

Do Iodine Water Purification Tablets Provide an Effective Barrier against *Cryptosporidium parvum*?

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U.S. Army Iodine Water Purification Tablets were tested to determine their efficacy against *Cryptosporidium parvum*, a protozoan resistant to chemical disinfection. Purified oocysts in phosphate-buffered water were treated with varying concentrations of iodine or with iodine tablets as per U.S. Army protocol. Neonatal mouse pups were then each inoculated with 10,000 treated oocysts, and 1 week later scored as infected or uninfected. Using this methodology, iodine tablets were found to be inadequate against *C. parvum* because the Army doctrinal dose of 560 mg min/L, calculated as 16 mg of I₂/L and 35 minutes of contact time, showed less than 1 log inactivation. A dose of 29 mg of I₂/L at the same contact time was required to achieve a 2 log inactivation.

Introduction

Soldiers require safe water—it is the fuel that keeps the war fighter powered. Today, American soldiers are still using iodine tablets to disinfect local water sources. Use of iodine was instituted in the early 1950s.¹ Although this practice is minimized through the use of Reverse Osmosis Water Purification Units and an aggressive logistics resupply doctrine; some units may find themselves still using iodine tablets as their only barrier of protection against a suite of unknown biological contaminants.

The biocidal efficacy of iodine against protozoa has been documented for *Giardia muris*.² However, the efficacy of iodine tablets against *Cryptosporidium* species has been reported in a limited number of studies. Using two different challenge waters and excystation to evaluate infectivity, Gerba et al.³ reported that iodine tablets inactivated only 10% of the oocysts tested given a 20-minute exposure and approximately 70% given a 240-minute exposure time. Black et al.⁴ report that infectivity was more reliable than in vitro excystation and vital dyes to measure infectivity in neonatal CD-1 mice.

Cryptosporidium is a genus of parasitic protozoa that contains several species capable of causing diarrhea that can sometimes be debilitating and for which there is no drug therapy or treatment. The disease is most commonly transmitted by the drink-

ing of water contaminated with infected feces. The transmission stage of *Cryptosporidium*, the oocyst, has been found to be ubiquitous in the waters of the United States; 65 to 97% of surface waters tested⁵ had an average concentration of 2.7 oocysts/L⁶, and *Cryptosporidium parvum* oocysts have been documented as the cause of several large waterborne outbreaks.⁷ In one outbreak, oocysts stored in municipal water aboard a Coast Guard cutter caused an outbreak of diarrhea in the crew of the vessel.⁸ It is believed that this agent is even more common in the developing world where sewage treatment is minimal in many communities. The regular infection of veterinary students who work with sick calves or foals infected with *C. parvum* suggests that healthy young adults who have not been previously exposed can be infected by this agent that routinely cycles in various animal hosts.⁹ People also develop disease when infected with the species *Cryptosporidium hominis*, which is transmitted between people.¹⁰ Previous mice infectivity studies have reported an infectious dose in which 50% of the tested population exhibit the sign of infection (ID₅₀) of 81.5 oocysts.¹¹ The number of individual oocysts required to attain an ID₅₀ in humans range from 9 to 1,042 oocysts.¹²

Iodine has been used as a disinfectant since World War I.¹³ After World War II, the current form of globaline tablets was first integrated into field uses for water treatment. The Army Iodine Water Purification Tablets release 8 mg/L iodine per tablet.¹⁴ Current guidelines specify two tablets per 1-quart canteen of water with a minimum contact time of 35 minutes to disinfect and prevent giardiasis.¹⁴

Among the species of iodine, elemental iodine (I₂) and hypoiodous acid (HIO) are the forms with the greatest biocidal efficacy.¹³ At pH 8.0 at 25°C, the concentration of I₂ and HIO are approximately equal.¹⁵ Several investigators have reported different levels of inactivation for I₂ based on the type of organism being studied. Initial reports indicate that I₂ may have an increased effect upon organisms protected by a membrane such as a spore or cyst.^{3,13} Iodine has also been reported to inactivate the eggs of common nematodes at concentrations greater than 100 mg/L.¹⁶ Wilson and Margolin¹⁷ reported that iodine, as 10% povidone, decreased excystation of oocysts, but did not affect the infectivity of the oocysts in an in vitro assay.

The objectives of the current study were: to assess the ability of globaline tablets to kill oocysts of *Cryptosporidium* when used at the concentration and contact time recommended by the current Army guidelines, to determine the required iodine concentration to induce a 2 log reduction in viable oocysts for 35 minutes of contact time, and to evaluate the tablets using a mice infectivity study.

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This manuscript was received for review in October 2003. The revised manuscript was accepted for publication in March 2004.

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Materials and Methods

Oocysts

Cryptosporidium parvum oocysts were obtained from naturally infected, 7- to 14-day-old calves in Tompkins County, New York. A sucrose/Percoll (Pharmacia, Uppsala, Sweden) flotation method was used to extract oocysts from calf feces.¹¹ After extraction, oocysts were stored at 4°C in water with antibiotics (100 U of penicillin G/sodium ml⁻¹, 100 µg of streptomycin sulfate/ml⁻¹, and 0.25 µg of amphotericin B/ml of suspension⁻¹) and were used within 1 month of collection.

Disinfection Procedure

Iodine stock solutions were prepared by combining 1.0 N standardized solution (Alfa Aesar, Ward Hill, Massachusetts) with phosphate buffer to make 1, 10, 50, 100, 500, and 1,000 mg of I₂/L. Army Disinfection tablets (NSN 6850-00-985-7,166, Jackson, Wisconsin) were ground and dissolved in phosphate buffer. Disinfection experiments were prepared by combining the iodine stock solution, oocyst stock suspension, and phosphate buffer in a 15-mL polypropylene centrifuge tube to attain the desired iodine concentration in a total volume of 10 mL. The resulting oocyst concentration was 10⁶ oocysts/10 mL and pH 7.06 and 7.15. The tubes were shaken at 25°C for 35 minutes, and the oocysts were collected by centrifugation (800 × g for 10 minutes). The supernatant was separated, and the pH and I₂ concentration (HACH, Iodine Method 8031) were measured.

Oocyst Preparation from Treated Water Samples

Treated oocysts were centrifuged at 800 × g for 10 minutes in 15-mL tubes. The tubes were decanted and the pellet was vortex mixed and resuspended with reverse osmosis water twice. The pellet was resuspended in 8 mL of reverse osmosis water, buffer A, and buffer B (1 mL of each) from a Dynabeads anti-*Cryptosporidium* kit (product no. 730.01/11, Dynal Biotech, Inc. Lake Success, New York). Resuspended Dynabeads (100 µL) were added to the tubes and rotated for 1 hour at room temperature. The tubes were placed onto a Dynal MPC-1 magnet and gently rocked by hand for 2 minutes. The tubes were opened and decanted while still attached to the magnet. The beads were released from the oocysts by the addition of 500 µL of 0.1 N HCl and the tube was vortex mixed for 10 seconds. The treated oocysts and beads were transferred to a clean siliconized microfuge tube and placed on a Dynal MPC-S magnet (without the magnetic strip in place) and allowed to stand for 5 minutes at room temperature. Each microfuge tube was removed and vortex mixed for 10 seconds. The tube was placed onto the Dynal MPC-S magnet. The magnetic strip was replaced in the tilted position and allowed to stand for 10 seconds. All liquid containing the released oocysts was then transferred separately into a clean microfuge tube.

Infective Dose Titration

Twenty timed-pregnant female CD-1 mice with litters (Charles River Laboratories, Wilmington, Massachusetts) were purchased and arrived on the day after delivery. One day later, the mouse pups (10 per litter) were inoculated with oocysts. For the dose titration curve in the mice pups, infective oocysts were diluted such that mice received 2 µL of water containing 0, 1, 10, 100, 1,000, or 10,000 oocysts. One litter received 0, 1, 10, 1,000, or 10,000 oocysts and two litters received 100 oocysts.

Inoculation of Mice to Test Effects of Treatment

Oocysts from the test samples were diluted in water such that there were a total of 5,000 oocysts/µL, and each mouse pup received 2 µL (10,000 oocysts). For the purpose of these dilutions, the concentration of oocysts in the microfuge tubes, in which the oocysts recovered from the test samples were placed, had been preweighed before sample addition. To determine the number of oocysts in each tube, individual tubes were placed onto a balance and the weight was brought to 1 g with water. A 10-µL sample was placed on a hemocytometer (Hausser Scientific Company, Horsham, Pennsylvania), counted, and recorded as number of oocysts per milliliter. The volumes in the microfuge tubes were corrected by centrifugation and the addition or subtraction of water using a micropipette on a gravimetric basis. Two litters of 10 mice each were given 10,000 oocysts that had been treated with 1, 10, 50, 100, 500, or 1,000 ppm of iodine. One litter of 10 mice each received 10,000 oocysts treated with the iodine tablet water.

Determination of Infectivity

Infectivity of oocysts was determined as described previously.¹¹ Briefly, 7 days after infection, mouse pups were euthanized for the collection of the cecum and colon. The contents of the cecum and colon were extracted and transferred to microfuge tubes containing 0.4 mL of specimen dilution buffer from the Alexon-Trend ProSpect *Cryptosporidium* Microplate Assay (Alexon-Trend, Ramsey, Minnesota). Samples were stored overnight at 4°C. From each tube, 0.2 mL was pipetted into individual wells of the Alexon-Trend ProSpect *Cryptosporidium* enzyme-linked immunosorbent assay plate. Plates were developed as described in the instructions. Positive wells turned yellow, and the plates' color change was recorded spectrophotometrically at 450 nm. Positive wells were considered those wells that were 2 SD above the background reading of negative control samples.

Statistical Analysis

The ID₅₀ for the batch of oocysts used was calculated based on a linear regression of the log of the oocyst dose against the proportion of mice infected using the generalized linear model procedure.¹⁸ Each mouse received at least 10,000 oocysts/mL to ensure an adequate number of oocysts was available for statistical analysis of the data. The Gompertz equation¹⁹ and nonlinear procedure¹⁸ were used to fit the observed data and to determine the relationship between iodine concentration and log decrease in infectious *C. parvum* oocysts. The one-way analysis of variance (ANOVA) assessing the efficacy of the globaline tablets versus various concentrations of free iodine was performed using MINTAB (Minitab Inc., State College, Pennsylvania).

TABLE I
INITIAL EXAMINATION OF EFFECTS OF GLOBALINE TABLETS

No. of Pups/Litter	Iodine Concentration	No. of Pups Positive	% Positive
6	Low dose (8 mg/L)	6	100
10	Medium dose (16 mg/L)	10	100
N/A	High dose (32 mg/L)	ND ^a	ND ^a

^aND, No data. Interference from inert ingredients prevented inoculation.

Results

Oocyst Infectivity

Based on a linear regression ($r^2 = 78.9$), a standard curve (log oocyst dose vs. proportion of mice infected) was prepared and indicated an ID_{50} of 79 oocysts. Of the 20 litters of 10 pups that each were given oocysts during the course of this trial, only 2 litters lost pups after inoculation. It is assumed that the pups expired and were subsequently ingested by the mother mouse. One of the two litters that received 100 oocysts lost two pups, and the litter receiving oocysts that had been treated with the globaline tablets lost one pup. Pup loss is not unexpected and appears to be a random factor associated with using neonatal mice that are handled as part of the experimental process.

Preliminary Experiment with the Globaline Tablets

A preliminary experiment using iodine tablets showed there was no effect on *C. parvum* when exposed to two tablets (16 mg of I_2/L) for 35 minutes (Table I). Higher concentrations could not be tested in this infectivity assay due to interferences from the inert ingredients (83.3% by manufacturer standards) in the tablets. In the subsequent studies, aqueous iodine solution was used to achieve the higher concentrations of iodine desired.

Comparison of Globaline Tablets to Free Iodine

The influence of iodine on *C. parvum* inactivation is presented in Table II. The oocyst survival as a function of iodine capsule is presented in Figure 1. The data indicated a sigmoidal form and was fit with the Gompertz equation to generate log calculated oocyst concentration: $F(t) = A_{exp}(-e^{\beta - \kappa x})$, where $A = 1$, $\beta = 5.950 \pm 0.1996$, $\kappa = 1.854 \pm 0.0591$, $F(t)$ is the proportion of mice infected by oocysts that was converted to log number of infectious oocysts, and x is the natural log of the iodine concentration.

Based on this nonlinear regression, ~30 ppm iodine resulted in 50% of the mice inoculated with a batch of 10^4 oocysts becoming infected, or a reduction of the viable oocyst concentration to ~79 oocysts, or at least a 2 log reduction of the original inoculum. The model predicts that an iodine concentration of

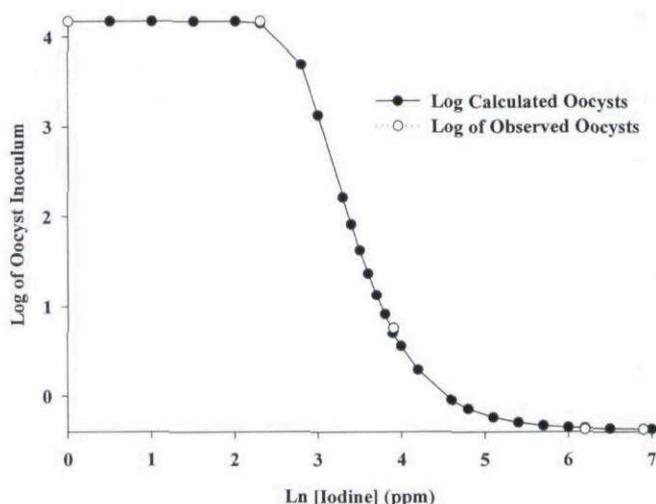


Fig. 1. Dose-response curve of mice infectivity data.

~123 ppm will reduce the number of infectious oocysts to less than 1; less than the range reported to cause human infection.¹²

The ANOVA comparing the percentage of mouse pups per litter given oocysts treated with globaline tablets or iodine revealed that the effects of the globaline tablets were not significantly different from oocysts treated with 0, 1, or 10 ppm iodine (Fig. 2, $p < 0.0005$). Oocysts treated with more than 50 ppm iodine demonstrated reduced infectivity. There was not an observed reduction in oocyst infectivity for concentrations greater than 100 ppm as demonstrated by the ANOVA procedure. The results of the ANOVA (Fig. 2) are in good agreement with the Gompertz equation (Fig. 1) in the vicinity of 30 ppm iodine.

Discussion

These studies have shown that the current Army guidelines for use of the globaline tablets for the treatment of drinking water will not inactivate the majority of oocysts that might be

TABLE II

EFFECTS OF IODINE CONCENTRATION ON THE PERCENTAGE OF POSITIVE MOUSE PUPS PER LITTER

Target ppm Iodine	Actual ppm Iodine ^a	CT (mg min/L)	No. of Positive Pups ^b	Total Pups Examined	Percentage of Positive Pups
0	0	0.0	10	10	100
1	0.42	14.7	10	10	100
1	0.46	16.1	10	10	100
10	1.0	35.0	10	10	100
10	1.52	53.2	10	10	100
50	11.90	416.5	3	10	30
50	14.18	496.3	2	10	20
100	30.6	1,071.0	1	10	10
100	34.4	1,204.0	0	10	0
500	228	7,980.0	0	10	0
500	260	9,100.0	0	10	0
1,000	598	20,930.0	0	10	0
1,000	504	17,640.0	0	10	0
16 ^c	4.3	150.5	8	9	88.9

^a Measured after the 35-minute reaction time.

^b Each mouse pup received 10,000 treated oocysts.

^c Globaline tablets.

Percentage of positive mouse pups given 10,000 oocysts

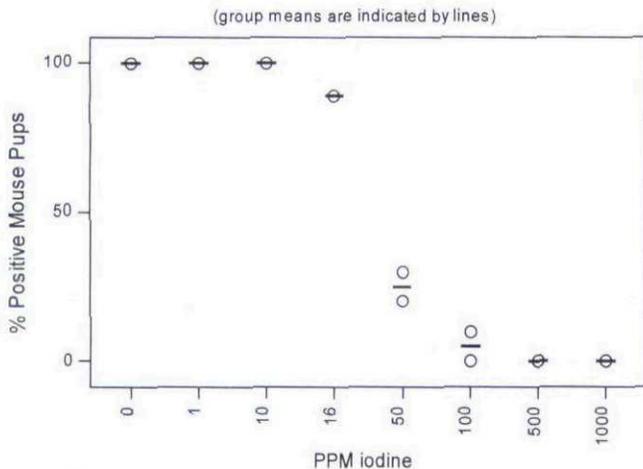


Fig. 2. ANOVA analysis of mice infectivity data. Circles represent the percent positive per liter.

present within contaminated water. Thus, the existing Army recommendations for water treatment may not be adequate to protect soldiers operating in an austere environment from developing infections from *Cryptosporidium* species. There was no effect observed on reducing infectivity using the tablets as prescribed by the manufacturer and the Army. This finding suggests that iodine tablets might be less effective in natural waters. An increase in iodine dose to at least 29 ppm (or 1,015 mg min/L) is required to achieve 2 log (99%) inactivation with iodine.

Gerba et al.³ reported that increased contact times do not significantly increase the inactivation of the oocysts from 10% inactivation with a 20-minute contact time to 66 to 81% inactivation at a 240-minute contact time. Simply increasing the contact time for the iodine to inactivate oocysts does not seem to be a feasible solution to provide adequate protection. Interestingly, Thitasut¹⁶ reported that the effects of iodine on the inactivation of eggs of the nematodes, *Ascaris lumbricoides*, *Toxocara canis*, and *Trichuris muris* was greater at 15°C than it was at the higher temperatures of 20°C, 25 to 30°C, or 37°C. The influence of temperature on inactivation of *C. parvum* oocysts merits further investigation. However, it is believed the applicability of temperature on inactivation may not be realistic since these tablets are used when soldiers are operating under austere conditions.

The ID₅₀ (79 oocysts) for the oocysts used in this work is within previously published ranges.¹¹ In this study, all mice that received 10,000 oocysts with no disinfection treatment did become infected with *C. parvum*; thus, the comparisons between the different iodine treatment levels were not affected by a lack of infectivity.

This work demonstrates that at least 29 ppm (or 1,015 mg min/L) is required to achieve 2 logs of inactivation with iodine. As increases in doses are considered, ancillary studies would be required to deter-

mine the impact of increased iodine concentrations upon sensitive populations such as those with existing thyroid conditions. Increased oocyst concentrations would be required to investigate the influence of iodine at higher concentrations. However, higher iodine concentrations are not typically used to disinfect drinking water.^{13,20} Because iodine may not be an effective means of inactivating *C. parvum*, additional treatment mechanisms, e.g., filtration, should be considered when using this disinfectant.

Acknowledgments

This work was funded by a grant from the U.S. Army Project Manager-Soldier's Systems. Statistical advice was given by Dr. Dwight Fisher, Range Scientist at the USDA-Agricultural Research Service, and J. Phil Campbell, Senior, Natural Resource Conservation, Watkinsville, GA.

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