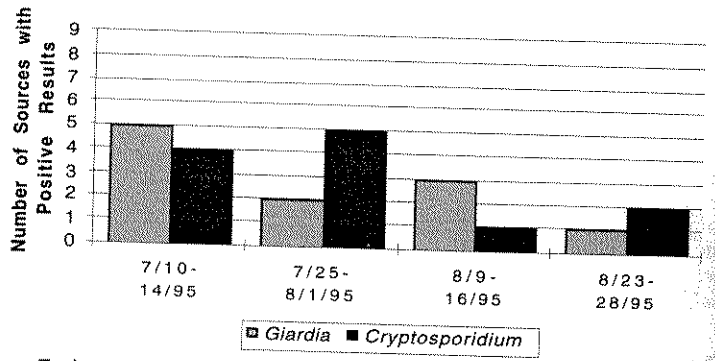


Figure 7 shows the cumulative number of *Giardia* and *Cryptosporidium* detected in each sample period in, and the number of sources testing positive in Figure 8. The charts are referenced to the *Giardia* and

Cryptosporidium result tables in APPENDIX A.

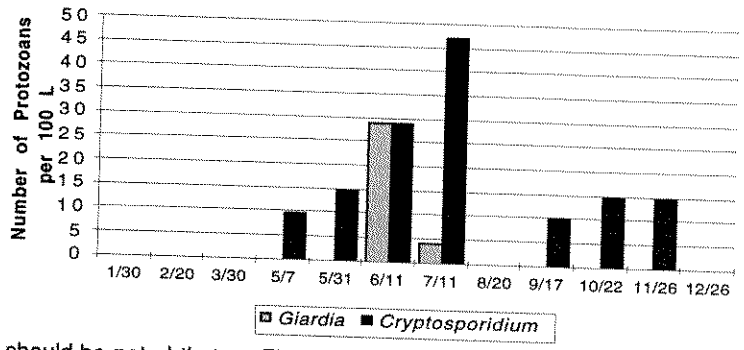
One of the goals of the second phase of the sampling program was to obtain samples during a full range of weather conditions that were expected to occur during the summer on the Arctic Coastal Plain. Several wind events did occur during the two month sampling program, in some cases delaying transport of samples back to the lab.

PROJECT 2: BUECI MONTHLY RAW WATER SAMPLING PROGRAM

The second project was with the Barrow Utilities and Electric Co-Operative (BUECI) which operates the drinking water treatment plant in Barrow. The raw water supply is taken from the Isatkoak Reservoir which is located between the City of Barrow and its suburb, Browerville. The reservoir is fed mainly from snow melt and tundra runoff during the spring thaw.

The BUECI raw water sampling program was instituted in January of 1995 to continue building a water quality data base for the Isatkoak Reservoir which could be used in the design of a new water treatment plant. Only those parameters which were found in previous monitoring to be of significance in this water supply were selected for monitoring. *Giardia* and *Cryptosporidium* were included in the monitored parameters. The samples were collected on a monthly basis to obtain a twelve month picture of how the protozoan population fluctuated throughout the seasons. Figure 9 outlines the results in the Isatkoak Reservoir for *Giardia* and *Cryptosporidium.*

Figure 9: *Giardia* & *Cryptosporidium* Results For Iksatkoak Reservoir, January - December 1995



It should be noted that on Figure 9 the spring thaw started around June 1 and the first ice cover was seen in mid-September. The only occurrence of *Giardia* was associated with spring thaw, while *Cryptosporidium* showed

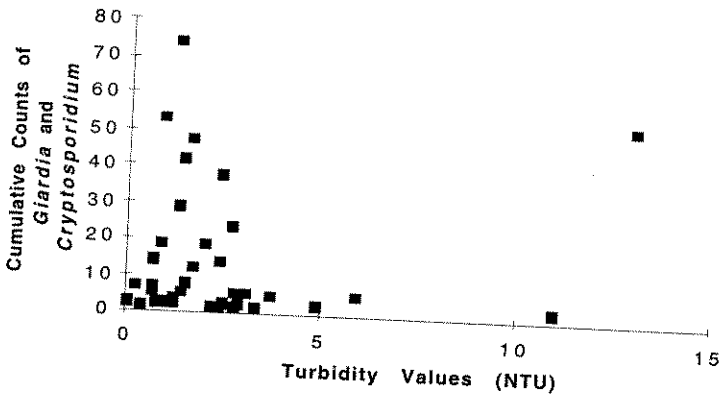
two occurrence intervals, spring thaw and shortly after the return of the ice cover.

CONCLUSION

These two projects represent the first significant regional data set for the parasitic protozoans *Giardia lamblia* and *Cryptosporidium parvum* that has been developed on Arctic surface water sources. At least one or both of these microorganisms were detected in each of the ten water sources during one or more rounds of sampling. Overall, eight of the sources had *Giardia lamblia* and eight had *Cryptosporidium* ssp. A declining trend for the NSB villages (FIGURE 7) in the total number of samples with positive occurrences of these protozoans over time was observed. The highest total occurrence of positive *Giardia* and *Cryptosporidium* data was immediately after spring thaw. The trend for BUECI peaked shortly after spring thaw, with recurrence of *Cryptosporidium* in late Fall.

Samples showed significantly higher turbidity and total particle counts during wind events in which the water quality was influenced by benthic sediments being stirred up into the water column by wind and wave action. The data shows no direct correlation (r=0.1) between higher turbidity and higher *Giardia* or *Cryptosporidium* counts (Figure 10).

Figure 10: Turbidity vs. *Giardia* & *Cryptosporidium*



The protozoans are of particular concern since they, along with a number of waterborne pathogenic bacteria and viruses, represent a significant and immediate public health threat. Furthermore, questions have arisen in the literature over the past several years about treatment efficiency for the removal and/or inactivation of *Cryptosporidium* by conventional filtration and disinfection. This is a significant treatment concern for rural Alaskan villages. The presence of *Giardia* and *Cryptosporidium* is an important

factor in the design and operation of all treatment plants even in Arctic climates.

APPENDIX A

(Data are Confirmed Counts per 100 Liters)

***Cryptosporidium* & *Giardia* Results for Nine North Slope Village Surface Water Supplies**

Village	Source	*Cryptosporidium*				*Giardia*			
		7/10-14/95	7/25-8/1/95	8/9-16/95	8/23-28/95	7/12/95	7/26/95	8/9/95	8/23/95
Atqasuk	Mead River	<5.3	<2.6	<1.3	<1.3	21	<2.6	4	<1.3
	Ikmakrak Lake	11	5.3	11	<1.3	42	69	2.6	<1.3
Kaktovik	Lab Sink (Raw tap)	0.5	5.3	<2.6	<1.3	2	<2.6	<2.6	<1.3
Nuiqsut	Raw Water	<2.6	<5.3	<1.3	2.6	<2.6	16	<1.3	<1.3
Point Lay	Raw Water Lake	<5.3	5.3	<2.6	11	<5.3	<5.3	<2.6	<2.6
Point Hope	Alternate Lake	16	32	<2.6	<5.3	<5.3	<11	<2.6	<5.3
	7 Mile Lake	<5.3	42	<2.6	<2.6	<5.3	<11	5.3	13
Wainwright	South Lake	21	<5.3	<1.3	<2.6	21	<5.3	<1.3	<2.6
	North Lake	<5.3	<5.3	<2.6	<53	26	<5.3	<2.6	<53
Number of sources w/positives:		4	5	1	2	5	2	3	1
Cumulative Sum		48.5	89.9	11	13.6	112	85	11.9	13

BUECI Monthly Raw Water Sampling Results

Microbiological Parameters:	1/31	2/21	3/31	5/8	6/1	6/12	7/12	8/21	9/18	10/23	11/27	12/27
Giardia	< 2.4	< 2.4	<2.4	<4.9	<4.9	29	4.3	<4.9	<4.9	<4.9	<4.9	<4.9
Cryptosporidium	< 2.4	< 2.4	<2.4	9.8	15	29	47	<4.9	10	15	15	<4.9

APPENDIX. REFERENCES

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