APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1996, p. 2866–2868 Vol. 62, No. 8
0099-2240/96/$04.00+0

Effect of Pasteurization on Infectivity of *Cryptosporidium parvum* Oocysts in Water and Milk

JAMES A. HARP,[1]* RONALD FAYER,[2] BRUCE A. PESCH,[1] AND GEORGE J. JACKSON[3]

Metabolic Diseases and Immunology Research Unit, USDA Agricultural Research Service, National Animal Disease Center, Ames, Iowa 50010-0070[1]; Immunity and Disease Resistance Laboratory, Beltsville Agricultural Research Center–East, Beltsville, Maryland 20705[2]; and Food and Drug Administration, Washington, D.C. 20204[3]

Received 25 March 1996/Accepted 16 May 1996

***Cryptosporidium parvum* is a major cause of diarrheal disease in humans and has been identified in 78 other species of mammals. The oocyst stage, excreted in feces of infected humans and animals, has been responsible for recent waterborne outbreaks of human cryptosporidiosis. High temperature and long exposure time have been shown to render oocysts (suspended in water) noninfectious, but for practical purposes, it is important to know if high-temperature–short-time conditions (71.7°C for 15 s) used in commercial pasteurization are sufficient to destroy infectivity of oocysts. In this study, oocysts were suspended in either water or whole milk and heated to 71.7°C for 15, 10, or 5 s in a laboratory-scale pasteurizer. Pasteurized and nonpasteurized (control) oocysts were then tested for the ability to infect infant mice. No mice (0 of 177) given 10^5 oocysts pasteurized for 15, 10, or 5 s in either water or milk were found to be infected with *C. parvum* on the basis of histologic examination of the terminal ileum. In contrast, all (80 of 80) control mice given nonpasteurized oocysts were heavily infected. These data indicate that high-temperature–short-time pasteurization is sufficient to destroy the infectivity of *C. parvum* oocysts in water and milk.**

Cryptosporidium parvum is a protozoan parasite shown to cause enteric infection and diarrhea in 79 species of mammals, including humans (4, 10). Disease is most prevalent in the young, such as children and bovine calves, and the immunocompromised, such as AIDS patients (2). In immunocompetent humans, cryptosporidiosis is relatively mild and self-limiting, but infection of AIDS patients often results in chronic and life-threatening illness (5, 13). Of the many drugs and treatment regimens that have been tested, none is presently available that is consistently effective against cryptosporidiosis (10, 13). Thus, avoidance of exposure to *C. parvum* by high-risk groups is desirable.

Cryptosporidiosis is spread through the fecal-oral route. This exposure may occur in many ways, including contact with infected farm animals, companion animals, or other humans (4, 10). Contaminated water is known to be a significant source of infection, as evidenced by recent large waterborne outbreaks of cryptosporidiosis (8, 11). Present water treatment standards do not guarantee removal of *C. parvum* from treated water, because of the small size and chlorine resistance of *C. parvum* oocysts (7, 12). An outbreak of cryptosporidiosis was recently described which resulted from ingestion of cider made from apples possibly contaminated with calf feces (9). Fecal contamination of milk could be a potential source of infection with *C. parvum* because of the high incidence of the parasite on dairy farms (6).

Treatment of milk by high-temperature–short-time (HTST) pasteurization is now a standard practice in developed countries and has eliminated the threat of several infectious diseases formerly transmitted via milk (1). In addition, heat-treated commercially bottled water offers an alternative source of drinking water to people who are at high risk of infectious disease, such as infants and immunocompromised persons. However, the efficacy of HTST pasteurization protocols in eliminating the infectivity of *C. parvum* oocysts has not been tested. Therefore, in the present study, we evaluated the infectivity of *C. parvum* oocysts after HTST pasteurization of water and milk.

MATERIALS AND METHODS

Source of oocysts. All oocysts used in this study were from a single batch of oocysts purified from feces collected from an experimentally infected calf. Diarrheic feces were collected into 2.5% potassium dichromate solution. This

TABLE 1. Infectivity of *C. parvum* for infant mice after pasteurization in water[a]

Treatment	No. of litters/trial		No. of mice		
	Trial 1	Trial 2	Total	Infected	Negative
71.7°C, 15 s	3	3	34	0	34
71.7°C, 10 s	3	3	32	0	32
71.7°C, 5 s	3	3	29	0	29
None	3	3	47	47	0

[a] Oocysts were recovered after pasteurization as described in Materials and Methods. Mice were orally inoculated with 10^5 oocysts per pup. Mice were categorized as infected or negative by microscopic examination of ileal sections as described in Materials and Methods.

TABLE 2. Infectivity of *C. parvum* for infant mice after pasteurization in milk[a]

Treatment	No. of litters/trial		No. of mice		
	Trial 1	Trial 2	Total	Infected	Negative
71.7°C, 15 s	3	3	29	0	29
71.7°C, 10 s	3	3	34	0	34
71.7°C, 5 s	2	3	19	0	19
None	3	4	33	33	0

[a] Oocysts were recovered after pasteurization as described in Materials and Methods. Mice were orally inoculated with 10^5 oocysts per pup. Mice were categorized as infected or negative by microscopic examination of ileal sections as described in Materials and Methods.

* Corresponding author. Phone: (515) 239-8533. Fax: (515) 239-8458. Electronic mail address: !A03RLMDIR@ATTMAIL.COM.

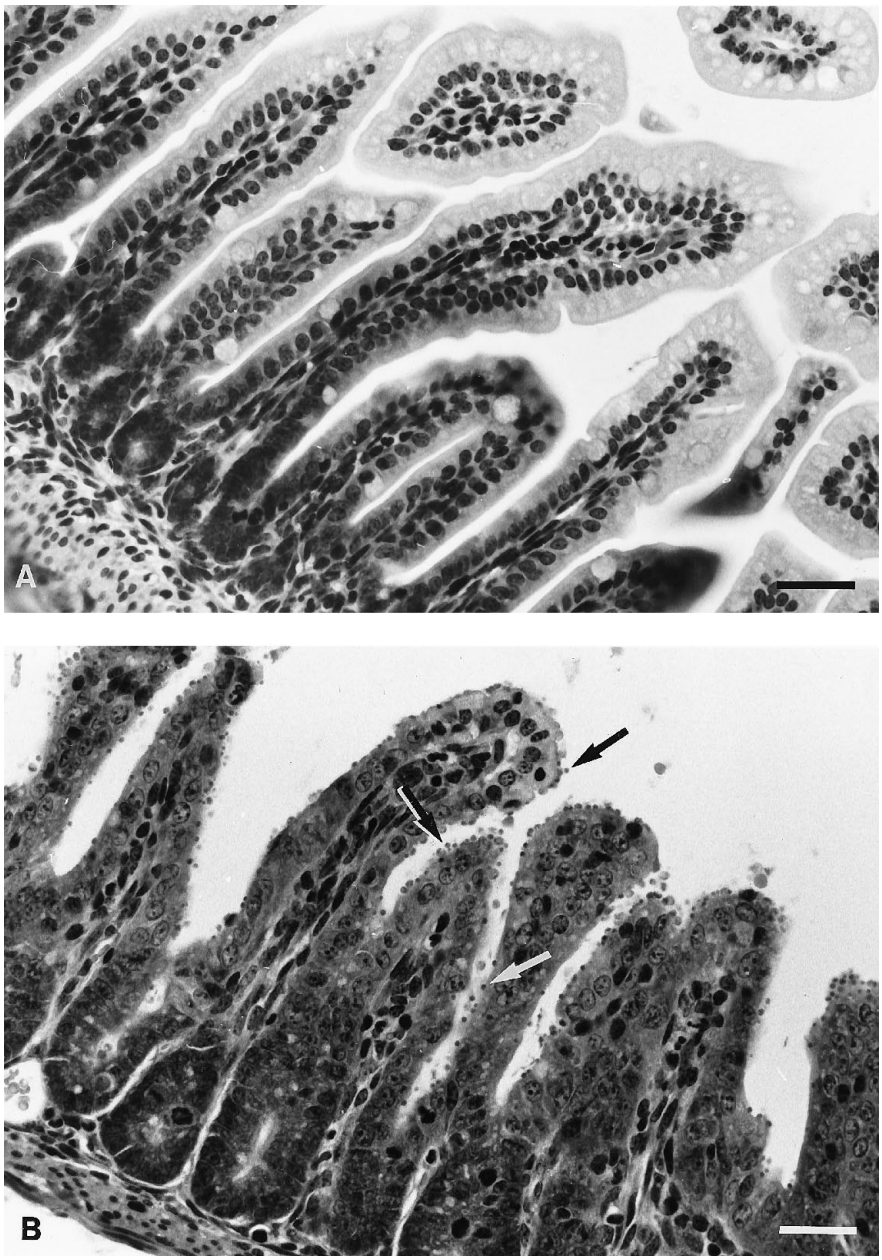

FIG. 1. Intestinal sections of ilea from mice which were inoculated at 1 week of age with *C. parvum* oocysts pasteurized in milk (A) or nonpasteurized control oocysts (B). Note the numerous developmental stages of *C. parvum* in panel B (arrows) compared with the total absence of *C. parvum* in panel A. Bars, 36 μm.

suspension was passed through a series of graded sieves to remove large particles and centrifuged, and the pellet containing oocysts was resuspended in potassium dichromate. Aliquots of this suspension were overlaid on Sheather's sucrose solution and centrifuged at 600 × *g* for 15 min, and then the interface layer (containing oocysts) was aspirated. Oocysts were washed three times by resuspension and centrifugation in phosphate-buffered saline solution and then counted with a hemacytometer.

Pasteurization of oocysts. Purified oocysts were suspended in distilled water or milk (commercial whole milk purchased at a local store) at a concentration of 10^8 oocysts per liter. Each suspension was processed through an Armfield FT 43A Laboratory Pasteurizer (Armfield, Ltd., Ringwood, Hampshire, United Kingdom) according to the manufacturer's instructions to simulate commercial pasteurization conditions. The temperature of the holding chamber was set at 71.7°C. The flow rate was adjusted to produce a retention time in the holding chamber of either 15, 10, or 5 s. For each replication, 2 liters of water or milk containing oocysts was passed through the apparatus, and the pasteurized effluent was collected. Prior to inoculation of mice, the pasteurized oocysts were concentrated by centrifugation of the effluent (15 min at 1,500 × *g*) and resuspended in phosphate-buffered saline solution at a concentration of 10^6 oocysts per ml.

Bioassay for infectivity. BALB/c mice from a colony maintained at the USDA Agricultural Research Service, National Animal Disease Center, were bred to produce litters of infant mouse pups for use in the assays. Infant mice were orally inoculated at 1 week of age with 0.1 ml (10^5 oocysts) each of the oocyst suspensions recovered after pasteurization of water or milk. Control pups received 0.1 ml (10^5 oocysts) each of oocyst suspensions from the same initial pool but not subjected to pasteurization. All pups were returned to their mothers for 96 h. Infant mice were then euthanized, and 2 to 3 cm of terminal ileum was removed from each pup. Tissues were fixed in 10% neutral-buffered formalin, paraffin embedded, and sectioned. The sections were stained with hematoxylin-eosin and examined microscopically for the presence of developmental stages of *C. parvum.*

Each retention period for both milk and water suspensions was replicated twice. At each replication, mouse pups received nonpasteurized oocysts as controls (Tables 1 and 2).

To determine the level of detection in the histologic sections, additional mouse pups were infected with 10^3, 10^2, and 10^1 nonpasteurized oocysts.

RESULTS

Prior to treatment in the pasteurizer, *C. parvum* oocysts used in these experiments were shown to have a 50% infective dose of $\sim 10^2$ oocysts for infant mice. Nine of 9 pups were infected at a dose of 10^3 oocysts per pup, 6 of 10 were infected at a dose of 10^2, and 0 of 7 was infected at a dose of 10^1. Results for infectivity of 10^5 *C. parvum* oocysts for infant mice after pasteurization in water are shown in Table 1. No mice (0 of 95) inoculated with oocysts held at 71.7°C in water for either 15, 10, or 5 s were infected with *C. parvum*. Similarly, no mice (0 of 82) inoculated with oocysts held at 71.7°C in milk for either 15, 10, or 5 s were infected with *C. parvum* (Table 2 and Fig. 1A). All (80 of 80) control mice inoculated with 10^5 nonpasteurized oocysts were heavily infected (Table 2 and Fig. 1B).

DISCUSSION

In a previous study, *C. parvum* oocysts remained infectious when exposed to a water temperature that rose to 67.5°C in 1 min, but they were noninfectious after exposure to a water temperature of 72.4°C or higher for 1 min (3). *C. parvum* oocysts were also noninfectious after exposure to 64.2°C or higher for 2 min or longer (3). Because this study did not test infectivity after exposure to HTST conditions used in commercial pasteurization (15 s at 71.7°C), the present study was undertaken to address this question and to determine if inactivation similar to that in water occurs in milk. Our results showed that exposure to a temperature of 71.7°C for 15 s rendered *C. parvum* oocysts noninfectious for infant mice. Similar results were seen when oocysts were held at 71.7°C for 10 or 5 s. This was true whether oocysts were treated in water or milk.

In order to maximize the likelihood of detecting a small fraction of oocysts present in water or milk that might survive pasteurization, we seeded samples at a concentration (10^8 oocysts per liter) far exceeding levels that would normally contaminate these products. We used purified oocysts to more closely approximate the condition of oocysts in processed drinking water or milk, which would have been subjected to treatments including filtration, sedimentation, and centrifugation. The nonpasteurized purified oocysts used in the experiments were shown to have a 50% infective dose of $\sim 10^2$ for infant mice. After pasteurization, a dose of 10^5 oocysts per pup was noninfectious. Thus, infectivity of *C. parvum* oocysts after pasteurization was reduced at least 3 log factors.

It was possible that the complex nature of milk would have a protective effect against heat inactivation of suspended oocysts, compared with oocysts suspended in water, but this was not the case under the conditions tested. Thus, it is reasonable to assume that oocysts in milk products with lesser fat content than whole milk would be similarly inactivated by these treatments.

In summary, these data indicate that conditions of commercial pasteurization, 71.7°C for 15 s, are sufficient to destroy infectivity of *C. parvum* oocysts present in either water or milk. Application of these findings to other beverages or food products has not been tested, but the results should remain consistent as long as the time and temperature requirements are met.

ACKNOWLEDGMENTS

We thank V. K. Bunning for helpful discussion of pasteurization protocols and Kathleen Kelderman for manuscript preparation.

REFERENCES

1. **Anonymous.** 1989. Grade "A" pasteurized milk ordinance, p. 78. *In* Public Health Service, Food and Drug Administration, publication 229. U.S. Government Printing Office, Washington, D.C.
2. **Crawford, F. G., and S. H. Vermund.** 1988. Human cryptosporidiosis. Crit. Rev. Microbiol. **16:**113–159.
3. **Fayer, R.** 1994. Effect of high temperature on infectivity of *Cryptosporidium parvum* oocysts in water. Appl. Environ. Microbiol. **60:**2732–2735.
4. **Fayer, R., C. A. Speer, and J. P. Dubey.** 1990. General biology of *Cryptosporidium*, p. 1–29. *In* J. P. Dubey, C. A. Speer, and R. Fayer (ed.), Cryptosporidiosis of man and animals. CRC Press, Boca Raton, Fla.
5. **Fayer, R., and B. L. P. Ungar.** 1986. *Cryptosporidium* spp. and cryptosporidiosis. Microbiol. Rev. **50:**458–483.
6. **Garber, L. P., M. D. Salman, H. D. Hurd, T. Keefe, and J. L. Schlater.** 1994. Potential risk factors for *Cryptosporidium* infection in dairy calves. J. Am. Vet. Med. Assoc. **205:**86–91.
7. **LeChevallier, M. W., W. D. Norton, and R. G. Lee.** 1991. *Giardia* and *Cryptosporidium* spp. in filtered drinking water supplies. Appl. Environ. Microbiol. **57:**2617–2621.
8. **MacKenzie, W. R., N. J. Hoxie, M. E. Proctor, M. S. Gradus, K. A. Blair, D. E. Peterson, J. J. Kazmierczak, D. G. Addiss, K. R. Fox, J. B. Rose, and J. P. Davis.** 1994. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. N. Engl. J. Med. **331:**161–167.
9. **Millard, P. S., K. F. Gensheimer, D. G. Addiss, D. M. Sosin, G. A. Beckett, A. Houck-Jankowski, and A. Hudson.** 1994. An outbreak of cryptosporidiosis from fresh-pressed apple cider. JAMA **272:**1592–1596.
10. **O'Donoghue, P. J.** 1995. *Cryptosporidium* and cryptosporidiosis in man and animals. Int. J. Parasitol. **25:**139–195.
11. **Pontius, F. W.** 1993. Protecting the public against *Cryptosporidium*. J. Am. Water Works Assoc. **85:**18–123.
12. **Smith, H. V., and J. B. Rose.** 1990. Waterborne cryptosporidiosis. Parasitol. Today **6:**8–12.
13. **Ungar, B. L. P.** 1990. Cryptosporidiosis in humans (*Homo sapiens*), p. 59–82. *In* J. P. Dubey, C. A. Speer, and R. Fayer (ed.), Cryptosporidiosis of man and animals. CRC Press, Boca Raton, Fla.