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Cryptosporidium and *Giardia* as Agents of Foodborne Disease

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ABSTRACT

Infections by the protozoan parasites of the genera *Cryptosporidium* and *Giardia* can be asymptomatic or cause gastroenteritis in immunocompetent people. However, in immunocompromised individuals, the infections can be more severe and even life threatening. Both parasites are common waterborne pathogens, but on occasion they may be foodborne or transmitted by body contact. In this review, several aspects of *Cryptosporidium* and *Giardia* are discussed including their life cycles, resistance to physical and chemical agents, routes of transmission to humans, the nature of the disease caused by the parasites, and detection of the organisms in water, feces, and food. Documented incidents in which *Cryptosporidium* or *Giardia* contaminated foods were implicated as cause of gastroenteritis are discussed to illustrate conditions leading to foodborne outbreaks and to suggest means of prevention and control of the parasites when present in foods.

Protozoan parasites are probably responsible for foodborne disease, yet yearly summaries indicate that few foodborne outbreaks due to protozoan parasites are reported. In the period 1973-1987, 140 out of 7,458 foodborne outbreaks (1.9%) were attributed to parasites (*12*). *Trichinella spiralis* accounted for 128 of those outbreaks, *Giardia* for 5, and other parasites (unlisted) accounted for 7. *Giardia* was responsible for 131 out of a total of 237,545 cases of foodborne disease for that time period. In contrast, during the period 1986-1988, protozoan parasites accounted for 10 of 50 outbreaks of waterborne disease. *Giardia* caused nine of the outbreaks and *Cryptosporidium* caused one (*74*). Out of a total of 25,846 waterborne disease cases for 1986-1988, *Giardia* accounted for 1,169 cases, whereas *Cryptosporidium* caused 13,000 illnesses. Comparison of the data for waterborne and foodborne outbreaks indicates that water transmission is the most important route of infection to humans, at least for *Giardia* and *Cryptosporidium*. However, on occasion these parasites do behave as foodborne pathogens.

CRYPTOSPORIDIUM

Species of the genus *Cryptosporidium* are protozoan parasites that infect a wide spectrum of animals, including humans (*28*). Cryptosporidiosis is one of the most common acute self-limiting gastroenteritic infections in immunocompetent people; however, infection in immunocompromised individuals and in children can be life threatening.

It has been estimated that 30-35% of the U.S. population is seropositive to *Cryptosporidium* (*129*). Seroprevalence rates in Europe and North America range from 25-35% but are believed to be considerably higher in less developed countries (*28*). There are 250-500 million cases of cryptosporidiosis annually in Asia, Africa, and South America (*28*). As many as 7% of the children in developing countries may be suffering from cryptosporidial diarrhea (*114*). Considering the estimated seroprevalence of the U.S. population in respect to *Cryptosporidium*, it would appear that cryptosporidiosis is not an inconsiderable infection even in the United States.

The organism

At the present time, *Cryptosporidium* species are classified as follows: subkingdom *Protozoa*, phylum *Apicomplexa*, order *Eucoccidiida*, suborder *Eimeriina*, family *Cryptosporidiida*. Previously, *Cryptosporidium* was believed to be host specific, and consequently, there was a large number of species; but today, the parasite is considered to be relatively nonspecific in terms of range of host infection, and many of the "species" are in doubt. Cryptosporidia that infect humans and other mammals are considered to belong to the species *parvum* (*28,114*).

The life cycle of *Cryptosporidium* is monoxenous, i.e., the development of the organism occurs within a single host. The infectious oocyst is shed in the feces of the infected host ($>10^8$ oocysts daily) and deposited into the environment where it is ingested or inhaled by a new host. In the gastrointestinal or respiratory tract, sporozoites are released from the oocysts by excystation. The sporozoites parasitize epithelial cells and differentiate into trophozoites. All stages of the organism (including sporozoites) appear to grow attached to the host cell; however, they are located in an intracellular extracytoplasmic parasitophorus vacuole located at the host cell surface. The trophozoites undergo asexual multiplication to form type I and type II meronts. Merozoites from type I meronts invade new tissue cells and differentiate into trophozoites which continue the infectious cycle. When merozoites from type II meronts invade uninfected tissue cells, they initiate sexual multiplication with the formation of male and female gametes. The zygotes produced by fertilization develop into unsporulated oocysts which sporulate (become infectious) in the host before they are excreted in the feces. Two types of oocysts are found. Thick-walled environmentally resistant oocysts are excreted to the outside and are responsible for transmission of the infection between hosts. Thin-walled oocysts rupture in the host and release sporozoites which invade uninfected epithelial cells; thus, they are responsible for autoinfection of the host. The presence of the

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autoinfective form of the oocyst and type I meronts which can recycle is probably responsible for the repeated infections seen in immunocompromised hosts (*28,41,58,114*).

Resistance of Cryptosporidium

Cryptosporidium oocysts do not maintain infectivity at extremes of temperatures; however, moist cool conditions do contribute to the maintenance of oocyst infectivity. Freeze-drying and exposure to temperatures below freezing or >65°C for 30 min destroys infectivity (*126*). Sherwood et al. (*109*) showed that *Cryptosporidium* species survived neither freeze-drying nor freezing even though a number of cryoprotectants were tested. There was a gradual loss of infectivity during storage at 4°C in distilled water, 5% bovine serum albumin, phosphate-buffered saline (pH 7.2) or 2.5% potassium dichromate. Infectivity was lost in phosphate-buffered saline in approximately 100 d at 4°C, 14 d at 15-20°C, and in 5 d at 37°C (*109*). *Cryptosporidium* oocysts present in calf feces or intestinal contents lost infectivity when warmed from 9°C to 55-60°C over 15-20 min or when held at 45°C for 20 min (*6*). Thus, it is probable that cryptosporidia infectivity is lost during pasteurization of raw milk. Infectivity was reduced or eliminated by drying contaminated calf feces at ambient temperatures for 1-4 d (*7*).

As long as the thick wall remains intact, cryptosporidia oocysts are quite resistant to most disinfectants commonly used in hospitals and laboratories. Infectivity was not affected by exposure of oocysts to 1 or 3% chlorine (as sodium hypochlorite) for up to 18 h (*96*). Treatment with 10% formal saline, 5-10% ammonia solution, or 3% hydrogen peroxide was necessary to inactivate oocysts (*20,23*).

When suspensions of *C. parvum* oocysts (10^4/ml) were treated with 2.25 ppm ozone for 8 min, no infectious oocysts were found; however, 0.43 ppm chlorine dioxide inactivated approximately 90% of the oocysts in 15-30 min (*89*). Greater than 90% loss of infectivity was found when phosphate buffer suspensions of *C. parvum* containing 10^8 oocysts were treated with 1 ppm ozone for 5 min (*69*). An exposure time of 60 min was necessary with 1.3 ppm chlorine dioxide and 90 min exposure to 80 ppm chlorine or monochloramine was necessary to inactivate 90% of the oocyst infectivity (*69*). *C. parvum* oocysts are 30X more resistant to ozone and 14X more resistant to chlorine dioxide than *Giardia* cysts. The works of Peeters et al. (*89*) and Korich et al. (*69*) indicate that disinfectants, except for ozone, will probably not completely inactivate *C. parvum* oocysts in drinking water.

Transmission of oocysts

Transmission of *Cryptosporidium* to humans may occur through direct or indirect contact with feces containing oocysts. Oocysts may be transmitted through contact via person-to-person or animal-to-human and through ingestion of fecally contaminated water, food, or air. Person-to-person exposure is probably the most important route of transmission. Infections have occurred in persons who live in a household having an infected individual, in children in day care centers, and in sexual contacts between homosexual men (*28,41,59,86,114*). Organisms also are probably spread by sexual contact by heterosexual partners, also.

Since a large number of domestic animals (including pets and animals raised for food), zoo animals, and other wild animals are susceptible to cryptosporidiosis (*41,86,126*), oocysts from infected animals can be transmitted directly to animal caretakers and farmers. Animals can contaminate the environment with oocysts in their feces and thereby infect humans who come in contact with those feces. Egger et al. (*35*) presented a case of intestinal cryptosporidiosis in a young child who had contacted the disease from a sick kitten excreting cryptosporidia oocysts.

Waterborne transmission of oocysts leading to cryptosporidiosis is also common and can cause large outbreaks of the disease (*10,23,28,40,110,115*). *Cryptosporidium* oocysts are not affected by current chlorination levels and filtration procedures do not necessarily remove all oocysts (*110*); therefore, drinking water may contain oocysts. Obviously, the use of conventional indicators for fecal contamination is useless in alerting people not to drink water contaminated with *Cryptosporidium*. Agricultural sources of oocysts are important since runoff waters from dairies, animal raising facilities, grazing lands, or farm lands, fertilized with animal or human manure, can contaminate surface waters and water reservoirs.

Foods may be a source of cryptosporidia oocysts (*59,114*). Oocysts may be present in raw milk, raw meat, and other raw foods. Handling raw meats or ingesting raw foods could expose an individual to oocysts. However, the role of foods in transmission of *Cryptosporidium* is sparsely documented.

The disease

The infectious dose for *Cryptosporidium* oocysts is quite low. As few as 10 oocysts caused cryptosporidiosis in infant nonhuman primates (*79*). Related studies indicated that the number of oocysts ingested did not influence the severity nor duration of the disease (*79*).

In humans and nonhuman primates, the individual's immunocompetency or age at time of primary exposure appears to have little influence on susceptibility to cryptosporidiosis. However, most reported infections are children less than 2 years old. The immune status did affect the length and severity of the disease. Immunocompetent individuals have a short-term, self-limited diarrhea, whereas the immunocompromised develop a prolonged diarrhea which progressively worsens and becomes life threatening (*28,86*).

In animals, however, age in combination with other factors does appear important. Neonates are more susceptible to cryptosporidiosis than adults and usually adults are refractory to the disease. This has been observed for mice, rats, cats, cattle, sheep, goats, and pigs as well as for other animals (*9,28,41,44,86,114,126*). Interestingly, both young and adult immunocompetent guinea pigs can be infected with *Cryptosporidium* oocysts (*26*). Thus, the guinea pig may be useful as an animal model for immunocompetent human infection. Immunosuppressed adult rats and hamsters can be infected with *Cryptosporidium* and should prove useful as animal models for cryptosporidiosis in immunocompromised humans (*17,97,107*).

Calves $\leq$1 month of age are very susceptible to cryptosporidiosis, but older calves and adult cattle are resistant to infection. When Harp et al. (*51*) raised calves in isolation and challenged them with *C. parvum* at ages ranging from 1 week to 3 months, the animals became diarrheic and shed oocysts regardless of age. On rechallenge after recovery, none of the animals became reinfected. Harp et al. (*51*) suggested that calf exposure to oocysts in the environment results in specific acquired immunity to *Cryptosporidium*. Thus, the reason why older animals are not infected is due to prior exposure to the parasite; it is the immune state of the animal that is important, not the age per se.

Cryptosporidium trophozoites develop in the brush border of epithelial cells in the gastrointestinal and respiratory tracts. Immunocompetent persons usually have a short self-limited, cholera-like, or flu-like gastrointestinal illness that spontaneously resolves in 1-2 weeks. Affected individuals have profuse watery diarrhea and may suffer abdominal cramping, nausea, vomiting, low grade fever, and headache among other symptoms (*28,127*). The illness in the immunocompromised is quite different; the disease is chronic with persistent diarrhea (due to continual sporozoite reinfection of the enterocytes). The loss in fluid with ensuing electrolyte imbalance can be life threatening. In addition, the immunocompromised individual may suffer other symptoms similar to those found in diseased immunocompetent people. The infection in the

immunocompromised is not limited to the gastrointestinal tract; epithelial cells of the respiratory tract, gall bladder, bile and pancreatic ducts may be invaded by *Cryptosporidium*, also (*28,127*).

Chemotherapeutic agents are lacking for the treatment of cryptosporidiosis in both immunocompetent and immunocompromised patients. Diseased individuals can be supported with oral or intravenous hydration along with parenteral nutrition (*28,127*). The disease in children is severe enough that therapeutic intervention would be desirable if it were available. When immunosuppressed individuals suffering from cryptosporidiosis are removed from suppressive therapy, they generally recover from the disease, thereby indicating that restoration of immune function allows resolution of the disease. Current and Garcia (*28*) discussed the potential role that immunologic intervention might play in the treatment of cryptosporidiosis in immunocompromised patients, but such studies are merely in the experimental stage at present. There is little that the clinician can do to help patients with severe cryptosporidia diarrhea other than rehydration and intravenous feeding.

More than 90 compounds—antibiotics, sulfonamides, coccidiostats, antihelminthics, antiprotozoan agents—are not effective as prophylactic or therapeutic agents against *C. parvum* infection in farm animals and other mammals (*44*). In cases of severe dehydration, animals can be given oral or parenteral hydration therapy along with antibiotics to prevent secondary infections.

Detection

Cryptosporidium can be diagnosed by identification of developmental stages of the parasite in stained biopsy tissue sections or by demonstrating, microscopically, oocysts in feces, sputum, or bile (*28,41*). Weber et al. (*132*) reported on the limitations of the oocyst detection methods and pointed out that these methods often fail to show evidence of cryptosporidiosis in both infected immunocompetent and immunocompromised individuals. At least 10,000 oocysts per g must be present in watery stool specimens for detection, whereas 50,000-500,000/g are necessary in formed stools. Prior exposure to *Cryptosporidium* can be detected by serologic techniques and serology can be used to diagnose and monitor infection (*28*).

Newer techniques described for the detection of *Cryptosporidium*. include polyclonal and monoclonal antibody indirect fluorescence for oocysts in fecal smears (*46,119*) and polyclonal and monoclonal enzyme-linked immunosorbent assays (ELISA) for oocysts in feces (*8,105,128*).

There are no specific techniques available to detect *Cryptosporidium* in foods. Hence, it will be necessary to adapt methodology that has proven useful in clinical settings. Detection of *Cryptosporidium* oocysts in foods will prove to be more difficult than their detection in stool specimens since the number of oocysts present in food will probably be much lower. Therefore, detection of oocysts in most foods by microscopic screening will be impractical.

Filtration and sucrose gradient centrifugation have been used to concentrate *Cryptosporidium* oocysts in water before microscopic examination (*10*). It may be possible to test milk and other fluid foods suspected of being contaminated with oocysts in a manner similar to water. Solid foods, however, will have to be assayed for oocysts in other ways. Monoclonal antibody ELISA for oocysts have a sensitivity of 10^5-10^6 oocysts per ml of feces (*8,105*) and may be useful in screening foods for the presence of high levels of oocysts. But it is probable that more sensitive techniques will have to be used with most foods. Such procedures would include DNA probe techniques (*64,130,135*). For food samples containing very low numbers of oocysts, the polymerase chain reaction (PCR) may be useful for amplifying target DNA (a nucleotide sequence from oocysts) which, after amplification, can be detected by conventional DNA probe techniques (*39,90*). Laxer et al. (*70*) developed a specific and highly sensitive procedure to identify and detect *C. parvum* DNA from oocysts by using PCR. There was no cross-reaction with *Giardia lamblia, Toxoplasma gondii*, or *Entamoeba histolytica*. The PCR technique was successful in detecting *C. parvum* DNA utilizing both stool and infected tissue specimens.

Cryptosporidium *as a cause of foodborne illness*

Undoubtedly, *Cryptosporidium* oocysts in foods can cause foodborne illness, but the cases are sporadic and foodborne outbreaks are rare or unrecognized.

A few incidents of foodborne illness attributed to *Cryptosporidium* are listed in Table 1. In Incident #l (Table 1), a U.S. traveler returning from Mexico presented to her physician with cryptosporidia diarrhea which was suspected to be due to eating a salad obtained from a street vendor; drinking hotel water was suspected, also.

TABLE 1. *Incidents of foodborne crytosporidiosis.*

Incident No.	Suspect food	No. ill	Reference
1	Salad	1	*116*
2	Raw cow milk	22	*36*
3	Raw cow milk, sausage	19	*24*
4	Frozen tripe	1	*21*
5	Raw goat milk	2	*137*

In incident #2 (Table 1), 22 of 25 high school students and teachers who had traveled from British Columbia to Mexico presented to their physicians with gastrointestinal disease which proved to be cryptosporidiosis. Questionnaires indicated that the individuals involved had drunk bottled water and soda while in Mexico, but most had used ice cubes and had drunk milk. The travelers may not have known that the ice cubes were probably prepared from tap water and that milk pasteurization may be lacking or substandard in less developed countries.

Incident #3 (Table 1) occurred in the Holywell area in northern Wales. This outbreak appeared to have five possible routes of infection: contact with animals or their excreta, consumption of contaminated milk, consumption of contaminated water, consumption of contaminated or infected food, and person-to-person spread. There was no significant relationship between illness and drinking raw milk; however, a high proportion of the local population did consume raw milk routinely. There was a positive association of sausage consumption with illness. The water supply was not implicated; however, source waters contained *Cryptosporidium* oocysts. Manure from farms in the area and manure spread on fields adjacent to homes of affected families contained oocysts. Local ponds used for recreational purposes (especially by children) were also positive. Some of the pets from affected households were found to have cryptosporidiosis. However, a definite source of the outbreak could not be determined.

In incident #4 (Table 1) from England, the suspect food was frozen tripe which the individual had thawed and cut up for pet food. The pet was not ill, and the patient explained that he had inadvertently tasted some of the tripe. Upon examination *Cryptosporidium* oocysts were found in the tripe. This case was interesting because other workers (*109,126*) have shown that freezing destroys infectivity of oocysts. However, it may be possible that oocysts present in foods are protected from the harmful effects of freezing.

An Australian mother and her 1-year-old child suffered severe diarrhea from cryptosporidiosis (Incident #5, Table 1) which was believed to be due to the drinking of unpasteurized goat milk. In a 2-year study of cryptosporidiosis in hospitalized children, Thomson et al. (*123*) found that a significantly greater number of infected children drank unpasteurized cow milk as compared to children

without cryptosporidiosis. In a study of cryptosporidiosis in England and Wales, 9% of the infected patients reported drinking raw milk in the month before onset of the disease (*94*). Casemore (*22*) reported that out of 75 cases of cryptosporidiosis, 27.7% of the patients were known to drink raw milk. Freidank and Kist (*45*) demonstrated a significant relationship between drinking raw milk and cryptosporidiosis. Thus, it appears that there may be a close association of cryptosporidiosis infection and drinking raw milk.

Even though food poisoning from *Cryptosporidium* does not appear to be a major problem, Hoskin and Wright (*59*) discuss the control and prevention of *Cryptosporidium* in foods and the food environment. They suggest that foods that undergo milk pasteurization temperatures (63°C for 30 min) should be free of infective oocysts since Anderson (*6*) has shown that oocysts are killed when held at 45°C for 20 min. Presumably, high-temperature short-time pasteurization will inactivate oocysts, but this does not seem to have been tested.

That market pigs may be infected with *Cryptosporidium* has been demonstrated by Tacal et al. (*121*). Five percent of rectal swabs taken from 200 pigs offered for sale at a livestock auction in southern California were positive for *Cryptosporidium* oocysts. These results suggest that animal carcasses could be contaminated by oocysts during the slaughtering process and cross-contamination of other carcasses could occur.

It is probable that dried, frozen, or freeze-dried foods will not contain infectious *Cryptosporidium* oocysts (*59*) since Sherwood et al. (*109*), Tzipori (*126*), and Anderson (*7*) have shown that oocysts do not survive freezing, freeze-drying, or drying. However, the foodborne case presented above where cryptosporidiosis was caused by frozen tripe would suggest that the effect of freezing on oocysts present in foods needs further study.

Hoskin and Wright (*59*) further point out that the food industry must be aware of and must consider *Cryptosporidium* when evaluating raw foods handling, food processing conditions, plant design, and equipment design. More effective sanitation procedures are needed to eliminate *Cryptosporidium* oocysts from water, raw foods, and the environment. Food processing conditions involving heat are probably adequate for eliminating oocysts; however, the effects of food fermentation and other nonheat food processes on the destruction of *Cryptosporidium* oocysts are unknown and studies should be initiated in these areas.

GIARDIA

Giardiasis can produce severe diarrhea in humans and is caused by *Giardia*, an anaerobic flagellated protozoan parasite. Healthy children and adults as well as immunocompromised individuals are susceptible to the disease. The organism is endemic in many areas of the world and has caused outbreaks of foodborne and waterborne disease in the United States (*12,74*). Populations that have high exposure to *Giardia* due to lack of proper sanitation in terms of food, water, or personal hygiene, generally have high seropositivity to the parasite. Miotti et al. (*81*) found that adults living on an Apache Indian reservation in Arizona had a seropositive prevalence level of 44%; people living in rural areas of Panama had a level of 48%, and 46% of the people in an urban area of Peru were seropositive for *Giardia*. However, people living in an urban area of Baltimore had a seropositive prevalence of only 18%. Children living in the less developed areas had higher levels of seropositivity than children from Baltimore (*81*). There was a lower prevalence of seropositivity to *Giardia* in lactating women from Houston (24%) as compared to 77% in lactating women from Mexico City (*80*). Adults in Washington, DC were seropositive at a level of 14% (*112*) as compared to 45% of adults in Dacca, Bangladesh (*60*). While the incidence is lower for the United States, these data indicate that giardiasis is prevalent in developed countries like the United States as well as in developing countries.

The organism

Giardia species are classified as follows: kingdom *Protozoa*, phylum *Sarcomastigophora*, subphylum *Mastigophora*, class *Zoomastigophora*, order *Diplomonadida*, suborder *Diplomonadina*, genus *Giardia*. *Giardia lamblia* (synonyms: *G. intestinalis, G. duodenalis*) infects the small intestine in humans and other animals with disease manifestations ranging from asymptomatic carriage (and excretion) of the organism to severe diarrhea.

The parasite has a trophozoite and a cyst stage; the dormant cysts infect the host and the trophozoite causes disease. The trophozoite is an obligate anaerobe lacking mitochondria, endoplasmic reticulum, and Golgi apparatus; however, lysosomes containing digestive enzymes are present (*65*). Recently, a Golgi apparatus has been reported in encysting trophozoites (*2*). There are four pair of flagella and two prominent nuclei of equal size; the DNA in the two different nuclei appears to be functionally equivalent. The ribosomal RNA of *Giardia* is unusual and appears to be more characteristic of prokaryotes than eukaryotes (*33,65*). The trophozoites reproduce by binary fission.

The mature cyst contains four prominent and equal-sized nuclei and the thick cyst wall ensures that it is environmentally resistant. In contrast, the trophozoite is quite fragile outside the host (*77*). It has been reported that the cyst wall is chitinous (*131*); however, the presence of chitin in the cyst wall has been disputed (*2*).

The ingested cyst travels to the stomach where the acid environment initiates excystation; under the influence of pancreatic proteases, the process of excystation is completed in the upper small intestine (*16*). At excystation, a quadrinucleate trophozoite, in the process of division, emerges from the cyst, completes division, and yields two binucleate trophozoites (*77,111*). The trophozoites attach to the luminal side of the small intestine epithelial cell membrane where they feed and replicate. *Giardia* is not normally considered to be invasive, but trophozoites can be invasive under some conditions since histological studies have shown the presence of trophozoites in the mucosa (*111*). As detached trophozoites move downwards, encystation takes place apparently stimulated by high pH, bile salts, and fatty acids (*47,98*). The encysted trophozoites undergo binary division so that the mature cyst has four nuclei. The cyst is then excreted. During diarrhea, the rapid movement of intestinal contents may not allow all trophozoites to encyst so that fecal contents may contain trophozoites as well as cysts. Encystation does not occur outside of the host, and the excreted trophozoites eventually disintegrate (*77*).

Trophozoites of *G. lamblia* undergo surface antigenic variation in vitro and in vivo (*82*). The loss of a particular surface antigen and the gain of a new one have been demonstrated in gerbils and humans (*4,85*) and probably occur in other animal species, also. Cyclical surface antigenic variation is a mechanism by which many organisms escape the host immune response. However, after the loss of the initial trophozoite surface antigen and appearance of a new surface antigen, further cyclical changes in *G. lamblia* surface antigens have not been demonstrated (*82,84*). The loss of the initial antigen is quite rapid which suggests that host immunological selection probably is not the mechanism of selection for new surface antigens (*84*). Why trophozoites change their surface antigens is unknown, but recent studies by Nash et al. (*84*) would suggest that the gain of a new surface antigen protects the *Giardia* trophozoite from host intestinal proteases.

Microbial symbionts, including viruses, mycoplasmas, bacteria and fungi, have been found in *Giardia* trophozoites and cysts (*42,43,61*). Aggarwal et al. (*5*) suggest that HIV-1 virus is taken up and replicated in trophozoites of *G. lamblia*; however, Brown et al. (*18*) were unable to demonstrate uptake of HIV-1 by the parasite. Whether or not *G. lamblia* can harbor HIV-1 obviously needs more study. The role of microbial symbionts in *Giardia* is unknown; however, the presence of microorganisms in trophozoites or cysts

suggests that *G. lamblia* may be a vehicle in the transmission of pathogens to a host infected by the parasite.

Similarly to other microbial species, *G. lamblia* appears to be infected by "phage." Tai et al. (*122*) discuss the double-stranded RNA virus, GLV, which infects *Giardia* trophozoites. Early during the course of viral infection, viral RNA appears in the cytoplasm, but during the later stages of infection, viral RNA is found in both the cytoplasm and nuclei. Eventually, the trophozoite nuclei disintegrate. At the present time, the impact of GLV infection of the trophozoite on the course of giardiasis is unknown.

Resistance of Giardia

Giardia cysts appear to be relatively stable in surface and ground waters. DeRegnier et al. (*30*) studied the viability of *G. muris* cysts suspended in lake, river, tap, and distilled waters. *G. muris* is not a pathogen for humans, and it has been shown to be more resistant to chlorine (*71,102*) than *G. lamblia* which suggests that *G. muris* may be the more environmentally stable species. *G. muris* cysts were nonviable after day 28 when suspended in lake water to a depth of 15 ft (457.2 cm) during fall weather (Minneapolis, MN) but were viable up to 56 d at 30 ft (914.2 cm). At fall temperatures, cysts were viable up to 28 d in river water. In winter, cysts were viable for 56-84 d in both lake (15 and 30 ft depth) and river waters (*30*). Cysts were not viable after 14 d in tap water but remained viable up to 56 d in refrigerated distilled water. Unfortunately, DeRegnier and his coworkers (*30*) did not study the stability of *Giardia* cysts in water during spring and summer months, but the survival of cysts would probably be less during those months. DeRignier et al. (*30*) stated that temperatures <10°C led to prolonged viability of *G. muris* cysts present in water.

Meyer and Radulescu (*78*) reviewed earlier work on the resistance of *Giardia* cysts to physical and chemical agents. The thermal death point of cysts was reported to be 62°C, and inactivation of cysts occurred when treated with phenol or lysol (2-5%) or with 3% ammonia. However, Meyer and Radulescu (*78*) criticized these studies on cyst destruction by pointing out that the criterion of cyst death was either not given or depended on an unreliable dye exclusion test.

The effect of temperature on viability of *Giardia* cysts was studied by Bingham et al. (*14*). These authors found that storage at 8°C in distilled water permitted survival up to 77 d, but cysts stored at 21°C remained viable for only 5-24 d; there was only 4 d survival at 37°C. Freezing and thawing resulted in almost complete loss of viability; however, <1% of the cysts survived at least 14 d which indicates that freezing and thawing of surface waters may not always lead to elimination of *Giardia*. Immersion of cysts in boiling water led to immediate death of the parasite (*14*).

The cysts of *G. lamblia* are quite resistant to UV irradiation. Rice and Hoff (*101*) suspended 6 x 10^5 cysts per ml in distilled water and found that doses of UV at 42,000 to 63,000 μW-s/cm^2 reduced cyst viability by less than 90%. *Escherichia coli* at a level of 1 to 3 x 10^7 CFU/ml in phosphate buffer were inactivated 99.9% at a dose of 3,000 μW-s/cm^2. Ozone used at a level of 0.17 mg-min/L at 25°C or 0.53 mg-min/L at 5°C reduced the viability of *G. lamblia* cysts (10^5/ml in distilled water) by 99% (*134*). The cysts were 2.4X more resistant to ozone inactivation than poliovirus and 26.5X more resistant than *E. coli*. Obviously, the treatment of waters with ozone or UV levels that would eliminate coliforms will not result in *Giardia*-free drinking water.

G. lamblia cysts isolated from sick people were resistant to chlorine at elevated pH or low temperatures (*63*). At 5°C, cysts did not survive a 10-min exposure to 8 mg/L chlorine at pH 6 or 7 or 30 min at pH 8. At 25°C, chlorine at 1.5 mg/L inactivated cysts in 10 min at all pH values. Leahy et al. (*71*) studied the inactivation of *G. muris* cysts by chlorine. At 25°C, 25.5-44.8 mg-min/L inactivated 99% of the cysts, whereas at 5°C, 449-1,012 mg-min/L chlorine was necessary. In order to compare chlorine inactivation of *G. lamblia* to *G. muris*, Leahy et al. (*71*) recalculated the data from Jarroll et al. (*63*) and determined that <15 mg-min/L chlorine inactivated *G. lamblia* cysts at 25°C and 90-170 mg-min/L was necessary at 5°C. They also calculated that chlorine at 0.02-0.24 mg-min/L inactivated *E. coli* by 99% at 5°C. These studies indicate that *Giardia* cysts are more resistant to chlorine at low temperatures, that *Giardia* is several times more resistant to chlorine than *E. coli*, and that *G. muris* is more resistant to chlorine than *G. lamblia*. *G. muris* should be a good model to use to demonstrate the efficacy of chemical treatment on survival of cysts in water supplies. It is apparent that chlorine levels that would render drinking water free of coliforms will not eliminate *Giardia*.

G. lamblia trophozoites were killed in vitro by normal human milk; 30 min exposure to 3% or 60 min to 1% normal human milk killed 50% of the trophozoites (*49*). Goat or cow milk did not give the killing effect. The *Giardia*-cidal activity is due to cholate-dependent milk lipase (*48,52*) releasing long-chain free fatty acids and other toxic lipolytic products (*99,106*). Addition of pure long-chain unsaturated fatty acids to *G. lamblia* trophozoites resulted in inactivation of the parasite (*106*). While the *Giardia*-cidal effect of free fatty acids can be demonstrated in vitro, it has not been demonstrated in vivo. However, breast feeding may be a means by which giardiasis is restrained in newborn children. Normal human milk, in vitro, prevented both the adherence and growth of *G. lamblia* (*27*). The milk effect on *Giardia* was dose dependent at concentrations ranging from 0.1 to 5.0%. In addition, infant feeding formulae containing either soy milk or cow milk suppressed adherence of the protozoan. Free fatty acids, in particular arachidonic, linoleic, and palmitic acids, inhibited adherence of *Giardia*, also (*27*). Since free fatty acids are often added to infant formulae, Crouch et al. (*27*) concluded that bottle-fed babies may be protected against *Giardia* infection if they are fed infant formulae.

Transmission of Giardia *cysts*

The infective cysts of *Giardia* are transferred to the mouth when fecally contaminated water or food is ingested or by direct person-to-person contact via the fecal-oral route. There is an inverse relationship between sanitary practices and behavior and the incidence of giardiasis.

Water is the major vehicle for the spread of giardiasis and a large number of waterborne outbreaks have been reported (*78*). In the United States, in the 3-year period, 1986-1988, there were nine reported waterborne outbreaks of giardiasis with 1,169 cases (*74*).

LeChevallier et al. (*73*) looked for *Giardia* and *Cryptosporidium* in the source waters of 66 surface water treatment plants in 14 states of the United States and one Canadian province. By means of immunofluorescence, *Giardia* cysts were detected in 81% of incoming raw water samples and *Cryptosporidium* oocysts were present in 87%. The density of the parasites was higher in source waters receiving industrial or sewage effluents, and there was a positive correlation between total bacterial counts and/or fecal coliform counts with parasite levels. When LeChevallier et al. (*72*) tested chemically treated and filtered drinking waters from these plants, they found *Giardia* cysts in 17% and *Cryptosporidium* oocysts in 27%; treatment plants with highly contaminated source waters were more likely to have the parasites in the finished drinking water. Microscopic examination suggested that most of the parasites were nonviable. Compliance of these plants to the Surface Water Treatment rule of the U.S. Environmental Protection Agency did not ensure that the treated waters were free of *Giardia* or *Cryptosporidium* since that rule requires that 99.9% of *Giardia* be inactivated or removed from surface water supplies. If the studies of LeChevallier et al. (*72,73*) give a true picture of the prevalence of *Giardia* and *Cryptosporidium* in drinking water sources, then it will be necessary to initiate strong treatment measures to ensure that drinking water does not become a means of transmission of either giardiasis or cryptosporidiosis.

Animal fecal excretion into water may be a source of giardiasis in humans. In four northeastern states and Minnesota, *Giardia* trophozoites were present in 95.9% of live-trapped muskrats and 13.7% of live-trapped beaver (*37*). The prevalence of infection in juvenile muskrats was 92.5% but was 23.2% in juvenile beaver. Similarly, 65% of fecal samples from small rodents in the state of Washington contained *Giardia* cysts (*88*).

G. lamblia has been found in domestic ruminants (*19*). The prevalence of infection was 17.7% in sheep and 10.4% in cattle. There was a higher prevalence of infection in calves (27.7%) and lambs (35.6%). Buret et al. (*19*) demonstrated that organisms infecting humans and ruminants are morphologically and antigenically similar. Cyst production and clinical signs in ovine infections resemble those of the human disease, and these workers suggested that sheep may be useful to use for studies on human giardiasis.

Erlandsen et al. (*38*) demonstrated that inoculating beaver and muskrat with *G. lamblia* cysts resulted in infection of the animals, and mongrel dogs have been experimentally infected with cysts or trophozoites of *G. lamblia* (*53*). However, Kirkpatrick and Green (*67*) found that cats <1 year of age were difficult to infect with *G. lamblia*. They did not test very young kittens, and studies by other workers indicate that young animals are more susceptible to giardiasis. Thus, it appears that animals, including pets, farm animals, and wild animals, can be infected with *G. lamblia*. Since animals can serve as reservoirs of the parasite, their excreta may contaminate water supplies. In addition to the possible contamination of water, animals may also infect humans by fecal contamination of the environment and may directly infect humans who are animal caretakers. It is probable, too, that humans can infect animals with *G. lamblia* (*19*).

Food has been implicated in giardiasis, generally due to infected individuals or asymptomatic carriers contaminating food with cysts. *Giardia* caused five of 7,458 foodborne outbreaks reported in the United States for the period 1973-1987 (*12*). Only 131 individuals were involved; thus, giardiasis is not a major foodborne disease in the United States.

Giardiasis may be transmitted from person-to-person via the fecal-oral route due to poor sanitary habits as found in children in day care centers (especially those that have not been toilet trained) and in institutionalized individuals (*92,117*). The disease is readily transmitted in family settings, especially if a small child is infected and in other situations involving close living arrangements or crowding. The sexual practices of adults, particularly in homosexuals, are considered to be important in the transmission of the parasite (*77,78*). Interestingly, *Giardia* infection of the vagina has been reported (*77*) which suggests that certain sexual behaviors in heterosexuals may pose a risk of giardiasis.

The disease

The infective dose for *Giardia* is low. Rendtorff (*100*) showed that of 13 healthy adult men who received oral doses of *G. lamblia* ranging from 10^2 to 10^6 cysts, all became infected while only eight of 22 men who received 10 to 25 cysts were infected. Infection was asymptomatic in most of the men and cyst excretion was inconsistent. Rendtorff's results (*100*) indicate that ingestion of as few as 10 cysts may lead to infection.

The organism infects all age groups, but infants and children are more readily infected than adults (*77,78,117*). The prevalence of giardiasis appears to be lower during the first 6 months of age which may be due to the protective effect of breast milk (*52*). Islam et al. (*60*), in a study conducted in Bangladesh, also noted that infection in infants <6 months of age was lower than in older children but were unable to demonstrate clear-cut protection against *Giardia* infection with breast feeding. They felt that breast-fed infants were less exposed to the parasite. In a study conducted in Vermont, Birkhead and Vogt (*15*) showed that the incidence of symptomatic *G. lamblia* infection during the years 1983-1985 was highest in children 1-4 years of age which was approximately 2.5 times that of the next highest group (30-39 years of age). The children aged 1-4 had an incidence approximately three times that of children <1 year of age. Similarly, Janoff et al. (*62*) found that children aged 1-4 years were more likely to have positive stool specimens than adults aged 20-39 years; this was true of both a population in Denver, Colorado, and Soongnern, Thailand.

The clinical spectrum of infection with *G. lamblia* ranges from asymptomatic carriage and excretion of cysts to persistent severe diarrhea with malabsorption, dehydration and loss of weight. AIDS patients and other immunocompromised individuals are more likely to have symptomatic giardiasis (*50*). Asymptomatic giardiasis appears to occur more often than illness and epidemiologically is probably more important. Asymptomatic giardiasis occurs in approximately 13% of adults and approximately 50% of children who are infected with the organism (*136*). Asymptomatic individuals are not normally detected, and they generally do not seek treatment. If the asymptomatic individual is a carrier, then he can disseminate the disease. *Giardia* carriers may excrete cysts for years (*77*). The asymptomatic carrier rate in the United States has been estimated to be 3 to 7% and may be as high as 20% in the southern part of the United States (*92*).

Malabsorption due to giardiasis can be quite severe and incapacitating to infants and children, the elderly, and to immunocompromised individuals. Malabsorption may occur in up to 60% of the patients who have diarrhea (*111*). Fat is the nutrient most frequently malabsorbed, but the uptake of sugars, vitamins, and proteins may be impaired, also. The mechanism by which the disease causes malabsorption is unknown but may involve prostaglandin secretion, villous atrophy, increased epithelial cell turnover, and/or brush border injury (*111*).

Individuals repeatedly exposed to *Giardia* have a lower incidence of infection and symptoms than newly exposed individuals, and immunocompromised individuals have increased prevalence of symptomatic giardiasis. It has been shown that a systemic antibody response against *Giardia* develops in patients with giardiasis, but since the parasite is located predominantly in the luminal portion of the upper intestine, gastrointestinal tract secretory antibodies are probably more important than circulating antibodies in protecting the individual against the disease (*92,111*).

Snider and Underdown (*113*), using a mouse model, demonstrated anti-*G. muris* IgA antibody in intestinal secretions of infected mice but did not find anti-*Giardia* IgG or IgM in the secretions. Expulsion of *G. muris* by the immunocompetent mice was closely associated with appearance of increasing levels of secreted IgA antibody. Both IgG and IgA anti-*G. muris* antibody was present in serum and remained at high levels for several weeks following clearance of the parasite.

Heyworth (*54*) showed that parasite-specific IgA and IgG bind to *G. muris* trophozoites present in the lumen of the intestine of immunocompetent mice and felt that these intestinal antibodies play a role in elimination of the infection. Trophozoites from T-cell-deficient mice show little evidence of antibody binding. Immunodeficient mice, due to inability to clear the organism from the intestines, had chronic giardiasis. While the works of Snider and Underdown (*113*) and Heyworth (*54*) do not agree in their entirety, they do indicate the importance of the intestinal secretory immune system in *Giardia* infections.

Cellular immune responses are important in eliminating *Giardia* trophozoites, also. T-lymphocyte-deficient mice are unable to eliminate trophozoites from the intestinal tract (*54*). Immunocompetent mice experimentally depleted of helper/inducer T-lymphocytes by the use of T-cell antibody were unable to eliminate *G. muris* (*54*). It is probable that helper/inducer T-cells play an important role in immunity against giardiasis.

Human mononuclear phagocytes, in the presence of heated anti-*G. lamblia* serum, ingested trophozoites, but in the presence of

unheated antibody, trophozoite ingestion increased 8-fold. Ingestion of parasites elicited an oxidative respiratory burst which led to destruction of ingested trophozoites (*56*). Lymphocytes obtained from Peyer's patches of *G. muris*-infected mice demonstrated a proliferative response to *G. muris* trophozoite antigen. The proliferation of lymphocytes correlated with clearance of infection (*55*). Macrophages from murine Peyer's patches ingested *G. lamblia* at low levels in the presence of nonimmune serum; however, in the presence of anti-*G. lamblia* serum, ingestion was enhanced. The interaction of macrophages and trophozoites was associated with an oxidative respiratory burst and destruction of the parasite (*57*).

Mice infected with *G. muris* were less responsive to intraperitoneally or intraduodenally administered sheep red blood cells, i.e., they were immunodepressed (*13*). Immunodepression was short-lived and was at a maximum during highest trophozoite density. The immunodepression was more pronounced in gut-associated lymphoid tissue than in systemic sites such as the spleen. Immunodepression in the gut lymphoid tissues would influence trophozoite establishment and proliferation, cyst production, and duration of the disease (*13*). It is probable that *G. lamblia* induces immunodepression in humans, and such parasite induced immunodepression may have serious effects: the parasitized host may become more susceptible to other infectious diseases, or the host may not respond effectively against *Giardia*.

As many as 86% of *Giardia*-infected patients spontaneously clear their infection (*29*), but chemotherapy should be considered in all symptomatic cases of giardiasis and in those asymptomatic cases who carry and shed parasitic cysts. Children with acute or chronic diarrheic giardiasis and who fail to thrive and/or exhibit malabsorption or other gastrointestinal symptoms should always receive chemotherapy (*92*). Treatment of children results in height and weight gains, and treatment of both adults and children reduces the human reservoir of infection with concomitant decreases in possible contamination of food and water as well as person-to-person spread (*117*).

Antigiardiasis drugs in common use include quinacrine (atabrine, an acridine derivative), furazolidone (a nitrofuran derivative), and metronidazole (an imidazole derivative). All of these compounds have side effects that make drug-taking compliance difficult, and they are either carcinogens or suspect carcinogens (*29*). Their use during pregnancy is contraindicated. The relatively nontoxic paromomycin has been recommended for treatment of giardiasis during pregnancy but appears to have been little used. Giardiasis during pregnancy should be treated after delivery of the child if at all possible.

A number of compounds have been tested for their ability to destroy trophozoites in vitro. Studies indicate that trophozoites of *G. lamblia* are sensitive to antihelminthic drugs such as albendazole and mebendazole (*34*) and to antiprotozoal 5-methylthioribose analogs (*104*). Since the rRNA of *Giardia* is unlike that of its eukaryotic host (*33*), protein synthesis inhibitors such as tetracyclines (*32*) and aminoglycosides (*31*) have been shown to be effective against trophozoites of *Giardia* in vitro, and one aminoglycoside, paromomycin, has had limited use against giardiasis during pregnancy (*29*).

There is no de novo synthesis or interconversion of purines in *Giardia*, and the parasite depends on the salvage of preformed purines from the environment (*11*). The purine nucleoside or base is taken up, the nucleoside is hydrolyzed, and then the base is phosphoribosylated to form the ribonucleotide. Baum et al. (*11*) demonstrated that ribonucleotide reductase is absent in *G. lamblia*, and thus, deoxynucleotides cannot be formed via reduction of the ribose moiety. However, they did find that preformed purine deoxynucleosides were incorporated into DNA via a purine deoxynucleoside kinase. Similarly, *Giardia* is dependent on salvage of exogenous pyrimidines (*2*). It is possible that the unique purine and pyrimidine metabolism of *Giardia* could form a basis for chemotherapy of the disease.

Developing noncarcinogenic antigiardiasis drugs with few toxic side effects should have research priority. Since vaccine therapy effective against giardiasis is currently not available, research is needed to develop effective vaccines that would protect high-risk individuals, i.e., travelers to *Giardia* endemic areas and children living in endemic areas (*117*).

There is no way to prevent infection once the *Giardia* cysts are ingested, but there are preventive measures that can be taken to reduce cyst ingestion. Meyer and Jarroll (*77*) have suggested that only cooked and peeled foods should be eaten, potable water suspected to contain *Giardia* should be boiled, and family pets should receive treatment if they have a *Giardia* infection. An additional preventive measure would be to initiate proper and thorough hand-washing procedures among family members, especially those involved in food preparation. And in fact, proper hand-washing practices in all food preparation establishments should be rigidly enforced as a means of preventing gastrointestinal infections.

Detection

Acute diarrhea caused by *Giardia* must be differentiated from diarrhea caused by viruses, bacteria, and other protozoan parasites (*136*). The physician should suspect giardiasis in patients with upper abdominal discomfort and distention, foul smelling stools, and gas. Blood and mucus are not normally present in the stool. The diagnosis of giardiasis may be confused, also, since the disease can mimic duodenal ulcer, hiatal hernia, gallbladder disease, or pancreatic disease (*136*).

It is much easier to suspect that the patient has giardiasis than it is to detect it in the laboratory. Routinely, giardiasis is diagnosed by finding cysts or trophozoites in diarrheic stools or cysts in formed stools. Three specimens collected over a week period are usually examined; however, since cysts are shed intermittently, it is easy to miss detecting them. The diarrheic specimens should be examined quickly after collection since trophozoite stability is of short duration outside of the host (*77*). If the stool specimens are negative, then aspiration and/or biopsy of the bowel should be done. An alternative test is the Entero-test in which a string is swallowed, allowed to remain in the upper bowel for a period during which trophozoites attach to the string. Upon retrieval, the string is washed and the washings examined for trophozoites (*92*) Detection of cysts in water or stools is made easier by immunofluorescence techniques (*103,108*).

ELISA has been used for the detection of trophozoites and cysts in both stool specimens and in water samples. Trophozoites are known to undergo surface antigenic variation in vitro and in vivo (*82*), but such variation does not appear to occur with cysts. Nonetheless, ELISA against trophozoite antigens present in stool samples appear to be as successful (*68,83*) as ELISA against cyst antigens (*118,120*) in detecting giardiasis. A commercial ELISA kit, ProSpecT/Giardia, can be used to detect *Giardia* in stool samples. The kit detects a *G. lamblia*-specific antigen (GSA-65) associated with both the cyst and the trophozoite and is more sensitive for diagnosis of giardiasis than microscopic examination (*3*).

A cDNA (complimentary DNA produced from a ribosomal RNA residue isolated from *G. lamblia* cysts) probe has been developed for the detection of *Giardia* cysts in water (*1*). The method appears to be comparable to the immunofluorescence technique. PCR has been used to detect *G. lamblia* cysts (*75*). The assay uses DNA from the giardin gene as the target and detects both living and dead cysts. However, as part of the PCR procedure, Mahbubani et al. (*75*) developed a method to differentiate between live and dead *Giardia* cysts. To determine the number of viable *Giardia*, giardin mRNA levels are determined before and after induction of excystation; during excystation (only viable cysts excyst), the amount of giardin mRNA increases significantly.

Giardin mRNA is detected by using reverse transcription to form cDNA which is then amplified by PCR (*75*). In a further study, Mahbubani et al. (*76*) have refined their PCR technique to differentiate between human and nonhuman species of *Giardia*. Thus, only the human pathogen, *G. lamblia*, is detected. The method is sensitive enough to detect a single *Giardia* cyst.

Giardia *as a cause of foodborne illness*

Foodborne giardiasis does not appear to be common but may occur more often than realized. Most cases are probably sporadic, and outbreaks may occur which are not recognized as due to giardiasis. Todd (*124*) estimates that 3,850 cases of foodborne giardiasis occur each year in Canada at an annual cost of $19.7 million. There were no reported fatalities. The extent of foodborne giardiasis in the United States is estimated to be 7,000 cases per year with a fatality rate of 0.0001% . The estimated annual cost to U. S. consumers is 36 million dollars (*125*).

TABLE 2. *Incidents of foodborne giardiasis.*

Incident No.	Suspect food	No. ill/total	Reference
1	Fruit salad	10/25	*93*
2	Sandwiches	88/312	*133*
3	Lettuce, onions, tomatoes	21/108	*25*
4	Noodle salad	13/16	*91*
5	Homecanned salmon	29/60	*87*

Some incidents of documented foodborne giardiasis are listed in Table 2. A party for 25 people (representing seven families) held at a private home in New Jersey was the setting for a giardiasis outbreak affecting 10 people (incident #1, Table 2). Nine of the infected individuals became ill within 6-12 d after the party, and *G. lamblia* was isolated from the stools of eight persons. A fruit salad, prepared by a woman who became ill after the party, was implicated as the vehicle of the parasite. Her 2-year-old child in diapers had cysts in her stool but was asymptomatic. Two days before the party, the child had been given a pet rabbit which subsequently was found to be shedding *Giardia*. *Giardia* cysts could have been transmitted to the food by the food preparer after diapering her child or after she had handled the rabbit (the rabbit was reported to have been in the kitchen during preparation of the salad). Lack of or improper handwashing after changing the child's diaper or after handling the rabbit was probably responsible for his food outbreak.

Incident #2 (Table 2) occurred in a combined nursing home-child care center in rural Minnesota. Cases were found in nursing home residents and staff and in children attending the day care center. Giardiasis was diagnosed in 88 people (15 children and 73 adults). Seventy-three percent of nursing home residents involved in an "adoptive grandparent" program (contact with preschoolers in the day care center) became ill as compared to only 25% who were not involved in the program. Waterborne transmission was ruled out. Consumption of sandwiches was significantly correlated with illness. Hand-washing facilities and practices appeared to be substandard throughout the nursing home and day care center. There was a good probability that ill food preparers had contaminated the sandwiches since 57% of the sandwich makers were shown to have *G. lamblia* in their stools. The outbreak probably proceeded as follows: the mother of an infected toddler in the day care center was infected by her baby. This individual was employed in the foodservice area of the nursing home, and she probably infected the other food handlers (either by food that she had prepared or by personal contact). These newly infected workers further spread the infection throughout the nursing home by the sandwiches they prepared. Concurrently, elderly patients in the "adoptive grandparent" program were infected by personal contact with children from the day care center. Corrective procedures initiated included proper training in hand-washing procedures (for foodservice, nursing, and child care staffs) and the removal of all infected individuals from foodservice operations.

Incident #3 (Table 2) took place at a dinner for members of a church group in Albuquerque, New Mexico. The foods served included tacos, corn, peaches, cupcakes, soft drinks, coffee, and tea. Water was not associated with the disease, and analysis indicated that the taco ingredients, particularly lettuce and onions, were correlated with illness. Lettuce and tomatoes were washed at the church kitchen sink and then lettuce, onions, and tomatoes were chopped on the same cutting board which was not washed between items. How the food became contaminated is not clear.

Incident #4 (Table 2) involved 13 of 16 people who attended a picnic. Thirteen family members of ill individuals who did not attend the picnic did not become ill, and one person who visited the picnic hostess the day after the picnic and who ate some of the leftover foods (including the suspect food) did contact giardiasis but was not counted as a picnic-related case. Water used for drinking or preparing food was not correlated with illness; however, eating cold noodle salad was significantly associated with illness. The salad was prepared on the day of the picnic and mixing of salad ingredients was done using bare hands. The preparer said that she did not wash her hands before the mixing operations. Significantly, the salad preparer did not attend the picnic and the next day, she became ill with gastrointestinal symptoms. It is tempting to suggest that she did not attend the picnic because she was not feeling well and could have been secreting *Giardia* cysts at the time the food was prepared. Her stool samples were negative, but stool samples from her children were positive even though the children were asymptomatic.

Twenty-nine of 60 employees of the Goodhue, Minnesota school system presented with giardiasis in incident #5 (Table 2). The results of a questionnaire indicated that salmon which was served in the employee's lounge was significantly correlated with illness. The salmon had been homecanned, and examination of remaining jars indicated that the product had been properly processed since the aerobic plate, total coliform, and *E. coli* counts were negative. *Giardia* cysts were not present. The wife of the employee who brought the salmon to the school employee's lounge had opened the cans, drained the juices, and placed the salmon in plastic containers. She could not remember whether she had touched or handled the salmon with her hands. She had diapered her diarrheic grandson before transferring the salmon and reported that she had washed her hands afterwards; the grandson's stools were later shown to contain *Giardia* cysts. The employee's wife did not eat any of the salmon; however, several days later, she suffered acute giardiasis probably as a result of personal contact with her grandson. While she may have washed her hands after diapering the child, thorough cleansing that would remove cysts from under her nails was probably not done. Why the infection did not spread to the student population was puzzling. Surely some of the infected employees worked in the school cafeteria; it might be expected that an outbreak could occur among the students who ate in the cafeteria serviced by infected employees.

Karabiber and Aktas (*66*) reported an outbreak of giardiasis in two Turkish families; however, the total number of ill individuals was not given. Sheep tripe soup was considered to be the vehicle of the outbreak. The authors suggested that *Giardia* cysts in deep layers of the tripe were protected from heat inactivation during soup preparation. A day-long meeting at a restaurant was associated with an outbreak of giardiasis in 27/36 people (*95*). Ice cubes but not water was implicated as the parasite vehicle. Two employees, one with asymptomatic giardiasis and the other with a *Giardia*-infected, diapered child, had served ice during breaks and lunch. The cause of the outbreak was probably due to inadequate hand washing before dispensing ice into drinks.

The incidents of foodborne giardiasis listed in Table 2 were probably due to preparation of food by persons with poor personal

hygiene and who were also infected with *Giardia* or were asymptomatic carriers. Food appears to be an effective means of transmitting *Giardia*: individuals who ate contaminated foods were infected at rates ranging from 19 to 81% with a mean rate of 44% (Table 2). Since the minimal dose for *Giardia* is quite low-approximately 10 cysts-it is surprising that there are not more outbreaks of foodborne giardiasis. It is probable that *Giardia* foodborne outbreaks are more common than realized but are simply not recognized as such.

In homes and restaurants, proper hand washing and nail cleansing by food preparers and the prohibition of infected individuals (who have diarrhea) and carriers from food handling areas and operations will prevent many cases of foodborne giardiasis. In institutional situations, strict sanitation is a must. Individuals who care for the institutionalized—food preparers, nurses, and other caretakers—must be trained in proper hygiene concerning food preparation as well as their own and their institutionalized patients' personal habits.

The concluding paragraph in the section on *Cryptosporidium* (see above) which discusses Hoskin and Wright's (59) concerns about *Cryptosporidium* in the food industry can be applied to *Giardia*, also. The food processor must consider the possibility of *Giardia* cysts, when evaluating raw foods handling, food processing conditions and operations, and plant and equipment design. Food processing conditions involving heat will probably eliminate *Giardia* cysts, but the effect of other food processing conditions such as fermentation and processes not involving heat on cyst destruction is not known. Studies are needed to ascertain that destruction of *Giardia* cysts is complete in nonheat processed foods that may contain the parasite.

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