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GIARDIASIS IN WASHINGTON STATE

by

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16. ABSTRACT

The objective was to determine the potential for transmission of giardiasis through approved drinking water supplies in Washington State. The project consisted of five studies: the first was conducted during trapping seasons (1976-1979) and resulted in examining of 656 beaver stool samples, 172 muskrat and 83 other animal stools. Positivity for beaver was 10.8%, for muskrat, 51.2%. No Giardia was found in other mammals. The second study contacted 865 Giardia-infected Washington state residents to identify likely sources or possible risk factors for infection. Clusters of cases were linked to day care centers, backpacker groups or sites for drawing water on outings and foreign travel. No excess cases were observed for users of surface drinking water supplies. The third study was a case-control study to identify risk factors for giardiasis. Factors which appeared to place a person at increased risk included consumption of untreated water, foreign travel and attendance at a day care center. The fourth study examined water filtering techniques for recovery of Giardia cysts from drinking water supplies and yielded improvements in recovery. The fifth study was a stool survey of children in Skagit and Thurston counties. Overall prevalence of infection was 7.1%. No differences in the prevalence were found by source of domestic water (surface filtered, surface unfiltered, well or spring).

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NOTICE

FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory conducts a coordinated environmental health research program in inhalation toxicology, genetic toxicology, neurotoxicology, developmental and experimental biology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, water pollution, non-ionizing radiation, environmental carcinogenesis, and the toxicology of pesticides and other chemical pollutants. The Laboratory participates in and provides data for the development and revision of criteria documents on pollutants for which national ambient air quality and water quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This report presents the results of several studies designed to obtain information on the epidemiology of giardiasis with specific attention given to the potential role of drinking water. With a better understanding of the epidemiology of this disease, informed decisions can be made on the need for standards, treatment requirements or guidance to prevent transmission of disease through our water supplies.

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Director
Health Effects Research Laboratory

ABSTRACT

This research project was initiated to determine the potential for transmission of giardiasis through approved drinking water supplies in Washington State. The project consisted of five separate studies.

The first study, a parasitological stool survey of commercially trapped aquatic mammals, was conducted during each trapping season from 1976 to 1979 and resulted in the examination of 656 beaver stool samples, 172 muskrat stools and 83 other animal stool samples. Positivity for beaver was 10.8% while positivity for muskrat was 51.2%. No *Giardia* was found in other trapped mammals (nutria, mink, raccoon, river otter, bobcat, coyote, lynx or mountain beaver).

The second study, a follow-up of human giardiasis cases identified through medical diagnostic laboratories, contacted a total of 865 *Giardia* infected Washington State residents and asked a series of questions designed to identify likely sources or possible risk factors for infection. Two outbreaks were identified which implicated domestic drinking water as the source. Other clusters of cases were linked to day care centers, backpacker groups or sites for drawing water on outings and foreign travel. No excess of cases was observed for customers of surface drinking water supplies.

The third study was a case-control study to identify risk factors for giardiasis. This study included 349 laboratory identified cases and 349 controls selected from directory assistance listings. Factors which appeared to place a person at increased risk of giardiasis incluued consumption of untreated water, foreign travel (for adults) and attendance at a day care center (for children under age 10).

The fourth study examined water filtering techniques for recovery of *Giardia* cysts from drinking water supplies. Initial application of the technique recovered cysts from several supplies not implicated in giardiasis outbreaks, however laboratory testing of the technique demonstrated very poor cyst recovery using the recommended filter application and analysis techniques. Changes in the application and analysis techniques (lower water pressure, use of a continuous flow centrifuge, different filter fiber washing techniques, 1 micron filter, etc.) yielded order of magnitude improvements in cyst recovery. As few as 3000 cysts in 500 gallons of water would be adequate for cyst identification under conditions of low to medium turbidity.

The fifth study was a stool survey of 1 to 3 year old children in

Skagit and Thurston counties. Children were randomly selected from birth certificate listings and parents were paid to submit 2 stool samples for analysis. Overall prevalence of infection was 7.1% for the children surveyed. No differences in the prevalence were found by source of domestic water (surface filtered, surface unfiltered, well or spring).

This report was submitted by the Washington State Department of Social and Health Services, Office of Environmental Health Programs, in fulfillment of Grant No. R-805809 from the U.S. Environmental Protection Agency. This report covers a period from July 1, 1978 to April 1, 1981 and work was completed as of December 31, 1981.

CONTENTS

FIGURES

TABLES

TABLES (continued)

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SECTION 1

INTRODUCTION

Although *Giardia* infections in man have been recognized for centuries, waterborne transmission of this parasite has only recently been recognized as a major mode of dissemination. Drinking water contaminated with human waste was thought to be the likely source of a giardiasis outbreak in Aspen, Colorado in 1966. Contamination of water by aquatic mammal waste was thought to be the likely source of outbreaks in Camas, Washington (1976) and Berlin, New Hampshire (1976). The latter outbreaks were of particular interest to water treatment engineers and public health officials since the treated water met both coliform and turbidity levels believed to protect against waterborne disease outbreaks. Furthermore, the conditions which resulted in the Camas outbreak were likely to occur commonly throughout Washington State and perhaps throughout much of the West.

Following the Camas outbreak of April and May 1976, the Washington State Department of Social and Health Services (DSHS) together with the Environmental Protection Agency (EPA) began a series of investigations to determine whether similar outbreaks were occurring elsewhere in Washington State and to estimate the potential for future outbreaks. The Camas outbreak was thought to be related to *Giardia* infected beaver residing in the watershed of the town's surface water supply. Problems with the Camas water filter system resulted in cysts (possibly excreted from beaver) passing through the filter. They were probably unaffected by the level of chlorination used at the time of the outbreak. The majority of Washington State residents are served by surface water supplies and many of these supplies use chlorination as the only means of disinfection. Since all of these watersheds are frequented by beaver, the presence of *Giardia* infected beaver could lead to similar outbreaks.

Information was required on both the potential for human exposure to *Giardia* as well as whether human illness resulted. To determine the extent of aquatic mammal infection with *Giardia*, stool surveys of commercially trapped animals were initiated in the fall of 1976 and continued through spring 1980. To begin assessing the extent of human illness resulting from giardiasis, a pilot human case follow-up was initiated in 1977 and extended to a statewide human follow-up in July 1978. To identify risk factors for human giardiasis, a case-control study was initiated in March 1979 and continued through March 1980 when case follow-up was also suspended. In July 1978 an investigation was initiated to estimate how frequently *Giardia* cysts could be recovered from drinking water supplies using a large volume water filtration technique developed by the Health Effects Research Laboratory (HERL) of the EPA. Due to problems with the technique, this aspect of the study was modified so that more effort was placed on evaluating alternative methods for cyst recovery. In September 1980 a human stool survey of one to three year old children was initiated to determine whether a difference in prevalence of infection existed between areas served by surface water supplies (Skagit county) and areas served by well water supplies (Thurston county).

SECTION 2

CONCLUSIONS

This project demonstrated a widespread potential for waterborne transmission of giardiasis in Washington State. During the four years of animal surveys, Giardia prevalence in beaver ranged from 6% to 19% and in muskrat from 0% to 85%. Infected beaver were found throughout the state in both protected and unprotected watersheds which provide drinking water for Washington State residents.

Statewide human giardiasis surveillance efforts and follow-up substantially increased the number of reported giardiasis cases, identified two outbreaks associated with domestic drinking water supplies, two outbreaks associated with consuming untreated drinking water, two day care center outbreaks, one outbreak associated with foreign travel and numerous smaller clusters of cases. From the case-control study, foreign travel, consumption of untreated water and attendance at a day care center (for children) were found to be significantly more common among giardiasis cases than controls. Among giardiasis cases with foreign travel, only travel to Third World countries was found to be associated with giardiasis.

The human case follow-up revealed that giardiasis follows a bimodal age distribution affecting both young children and young adults. Evidence of secondary transmission was observed, especially within households having young children. No excess of cases was observed among customers of surface water supplies, even after eliminating individuals with other likely sources of infection (homosexuals, those who consumed untreated surface water, day-care center attendees, persons with a history of recent foreign travel, and case clusters with a likely common exposure).

Results of the stool survey of one to three year old children generally supported the findings of the case-control study and the human case follow-up. No difference in Giardia prevalence was observed for children served by deep well water supplies and surface supplies. In both cases one to three year olds were found to have a 7.1% prevalence of Giardia. An increased risk of infection was found for children with exposure to untreated surface water and for children with more than two siblings between the age of three and ten. No increased risk was found for children attending day care centers, contradicting results of the case-control study.

Environmental sampling to recover Giardia from natural waters proved to be disappointing. Of the 77 water filter samples examined, only 5 were positive for *Giardia* and 3 of these were taken in response to a reported outbreak. An examination of recovery efficiency was begun early in the project to test the filter both in the field and in the laboratory. Initial recovery of one cyst out of 30,000 cysts was followed by changes in both the application and analysis procedures. These changes (lower water pressures, more agitation to remove cysts from the filter fibers, and the use of an Foerst centrifuge) resulted in recovery of nearly 10% of the experimentally added cysts. Concentration techniques using sucrose or zinc sulfate were examined but did not provide noticeable improvements when used on filter samples.

The implications of these findings for waterborne transmission of giardiasis in Washington state are: 1) *Giardia* infection among aquatic mammals in Washington is widespread, including animals in the most remote and protected watersheds. 2) Although recovery of cysts from water implicated in an outbreak has usually been possible, recovery of cysts from other surface water was only occasionally possible. Although animal trapping results suggest that cysts should be commonly found in surface waters, the concentration of cysts required for filter recovery is seldom observed. 3) With the exception of several outbreaks, Washington's surface water supplies did not provide an increased risk of giardiasis or *Giardia* infection for their customers. The suspected excess level of disease in communities served by surface water supplies was not observed. Consumption of untreated surface water, person to person transmission (primarily among children), and travel to Third World countries were the most important risk factors associated with giardiasis.

SECTION 3

RECOMMENDATIONS

Waterborne giardiasis does not appear to be a significant public health problem in Washington State, despite the widespread potential for water supply contamination. The waterborne outbreaks detected were associated with operational problems (Leavenworth) and with inadequate design (Boistfort) of treatment plants. No outbreaks were detected in cither Tacoma or Seattle, even though infected animals were trapped from the watersheds of the surface water supplies and the only treatment provided.is chlorination.

In contrast, untreated surface water does present a significant public health problem. Consumption of untreated water was recognized as a risk factor for giardiasis in all age groups and was also associated with Giardia infection among stool survey participants.

Orlon-wound filters proved to be useful in recovering cysts from water supplies implicated in a human giardiasis outbreak but did not yield useful information on water supplies randomly selected. Laboratory evaluation of filter analysis procedures suggests that improvements in recovery and reductions in cost can be achieved by using an algal (Foerst) centrifuge rather than the series of screens recommended by earlier studies.

Results of the stool survey suggest that water contamination may interact with other risk factors by providing an initial infection. The number of children in a household appeared to be a risk factor, however the risk was only increased among families with a history of untreated water consumption.

SECTION 4

ANIMAL SURVEYS

The first of the five projects to be described is a series of animal surveys begun in November 1976 and continued through the 1979-80 trapping season.. The surveys focused primarily on aquatic mammals and were made possible with the co-operation of the commercial trappers and the State Game Department.

Cross-transmission of Giardia from infected beaver to humans was postulated as the likely source of a giardiasis outbreak in Camas, Washington in 1976 (1). The Boulder and Jones Creek watersheds, supplying water to Camas, are characterized by extremely limited human activity and the presence of varied wildlife species, including beaver. During the outbreak investigation, Giardia cysts were recovered from both raw and treated water. A total of twelve animals of various species were trapped in each watershed and examined for Giardia. Three Giardia-infected beaver were detected from areas above the intakes.

MATERIALS AND METHODS

Commercial trappers were recruited with the assistance of the State Game Department to provide stool samples from trapped animals. Those who agreed to participate were supplied with sampling kits containing vials, mailing containers, instructions on how each sample was to be obtained and a survey form. Samples were collected from the large intestine or rectum of the animal using a stick included with each kit. The sample was then placed in the vial and mailed to the State Public Health Laboratory. During the second and third years, the vials contained either 2.5% formalin or distilled water and, during the fourth year, 5% buffered formalin to preserve the samples. One sample per animal was taken.

Trappers were encouraged to submit samples from municipal watersheds. Special arrangements were made for trapping in protected watersheds. During the first year only beaver samples were requested. During the second, third and fourth years samples from beaver, muskrat, mink, raccoon and river otter were requested. Stool samples from other mammals were also accepted and examined.

The stool samples were sent by mail to the State Public Health Laboratory for analysis. All samples received were processed by the formalin-ether (FE) sedimentation technique (2). If a delay occurred between receipt and processing of the samples, they were refrigerated at 4° to 7° C. until processed. In addition to the FE technique, the zinc sulfate (3) and sucrose (4) centrifugal flotation techniques were used to examine samples received during the fourth year of the survey. This was done to determine if the analysis techniques might improve the estimated prevalence of infection. Zinc sulfate (1.22 specific gravity) with 3 minutes of centrifugation at 1000 rpm (180g) and sucrose (1.15 specific gravity) with 5 minutes of centrifugation at 2000 rpm (750g) were utilized in the respective flotation techniques. A 22 mm square coverslip was superimposed on each 15 ml centrifuge tube in contact with the flotation fluid prior to centrifugation. Samples processed by all techniques were examined microscopically following staining with Lugol's iodine. Coverslips were scanned at 10x and suspect objects were examined at 45x. All parasites and eggs observed in samples submitted during the third and fourth seasons were noted.

Samples were processed in numerical order in this fashion until all samples had been examined. Samples positive for Giardia cysts were evaluated semi-quantitatively using the following scale: 1+ for 1-50 cysts/coverslip, 2+ for 50-200 cysts/ coverslip, 3+ for 200-500 cysts/coverslip, and 4+ for 500+ cysts/coverslip.

Statistical analysis of the data was performed using t-tests and Chi-square tests.

RESULTS

A total of 911 animal stool samples were submitted over the four trapping seasons. Of these, 656 were from beaver, 172 from muskrat, 7 from nutria, 12 from mink, 28 from raccoon, 19 from river otter, 8 from bobcat, 2 from coyote, 1 from a lynx, and 6 from mountain beaver. Samples were received from 31 of 39 counties in Washington.

Parasites were found in the stools of all species of animals from which samples were submitted. However, only beaver and muskrat specimens were positive for Giardia (Table 1). The overall prevalence of Giardia noted in beaver specimens ranged from 6% to 19%, while that in muskrat ranged from 0% to 85%. Giardia prevalence in muskrat specimens was higher than in beaver specimens submitted during the third and fourth trapping seasons and, also when the data from the four trapping seasons were pooled ($p < 0.01$). A higher percentage of juvenile beaver specimens were positive for Giardia than were specimens from adult beaver. The difference was significant for the second, third and fourth trapping seasons and, again, when the data from all four surveys were pooled ($p < 0.01$) (Table 2). For muskrat no significant difference in prevalence by age class was observed. The distributions of positive specimens by month trapped, trapping season and animal species revealed no consistent trends from year to year (Table 3). Positive animals were detected in 24 of 31 counties surveyed over the four trapping seasons. Positive animals were detected in watersheds regardless of the level of watershed protection (Table 4).

No significant differences were detected in the prevalences of other parasitic infections among Giardia-positive versus Giardia-negative beaver (Table 5). Inconsistent differences in the prevalences of other parasitic infections were observed for Giardia-positive versus Giardia-negative muskrat samples submitted during the second and third trapping season.

Analyses of the fourth year's data revealed no statistically significant differences in the diagnostic powers of the formalin-ether sedimentation, zinc sulfate centrifugal flotation and sucrose centrifugal flotation methods with respect to detection of Giardia cysts (Table 6). The only significant observed difference occurred in the diagnosis of trematode eggs. The formalin-ether technique recovered trematode eggs, while the flotation procedures did not. There was some indication that the sucrose technique was superior to the formalin-ether technique in the recovery of coccidia cysts.

DISCUSSION

Giardia appears to be a common intestinal parasite of beaver and muskrat in Washington. The surveys conducted cannot be considered to have randomly sampled either the beaver or muskrat population. The manner in which specimens were obtained did not give equal probability of inclusion for all animals in the state. If cyst excretion is as variable in infected beaver and muskrat as it is in man, the use of a single stool specimen per animal can be expected to underestimate the actual prevalence of Giardia in these mammals. Thus, the prevalences estimated should be considered as minimum prevalences of infection. Nevertheless, the data obtained indicate a widespread distribution of Giardia infected aquatic mammals across the state and therefore the potential for water contamination by aquatic mammals. This may occur even in "protected" watersheds.

The data collected suggest that aquatic mammals in Washington can obtain Giardia infections in the absence of human involvement. It is possible that an influx of infected animals into an area is a component of the apparent sylvatic cycling of the parasite with the immigrating infected animals infected via human- or animal-source contamination. However, cross-transmission of Giardia may occur between various animal species in the same ecosystem (5). It is also possible that once infected by whatever means, these mammals maintain their infections, shedding cysts periodically in response to undetermined factors, but frequently enough to infect offspring. Beaver infected as juveniles might then infect animals in other areas when they migrate as two-year-olds to establish their own territories and colonies. Thus, the higher prevalence of infection or increased frequency of cyst shedding noted in juvenile versus adult beaver may be of importance in parasite transmission.

The observed difference in prevalence of infection in juvenile versus adult beaver cannot be totally explained from the collected data. Possible explanations include loss of infection in adulthood, decreased susceptibility to infection with increased age, development of latent infections, longer periodicity in cyst shedding, or lower levels of cyst shedding increasing the difficulty of cyst detection. To further investigate these possibilities, it would be necessary to collect intestinal tracts from animals sampled and examine them for trophozoites or to monitor infected animals in captivity from the time they are juveniles. A possible difference in prevalence with age in muskrats may become apparent with larger sample sizes. It should be noted that the individual trappers were responsible for determining the age classes of the animals trapped and, therefore, some errors may have been incorporated into the results.

It is unclear why both juvenile and adult muskrat demonstrated a higher percentage of Giardia-positive stools than the corresponding age classes of beaver. Perhaps muskrat more often shed cysts. Alternatively, a greater proportion of areas containing Giardia-shedding animals may have been sampled for muskrats than for beaver.

The results of the technique comparisons made during the fourth year of the study suggest that using the formalin-ether sedimentation technique did not affect the estimated prevalence of Giardia infection. In fact, it may be the technique of choice for this type of work because it concentrates a wide range of parasites and, based on this limited study, is as sensitive as zinc sulfate and sucrose for identifying Giardia.

TABLE 1. NUMBER OF ANIMALS EXAMINED AND PERCENT *GIARDIA*-POSITIVE BY SPECIES AND TRAPPING SEASON

Species	Parasites	Number Submitted (% Giardia-positive)			
		1976/1977	1977/1978	1978/1979	1979/1980
Beaver	G,C,Tr,E,N	173 (6%)	178 (7%)	179 (19%)	126 (12%)
Muskrat	G,C,Tr,E,N,Ch,Tc	1	17 (35%)	114 (42%)	40 (85%)
Nutria	C,Tr		2	5	
Mink	Tr,N,Tc		5	7	
Raccoon	C,N,Tc		4	24	
River Otter	Tr	1	2	10	6
Bobcat	To			7	1
Coyote	To			2	
Lynx	To			1	
Mountain Beaver					6

G = *Giardia* sp.
C = *coccidia*
E = *Entamoeba muris*
Ch = *Chilomastix* sp.
Tr = trematode eggs
N = nematode eggs
To = *Toxocara* sp.
Tc = *Trichuris* sp.

TABLE 2. PREVALENCE OF GIARDIA IN BEAVER AND MUSKRAT BY AGE AND TRAPPING SEASON

		Juvenile			Adult			Unknown		
Animal	Trapping Season	G+	(%)	Total#	G+	(%)	Total#	G+	(%)	Total#
Beaver	1976-77	5	(12)	43	6	(5)	123	0	(0)	7
	1977-78	8	(17)	47	4	(3)	127	0	(0)	4
	1978-79	17	(38)	45	14	(11)	129	3	(60)	5
	1979-80	8	(31)	26	7	(7)	97	0	(0)	3
		38	(24)	161	31	(7)	476			
Muskrat	1976-77	0	(0)	0	0	(0)	1	0	(0)	0
	1977-78	0	(0)	0	5	(36)	14	1	(33)	3
	1978-79	12	(71)	17	36	(38)	95	0	(0)	2
	1979-80	3	(75)	4	31	(86)	36	0	(0)	0
		15	(71)	21	72	(49)	146			

TABLE 3. GIARDIA-POSITIVE SPECIMENS BY MONTH TRAPPED, TRAPPING SEASON AND ANIMAL SPECIES

		Beaver			Muskrat		
Month	Trapping Season	#Examined	#Giardia+	(%)	# Examined	#Giardia+	(%)
Sept.	1976-1977[1]	-	-		-	-	
	1977-1978[2]	1	0	(0)	-	-	
	1978-1979[3]	-	-		-	-	
	1979-1980[4]	-	-		-		
Oct.	1976-1977	-	-		-	-	
	1977-1978	-	-		-	-	
	1978-1979	3	1	(33)	-	-	
	1979-1980	-	-		-	-	
Nov.	1976-1977	16	0	(0)	-	-	
	1977-1978	61	4	(7)	5	2	(40)
	1978-1979	81	19	(23)	56	30	(54)
	1979-1980	48	8	(17)	14	11	(79)
Dec.	1976-1977	97	7	(7)	1	0	(0)
	1977-1978	73	5	(7)	4	2	(50)
	1978-1979	77	13	(17)	33	12	(36)
	1979-1980	74	7	(9)	8	5	(62.5)
Jan.	1976-1977	12	0	(0)	-	-	
	1977-1978	12	1	(8)	1	0	(0)
	1978-1979	8	0	(0)	6	2	(33)
	1979-1980	3	0	(0)	-	-	
Feb.	1976-1977	11	0	(0)	-	-	
	1977-1978	1	0	(0)	3	0	(0)
	1978-1979	6	1	(17)	9	1	(11)
	1979-1980	-	-		-	-	
Mar.	1976-1977	5	0	(0)	-	-	
	1977-1978	5	0	(0)	-	-	
	1978-1979	3	0	(0)	10	3	(30)
	1979-1980	1	0	(0)	18	16	(89)

(continued)

TABLE 3. (CONTINUED)

Month	Trapping Season	Beaver #Examined	Beaver #Giardia+	Beaver (%)	Muskrat # Examined	Muskrat #Giardia+	Muskrat (%)
Apr.	1976-1977	5	1	(20)	-	-	
	1977-1978	3	0	(0)	-	-	
	1978-1979	-	-		-	-	
	1979-1980	-	-		-	-	
Unknown	1976-1977	27	3	(11)	-	-	
	1977-1978	12	2	(17)	4	2	(50)
	1978-1979	1	0	(0)	-	-	
	1979-1980	2	0	(0)	-	-	

(1) General Open Season: Eastern Washington: Beaver and Mink, 11/13/76-12/31/76; Muskrat 11/13/76-3/31/77; Raccoon, 11/13/76-2/28/77. Western Washington: Beaver, Mink, River Otter, 11/20/76-12/31/76; Muskrat and Raccoon, 11/20/76-2/28/77.

(2) General Open Season: Eastern Washington: Beaver and Mink, 11/12/77-12/31/77; Muskrat, 11/12/77-3/31/78; Raccoon, 11/12/77-1/31/78. Western Washington: Beaver, Mink, River Otter, 11/19/77-12/31/77; Muskrat and Raccoon, 11/19/77-2/28/78.

(3) General Open Season: Eastern Washington: Beaver and Mink, 11/11/78-12/31/78; Muskrat, 11/11/78-3/31/79; Raccoon, 11/11/78-1/14/79. Western Washington: Beaver and River Otter, 11/18/78-12/31/78; Muskrat, 11/18/79-2/28/79; Mink and Raccoon, 11/18/78-1/31/79.

(4) General Open Season: Eastern Washington: Beaver and Mink, 11/10/79-12/31/79; Muskrat, 11/10/79-3/31/80; Bobcat, 12/1/79-12/31/79. Western Washington: Beaver and River Otter, 11/17/79-12/31/79; Muskrat, 11/17/79 -2/29/80; Bobcat, 11/17/79-1/20/80.

TABLE 4. GIARDIA-POSITIVE ANIMALS BY WATERSHED AND TRAPPING SEASON

Means of Watershed Protection	County	Watershed	76-77	77-78	78-79	79-80
Fences, gates, patrolled	King	Cedar River			X	X
	King	N.F. Tolt River				X
	King	Green River			X	
	Skagit	Judy Reservoir		X		
Signs posted, gates	Clark	Jones Creek	X			
	Clark	Boulose Creek	X			
	Grays H.	Wishkah River	X			
	Grays H.	Davis Creek		X		
	Lewis	N.F. Newaukum River				X
	Pierce	So. Prairie Creek				X
Open	Columbia	Touchet River	X		X	X
	Kittitas	Yakima River		X	X	X
	Pend Or.	Pend Or. River			X	X
	Snohomish	Pilchuck River	X			X
	Snohomish	Sultan Munic.			X	
	Yakima	Naches River			X	X

TABLE 5. PARASITE ASSOCIATIONS OF BEAVER AND MUSKRAT BY TRAPPING SEASON AND PARASITES

	Giardia-positive		*Giardia*-negative	
Species	1978-79	1979-80	1978-79	1979-80
Beaver				
Number examined	34	15	145	111
No other parasites	67.6%	46.7%	57.9%	48.6%
Coccidia	11.8%	46.7%	19.3%	24.2%
Trematodes	11.8%		15.9%	8.1%
Coccidia, trematodes	8.8%	6.7%	6.9%	6.3%
Coccidia, *E. muris*				0.9%
Nematode eggs				1.8%
Muskrat				
Number examined	48	34	66	6
No other parasites	16.7%	32.4%	42.4%	16.7%
E. muris	25.0%	2.9%	12.1%	
Trematode	16.7%	5.9%	24.2%	
Coccidia	2.1%	11.7%		33.3%
Nematode eggs		5.9%	1.5%	16.7%
E. muris, other parasites	20.8%	29.4%	13.6%	16.7%
Other combinations	18.7%	11.8%	6.1%	16.7%

TABLE 6. NUMBER OF INFECTIONS MISSED BY TECHNIQUE * (%)

Animal	Technique	*Giardia*	Coccidia	*E. muris*	Trematode eggs	Nematode larvae	Trichuris-type egg
Beaver							
	Formalin-ether	2 (13%)	25 (47%)	1 (100%)	0 (0%)	2 (100%)	--
	Zinc sulfate	3 (20%)	20 (38%)	0 (0%)	17 (100%)	0 (0%)	--
	Sucrose	6 (40%)	15 (28%)	1 (100%)	17 (100%)	1 (50%)	--
Muskrat							
	Formalin-ether	2 (6%)	9 (75%)	4 (31%)	0 (0%)	2 (20%)	0 (0%)
	Zinc sulfate	8 (24%)	6 (50%)	3 (23%)	13 (100%)	5 (50%)	1 (100%)
	Sucrose	4 (12%)	3 (25%)	9 (69%)	13 (100%)	6 (60%)	1 (100%)

* Each sample was examined by each technique

SECTION 5

CYST RECOVERY METHODS

Recovery of *Giardia* cysts from a water supply implicated in a giardiasis outbreak first occurred in Rome, New York in 1975 (6) and later during outbreak investigations in Camas, Washington in 1976 (7) and Berlin, New Hampshire in 1976 (8). *Giardia* cysts were also recovered from two Washington State water supplies (Everett and Hoquiam) not implicated in outbreaks.

The recovery of cysts from water supplies not implicated in giardiasis outbreaks, together with widespread distribution of *Giardia* positive beaver and muskrat found in aquatic mammal surveys (9), suggested likely *Giardia* contamination of drinking water supplies. To ascertain the extent of the problem, a project was initiated to evaluate cyst recovery methods and then apply cyst recovery techniques to water supplies throughout Washington state. The project began by filtering water with known cyst concentrations to estimate cyst recovery. Two filters (7 um and 1 um) and two analysis procedures were examined. The project then applied the technique to 18 surface water supplies in Washington state.

MATERIALS AND METHODS

Evaluation of cyst recovery required a cyst supply and an experimental apparatus to simulate field conditions. Since only a few parameters could be examined, due to the considerable time each filter run involved, it was decided to compare recovery using the 7 um versus the 1 um filter on three cyst concentrations and compare two analysis techniques (the Foerst centrifuge versus gravity fed filters) on split samples from each filter run.

Preparation of Cysts

Approximately 3 ml of formalized feces from at least two human donors were comminuted with 8 ml of distilled water in a 15 ml pointed centrifuge tube. The suspension was filtered through two layers of cheese cloth. The filtrate was resuspended in 8 ml of distilled water and centrifuged for 130 seconds at 2400 rpm (including 30 seconds acceleration and 10 seconds braked deceleration). The supernatant was poured off and then resuspended in 8 ml of distilled water. This process was repeated a total of 10 times or until the supernatant appeared to be clear. The sediment was finally resuspended in 8 ml of distilled water and centrifuged for 20 seconds at a maximum of 1000 rpm. The function of this procedure was to throw down most of the more dense debris while retaining the *Giardia* cysts in suspension.

The sediment was reprocessed (twice) to increase cyst yield while the supernatant was collected, resuspended in distilled water and centrifuged for 130 seconds at 2400 rpm with the cysts concentrated in the sediment and ready for future use.

Determination of Cyst Density

The collected sediment was suspended in 15 ml distilled water. A 0.01 ml aliquot was pipetted from the cyst concentrate and placed on a glass slide with a drop of Lugol's iodine. The aliquot was covered by a 22 X 22 mm No. 1 coverslip. Using bright field microscopy at 100X, all of the cysts observed were counted. This was repeated until twelve 1.0 ml aliquots had been examined. The number of cysts per 1.0 ml was estimated by the average of the twelve 0.01 ml aliquots multiplied by 100. Distilled water was added until a suspension of approximately 36,000 cysts per ml was obtained.

Laboratory Filtering Apparatus

Giardia cysts were placed in 4.5 liters distilled water contained in a side-arm carboy and pumped into a water line feeding the filter by a proportioning pump. The suspension of cysts in the carboy was placed on a mixer until the suspension was exhausted. To reduce pressure across the filter, a pressure reducer was placed in the line prior to the filter holder. A pressure gauge and flow meter were installed to monitor the filter and measure flow. Experiments were run with both 7 um and 1 um orlon filters. The experimental apparatus is shown in Figure 1. The water pressure was set to be no higher than 10 psi.

Analysis Procedure

The filter yarn was unwound from the stainless steel filter core, cut in 4 foot lengths, and placed, with the excess water from the filter, into a 3500 ml beaker. Distilled water and 25 ml of formaldehyde were added to bring the volume to 1000 ml. This suspension was placed into a one gallon paint can and mixed in a paint shaker for ten minutes. The filter material was removed, the fluid squeezed out, and the filters discarded. The formalin suspension from the paint can was separated into two 500 ml aliquots, one to be processed by a Millipore filter technique and one to be used in a algal (or Foerst) centrifuge.

Millipore Filter Procedure

This 500 ml of homogenate was filtered by gravity through a 45 um and then a 30 um porosity nylon screen. The material retained by these screens was discarded. The homogenate was next filtered by gravity through a 7 um Millipore filter. The material retained in this filter was washed in a beaker using 2 ml distilled water. Five slides (.01 ml each) of this filter debris were examined microscopically under low and high dry power using Lugol's iodine as a stain. The Giardia cysts recovered were counted and the results recorded.

Algal Centrifuge Procedure

The other 500 ml homogenate was processed through a high speed algal centrifuge at 1000 g. The material collected in the centrifuge was re-suspended and mixed with 2 ml of distilled water making 7 ml of suspension. Five slides (0.01 ml each) were examined microscopically under low and high dry power using Lugol's iodine as a stain.

RESULTS

Cyst recoveries for the six experiments are presented in Table 7. In three experiments a 7 um orlon filter was dosed with 36,000, 12,000 and 6,000 cysts respectively. In the other three experiments a 1 um orlon filter was dosed with 40,000, 20,000 and 10,000 cysts respectively. In each experiment half the homogenate was concentrated using the algal centrifuge and half by the Millipore filter technique. Results from the algal centrifuge technique were comparable to Millipore filter technique in cysts per ml of homogenate, however in all six experiments the percent recovery was much higher for the algal centrifuge. The actual cyst counts per ml of homogenate were also slightly higher for the algal centrifuge. The 1 um filter yielded higher cyst counts than did the 7 um filter.

A 7 um and 1 um orlon filter were then installed in series so that the 1 um filter would receive effluent of the 7 um filter. These filters were dosed with 40,000 cysts. Sediment was processed with the algal centrifuge technique. Recoveries were 11 cysts from the 7 um filter and 56 from the 1 um filter.

The filter technique was applied to 18 surface water supplies or streams in the state (Table 8). Giardia positive filters were identified from the cities of Sultan in Snohomish county and Dayton in Columbia county, neither of which reported an excess number of human giardiasis cases. Three positive filters were identified from a stream implicated in a series of backpacker illnesses. This cluster of human cases is described later in the human case follow-up section (Big Four Ice Caves Trail).

DISCUSSION

Percent recoveries of Giardia cysts using 7 um and 1 um orlon filters were estimated using known cyst concentrations and two methods of filter analysis. In each of the 6 experiments the algal centrifuge method gave higher recovery percentages, in part because it produced a larger volume of sediment material. However, even the concentrations per ml of sediment were slightly higher for the algal centrifuge method. Both analysis procedures produced higher recoveries with the 1 um orlon filter than with the 7 um filter. When the 7 um and 1 um filters were connected in series, more cysts were detected on the smaller porosity filter. This suggests failure of the 7 um filter to retain significant numbers of cysts and indicates that a smaller porosity filter should be used to recover Giardia from water samples.

Application of the filter to 18 water supplies or water sources detected only two with *Giardia* cyst contamination. Since approximately 18% of beaver and 33% of *muskrat* were found to excrete *Giardia* during earlier stool surveys, and since *Giardia* positive animals were distributed throughout the state, failure to detect more than 2 of 18 systems tested as *Giardia* contaminated was unexpected. Perhaps *Giardia* cysts are seldom found in concentrations necessary to assure detection.

To estimate ranges of possible cyst concentrations in natural settings, cyst density calculations based on *Giardia* prevalence among beaver, cysts/gram of stool in positive beaver, total stool output and total water flow were made for three locations in each of two watersheds (Cedar and Upper Yakima). Beaver population estimates were based on Dept. of Game trapping data for these watersheds (assuming a 10% harvest and 50% reporting of harvest). Stream flows were based on high, low and mean flow measurements of the U.S.G.S. *Giardia* prevalence (15.8% - Cedar River, 26.3% - Upper Yakima River) and cyst density/gram stool (2/gm - low,1599/gm - mean, 16,900/gm - high) were based on beaver stool survey results. Total daily stool output per beaver was assumed to be 100 grams.

The results of the calculations are as follows:

	Max.flow, Min cysts	Min flow, Max cysts
Cedar River Watershed		
Upper	.0000014 cysts/gal.	5.59 cysts/gal.
Taylor Creek	.0000048 cysts/gal.	245.22 cysts/gal.
Landsburg	.0000093 cysts/gal.	1.34 cysts/gal.
Upper Yakima Watershed		
Martin Creek	.0000053 cysts/gal.	.98 cysts/gal.
Cle Elum River	.0000021 cysts/gal.	.35 cysts/gal.
Cle Elum City	.0000015 cysts/gal.	9.71 cysts/gal.

These crude calculations suggest that cyst density only rarely reaches levels which are detectable by current water filtration technology, possibly explaining the poor cyst recovery results with the orlon filter.

However the results of both the experimental analysis and field application suggest that the 7 um orlon filter can be useful in an outbreak investigation, even if not sensitive enough for routine monitoring of water supplies. Although the 1 um improves recovery, insufficient field experience is available to determine the operational significance of this improvement.

FIGURE 1

SCHEMATIC OF LABORATORY CYST CONCENTRATION EXPERIMENT

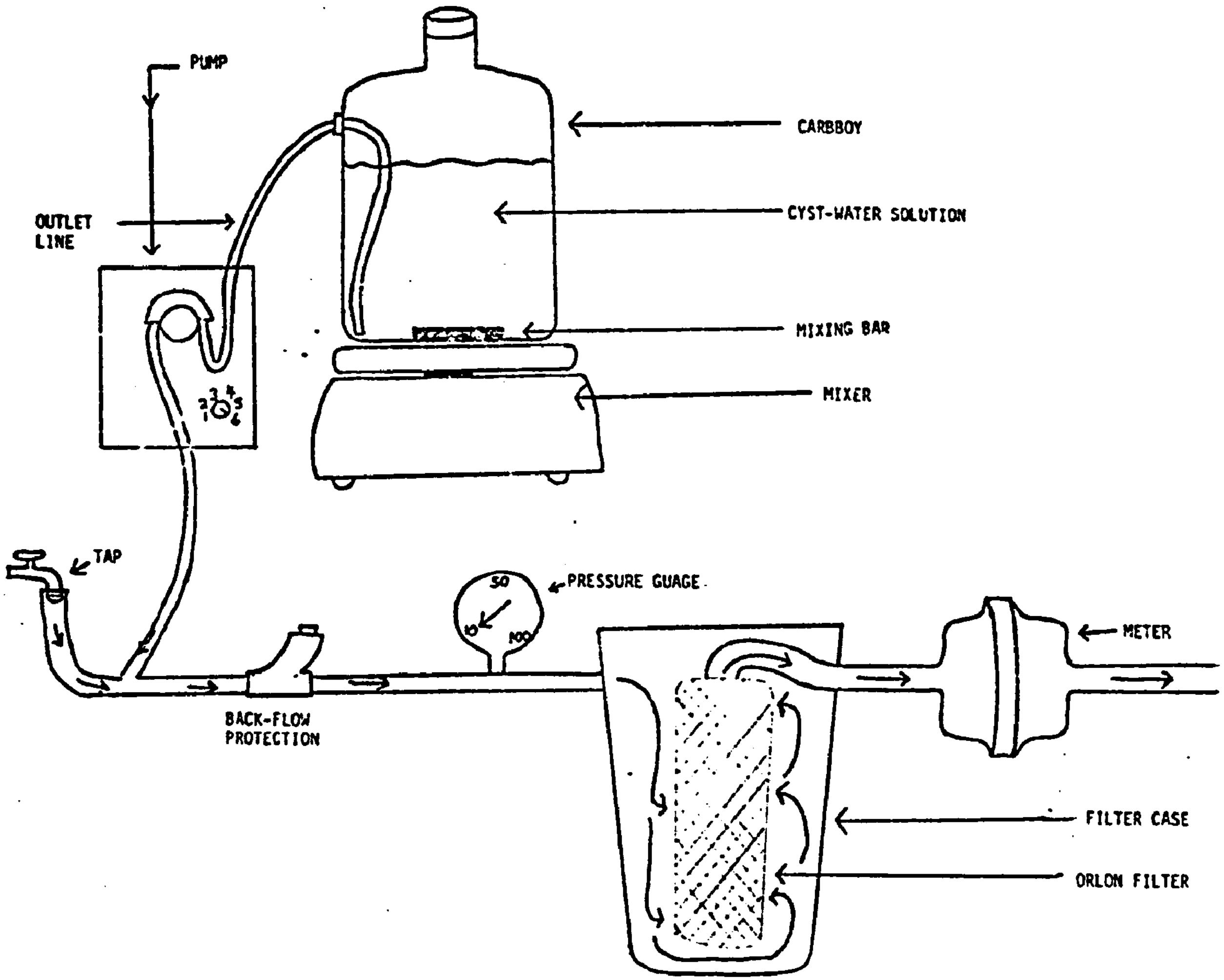

TABLE 7. CYST RECOVERY BY TECHNIQUE AND FILTER PORE SIZE

Cysts dosed	36,000	12,000	6,000	36,000	12,000	6,000
Filter size	7 um	7 um	7 um	7 um	7 um	7 um
Sediment vol.	7 ml	7 ml	7 ml	2 ml	2 ml	2 ml
Cysts recovered per 1/100 ml *	31	14	3	28	12	1
Total cysts recovered **	4200	1960	420	1120	480	40
% Recovery	11.5%	16.0%	7.0%	3.1%	4.0%	7%
Cysts dosed	40,000	20,000	10,000	40,000	20,000	10,000
Filter size	1 um	1 um	1 um	1 um	1 um	1 um
Sediment vol.	10 ml	18 ml	18 ml	10 ml	18 ml	18 ml
Cysts recovered per 1/100 ml	49	19	7	41	8	3
Total cysts recovered	10,000	7,200	2,520	8,000	36,000	1,080
% Recovery	25%	36%	25%	20%	18%	10%

* Estimates based on at least five 1/100 ml samples examined
** Based on cysts per ml and total sediment volume

TABLE 8. RESULTS OF FIELD APPLICATION OF ORLON FILTER

SOURCE	TREATMENT*	DATE	GALLONS	FINDINGS
Lake Whatcom	None	11-30-78	480	Neg
Ryderwood-stream	None	12-13-78	599	Neg
Ryderwood-stream	None	12- 3-78	465	Neg
Lake Whatcom	None	12-13-78	<500	Neg
Lake Whatcom	None	12-13-78	<500	Neg
Lake Whatcom	None	13-14-78	<500	Neg
Lake Whatcom	None	12-14-78	<500	Neg
Lake Whatcom	None	12-14-78	396	Neg
Lake Whatcom	None	12-19-78	<500	Neg,Hookworm lar
Ryderwood	None	12-26-78	<500	Neg
Lake Samish	None	1- 2-79	475	Neg,Hookworm lar
Lake Whatcom	None	1- 2-79	501	Neg
Hoquiam	None	1-30-79	213	Neg,Hookworm lar
Hoquiam	None	1-30-79	206	Neg,Hookworm lar
Hoquiam	None	1-24-79	194	Neg,Hookworm lar
Hoquiam	None	1-22-79	241	Neg
Centralia	None	1-29-79	517	Neg,Hookworm lar
Centralia	None	2- 1-79	146	Neg,Hookworm lar
Sequim	None	2- 8-79	546	Neg
Sequim	None	2- 7-79	683	Neg,Hookworm lar
Sequim	None	2- 6-79	645	Neg
Sequim	None	2- 5-79	261	Neg
Port Townsend	None	2- 5-79	454	Neg,Hookworm lar
Port Townsend	None	2- 6-79	580	Neg
Port Townsend	None	2- 7-79	592	Neg
Port Townsend	None	2-14-79	477	Neg
Mrs. Theirs	None	2-14-79	<500	Neg,Hookworm lar
Camas	None	2-28-79	464	Neg,Hookworm lar
Camas	None	2-27-79	491	Neg,Hookworm lar, ascaris eggs
Stevenson	None	2-26-79	466	Neg
Stevenson	None	3- 1-79	575	Neg
White Salmon	None	2-27-79	613	Neg
White Salmon	None	2-28-79	602	Neg
Carson	None	2-28-79	513	Neg
Carson	None	3-14-79	454	Neg
Camas	None	3- 1-79	607	Neg
Carson	None	2-26-79	476	Neg,Hookworm lar
Carson	None	3-20-79	666	Neg
Camas	None	3-21-79	447	Neg
Stevenson	None	2-28-79	557	Neg,Hookworm lar
White Salmon	None	3- 1-79	607	Neg
Stevenson	None	3-27-79	468	Neg
Hoquiam	None	4-11-79	475	Neg

(continued)

TABLE 8. (continued)

SOURCE	TREATMENT	DATE	GALLONS	FINDINGS
Hoquiam	None	4-12-79	612	Neg
Aberdeen	None	4- 9-79	47	Neg. Hookworm lar
Aberdeen	None	4-12-79	361	Neg
Hoquiam	None	4-10-79	236	Neg, Hookworm lar
Hoquiam	None	4-11-79	620	Neg
Hoquiam	None	4-12-79	606	Neg
Hoquiam	None	4- 9-79	573	Neg
Aberdeen	None	4-11-79	361	Neg, Hookworm lar
Aberdeen	None	4-10-79	360	Neg
Sultan	None	4-16-79	631	Neg, Hookworm lar
Everett	None	4-16-79	555	Neg
Everett	None	4-19-79	546	Neg
Sultan	None	4-19-79	750	Neg
Snohomish	None	4-19-79	508	Neg, Hookworm lar
Sultan	None	5- 1-79	639	Pos-*Giardia*
Snohomish	None	4-18-79	500	Neg
Shohomish	None	4-18-79	569	Neg
Everett	None	4-18-79	409	Neg
Everett	None	4-18-79	471	Neg
Sultan	None	4-18-79	330	Neg
Granite Falls	None	7- 5-79	<500	Pos-*Giardia*
Granite Falls	None	7- 5-79	<500	Pos-*Giardia*
Granite Falls	None	7-10-79	475	Pos-*Giardia*
Granite Falls	None	7-10-79	392	Neg
Walla Walla	None	7- 2-79	475	Neg
Walla Walla	None	7- 5-79	580	Neg
Walla Walla	None	7- 1-79	406	Neg, diatoms
Walla Walla	None	7- 5-79	542	Neg, diatoms
Walla Walla	None	7- 5-79	510	Neg, diatoms
Dayton	None	7-13-79	<500	Pos-*Giardia*
Dayton	None	7-11-79	<500	Neg, diatoms
Dayton	None	7-12-79	<500	Neg, diatoms
Dayton	None	7-14-79	<500	Neg, diatoms
Dayton	None	7-14-79	<500	Neg, diatoms

* Treatment provided to the sample - not necessarily the system

SECTION 6

CASE FOLLOW-UP AND CASE CONTROL STUDY

Having established the potential for Giardia contamination of water supplies by infected aquatic mammals, interest centered on whether human giardiasis was associated with domestic surface water supplies. In addition, should water supplies not be a primary contributor to an increased risk of giardiasis, identifying factors responsible for the high prevalence of Giardia found in diagnostic stool specimens was a secondary objective.

To identify giardiasis outbreaks as well as to determine whether an excess number of laboratory confirmed giardiasis cases were occurring in cities served by surface water supplies, a statewide case follow-up program was initiated in June 1978. By August 1978, 103 laboratories had been contacted and statewide reporting had begun. To identify risk factors for giardiasis, a case-control study was begun in March 1979. Both the statewide follow-up and the case-control study were concluded on March 1, 1980.

METHODS

All clinical diagnostic laboratories providing parasitological services in Washington State were identified and asked to submit to the State Public Health Laboratory the patient and physician name of all confirmed giardiasis cases identified during the study period (June 1978 to March 1980). Of 103 laboratories providing parasitological services, 93 agreed to participate in the study. Based on workload estimates, the participating laboratories accounted for 96% of parasitological examinations statewide. Alternatively, assuming an estimated stool positivity of 3.3% (the average positivity seen in several large reporting laboratories) the reported cases accounted for 65% of the cases which may have been expected (by applying 3.3% positivity to the total laboratory workload).

There were 1241 reports of laboratory confirmed giardiasis from diagnostic laboratories during the study period. Persons were excluded from the study if they were non-residents (16 cases), migrants (161 cases), or recent immigrants (187 cases). Of the 877 eligible cases 99 could not be found or contacted for a variety of reasons. The physician was contacted prior to interviewing the patient and the physician recommended against contacting the patient in 8 cases. In another 5 cases the patient refused to be interviewed. A total of 765 cases (87% of eligible cases) were followed.

During the period of the case-control study (March 1, 1979 to March 1, 1980) 488 of the 765 cases were followed. Since controls were to be selected from directory assistance listings, cases were excluded (from the 488 followed) if they did not have a listing. There were 113 persons excluded for this reason with 34 having unlisted telephone numbers, 18 having no phone, 41 with a phone under another name (e.g. dormitory) and 20 with no explanation for being unlisted.

Controls were selected from the same phone book as the cases by randomly choosing a page and a name from the page, or from directory assistance by prompting a name not listed in the phone book but similar to a randomly selected directory assistance name. The proportion of controls selected in the latter fashion was determined by the proportion of cases with a directory assistance listing but with no phone book listing. Controls were matched to the cases on the basis of sex (for cases over age 9), age (0 to 4, 5 to 9, 10 to 29, 30 to 49, 50 to 64, and over age 64), and location of residence (same phone book service area). Controls were followed as soon after the case report as possible to avoid seasonal differences in the responses of cases and controls. Of the 375 cases eligible for inclusion, 349 were actually matched within 2 months of the report. The remainder of cases were excluded from the case-control study.

Both cases and controls were asked about illness histories. Cases were asked a more detailed set of questions to accurately depict the illness and to determine the interval between potential exposure and the onset of symptoms. A number of exposure probabilities were explored including pets, travel (foreign, U.S., Washington State), recreation, residence history, domestic water supply, waste treatment and family characteristics. Questions were also included on the use of restaurants, occupation and the number of bathrooms in the home. The latter two questions were used to help ascertain the economic status of the people interviewed. Access to medical care was measured by the interval since the last routine physical examination and interval since the last physician visit for a problem other than giardiasis. The case and control interview forms are included in appendix A.

RESULTS

Case Clusters

Cases often appeared in clusters. These clusters were composed of people who shared a common exposure or who had been exposed to another positive person.

Several of the clusters each involved many people. There were 10 reported cases where the people had consumed water from a stream while hiking. The stream water was later found to be contaminated by Giardia, possibly of animal origin. Fourteen cases had a common exposure to a Forest Service work camp. All had consumed water taken from a stream which was later found to be contaminated with Giardia cysts. There were 11 cases which were exposed through a community water system, 12 cases which had recently returned from Puerto Vallarta, Mexico, 17 cases from one day-care center, and 8 from another. These will be discussed later.

The remaining 76 clusters were composed of 7 or fewer people accounting for 205 cases or an average of 2.6 cases per group. Twelve of the groups (33 cases) were composed of people who had traveled together to foreign countries. One foreign country case was associated with 2 non-traveling family members who became _Giardia_ positive after her return home. Twenty of the groups (43 cases) were composed of people who had consumed untreated water in Washington while on an outing as a group or during their work (loggers, game wardens, campers, etc.). In 12 of the clusters only 1 person in each cluster drank untreated water, but a total of 29 cases were involved. The 17 cases not exposed to untreated water were diagnosed after the index case for the group (the person exposed to untreated water). Persons in 2 groups (4 cases) volunteered that they were homosexuals. One group (4 cases) was from a school for emotionally disturbed children.

In 10 of the groups (24 cases) 13 children had attended a nursery school or day-care home (10 different locations). Associated with these 13 children were 7 _Giardia lamblia_ positive adults, 2 _Giardia lamblia_ positive siblings and 2 positive playmates.

The remaining 21 groups involved 73 cases. Fifteen of the 21 groups (51 cases) were composed of 1 or more positive adults and 1 or more positive or non-positive children. Of these 15 groups, 12 (80%) had a child two years or younger, 9 (60%) had a child age three to five years, 8 (53%) had a child age six to eight years, and 4 (29%) had a child age nine to ten years. Of the 51 cases, 14 were aged two years or younger, 7 aged three to five years, 7 aged six to eight years, 1 aged nine to ten years, 1 aged eleven to nineteen years, and 21 were over the age of nineteen. Of the 21 adults from these clusters (over age nineteen), 13 were female and 6 were male. Four groups (9 cases had no positive cases over the age of twelve. Two groups ([illegible] cases contained no childrenunder the age of twelve in the family.

From the total 79 clusters of cases, 7 households had at least 1 member with a repeat positive. In 1 household, the positive people had not been treated. In the other 6 they had been treated. In 6 of the 7 households there was a _Giardia_ positive child age two or less, although in 5 of these 6 households the repeat positive was an adult.

In the 79 clusters of cases there were 34 instances of both a child and an adult diagnosed positive for _Giardia_. In 18 of the instances the adult was diagnosed first, in 7 the child was diagnosed first, and in 9 they were diagnosed at the same time.

Big Four Ice Caves Trail, Snohomish County

During the summer of 1979, 10 confirmed and 7 suspected cases of giardiasis were identified where the positive persons had a common exposure to the Big Four Ice Caves Trail in the Monte Cristo area of the Cascades. Of the 17 confirmed or suspected cases, 9 were between age twenty and twenty-nine, 5 were between age thirty to thirty-nine, and 3 were less than age three. Seven were females and 10 were males. The dates of onset of symptoms spanned the entire summer with the earliest onset being May 24 and the latest being September 18. All confirmed or suspected cases drank water from the same stream. One pet dog who also drank water from the stream developed symptoms.

A survey of the immediate area where water was drawn revealed no aquatic mammals. However directly upstream were a series of beaver dams and lodges. In early August, a 500-gallon water sample of the beaver pond was filtered through a 7 um orlon filter. Analysis revealed the presence of Giardia cysts in the water.

Although warning signs were posted by the Forest Service in August, cases were diagnosed until September. This was possibly due to multiple access trails to the area, not all of which could be posted.

Forest Service Outbreak Near Forks, Washington

In August 1978, a cluster of cases was noticed from a Forest Service work camp. All of the Giardia positive persons in this cluster were employed by the Forest Service and most worked on brush clearing activities. An inspection of the camp's water supply and food service revealed no likely sources of infection. Investigation revealed that during the month of August, approximately 50% of the employees in this work camp developed symptoms suggestive of giardiasis. Thirty-nine of the workers submitted stool samples for diagnosis; 14 were positive for Giardia. Another 9 workers were diagnosed on the basis of their having three or more of the following symptoms: diarrhea, flatulence, nausea, weakness, loss of appetite, abdominal cramps and easy fatiqueability.

Many of the positive people and others with diarrhea had consumed water from rivers and streams in the area in addition to water from the camp supply. Many had consumed water from a tanker truck while on a brush clearing crew working near Forks, Washington. These workers used the truck to supplement the water brought from the camp. The tanker truck obtained its water from a small stream in the area and was present at the work site for purposes of fire control.

Water filtration was initiated on the stream used by the tanker trucks. Three Giardia cysts were recovered using a 10 micron orlon filter. In the fall of 1978, a Giardia positive beaver was trapped from the general vicinity of the outbreak.

Puerto Vallarta, Mexico

During the winter of 1979-80, 12 confirmed and 7 suspected giardiasis cases reported recent travel to Puerto Vallarta, Mexico. Most of the cases were tourists who traveled to Puerto Vallarta with organized tour groups for a one week visit. Seven of the 12 cases had stayed at the same hotel. Illness occurred throughout the winter with the first case reporting travel between October 15 and 22, while the last case traveled between February 1 and 15. Seven of the cases traveled in November with 1 identified from the trip of November 5 to 11 and 2 cases from trips during each succeeding week in November. The problem of giardiasis may be associated with a larger problem of diarrheal diseases among travelers to Puerto Vallarta during this time period. It was not possible to identify a common exposure which distinguished cases from non-cases.

Boistfort Water System, Lewis County

During the fall and winter of 1979-1980, 11 cases of giardiasis were reported from the Boistfort Valley area of Lewis County. Ten of the cases were adults, 8 between the ages twenty and twenty-nine, and 2 over age sixty. There was 1 positive child. Among the adults, 4 were male and 6 were female. None of the cases reported having any foreign travel in the two months prior to onset of symptoms. One case drank untreated water from a stream in the prior two months and 2 reported having gone swimming. All of the reported cases were served by the Boistfort Water System or reported drinking water from the system.. Nine of the cases received their water from the system, 1 person was a visitor who became ill one week after staying with a family served by the system, and the other person worked at a dairy farm served by the system.

Four additional cases of giardiasis were reported from Lewis County during the winter months, but they did not report any consumption of water from the Boistfort water system.

A telephone survey was initiated when the diagnosed persons reported multiple cases of diarrheal illness among family members, neighbors and friends on the Boistfort Water System. Of approximately 448 households on the system, 174 were interviewed and asked questions about any diarrheal illness, possible exposures to Giardia if they reported illness, and water pressure problems in their homes.

There were 534 persons in the 174 households surveyed. In 37 households, at least 1 member had diarrhea that lasted longer than one week. Sixty-eight people (12.7%) had giardiasis-like symptoms. Combining the households of symptomatic persons with the households of the 11 positive persons, 14.9% of the people interviewed were symptomatic or positive for giardiasis.

Two surrounding communities were also surveyed for diarrheal illness during the same time period. A total of 65 households from the towns of

Winlock and Pe Ell were surveyed. These included 168 persons of whom 8 (4.8%) reported diarrhea lasting longer than one week. These symptomatic persons came from 4 households and none reported consumption of water from the Boistfort system.

The peak of the Boistfort outbreak appeared to have occurred during December and January. The first diagnosed case reported onset of symptoms as early as July, but 5 of the 11 became ill either in December or January. The 68 surveyed cases with giardiasis-like illness reported the month of onset of symptoms as ranging from September through February, with a cluster of 21 cases in December and 29 in January. The customers on the system were surveyed in February and the reports for onset of illness may be somewhat inaccurate due to the length of time passed.

Water filtration efforts using 1 um orlon filters were unsuccessful in recovering cysts of Giardia from the Boistfort raw water. Stools of beaver acquired from beaver ponds in the watershed were also negative for Giardia.

Considerable potential for water cross-connections were present. Many dairy farms had dual water sources, one from the Boistfort supply and one from wells on their property. Periods of low water pressure were reported by customers throughout the system. Under negative pressure conditions, cross-connections of these dual systems could have contaminated the system or a portion of it. Cases of confirmed giardiasis and persons with persistent diarrhea were reported throughout the area served by the system, however, and water pressure recorders were unable to detect negative pressures at the locations tested.

Chlorine levels were increased on February 1, 1980 so that 0.6 mg/l free available chlorine could be measured throughout the system. Previously no free chlorine could be measured at most places on the system. A boil water recommendation was issued at the same time. As of May 1, 1980, no further confirmed cases of giardiasis had been reported from the Boistfort area except 1 case whose onset of symptoms was within two weeks of the chlorine residual increase and the boil water order. The boil water order has since been lifted; however, high free chlorine levels remain. A cross-connection control program has also been implemented.

Day Care Centers, Skagit County

In December 1978, an Anacortes physician diagnosed giardiasis as the cause of a four-year-old child's "failure to thrive". The physician noted that two sisters had also experienced diarrhea, abdominal pain and nausea since November 1978. On examination they were found to be positive for Giardia. As their mother was a licensed day-care mother, the physician directed her to notify the families of the children enrolled of their exposure to giardiasis. Subsequently, 7 additional positive children with exposure to the day-care home were identified.

The cluster of cases from Anacortes was noted by Giardia Project staff. The Skagit County Health Department and the State Office of

Epidemiology were notified after it became apparent from interviews with the physician and parents that the day-care home was the common exposure. The Skagit County public health nurse for the area interviewed the day-care mother and obtained the names of children who had attended the day-care home in the past five months and the names of children in the neighborhood who had played with the positive cases. She arranged for family members of the positive cases and for children exposed to the day-care home to be tested for Giardia.

At that time, 19 children were examined for Giardia. Ten children were found to be positive. Of these, 6 children lived in or attended the day-care home, 3 children were neighbors who played with the day-care home children, and 1 was a sibling of a day-care home child. Stool samples submitted by adults living in the same households as the positive children were all negative for Giardia. Of the 10 positive children, 4 were symptomatic and 6 were asymptomatic for giardiasis.

The number of asymptomatic children involved made it difficult to determine a possible source of infection for the outbreak. Five of the children had picnicked often during the summer in Whatcom and Skagit Counties and swam in several local lakes. One asymptomatic child had been exposed to Giardia positive friends in Hawaii 11 months before the outbreak.

In the year following the investigation, 7 more children associated with the day-care home were diagnosed positive for giardiasis. Of these, 2 children attended the day-care home, 4 were neighbor children, and 1 was a sibling of an earlier cases.

The second day-care home outbreak was also in Anacortes and may have been connected to the first outbreak as 2 of the children found positive for Giardia had earlier attended the first day-care home. Eight people were diagnosed positive for giardiasis as a result of the second investigation. Seven of the cases were children who attended the home and 1 was a parent. Again it was difficult to determine the origin of the outbreak. The parent was the first case diagnosed and may have been responsible for introducing the parasite to the day-care home through her 2 children. The 2 children who had attended the first day-care home had not been positive when tested during the first outbreak. They may not have submitted enough stool samples for adequate diagnosis at that time or they may have acquired the parasite after being tested.

The second day-care mother was not as cooperative in providing information to the Skagit County Health Department as the first had been, so it was not possible to test as many children or parents as in the first outbreak.

Case Follow-up and Case-Control Study

An effort was made to make the questions identical for cases and controls. Despite these efforts, two questions appear to have been interpreted differently by cases and controls. These were the amount of Washington State and other U.S. travel. For both questions, the controls reported more frequent travel than did the cases. For Washington travel, controls reported 3.5 times that of cases. For other U.S. travel, controls reported 1.6 times that of the cases. Yet Canadian and European travel were nearly identical and the cases reported considerably more outdoor activity than did controls.

The differences in reported travel between cases and controls may have been real or may have resulted from confusion on the part of controls over the time period of interest. Unlike cases, the controls did not have a traumatic event to define the time interval of interest. On the other hand, cases may have under-reported travel which they felt to be unimportant or unrelated to their disease.

Answers to the remaining questions were recoded and condensed to 26 variables. Not all of these questions were risk factors of interest. Six questions were potential confounders of risk factors and disease while 7 questions related to potential effect-modifying conditions.

Several questions relating to income were collected as potential confounding variables, such as the number of bathrooms and the occupation of the person and his or her spouse. The occupations were ordered by estimated income and the higher occupation score (the person or spouse) was taken as the occupation variable. Several questions relating to medical care were asked and recoded to give the number of months since the last physician visit (other than for the diagnosis of giardiasis for the cases). This was also considered a potential confounding variable.

An "effect modifying" variable should identify a subset of cases where the effects of exposure may be different than for the majority of the population. This could happen here if a subset of the population had a reduced immunity to Giardia either because of a medical condition (hypogammaglobulinemia) or because of no prior infections with Giardia. The effect on these people of a first time exposure to Giardia could be more substantial than the same exposure for someone previously infected with Giardia. For this reason, questions were asked on previous enteric problems and on length of residence in Washington and at the current location. Wright (10) suggested that long term residence in Colorado may be protective, perhaps due to prior Giardia exposure. The questions on place of birth and length of residence should provide similar information for this study. Since domestic water might also be a source of prior exposure, the source and type of treatment of domestic water (surface filtered, surface unfiltered or well) and size of system (number of connections) were also included.

Since controls were selected from lists of phone numbers rather than from lists of people, family size cannot be considered a risk factor. Larger families were over-represented in the cases because larger families have a greater risk of at least one member becoming ill than do small families. Yet large families have an equal chance of inclusion in the control population. Adjustment for family size must be made when estimating the importance of exposures related to family size.

The risk factors surveyed were exposure to household pets, outdoor pets, restaurant visits (sit-down, fast-food) swimming, untreated water consumption, outdoor activity, number of glasses of water consumed per day, foreign travel, exposure to children less than age three, exposure to children age three to ten, and attendance of nursery schools for children.

Variables were coded to provide for increasing values of the codes to correspond to expected increasing or decreasing effects for the variable. Thus, indoor pets were recoded so that zero was assigned to no pets and three was assigned to the presence of young animals in the house. Code one was assigned to a single adult indoor pet and two was assigned to multiple adult indoor pets. This coding was based on the hypothesis that young animals excrete cysts more frequently than do older animals (9). Coding instructions for the case-control study are presented in Appendix B.

Analysis of the Case-Control Data

There were four possible outcomes for each case-control pair on any dichotomous exposure: (1) both the case and the control were exposed, (2) both were unexposed, (3) the case was exposed and the control unexposed, and (4) the case was unexposed and the control exposed. The number of case-control pairs falling into each of these categories were represented as z(i,j) where i=1 if the case was exposed and 0 otherwise and j=1 if the control was exposed and 0 otherwise. The four possibilities were represented as:

	Control	
	Exposed	Unexposed
Exposed	z(1,1)	z(1,0)
Unexposed	z(0,1)	z(0,0)

To determine whether the exposure occurred more commonly than expected among the cases, a variety of measures have been developed. The relative risk was the most commonly used measure because it can easily be obtained from case control studies, it approximates the ratio of the probability of disease given exposure divided by the probability of disease given no exposure (the odds ratio), and it occurs as a parameter in more complex statistical models for the analysis of case-control data. For these reasons, considerable use will be made of the relative risk in the following analysis.

The relative risk (μ) is estimated from matched case-control studies as the number of unexposed controls whose case is exposed (z(1,0)) divided by the number of exposed controls whose case is unexposed (z(0,1)).

$$\mu = z(1,0)/z(0,1)$$

This ratio should be close to one when there is no association between exposure and disease risk. When the exposure increases the risk of disease, the relative risk is greater than one and when the exposure is protective, the relative risk is less than one. Note that the number of case-control pairs with identical exposure does not enter into this calculation, although it does affect the variance of the estimates.

Relative risks are calculated for each potential risk factor, confounding variable, and effect-modifying variable in each of three age groups (0-9, 10-39, and 40+ years). These estimates are given in Tables 9, 10 and 11. The confidence intervals are large sample estimates of actual confidence intervals.

Several factors stand out as possibly important risks. Among these are foreign travel, except possibly among children, untreated water consumption and nursery school exposure for children zero to nine years old. Presence of an infant in the household may be important for cases under 40. Swimming has a moderately increased relative risk for each age group.

Whether each of these factors produces an independent effect on the risk of disease cannot be answered from the above relative risk estimates. For example, if two activities are related, such as restaurant visits and foreign travel, then both might appear as risk factors even though only one may be related to disease. Also, another factor, such as income, may confound a relationship as illustrated previously. Third, the importance of effect modifying variables must also be tested.

A statistical method for coping with multivariate problems from matched case-control studies was developed by Breslow and others (11). This technique provides estimates for the risk of disease given several exposures and adjusts for both confounding and effect modifying variables.

The contribution of a risk factor can be tested as twice the difference between the log-likelihood of the equation when the factor is present and the log-likelihood when the factor is absent (and all other variables are the same). This difference has a Chi-squared distribution with one degree of freedom (approximately). For a more detailed account of the technique and its mathematical derivation, see (12).

A computer program for performing this analysis was developed by Kathy Halverson and modified by Brian Plikaytis of the Center for Disease Control. The program was further modified for this study and adapted to a Univac 1182.

Risk factors which had a relative risk for giardiasis significantly different from one were combined with other potentially important variables (confounding and effect modifying) to produce a multivariate logistic regression equation which predicts the probability of disease. Each factor was then removed one at a time and the change in the log-likelihood tested. The estimated parameters (b_k) were also compared for the variables still in the equation to insure that the elimination of the variable did not substantially alter other estimates.

Once a set of factors were identified which either made a contribution to the likelihood equation or which altered estimates when absent, each variable not in the equation was added one at a time and the loglikelihood and b_k's tested to see if the variable made a contribution.

TABLE 9. RELATIVE RISK ESTIMATES - 87 CASES AGE 0 to 9 (unadjusted)

Exposure	Estimated Relative Risk	Lower/Upper 95% Confidence Interval
Years at present residence	1.00	(.77, 1.30)
Washington residence	.79	(.57, 1.09)
Place of birth	1.21	(.70, 2.10)
Previous enteric problem	.83	(.42, 1.75)
Family size	1.34	(1.01, 1.77)
Occupation	1.04	(.88, 1.24)
Number of bathrooms	1.32	(.89, 1.95)
Months since last M.D. visit	1.94	(1.4, 1.21)
Indoor pets	1.19	(.85, 1.65)
Pet sleeping place	1.04	(.81, 1.34)
Outdoor animals	.64	(.28, 1.49)
Infant in household	2.42	(1.27, 4.58)
3-10 year old in household	1.62	(1.06, 2.47)
Nursery school	3.50	(1.56, 7.68)
Sit-down restaurants	1.41	(.72, 2.76)
Fast-food restaurants	.69	(.45, 1.06)
Foreign travel	3.00	(.31, 28.80)
Swimming	1.53	(1.02, 2.29)
Untreated water	10.60	(2.51, 44.86)
Outdoor activity	.91	(.50, 1.67)
Number of glasses of water	1.44	(.91, 2.26)
Domestic water	.75	(.46, 1.21)
Age	8.83	(2.71, 28.80)

TABLE 10. RELATIVE RISK ESTIMATES - 180 CASES AGE 10 to 39
(Unadjusted)

Exposure	Estimated Relative Risk	Lower/Upper 95% Confidence Interval
Years at present residence	.99	(.88, 1.11)
Washington residence	.94	(.83, 1.05)
Place of birth	1.16	(.83, 1.61)
Previous enteric problem	1.11	(.71, 1.71)
Family size	1.28	(1.07, 1.53)
Occupation	1.02	(.91, 1.16)
Number of bathrooms	1.31	(.97, 1.76)
Months since last M.D. visit	1.18	(.94, 1.47)
Indoor pets	.91	(.72, 1.14)
Pet sleeping place	1.04	(.84, 1.31)
Outdoor animals	.55	(.26, 1.20)
Infant in household	2.46	(1.43, 4.23)
3-10 year old in household	1.95	(1.32, 2.88)
Sit-down restaurants	1.12	(.80, 1.57)
Fast-food restaurants	1.00	(.73, 1.38)
Foreign travel	10.33	(3.16, 33.79)
Swimming	1.68	(1.28, 2.22)
Untreated water	7.13	(3.68, 13.80)
Outdoor activity	.90	(.58, 1.40)
Number of glasses of water	1.12	(.85, 1.46)
Domestic water	.86	(.60, 1.21)

TABLE 11. RELATIVE RISK ESTIMATES - 82 CASES OVER AGE 39
(Unadjusted)

Exposure	Estimated Relative Risk	Lower/Upper 95% Confidence Interval
Years at present residence	.91	(.77, 1.08)
Washington residence	.96	(.79, 1.17)
Place of birth	.61	(.34, 1.09)
Previous enteric problem	.70	(.39, 1.27)
Family size	1.24	(.92, 1.68)
Occupation	.80	(.66, .97)
Number of bathrooms	1.22	(.82, 1.81)
Months since last M.D. visit	1.21	(.85, 1.72)
Indoor pets	.93	(.64, 1.35)
Pet sleeping place	1.01	(.73, 1.40)
Outdoor animals	1.00	(.14, 7.10)
Infant in household	1.34	(.29, 6.14)
Sit-down restaurants	.84	(.53, 1.31)
Fast-food restaurants	3.22	(1.40, 7.42)
Foreign travel	6.00	(1.77, 20.36)
Swimming	2.38	(1.31, 4.32)
Untreated water	3.61	(1.48, 8.79)
Outdoor activity	2.74	(1.22, 6.18)
Number of glasses of water	1.35	(.92, 1.97)
Domestic water	1.17	(.67, 2.02)

TABLE 12. MULTIVARIATE EQUATIONS

FACTOR	RR*	X^2_1, P*	C.I.*
0-9 Year Old Cases - 87 Cases			
Untreated water	43.3	22.6 (p <.0005)	(4.8, 387.6)
Nursery school	5.7	10.1 (p <.005)	(1.7, 19.7)
Swimming	1.7	2.3 (p >.1)	(.8, 3.3)
Infant in household	2.7	3.6 (p <.1)	(.9, 7.8)
Foreign travel	1.5	.1 (p >.1)	(.1, 19.7)
Sex	.6	1.0 (p >.1)	(.2, 1.8)
Age	.1	16.4 (p <.0005)	(0.0, .5)
Family size	1.3	1.3 (p >.1)	(.8, 2.0)
10-39 Year Old Cases - 180 Cases			
Foreign travel	14.9	24.3 (p <.0005)	(3.8, 59.2)
Untreated water	5.0	37.6 (p <.0005)	(2.7, 9.4)
Infant in household	2.8	8.8 (p <.005)	(1.3, 5.7)
Indoor pets	.8	2.3 (p >.1)	(.6, 1.1)
3-10 year old in household	1.9	3.9 (p <.05)	(1.0, 3.6)
Swimming	1.4	2.6 (p >.1)	(.9, 2.0)
Family size	1.0	.1 (p >.1)	(.8, 1.4)
Age	.8	.9 --	(.5, 1.3)
Sex	--	.1 --	--
40+ Year Old Cases - 82 Cases			
Foreign travel	14.1	14.1 (p <.0005)	(2.5, 80.0)
Swimming	2.0	2.3 (p >.1)	(.8, 5.0)
Untreated water	6.9	12.8 (p <.0005)	(1.9, 24.8)
Outdoor activity	5.5	9.0 (p <.005)	(1.5, 19.2)
Place of birth	.4	3.5 (p <.1)	(.2, 1.1)
Number of fast-food restaurant visits	2.6	3.9 (p <.05)	(.9, 7.4)
Years at present residence	.7	6.5 (p <.025)	(.6, .9)

(continued)

TABLE 12. (continued)

* χ_1^2 = 2 (loglikelihood with factor - loglikelihood without factor)

P = probability associated with the Chi-squared value

R.R. = estimated relative risk exp (B_k)

C.I. = 95% confidence interval based on variance estimates derived from the inverse of the Fisher Information Matrix

TABLE 13. RELATIVE RISK ESTIMATES - 87 CASES AGE 0 to 9 (Adjusted*)

Exposure	Estimated Relative Risk Unadjusted	Adjusted	χ_1^2,P	Lower/Upper 95% Confidence Interval*
Years at present residence	1.00	1.16	.4 (p >.1)	(.72, 1.88)
Washington residence	.79	1.07	0.0 (p >.1)	(.56, 2.04)
Place of birth	1.21	1.75	1.1 (p >.1)	(.58, 5.35)
Previous enteric problem	.83	.97	0.0 (p >.1)	(.32, 2.93)
Family size	1.40	1.28	1.3 (p >.1)	(.82, 2.00)
Occupation	1.04	1.04	.1 (p >.1)	(.79, 1.37)
Number of bathrooms	1.32	.88	.1 (p >.1)	(.44, 1.78)
Months since last M.D. visit	1.94	2.24	5.1 (p <.05)	(1.01, 4.97)
Indoor pets	1.19	1.11	.1 (p >.1)	(.85, 1.65)
Pet sleeping place	1.04	.95	.1 (p >.1)	(.63, 1.43)
Outdoor animals	.64	1.60	.4 (p >.1)	(.36, 7.16)
Infant in household	2.42	2.68	3.6 (p <.1)	(.93, 7.77)
3-10 year old in household	1.62	1.67	.8 (p >.1)	(.55, 5.11)
Nursery school	3.50	5.74	10.1 (p <.005)	(1.67, 19.74)
Sit-down restaurants	1.41	1.24	.2 (p >.1)	(.43, 3.60)
Fast-food restaurants	.69	.74	.9 (p >.1)	(.39, 1.40)
Foreign travel	3.00	1.45	.1 (p >.1)	(.11, 19.70)
Swimming	1.53	1.67	2.3 (p >.1)	(.85, 3.28)
Untreated water	10.60	43.33	22.6 (p <.0005)	(4.84, 387.60)
Outdoor activity	.91	4.42	3.9 (p <.05)	(.88, 22.22)
Number of glasses of water	1.44	1.76	2.4 (p >.1)	(.82, 3.78)
Domestic water	.75	.57	1.9 (p >.1)	(.26, 1.27)

*Adjusted by a logistic model with factors given in Table 11

TABLE 14. RELATIVE RISK ESTIMATES - 180 CASES AGE 10 to 39 (Adjusted*)

Exposure	Estimated Relative Risk Unadjusted	Adjusted	X_1^2, P	Lower/Upper 95% Confidence Interval*
Years at present residence	.99	1.03	.1 (p >.1)	(.87, 1.22)
Washington residence	.94	1.12	1.7 (p >.1)	(.94, 1.34)
Place of birth	1.16	.82	.6 (p >.1)	(.49, 1.37)
Previous enteric problem	1.11	.78	.6 (p >.1)	(.42, 1.46)
Family size	1.28	1.03	1.1 (p >.1)	(.76, 1.40)
Occupation	1.02	1.06	.4 (p >.1)	(.89, 1.25)
Number of bathrooms	1.31	1.08	.1 (p >.1)	(.70, 1.66)
Months since last M.D. visit	1.18	1.35	3.6 (p <.1)	(.98, 1.84)
Indoor pets	.91	.79	2.3 (p >.1)	(.57, 1.10)
Pet sleeping place	1.04	.97	0.0 (p >.1)	(.68, 1.37)
Outdoor animals	.55	.58	.9 (p >.1)	(.19, 1.76)
Infant in household	2.46	2.75	8.8 (p <.005)	(1.32, 5.71)
3-10 year old in household	1.95	1.88	3.9 (p <.05)	(.99, 3.60)
Sit-down restaurants	1.12	.99	0.0 (p >.1)	(.63, 1.55)
Fast-food restaurants	1.00	1.13	.4 (p >.1)	(.74, 1.74)
Foreign travel	10.33	14.93	24.3 (p <.0005)	(3.76, 59.24)
Swimming	1.68	1.35	2.6 (p >.1)	(.93, 1.97)
Untreated water	7.13	5.03	37.6 (p <.0005)	(2.68, 9.43)
Outdoor activity	.90	.54	3.2 (p <.1)	(.28, 1.07)
Number of glasses of water	1.12	1.09	.2 (p >.1)	(.76, 1.58)
Domestic water	.86	.85	.4 (p >.1)	(.52, 1.40)

*Adjusted by a logistic model with factors given in Table 11.

TABLE 15. RELATIVE RISK ESTIMATES - 82 CASES OVER AGE 39
(Adjusted*)

Exposure	Estimated Relative Risk Unadjusted	Adjusted	χ^2_1, P	Lower/Upper 95% Confidence Interval*
Years at present residence	.91	.75	6.5 (p <.025)	(.59, .95)
Washington residence	.96	1.01	0.0 (p >.1)	(.72, 1.42)
Place of birth	.60	.43	3.5 (p <.1)	(.16, 1.12)
Previous enteric problem	.70	.78	.3 (p >.1)	(.33, 1.86)
Family size	1.24	1.18	1.1 (p >.1)	(.81, 1.70)
Occupation	.80	.82	1.6 (p >.1)	(.60, 1.12)
Number of bathrooms	1.22	.66	1.3 (p >.1)	(.32, 1.37)
Months since last M.D. visit	1.21	1.09	0.0 (p >.1)	(.61, 1.94)
Indoor pets	.93	1.03	0.0 (p >.1)	(.57, 1.88)
Pet sleeping place	1.01	1.01	0.0 (p >.1)	(.61, 1.98)
Outdoor animals	1.00	12.76	2.6 (p >.1)	(.58, 283.22)
Infant in household	1.34	1.56	.2 (p >.1)	(.20, 12.13)
3-10 year old in household	1.00	.95	0.0 (p >.1)	(.29, 3.12)
Sit-down restaurants	.84	.94	0.0 (p >.1)	(.49, 1.82)
Fast-food restaurants	3.22	2.60	3.9 (p <.05)	(.92, 7.35)
Foreign travel	6.00	14.14	14.1 (p <.0005)	(2.50, 80.00)
Swimming	2.38	1.97	2.3 (p >.1)	(.77, 5.02)
Untreated water	3.61	6.92	12.8 (p <.0005)	(1.94, 24.75)
Outdoor activity	2.74	5.45	9.0 (p <.005)	(1.54, 19.23)
Number of glasses of water	1.35	1.70	2.9 (p <.1)	(.89, 3.22)
Domestic water	1.17	.81	.2 (p >.1)	(.33, 1.94)

*Adjusted by a logistic model with factors given in Table 11

Separate regression equations were developed for the three age groups (0-9 years, 10-39 years and 40 plus years). The estimated relative risks, Chi-squared statistic for removal and p-value of the Chi-squared statistic are given in Table 12.

Untreated water consumption was an important risk factor for each age group. Foreign travel was also important for cases over age nine. Swimming was not statistically significant for any age group, but the estimated relative risk was greater than one for each age group.

Among children, nursery school exposure predicted an increased risk of disease. Cases in this age group also tended to be younger than controls (despite being matched by age groups 0 to 4 and 5 to 9).

Among cases ten to thirty-nine years old, the presence of an infant and the presence of a person age three to ten years old in the household predicted an increased risk of disease. Outdoor activity, Washington State as the place of birth, number of fast-food restaurant visits per week and short duration of residence at the present location predicted an increased risk for cases over forty years of age.

Variables not present in the equations of Table 12 were then added one at a time. The effect of each variable as a separate risk factor (unadjusted) and in the presence of other risk factors (adjusted) are given in Tables 13, 14 and 15.

For each age group, the length of time since the last physician visit appeared to be important. The estimated relative risks suggest cases saw physicians much less often than did controls. This is the opposite of what was expected and might be due to difficulty handling unknown or missing values. All of the missing values for this variable occurred among cases. Missing values were recoded to the mean value of the variable for cases. Alternatively, this effect may have resulted from excluding all visits by the cases for giardiasis related problems. This means that for cases with a long duration of symptoms, many recent physician visits would have been excluded.

The effect of outdoor activity (a combination of all forms of outdoor recreation other than swimming) was significant even after adjusting for consumption of untreated water. However, the effect of this variable was reversed for cases over forty, with these cases showing a reduced amount of outdoor activity relative to controls. The cases under age forty had an increased amount of outdoor activity relative to the controls.

None of the potential effect modifying variables (place of birth, duration of Washington State residence and present residence, domestic water source, and previous enteric problems) were significant except for duration of residence at the present location and the place of birth for cases over age forty. Family size, occupation and the number of bathrooms were thought to be potentially confounding variables, but they made little contribution to the likelihood and had no effect on the estimates of other relative risks. Among children, age was a confounding variable which significantly affected the likelihood equation and relative risk estimates for other variables.

Foreign travel was examined in more detail to determine the difference between cases and controls. The foreign travel variable had been recoded to exclude Canadian travel, which was comparable for cases and controls. Table 16 shows the place of travel for cases and controls. It is apparent that cases travelled more often to countries sometimes called Third World or Developing Countries. European travel was comparable for cases and controls.

TABLE 16. FOREIGN TRAVEL DESTINATION FOR CASES AND CONTROLS

Destination	Frequency Cases	Frequency Controls
Canada	22	24
Europe	1	1
Mexico	28	3
South or Central America	12	0
Middle East or North Africa	3	0
Southeast Asia	5	0
Africa	1	0
Northern Asia	2	0
Multiple country	3	4

TABLE 17. RELATIVE RISK ESTIMATES CALCULATED WITH (87 CASES) AND WITHOUT (74 CASES) THE 11 DAY CARE CHILDREN

Exposure	Relative Risk (87 Cases)	Relative Risk (74 Cases)	X_1^2, P (74 Cases)
Untreated water	43.3	22.2	16.8 ($p < .0005$)
Nursery school	5.7	4.2	6.5 ($p < .05$)
Swimming	1.7	1.5	1.3 ($p > .1$)
Infant in household	2.7	1.6	.7 ($p > .1$)
Foreign travel	1.5	.9	0.0 ($p > .1$)
Sex	.6	.3	--
Age	.1	.1	12.1 ($p < .0005$)
Family size	1.3	1.2	.7 ($p > .1$)

Biases

The methods of ascertaining cases or of selecting controls may also affect the estimated relative risks. Two such issues are considered. One relates to the method of ascertaining cases among children attending day-care centers and the other to the method of selecting controls for adult cases.

Giardia infections in children who attend a day-care center may be ascertained more completely than Giardia among other children simply because physicians expect a relationship between nursery schools and intestinal parasites. If so, this expectation could generate the relationships observed in the data. To partially adjust for ascertainment biases, an analysis of 74 cases which were not part of any known day-care center outbreak were compared to their matched controls (Table 17). The estimated relative risks for untreated water consumption and nursery school exposure were reduced slightly but were still significant.

The over-representation of children in the families of adult cases may have resulted from the over-representation of children among the cases. The excess number of children among the cases made many control calls necessary to obtain matches. Many of these control calls generated potential matches for other cases in the area. However, these potential controls might not have young children in the household. Some of these controls were matched to adult cases. To determine whether the matching process had an effect on the estimated relative risk for an infant in the household, all control families were again examined. When a potential match to a case in the twenty to twenty-nine years age group or the thirty to thirty-nine age group was found, a tally was made for the presence of an infant in the household. These tallies were then compared to the percent of cases in the respective age groups with a child under age three in the household. Excluded from the cases in this group were people with recent foreign travel or recent consumption of untreated water.

For potential controls in the twenty to twenty-nine year age group, 32.3% had a child under age three in the household. This compared with 16.3% of cases in the age group who had no foreign travel or untreated water consumption. Among potential controls in the thirty to thirty-nine age group, 19.8% had a child under age three while 32.0% of the cases in this age group (without foreign travel or untreated water consumption) had a child under age three.

The results of this analysis suggest that the relative risk of having an infant in the household was probably over-estimated for the entire age group. There is no evidence that cases twenty to twenty-nine with a child in the household have an increased risk of giardiasis.

Comparison of Cases Matched and Unmatched

The exclusion of persons without a phone, with unlisted phone numbers or with group residences was necessary to insure that cases and controls were comparable (that is, drawn from the same population). The exclusion of these cases may reduce the estimated importance of risk factors which occur more commonly among people excluded. To determine if this had happened and to identify risk factors which differ between matched and unmatched cases, a comparison of characteristics of cases matched and cases not matched was done.

For each of the 23 items used in the analysis of the case-control study and for three items relating to characteristics of the domestic water supply of cases, a Chi-squared statistic was calculated to determine if the factor had a comparable distribution for matched and unmatched cases. The results of the tests are given in Table 18.

Since the age distribution was demonstrated to be different for matched and unmatched cases ($p < .001$), a comparison of characteristics within age groups was done (age groups 0-9, 10-39, 40+). Thirty-seven percent of the unmatched cases were under age ten while 25% of the matched cases were under age ten.

As expected, the length of time at the present residence differed for matched and unmatched cases for each of three age groups considered (age 0-9, $p = .016$; age 10-39, $p = .000$; age 40+, $p = .036$). In each age group the proportion of unmatched cases residing at the current residence for one year or less was greater than the proportion of matched cases (59% vs. 47%, 72% vs. 42%, and 25% vs. 13% for the three respective age groups). The total duration of residence in Washington did not differ for unmatched and matched cases after adjusting for the age differences between the matched and unmatched cases.

No other differences were observed uniformly across all age groups. For the ten to thirty-nine age group and the forty plus age group, differences in the number of sit-down restaurant visits per week ($p = .017$ for 10-39 year old cases, $p = .0025$ for 40+ year old cases) and the number of fast food restaurant visits per week ($p = .062$ for cases 10-39 years old; $p = .009$ for cases 40+) were observed. For both age groups, the unmatched cases visited more restaurants.

For the 10-39 year group, differences in the number of glasses of water per day ($p = .011$), swimming ($p = .023$), occupation ($p = .003$), infant in household ($p = .045$), outdoor activity ($p = .046$) and number of bathrooms ($p = .000$) were observed. The matched cases drank more water, went swimming less often, were more often from a professional or managerial occupation group, had fewer infants in the household, had more outdoor activities and more bathrooms in their homes.

An analysis was done on the reasons for non-matching. In 34 cases the person had an unlisted number. In 18 cases there was no phone. In 41 cases the phone was listed under someone else's name or under a group residence name such as a dormitory. In 26 instances the case was matchable, but a match could not be obtained within the seasonal constraints. In 20 instances there was no phone listing in directory assistance and no explanation could be obtained. In each of these instances the case did have a phone.

The length of residence in Washington was shorter for cases with no phone than for other cases. No difference in length of Washington residence was observed for the other groups. The length of time at the present residence was comparable for matchable people and for people with unlisted phone numbers but was over one year shorter on the average for other groups (p =.04). The occupation status (based on the artificial scale in Appendix C) showed that persons with unlisted phones had a higher score than matchable cases and that persons with no phone had a lower score. As mentioned earlier, when these groups were combined, the group of unmatched cases (as a whole) had a comparable occupational distribution to that of matched cases.

The age distribution for unmatched cases also differed by reason for non-matching (p =.04). Fewer children were observed in the group of cases with phone listings under another name and where the phone number was unlisted for no apparent reason.

TABLE 18. MATCHED VS. UNMATCHED CASES

RISK FACTOR		ALL CASES	0-9 YEAR	10-39 YEAR	40+ YEAR
Sex	X^2_1=	.2	0	3.1	2.2
	p=	.647	1.0	.077	.142
Age	X^2_1=	20.1	1.8	.4	0
	p=	.001	.184	.504	1.0
Family size	X^2_1=	6.7	7.9	4.4	9.2
	p=	.250	.096	.496	.100
Wash. resident	X^2_1=	13.3	10.2	5.9	9.5
	p=	.065	.069	.555	.211
Present resid.	X^2_1=	70.5	13.9	38.4	15.0
	p=	.000	.016	.000	.036
Indoor pets	X^2_1=	.8	.2	1.4	1.2
	p=	.848	.980	.708	.752
Foreign travel	X^2_1=	1.0	1.4	1.2	0
	p=	.321	.239	.265	1.0
# of glasses	X^2_1=	14.1	7.5	13.0	5.7
	p=	.007	.111	.011	.125
# of sit-down restaurants	X^2_1=	14.1	3.9	10.2	14.3
	p=	.003	.268	.017	.002

(continued)

TABLE 18 (continued)

RISK FACTOR	ALL CASES X^2_1=	p=	0-9 YEAR X^2_1	p	10-39 YEAR X^2_1	p	40+ YEAR X^2_1	p
# of fast food restaurants	11.7	.011	1.7	.637	7.3	.062	11.6	.009
Swimming	5.3	.070	1.6	.479	7.6	.023	.1	.955
Untreated water	.614	.433	.01	.936	0	.963	.8	.378
Occupation	57.9	.000	8.2	.147	15.6	.003	8.2	.147
Place of birth	1.82	.610	.4	.934	2.0	.571	.9	.651
Infant in household	2.01	.366	.4	.821	6.2	.045	0	1.0
3-10 year old in household	7.65	.022	2.9	.236	1.1	.566	1.3	.523
Months since last M.D. visit	1.39	.707	3.1	.370	1.0	.803	1.2	.752
Previous enteric problem	0	1.0	0	1.0	.6	.450	0	.880
Giardia contact	.1	1.0	2.4	.295	1.3	.513	.3	.874
Pet sleeping place	4.3	.231	5.7	.128	4.1	.253	1.2	.746
Outdoor animals	0	.958	.4	.518	.2	.642	1.4	.235
Outdoor activity	1.4	.231	0	.959	4.0	.046	0	1.0
# of bathrooms	29.5	.000	5.3	.152	18.4	.000	4.4	.111
Nursery school exposure	5.6	.182	.2	.696	--		--	
Surface unfiltered	.2	.661	.1	.769	.3	.595	.6	.441

An Examination of Cases Without Apparent Exposures

The analysis of case clusters and the case-control study identified several exposures which appear to place the individual at an increased risk of giardiasis. The exposures include drinking untreated surface water within two months of symptoms onset, foreign travel within two months of symptoms onset, attendance at a day-care center (for children) and likely secondary transmission where the index case had one of these exposures.

An examination of all cases followed reveals that 323 of the 765 cases (42%) consumed untreated surface water within two months of symptoms onset and that 146 of 765 cases (19%) had recently travelled to a foreign country (other than Canada). Among children less than age six, 84 of 113 cases (74%) attended a day-care center. (For 43 children day care center attendance was unknown.)

Removing these people and positive persons with exposure to one of these people and persons identifying themselves as homosexuals leaves 207 of the original 765 cases (27%). There are 108 males and 99 females in this group. The age distribution shows an excess number of cases in the 0-4, 20-29 and 30-39 year age groups (Table 19).

The presence of an infant in the household was examined by the age and sex of the case (Table 20). The percent of persons in this group who were 20-29, and 30-39 with an infant in the household was compared to the percent expected for the age group. For the 20-29 year old cases, the observed and expected percents were nearly identical, while for the 30-39 year old cases the percent observed was nearly twice that expected (Table 21). This result is in general agreement with that of the case-control study which considered matched cases followed during a 12 month period rather than cases without other exposures followed during the total 20 month period of the project. These results cannot be considered to be completely independent, however, because of some overlap in cases.

Swimming was also a common exposure among these cases. Thirty-nine percent of the males and 53% of the females reported swimming within two months prior to the onset of symptoms. Over half of the swimming was done in natural water (other than pools) (Table 22). Exposure to swimming occurred less often between October and December of each year, but otherwise showed no seasonal difference (Table 23).

The distribution of cases by source of domestic water supply did not demonstrate a seasonal trend (Table 23) nor were there remarkable clusters by county or by month of onset (Table 24). Most of these cases occurred as isolated events with few county-month of onset cells having more than one occurrence and few consecutive months having case reports for a particular county (except for King County).

TABLE 19. AGE-SEX DISTRIBUTION (RESIDUAL CASES*)

Age	Male	Female	Total	% Total	1979 State Population
0-4	20	8	28	14%	7.3%
5-9	5	4	9	4%	7.6%
10-19	10	11	21	10%	17.5%
20-29	32	26	58	28%	19.4%
30-39	23	22	45	22%	14.1%
40-59	10	21	31	15%	19.7%
60+	8	7	15	7%	14.6%
TOTAL	108	99	207	100%	100.2%

*Residual cases include persons without foreign travel, untreated water or nursery school exposure, who did not state they were a homosexual and were not in contact with a *Giardia* positive child or adult.

TABLE 20. PRESENCE OF AN INFANT IN THE HOUSEHOLD
(Residual Cases)

Age	Male Cases -Infants*				Female Cases -Infants*			
	0	1	2	3	0	1	2	3
0-4	7	11	2		3	5		
5-9	4	1			2	2		
10-19	10				10	1		
20-29	23	7	2		16	9	1	
30-39	17	4	2		16	2	3	1
40-59	8	2			21			
60+	8				7			
TOTAL	77	25	6		75	19	4	1

*Child less than age 3, other than the case.

TABLE 21. OBSERVED AND EXPECTED INFANTS IN THE HOUSEHOLD
(Residual Cases)

Age	Case	Number Cases With Child < Age 3 In Household	Percent Total	Percent Expected*
20-29	58	19	32.8%	31.5%
30-39	45	12	26.7%	13.7%

*The expected percents are based on an estimated number of births to the current state population of females in each age class over the past three years. This assumes that there is only one birth per woman and one woman per household and ignores adoptions to derive an estimate of the percent of households with a child less than age three. Changes in the birth rate, multiple births to a woman during the three years, and multiple women in a household would most likely reduce the estimated expected values somewhat.

TABLE 22. SWIMMING BY AGE AND SEX
(Residual Cases)

	Males			Females		
Age	None	Pools	Other	None	Pools	Other
0-4	8	3	9	2	1	5
5-9	9	3	2	1	3	0
10-19	4	1	5	2	3	6
20-29	19	4	9	10	7	9
30-39	13	9	1	11	6	5
40-59	8	2	0	17	2	2
60+	8	0	0	4	2	1
TOTAL	60	22	26	47	24	28

TABLE 24

COUNTY BY MONTH OF ONSET (RESIDUAL CASES)

County	County Total	1978 <6	1978 6	1978 7	1978 8	1978 9	1978 10	1978 11	1978 12	1979 1	1979 2	1979 3	1979 4	1979 5	1979 6	1979 7	1979 8	1979 9	1979 10	1979 11	1979 12	1980 1	1980 2	Unknown Month
Benton	9					1			1	1		2			1				1					2
Chelan	2					1			1															
Clallam	4						1		1			1		1										
Clark	3	1	1	1																				
Cowlitz	1																							1
Douglas	3				1	1											1							
Grant	2					1												1						
Grays Harbor	4				1												1	1					1	
Island	5	1						1								1						1		1
Jefferson	1					1																		
King	76	4	1	1	4	10	5	4	5	2	1	3	3	4	1	2	5	7	3	2	3	4		2
Kitsap	4		1						1		1			1										
Kittitas	3			1				1												1				
Lewis	2					1															1			
Mason	2						1								1									
Okanogan	2					1				1														
Pierce	14									1	3		[illegible]		1	1	2	1		2		1		1
San Juan	1				1																			
Skagit	7		1		1			2		1		1												1
Snohomish	15	2	1			3				1				1		2		1				2		2
Spokane	17	2	1	1	1	2	1		1	1	1	1			1	1	0							3
Stevens	2		2																					
Thurston	10			1		2		1	1						1	1				1	2			
Walla Walla	3	1	1																1					
Whatcom	2			1																1				
Whitman	1																		1					
Yakima	5			1	1						1	1									1			
Unknown	7				2	2	1																	2
TOTAL	207	11	S	7	12	26	9	9	11	8	7	9	4	7	6	8	9	11	6	7	7	8	1	15

TABLE 23. EXPOSURE BY SEASON OF SYMPTOMS ONSET
(Residual Cases)

Time Period	Cases	% Total	% of Cases With Exposure to: Swimming	Domestic Surface Water
Prior to 6/78	11	5%	64%	36%
6/78-9/78	54	26%	59%	44%
10/78-1/79	37	18%	24%	68%
2/79-5/79	27	13%	56%	63%
6/79-9/79	34	16%	50%	56%
10/79-1/80	28	14%	21%	54%
2/80	1	0%	0%	0%
Unknown	15	7%	93%	47%
TOTAL	207	99%	48%	54%

A comparison of cases with identified exposures likely to cause the infection and cases without such exposures shows that a similar percent of both groups were served by surface unfiltered water supplies (Table 5-7). Should surface unfiltered water supplies contribute substantially to the risk of giardiasis, then the percent of cases without obvious exposures served by surface unfiltered water supplies should have been greater. A comparison of the exposure groups by size of the water utility (system class) shows that a comparable percent of both groups were served by class 1 water utilities.

TABLE 25. SOURCE OF DOMESTIC WATER SUPPLY BY EXPOSURE GROUP

Source or Type of Supply	Total Cases (765 cases)	Identified Exposures (558 cases)	Residual Cases (206 cases)
Surface unfiltered	337	45%	41%
Surface filtered	69	9%	10%
Well or spring	359	46%	49%
Total	765	100%	100%
Class 1	577	73%	80%

SECTION 7

DISCUSSION

The concerns which motivated this study focused on waterborne transmission of giardiasis. The waterborne outbreak in Camas in 1976 and the subsequent finding of a high prevalence of Giardia sp. infection in aquatic mammals throughout the state (9) suggested a potential for widespread outbreaks of giardiasis through contaminated water supplies.

Despite an intensive surveillance of laboratory confirmed giardiasis in Washington State, only two likely waterborne outbreaks were identified. Other exposures appeared to be more common sources of infection.

The results of the study establish that giardiasis is a relatively common parasitic infection in Washington State. Considering the difficulty in obtaining stool samples from ill persons, problems in identification of the parasite when an infection is present, and the potentially large number of asymptomatic or moderately ill persons who do not submit stool samples for analysis, the true incidence of giardiasis is likely to be much higher than that reported here. It should also be recognized that these unreported cases could substantially bias the results of this study.

Among cases reported, giardiasis has a bimodal age distribution more often affecting young children and young adults. Teenagers and adults over age 40 were represented less commonly among the cases than expected. Males were affected more often than females.

The bimodal age distribution of giardiasis remains even after removing cases with identified likely sources of infection. To what extent the age distribution of reported cases is determined by factors affecting the onset of symptoms rather than acquisition of infection is unclear. A larger number of asymptomatic children were identified than asymptomatic adults, suggesting that either more infections went undetected among children or that the case ascertainment was better for children.

Risk factors appear to include nursery school exposure for children, foreign travel for adults and consumption of untreated water for all age groups. The relationship between giardiasis and nursery school exposure has recently been established (13) and was seen both in the case-control study and in two outbreak investigations in Skagit County. For cases over age 10, foreign travel placed the individual at an increased risk of disease. The relationship between foreign travel and giardiasis was supported by the occurrence of disease usually within one month of the trip and by the increased relative risk of foreign travel for cases in the case-control study. The entire excess of foreign travel for cases in the case-control study resulted from travel to Third World countries.

Consumption of untreated water was an important risk factor for all age groups. Over 40% of the cases had recently consumed untreated water. This exposure occurred more commonly among adult males, but was observed in both sexes and in all age groups. It occurred throughout the year, but more commonly during the summer. An examination of the place of untreated water consumption showed that 31 of 39 counties were represented.

Evidence of secondary transmission (most likely person-to-person) was obtained more commonly than expected. The literature provides few examples of secondary transmission of Giardia. Although it is impossible to conclusively establish that secondary transmission occurred, the later occurrence of giardiasis among family members of an index case and the failure to find exposures of significance among any members of the family other than the index case is suggestive of secondary transmission. If these families are examples of secondary transmission, then disease was spread from adults to children, from children to adults and between adults.

The role of children in the household appeared to be important for cases age 10-39 years from results of the case-control study, but this may have been an artifact of the control selection procedure. When compared to all potential controls, rather than the controls matched to cases, no excess of infants was observed for cases age 20-29. A higher level of exposure to children under age three was observed for cases age 30-39 when compared to all potential controls or when compared to an expected level of exposure for the population (based on the number of births to women in this age group). Why an excess should occur for one age group and not for another is unclear.

The occurrence of giardiasis case clusters among groups of children may support the role of children in the secondary transmission of giardiasis or may simply be related to an increased susceptibility of children to the infection. The number of repeat positive adults with children under age three in their household does support the role of children in transmitting the disease, but the number of such cases is small. Treatment failure rather than re-infection could explain this observation.

The role of other factors for increasing or modifying the risk of giardiasis is, at best, less important than the risk factors discussed above. Homosexual activities were not surveyed for cases due to the implication of even asking these questions and the limited need for the information. Some homosexual activity was reported, however. Swimming demonstrated a slightly increased relative risk for each age group, but was not significant for any age group. Outdoor activity was significant for every age group, but the effect was reversed for cases over age 40 compared to cases under age 40. For the younger cases, outdoor activity increased the risk of giardiasis, even after adjusting for consumption of untreated water. For cases over age 40, outdoor activity reduced the risk. Since many water supplies at recreational sites are untreated or marginally treated, it was expected that outdoor activity would increase the risk of giardiasis even though the case did not report consumption of untreated water. The results of the study do not conclusively support this hypothesis.

Factors which do not predict disease or modify the effects of other variables include place of birth and length of residence at the present location for cases under age 40 and length of Washington State residence. Source of domestic water supply was also unimportant in modifying the effects of other risk factors. These results contradict the finding of Wright (10) in Colorado where length of residence in the state predicted a reduced risk of giardiasis. The exclusion of controls without directory assistance listings and the procedure of drawing controls from both directory assistance listings and from telephone books rather than strictly from telephone books may have caused this different conclusion. Wright's study compared laboratory diagnosed cases to controls selected from the telephone book. Cases with or without phone book listings were matched to controls selected from the telephone book. By selecting controls from the telephone book, one insures a minimum residence for controls of approximately six months. The actual minimum length of residence depends on the age of the telephone book. This bias is amplified when a large number of people move into an area (such as either Colorado or Washington State).

The role of domestic water supplies was examined for cases without any of the established exposures mentioned. Of the 765 cases followed, only 207 remained after cases with foreign travel, untreated water consumption (springs or creeks etc.), nursery school exposure, homosexuality and case clusters where the index case had one of the above exposures were excluded. These exposures accounted for 73% of the cases followed. The residual cases were analyzed to determine if more than expected came from counties served primarily by surface water supplies, whether these cases clustered by time or by geographic location and whether they occurred more commonly than expected from customers of surface water supplies. No evidence was developed to support a role of surface water supplies in increasing the risk of giardiasis. Fifty-one percent of all cases reported were served by surface water supplies. After removing the 73% of cases with likely sources of infection, 54% of the residual cases were served by surface water. These figures compare to 46% expected if statewide reporting was uniform and there was no water supply effect.

The conclusion that no evidence was developed to support a role of public water systems in increasing the risk of giardiasis must be qualified. In one outbreak during the project period a public water system was implicated in transmission of giardiasis. It is possible that other outbreaks went undetected. Clearly not all giardiasis cases in the state were identified by the project. Further there may be biases in the identification of cases which work against identifying these outbreaks. For example, small water utilities are of special concern for the transmission of giardiasis because their treatment facility is often both poorly equipped and inadequately operated. The customers of these utilities often have low incomes, are isolated from access to good medical care (both physically and financially) and are either unaware of the health department or distrustful of government. Consequently, outbreaks in these populations may be both more likely and less detectable than outbreaks elsewhere.

SECTION 8

HUMAN STOOL SURVEY

Since many Giardia infections are likely to result in asymptomatic infections rather than disease, the failure to observe an increased risk of giardiasis among customers of surface water supplies does not necessarily imply that public water systems using surface water do not increase the risk of infection. To examine the possibility that surface water supplies increase the risk of Giardia infection, a stool survey of one to three year old children was conducted in two Washington state counties. This was done to compare the prevalence of Giardia infections among people served by public water supplies using surface water to persons served by deep wells or springs, as well as to estimate Giardia prevalence for young children.

METHODS

Two Washington State counties (Thurston and Skagit) were selected for the survey. Thurston County includes the state capitol, Olympia, and adjoining cities of Lacey and Tumwater. The county includes both an urban population composed primarily of state employees as well as a rural and small-town population engaged primarily in the forest products industry. Olympia, Lacey and Tumwater residents are served by artesian springs or deep wells for their water supply. The remainder of the county is served by small municipal and individual wells. There are no surface water supplies in the county.

Skagit County contains a number of small to medium-sized towns and a substantial rural population. The economy is diversified, being based on farming, fishing, logging and manufacturing. Most drinking water is obtained from surface sources. Several smaller communities and most private systems are supplied by wells.

Children were selected from birth certificate records. A total of 1,349 certificates were randomly selected from the two counties. To be eligible, the child had to be between the ages of one and three at the time of the selection. Of the selected certificates, exclusions were made if the child was deceased (17 children), if the child had a serious reported birth defect or a birth weight less than 1,500 grams (25 children), if the mother was less than age 20 and no father was listed (57 children) and if the mother was an immigrant (15 children). Another 545 children were excluded because the family could not be traced from the information provided on the birth certificate, through directory assistance, or from the county telephone book.

The parents of the remaining 690 children were sent letters explaining the study and requesting their participation. The letter was followed by a telephone call, at which time the parent, if agreeing to participate, was interviewed. Some parents with unpublished telephone numbers were mailed the interview questionnaire. Of the 69 households receiving letters, 91 refused to take part in the survey. Thirty two agreed to participate but did not send in the required stool specimens. Fifty families were sent letters but could not be reached by telephone.

A total of 518 parents (74.9% of those sent letters) were surveyed with 295 having children born in Skagit County and 223 in Thurston County. Skagit County was over-sampled to gain a comparable number of persons on well water supplies as on surface water supplies.

Only the child on the selected birth certificate was included in the survey. However, family members were examined if the index child was positive for any parasite. Participants were paid $5.00 for each of two samples taken at least one day apart. Stool samples were submitted by mail to the State Public Health Laboratory in 5% buffered formalin preservative. When received in the laboratory, samples were examined by direct smear and by formalin-acetate sedimentation. The results of the stool examination were sent to the parent, and to the physician when requested by the parent.

RESULTS

Of the 518 children surveyed, 271 were male and 247 female. Ages ranged from 16 to 34 months. Giardia prevalence was 7.1% (37 cases) for all children in the survey. With the exception of one Trichuris trichiura, Giardia was the only potentially pathogenic intestinal parasite found. Non-pathogenic parasites were more commonly found among Giardia positive than Giardia negative children (p =.001). Nine Giardia negative children carried nonpathogenic parasites; eight had Entamoeba coli, one had Entamoeba hartmanni as well as Endolimax nana. Six Giardia positive children had nonpathogenic parasites; three had E. coli, two had E. nana and one had E. hartmanni. Among the 47 asymptomatic family members of Giardia positive children who submitted stool specimens, 10 (21.3%) were positive for Giardia, six had E. nana, three had E. coli and one had E. hartmanni.

There were no statistically significant differences in Giardia prevalence by type of domestic water (surface filtered, surface unfiltered, well or spring) or by county of birth (Table 26). Combining the results from both counties revealed no statistically significant increase in prevalence for working women, children in day care settings or for families which engaged in many outdoor activities (boating, camping, swimming or hiking) (Table 27). Significant increases in prevalence were observed, however, if the child had a history of drinking untreated surface water (from streams or lakes) (p=.002) or if the family had two or more children between the ages of three and ten (p=.01) (Table 28). No increase in prevalence was observed for children with a sibling under age three.

Additional risk factors were not found when children with either untreated water or with more than two siblings between ages three and ten were eliminated.

To estimate the effect of the exclusions and the children not followed, the occupational distribution of survey participant fathers (1978 births) were compared to a random sample of Thurston and Skagit counties 1980 births. The 1980 comparison was necessary since occupation was not previously recorded on the birth certificate. The results (Table 29) show that the survey included a higher percentage of professional and administrative occupations and a lower percentage of laborers, clerical, sales and service workers than the random sample (p=.00). No difference was observed in Giardia prevalence by occupation (p=.72).

DISCUSSION

Although no differences in Giardia prevalence was found between surface and well drinking water sources, a higher than expected Giardia prevalence was observed. Increases in risk of infection appeared for children consuming untreated surface water and for children with more than one three-to-ten year old child in the household. This latter finding, together with the high prevalence of non-pathogenic parasites in Giardia positive children and the high prevalence of Giardia among household members of Giardia positive children, supports the hypothesis of person to person transmission in these families. The failure to find an increased risk among children attending a day-care center was unexpected in light of family-associated risk factors. Giardia infection was not associated with a recent history of illness, suggesting that many, if not most, infections in this age group are asymptomatic.

The survey did not constitute a random sample of the population of one to three year old children in these counties. The differences in the paternal occupation distribution of survey participants and the sample of 1980 birth certificates was expected from the design of the survey. Since no differences in Giardia prevalence were observed by paternal occupation, the importance of this bias is unclear.

The survey findings suggest that intestinal parasites continue to be a common occurrence among young children despite advances in sanitation and personal hygiene. The uniformly high prevalence of Giardia in children of all paternal occupation groups suggests that the problem is not restricted to any socio-economic segment of the population. Since exposure to untreated surface water occurred commonly among children surveyed, these prevalence estimates may be higher than would be observed elsewhere. If, however, these estimates correctly indicate a generally high prevalence of Giardia infection among children, then the value of treating an asymptomatic child to reduce the risk of exposing others is questionable.

TABLE 26. POSITIVITY BY TYPE OF WATER SOURCE

Type of Water Supply	Number Surveyed	% Giardia Positive
Surface unfiltered	188	7.4%
Surface filtered	38	5.5%
Well or spring	292	8.1%
Total	518	7.1%

p=.87

TABLE 27. POSITIVITY BY TYPE OF RECREATION

Type of Activity	Number Surveyed	% Giardia Positive	p Value
Boating	41	9.8%	p > .1
Camping	101	5.9%	p > .1
Wading pool	221	9.0%	p > .1
Swimming in pool, lake or river	204	8.3%	p > .1
Hiking	32	9.3%	p >. .1

TABLE 28. NUMBER OF CHILDREN AND UNTREATED WATER CONSUMPTION

Exposure	# Surveyed	% Giardia Positive
Number of 3-10 year old children in household		
0	232	6.0%
1	207	5.3%
2+	79	15.2%
p=.01		
Untreated water consumption		
yes	106	15.1%
no	410	5.1%
p=.0018		

TABLE 29. OCCUPATION OF PARTICIPANTS AND CONTROLS

Occupation Group	1980 Birth Certificate Sample	% Total	Number Surveyed	% Total	% Giardia Positive
Prof., administ.	64	6%	140	27%	7.6%
Crafts, sales (insur., real estate, etc.)	591	59%	294	57%	5.9%
Laborers, clerical, sales, services	230	23%	36	7%	8.3%
Students, unemployed	24	2%	25	5%	8.0%
No father	91	9%	22	4%	13.6%

Occupation - sample vs survey p=.00
Prevalence by occupation p=.72

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Appendix A

Case and Control Questionaire

Date Time Results

Reference Number ______________

Lab. slip date ______________

Phone book entry ______________

Physician name ______________

Physician phone ______________

Interview date ______________

Interviewer name ______________

Patient Name ______________

Patient Address ______________

____

Phone (Home) ________ (Work) ________

Occupation ______________

Spouse Occupation ______________

Place of Birth ______________

Length of residence at present address ______________

City of ______________

County of ______________

Length of residence in Wash. ________

Interviewee ______________

Relationship to patient ______________

Patient's Sex ______ Age ______

Ht. ______ Wt. ______

Number in household ________

sex	age
____	____
____	____
____	____
____	____
____	____
____	____

Former residences: City/State ______________ Length ______
(3 years) City/State ______________ Length ______
City/State ______________ Length ______

Illness History

Date of diagnosis ______________ Date illness began: ______________

Duration of Illness ______________ Date of first visit to physician ________

Indicate which, if any, of the following symptoms you experienced:

Nausea ____ Vomiting ____ Pain in upper abdomen ____ Loose stools ____ Cramps ____

Constipation ____ Increase in number of bowel movements ____ Gas ____

Loss of appetite ____ Wt. Loss ____ Bloating ____ Weakness ____ Fatigue ____

Fever/Chills ____ Other symptoms ______________

If no symptoms, reasons for submitting stool sample ______________

Which drug were you given: Flagyl ___ Metronidazole ___ Atabrine ___ Don't Know ___

Other (specify) _________ No drug (reason?) ______________________________

How long between beginning of treatment and end of symptoms? ______________

Were you being treated for any other illness at that time? ________________

If so, what type? ____

When did you last have a routine check-up? _____ ______________________

Have you seen a doctor for a stomach or intestinal problem other than Giardia? ____

If so, what ____

When did you last see a doctor for any illness or health problem other than Giardia? ____

Were other family members or friends affected? Yes _____ No _____

If so, when _______	Relationship _____________	Name ___________	Ref. # ____
when _______	Relationship _____________	Name ___________	Ref. # ____
when _______	Relationship _____________	Name ___________	Ref. # ____

<u>Pets</u> Do you have indoor pets? ________ yes/no

If so, what type (1) _____________ Age (1) _________

(2) _____________ (2) _________

Where do they sleep? ______________________________

Do you have outdoor pets or other animals? ____________________

Stool sample kits for pets sent: Number _____ Date __________________

<u>Results</u>: #1 _____________	- animal _____________	indoor? _____
#2 _____________	- animal _____________	indoor? _____
#3 _____________	- animal _____________	indoor? _____

<u>Travel</u> During the 2 months prior to onset of symptoms, did you do any

a. <u>Foreign travel</u> (outside U.S.) ________ yes/no When __________________

If so, where? ______________________________________

Did symptoms begin there? ______________________________

If not, how long after return did they begin? ______________

What was the source (usual) for drinking water? ______________

b. U.S. (outside Wash.) ______ yes/no When? ____________________________

Where? ____

Did symptoms begin there? ____

If not, how long after return did they begin? __________________________

Did you engage in any outdoor activities while there? Circle: No Day hikes

Camping Backpacking (overnight hikes) Fishing Hunting Boating

Swimming Water Skiing Picnicking Snow skiing Other ________

What was the source of your drinking water? ___________________________

Did you drink water from lakes, streams, etc.? _________________________

---

c. Within Wash. _____ yes/no When? ____________________________

Where? ____

Did symptoms begin there? ____

If not, how long after return did they begin? __________________________

Did you engage in any outdoor activities while there? Circle: No Dayhikes

Camping Backpacking (overnight hikes) Fishing Hunting Boating

Swimming Water Skiing Picnicking Snow skiing Other ________

What was the source of your drinking water? ___________________________

Did you drink water from lakes, streams, etc.? _________________

If yes, did you treat the water before drinking it? ____________________

Domestic Water Supply

a. What is the source of home water?

City ________________ Well ________________ Other __________________

b. Do you purchase irrigation water? _____________________________________

c. What type of sewage system is used in your home?

Sanitary sewer system _____ Septic tank _____ Other ___________________

d. How many bathrooms do you have in your home? _____________

Public Exposures - Other

a. Are you a vegetarian?________________

b. Did you go swimming in the 2 months prior to the onset of the symptoms?

__________ yes/no Pool ____

Streams, lakes, rivers ____

c. If a child, has he/she attended a nursery school or day care center within the 2 months prior to the onset of symptoms? _______ yes/no

Where? ____

d. If under the age of 10, number of different playmates per week (excluding nursery school or day care center playmates) _______________

e. If an adult, have you had contact with children under the age of 3 ____ yes/no
Under the age of 10? ______ yes/no

If yes, what was that contact? (e.g. babysitting, youth group activities, neighbor children, relative?) _____________________________________

f. Other possible exposures:

CONTROL

Name of
Phone Book Entry ______________________
Name of
Matched control ______________________

Address ______________________

____

Phone ______________________

CASE

Case Reference # ____________

Age of Case ____________

Sex of Case ____________

Date of report
of illness ____________

Date of follow-up ____________

Date of Control Interview ______________________

Age ______ Sex ______ Ht. ______ Wt. ______

Place of Birth ______________________

Occupation ______________________

Spouse Occupation ______________________

Length at Present Address ______________________

Number in Household ____________

Sex	Age
______	______
______	______
______	______
______	______
______	______
______	______
______	______

Former Residence: City ____________ State ____________ Length ______
(3 years) City ____________ State ____________ Length ______
City ____________ State ____________ Length ______

Total Length of Residence in Washington ________

Illness History

When did you last have a routine check-up? ______________________

When did you last see a doctor for any illness or health problem. ______________________

Have you ever seen a doctor for a stomach, intestinal or bowel problem? ______ yes/no

If so, what was it? ______________________ (e.g. colitus, ulcer)

Have you ever suffered with chronic diarrhea (more than one weeks duration)? ______ yes/no

Travel - Foreign

Have you traveled outside of the United States in the last 2 months? _________ yes/no

If so, where? ____________________ When? ____________

Did you suffer from stomach problems or diarrhea while you were there? ____________

Did you suffer from any diarrhea within 3 weeks of returning home? ____________

Travel - United States

Have you traveled outside of Washington State in the last 2 months? _________ yes/no

If so, where? ____________________ When? ____________

Did you engage in any outdoor activities while there? Please circle: Day hikes

Camping Hunting Boating Swimming Water skiing Backpacking (overnight hikes)

Picnicking Snow skiing Fishing Other ____________ (specify)

Did you drink water from lakes, streams, rivers, etc.? ____________

Did you treat the water before drinking it? __________ yes/no How? __________

Travel - Washington

Have you traveled within Washington State in the last 2 months? _________ yes/no

If so, where? ____________________ When? ____________

Did you engage in any outdoor activities in the last 2 months in Washington State?

Please circle: Day hikes Camping Hunting Boating Swimming Water skiing

Backpacking (overnight hikes) Picnicking Snow skiing Fishing

Other ____________ (specify)

If so, where? ____________________ When? ____________

Did you drink water from lakes, streams, rivers, etc.? ____________

Did you treat the water before drinking it? __________ yes/no How? __________

Water Supply

How many glasses of the following liquids do you drink per day:

Water ______ Juices/drinks made with water ______ (e.g., lemonade, iced tea, Kool Aid)

Milk ______ Coffee/tea ______ Canned or bottled beverages ______

What is the source of home water?

City ________ Well ________ Other ____________________ (specify)

What type of sewage system is used in your home?

City Sewer System ________ Septic Tank ________ Other ____________ (specify)

How many bathrooms do you have in our home? ______________

When hiking or traveling in remote mountain areas would you drink water from clear flowing streams? ______________ Or would you bring your own water? _______________

Pets

Do you have indoor pets? ______________ yes/no

What type and age ____

____

____

Where do they sleep? ____

Do you have outdoor pets? ______________ yes/no What kind? ________________________

Other Exposures

How many times per month do you eat in a restaurant?

Sit down type restaurant ______________

Limited menu or fast food _____________ (e.g., Skippers, Kentucky Fried, McDonalds)

Did you go swimming in the past 2 months? ______________ yes/no

Streams, lakes, rivers ________________________ Pool __________________________

If a child, has he/she attended a nursery school or day care center within the last 2 months? ________________ yes/no

If under the age of 10, how many different playmates does the child have per week (excluding nursery school or day care center playmates)? __________________________

Appendix B

Coding Instructions

CODING USED FOR VARIABLES

VARIABLE DESCRIPTION	CODING
Sex	M male F female
Age	0 <1 year Actual number 1 year or more
County/City	Federal codes
Family Size	Actual number
Washington Residence/Present Residence	0 <1 year Actual number 1 year or more
Symptoms vomiting nausea pain in abdomen loose stools cramps constipation change in bowel movements gas loss of appetite weight loss bloating weakness fatigue fever/chills other symptoms	1 yes 2 no
No Symptoms/Reason for Submitting Stool Sample	1 foreign travel 2 exposed to positive person 3 recent immigrant 4 exam for other health problem 5 exposed to *Giardia* source
Drug Prescribed	1 Flagyl 4 don't know 5 Atabrine 6 other drug 7 no drug 8 combination/multiple prescription of drugs
Indoor Pets	0 none 1 young dog 2 adult dog 3 multiple dog 4 young cat 5 adult cat 6 multiple cat 7 combination cat and dog 8 other animal

CODING USED FOR VARIABLES (CONTINUED)

VARIABLE DESCRIPTION	CODING
Foreign Travel/ Out of State Travel/ Washington Travel	1 yes 2 no
Occurrence of Illness Since Travel	0 while away 1 one week or less Actual number >1 week 97 daily/frequent exposure 98 no symptoms
Glasses of Water Consumed	Actual number
Method of Waste Disposal	1 sewer 2 septic tank 3 other
Restaurant Visits Per Month	Actual number
Fast Food Restaurant Visits Per Month	Actual number
Swimming	0 none 1 private pool 2 public pool 3 river 4 lake 5 combination pool and natural 6 combination of pools 7 combination of natural 8 salt water
Consumption of Untreated Water	1 yes 2 no
Immigrant/Migrant	1 yes 2 no
Occupation/Spouse Occupation	01 professional, technical 02 managers, administrators 03 sales workers 04 clerical 05 craftsmen 06 operatives 07 transport equipment operatives 08 laborers except farm 09 farmers and farm managers 10 farm laborers 11 service workers 12 private household workers 13 homemaker 14 retired 15 institutionalized 16 student 17 unemployed 18 other

VARIABLE DESCRIPTION	CODING
Place of Birth	See Travel Place below
Household Infants 0-2 Years	0 none Actual number 1-7 infants 8 eight or more infants
Household Children 3-10 Years	0 none Actual number 1-7 children 8 eight or more children
Date Illness Began	Actual month and year
Duration of Illness	1 one week or less Actual number >1 week 98 ninty-eight weeks or more
Months Since Last Check-up	1 one month or less Actual number >1 month 98 ninety-eight or more
Previous Enteric Problems	1 yes 2 no
Months Since Last Physician Visit For Illness	1 one month or less Actual number >1 month 98 ninty-eight months or more
Contact With Positive Person	1 confirmed case of giardiasis 2 suspected case/symptomatic 3 none
Pet Sleeping Place	1 indoors 2 outdoors 3 indoors, confined space
Own Outdoor Farm Animals	1 yes 2 no
Travel Place-County	001 Adams 003 Asotin 005 Benton 007 Chelan 009 Clallam 011 Clark 013 Columbia 015 Cowlitz 017 Douglas 019 Ferry 021 Franklin 023 Garfield 025 Grant 027 Grays Harbor

VARIABLE DESCRIPTION	CODING
Travel Place-County (Continued)	029 Island 031 Jefferson 033 King 035 Kitsap 037 Kittitas 039 Klickitat 041 Lewis 043 Lincoln 045 Mason 047 Okanogan 049 Pacific 051 Pend Oreille 053 Pierce 055 San Juan 057 Skagit 059 Skamania 061 Snohomish 063 Spokane 065 Stevens 067 Thurston 069 Wahkiakum 071 Walla Walla 073 Whatcom 075 Whitman 077 Yakima 078 Multiple 079 Unknown 222 No county travel
Travel Place-State	801 Alabama 802 Alaska 803 Arizona 804 Arkansas 805 California 806 Colorado 807 Connecticut 808 Delaware 809 District of Columbia 810 Florida 811 Georgia 812 Hawaii 813 Idaho 814 Illinois 815 Indiana 816 Iowa 817 Kansas 818 Kentucky 819 Louisiana 820 Maine 821 Maryland 822 Massachusetts 823 Michigan 824 Minnesota 825 Mississippi

CODING USED FOR VARIABLES (CONTINUED)

VARIABLE DESCRIPTION	CODING
Travel Place-State (Continued)	826 Missouri 827 Montana 828 Nebraska 829 Nevada 830 New Hampshire 831 New Jersey 832 New Mexico 833 New York 834 North Carolina 835 North Dakota 836 Ohio 837 Oklahoma 838 Oregon 839 Pennsylvania 840 Rhode Island 841 South Carolina 842 South Dakota 843 Tennessee 844 Texas 845 Utah 846 Vermont 847 Virginia 848 Washington 849 West Virginia 850 Wisconsin 851 Wyoming 888 Multiple State 222 No state travel
Travel Place-Foreign Country	081 U.S. Possessions 082 Canada 083 Mexico 084 South and Central America 085 Europe-Western 086 Europe-Eastern 087 Middle East and Northern Africa 088 Southeast Asia 089 Russia 090 Africa 091 Australia and South Pacific 092 Northern Asia 093 Multiple foreign country 222 No foreign travel
Outdoor Activities day hikes camping backpacking fishing boating water skiing picnicking snow skiing other	1 yes 2 no

CODING USED FOR VARIABLES (CONTINUED)

VARIABLE DESCRIPTION	CODING
Domestic Water Source/Class	1 municiple >100 residences 2 community >10 residences 3 recreation/commercial 4 community <10 residences 5 private, single residence
Domestic Water Source surface unfiltered surface filtered well spring	1 primary water source 2 secondary water source 7 not used as water source
Number of Bathrooms in Home	Actual number
Nursery School/Day Care Home Attendance	1 yes 2 no
Playmates	Actual number
All Unknown Information	9

Appendix C

Occupation Status Codes