Comparison of Animal Infectivity and Excystation as Measures of
Giardia muris Cyst Inactivation by Chlorine

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In this study, in vitro excystation and mouse infectivity were compared as methods for quantitatively
determining the viability of Giardia muris cysts before and after exposure to free residual chlorine. The mouse
infectivity results show that very few cysts (1 to 15) constitute an infectious dose. The results of the inactivation
studies indicate that in vitro excystation is an adequate indication of G. muris cyst infectivity for the host and
can be used to determine the effects of disinfectants on cyst viability.

Over the past several years, giardiasis has consistently been one of the most frequently occurring waterborne dis-
ease transmitted by drinking water in the United States (3). Waterborne transmission occurs via the cyst stage of the
etiologic agent Giardia lamblia. Most outbreaks occur in drinking water systems that rely only on disinfection for
treatment. To assess the adequacy of this treatment, information on the effects of disinfectants on the viability of cysts
is needed.

The development of in vitro methods for measuring cyst viability (2, 12) has made it possible to determine the effects
of chlorine (5, 11) and other disinfecting agents (6, 10) on cyst viability. Difficulties encountered in obtaining adequate
supplies of G. lamblia cysts for such studies led to the use of a model cyst system, employing the cysts of Giardia muris,
which are infective for mice (13). Modified excystation procedures for G. muris cysts have been developed (14) and
comparative disinfection data on G. lamblia and G. muris have been reported for chlorine (11) and other disinfectants
(8, 18). The adequacy of in vitro excystation for measuring loss of animal infectivity of cysts after exposure to disinfect-
ants has not been established. This study was undertaken to compare the two methods and, thus, to assess the validity of
in vitro excystation as an indicator of cyst inactivation by disinfectants.

G. muris cysts (initially isolated from infected mice supplied by David Stevens, Department of Medicine, University
Hospital, Cleveland, Ohio) were produced in outbred female Swiss albino mice (CF-1). These cysts were used as the
initial inoculum and were subsequently passaged at weekly intervals. Cysts present in fresh stools obtained from
infected mice were separated by flotation on 1 M sucrose, washed twice by centrifugation, and stored in distilled water
at 5°C. Cyst densities were determined by hemacytometer count and adjusted to appropriate concentrations for use in
the disinfection experiments.

All disinfection experiments were conducted at 5°C at pH 7.0 and a free chlorine residual of 1 mg/liter. Stock chlorine
solutions were prepared from sodium hypochlorite as described previously (11). Chlorine determinations were done at
the beginning of each experiment and at the end of each exposure time using the N,N-diethyl-p-phenylenediamine
colorimetric method (1). Chlorine levels remained constant throughout the course of the experiments. The disinfection

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cysts). For each experiment, the chlorinated sample showing ~90% or more cyst inactivation by excystation was selected for determination of mouse infectivity by determination of the 50% infective dose (ID$_{50}$).

For the animal infectivity studies, the cyst concentration of the selected reconstituted chlorinated-exposed cyst suspension was adjusted to contain $5 \times 10^5$ cysts per ml. Serial decimal dilutions were prepared to give inocula of 0.2 ml containing $10^2$ (undiluted), $10^3$, $10^4$, $10^5$, $10^6$, and $10^7$ cysts per mouse. Ten mice were each inoculated per os (by intubation with a 22-gauge cannula) with each dilution. Each mouse was caged individually. Individually caged negative control mice were evenly dispersed among the other cages to check for intercage contamination. Control cyst suspensions, not exposed to chlorine were prepared in a similar manner and inoculated into mice at doses of $10^3$, $10^4$, $10^5$, $10^6$, and $10^7$ cysts per mouse. ID$_{50}$ titers of control and chlorine-exposed cyst suspensions were determined by using the Spearman-Karber (4) method. Since the number of cysts in each dose was known through microscopic count, the actual number of cysts constituting one ID$_{50}$ could be calculated. The percentage of cysts inactivated by chlorine was determined by dividing the number of control cysts needed for one ID$_{50}$ by the number of exposed cysts required for one ID$_{50}$.

Unexposed cyst viability (as determined by percent excystation) is compared with mouse infectivity (as determined by number of cysts per ID$_{50}$) in Table 1. It is evident that excystation percentages were higher using the modified procedure (14) than when using the method of Bingham and Meyer (2), which was originally developed for G. lamblia excystation. The percent excystation attained with the modified method ranged from 91 to 99%. In this same set of samples, the number of cysts required for one mouse ID$_{50}$ ranged from 0.13 to 15.85 cysts, with a mean of 5.02 cysts. No relationship between percent excystation and number of cysts per ID$_{50}$ is evident. The ID$_{50}$ results show greater variability than the excystation results because of larger error terms inherent in the ID$_{50}$ method.

The G. muris cyst infectivity data are in good agreement with the results of earlier much more limited studies involving feeding of G. lamblia cysts to humans (9). These data indicated that the G. lamblia ID$_{50}$ for humans was $\leq$10 cysts. More recent studies with G. muris cysts (15), also based on very limited data, also indicated an infectious dose of $\sim$10 cysts. Thus, our results substantiate the concept that the minimal infectious dose for these pathogens is very low.

The results of a series of seven experiments comparing cyst inactivation by chlorine as determined by excystation and by mouse infectivity are shown in Table 2. The results show that the exposure to chlorine under the experimental conditions used resulted in similarly large reductions in both mouse infectivity and percent excystation. The percent inactivation ratios (excystation/infectivity) of the two methods are near unity, and neither method consistently showed a higher degree of inactivation than the other method. Kasprzak and Majewska (7), in studies of G. muris survival in stored water, pointed out that the G. muris system, because of the higher in vitro excystation percentages attainable, offered a good approach to the in vitro determination of Giardia cyst viability. Some of their data, however, based on cyst infectivity for rats, indicated that even when in vitro excystation levels were zero, infection of some rats occurred. Their studies did not involve the use of disinfectants.

Stringer (16) developed a viability assay based on in vitro excystation for Entamoeba histolytica. He noted the advantages of this assay system over others used earlier, including staining methods that lacked specificity and quantal assay culture techniques that were tedious and time consuming. In later, more extensive studies, Stringer et al. (17) used the excystation method to determine the effectiveness of halogen disinfectants for inactivation of E. histolytica cysts. To our knowledge, excystation and animal infectivity for determining viability of E. histolytica cysts exposed to disinfectants have not been compared. The E. histolytica data apparently were implicitly accepted and their validity borne out by field experience.

The excystation bioassay system offers a simple, reliable method for determining percent viability in a population of

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>No. of cysts/ID$_{50}$</th>
<th>% Excystation</th>
<th>% Inactivation determined by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Chlorine exposed</td>
<td>Control</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>2.51</td>
<td>12.59</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>15.85</td>
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<tr>
<td>6</td>
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<tr>
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</tr>
<tr>
<td>10</td>
<td>60</td>
<td>2.51</td>
<td>12.59</td>
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$^a$ Corrected (see text).
cysts in contrast to animal infectivity bioassays, which are much more complex, expensive, and time consuming. The results of our study provide substantial support for the validity of using excystation data to indicate the inactivation of *Giardia* cysts by drinking-water disinfectants such as chlorine.

LITERATURE CITED


