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Determination of *Giardia lamblia* Cyst Infective Dose for the Mongolian Gerbil (*Meriones unguiculatus*)

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The purpose of this study was to determine the 50% infective dose for *Giardia lamblia* (CDC:0284:1) cysts in Mongolian gerbils (*Meriones unguiculatus*). The $\log_{10}$ 50% infective dose results calculated by probit analysis and the Spearman-Karber method were 2.45 and 2.50, respectively.

Giardiasis is a frequently occurring waterborne disease transmitted by drinking water in the United States (2). Waterborne transmission occurs via the cyst stage of the etiologic agent, *Giardia lamblia*. Methods for detecting cysts presently do not determine viability. An approach for determining viability has employed a Mongolian gerbil model (1). The purpose of this study was to determine the 50% infective dose (ID_{50}) for a *G. lamblia* isolate that routinely infects gerbils and compare it with human infectivity data.

Female Mongolian gerbils (*Meriones unguiculatus*), 4 to 6 weeks of age, were obtained from Tumblebrook Farm (West Brookfield, Mass.). All animals used in this study were quarantined in a separate room until exposure to the infective agent. *G. lamblia* CDC:0284:1 cysts were obtained from Govinda S. Visvesvara (Division of Parasitic Diseases, Centers for Disease Control, Atlanta, Ga.) and produced in Mongolian gerbils (1). This study was conducted with a single cyst preparation isolated from infected gerbil feces by flotation on 1 M sucrose. Further purification of the cysts included an additional float on 0.85 M sucrose followed by velocity sedimentation on a Percoll gradient (6). The cysts were stored in 0.01% (vol/vol) Tween 20 at 4°C for 6 days prior to use in the infectivity study.

Cyst densities were determined by hemocytometer counting. For the infectivity studies, dilutions of the stock cyst preparation were made in 0.01% (vol/vol) Tween 20 to yield the required number of cysts in a 0.2-ml total volume. Ten gerbils were inoculated per os with a 22-gauge feeding cannula for each dose. After exposure, all animals were caged individually. Negative control gerbils, which received 0.2 ml of saline rather than cysts, were randomly dispersed among the other cages to check for intercage contamination. Seven days postexposure, each gerbil was examined for fecal cyst production by zinc sulfate (specific gravity, 1.18) flotation. Fourteen days postexposure, the gerbils were again examined for fecal cyst production. Those animals failing to show fecal cysts after the second examination were sacrificed to check for the presence of *G. lamblia* trophozoites in the small intestine. The entire small intestine, which was transferred to a 100-mm-diameter petri plate containing 15 ml of 1× Hanks' balanced salt solution at 4°C, was cut open longitudinally with iris scissors. After the dish was chilled for a minimum of 10 min at 4°C in a refrigerator, the small intestine was agitated in the dish with a tissue probe. The dish was brought to room temperature for a minimum of 10 min before being observed for trophozoites with an inverted phase-contrast microscope at a magnification of 100×. Gerbils were considered to be infected when trophozoites were found in the small intestine and/or cysts were found in the fecal material.

The results of the infectivity study are summarized in Table 1. None of the control animals became infected. The ID_{50} is defined as the dose which produces an infection in 50% of the population. The ID_{50} was calculated by two commonly used methods, probit analysis and the Spearman-Karber method (3). Probit analysis yielded a $\log_{10}ID_{50}$ of 2.45, with 95% upper and lower confidence intervals for the $\log_{10}ID_{50}$ of 1.78 and 3.04, respectively. The $\log_{10}ID_{50}$ as determined by the Spearman-Karber method was 2.50, with 95% upper and lower confidence intervals of 2.20 and 2.80, respectively. These values are much larger than the $\log_{10}ID_{50}$ reported for *G. muris* cysts. In the murine model, only 1 to 15 cysts ($\log_{10}ID_{50}$ of 0 to 1.18) are needed to produce infection (4). There is a similar disparity when these results are compared with those of limited studies involving feeding *G. lamblia* cysts to humans (5). The human feeding studies indicated that the minimal infective dose for *G. lamblia* in humans is ≤10 cysts. The higher number of *G. lamblia* cysts required for gerbil infection appears to be related to the use of the gerbil as an aberrant host which does not readily become infected and thus does not appear to mimic what occurs in the natural host.

A least-squares regression analysis, with 95% confidence intervals, illustrates the relationship between the number of cysts inoculated and the percent of infection (Fig. 1). The estimated $\log_{10}ID_{50}$ was 2.50, with a coefficient of correla-

TABLE 1. Infectivity of *G. lamblia* cysts serially diluted and inoculated into Mongolian gerbils

No. of cysts inoculated	No. of animals infected	No. of animals not infected	Infectivity ratio
0	0	10	0/10
10	1	9	1/10
20	0	10	0/10
40	1	9	1/10
80	4	6	4/10
100	4	6	4/10
160	4	6	4/10
320	5	5	5/10
640	6	4	6/10
1,000	6	4	6/10
10,000	10	0	10/10

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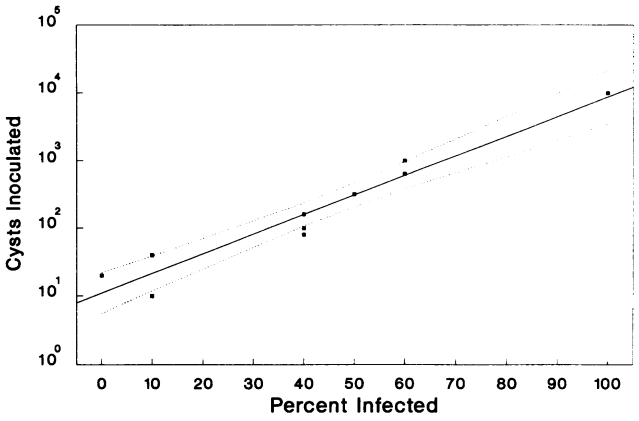

FIG. 1. Regression analysis of *G. lamblia* cyst inoculum versus percent gerbil infection (r = 0.9689). Dotted lines indicate 95% confidence intervals.

tion (r) of 0.9689. The $\log_{10}ID_{50}$ derived by this method is similar to those calculated by the other two methods used for calculating the 50% endpoint. At the 10^4-cyst inoculum level, 100% of the animals were infected. This level of inoculum is routinely used for maintenance of the parasite cycle in the gerbil model (1).

Excystation and animal infectivity have been used to evaluate chemical inactivation of *G. muris* cysts (4). There is a desire and need to work with *G. lamblia* cysts, the human etiologic agent, rather than *G. muris* cysts in chemical inactivation studies. However, the ID_{50} for this particular strain of *G. lamblia* in the gerbil host is in sharp contrast to the minimal infective dose reported for the human host. This discrepancy is apparent in spite of the wide confidence intervals inherent in such animal infectivity studies. Recent studies also have shown that not all *G. lamblia* isolates can infect gerbils (7). These findings call into question the use of gerbils as a model system for human giardiasis. Further research is necessary to determine whether other strains that infect humans have similar ID_{50} values in the gerbil system. Strains with ID_{50} values similar to those seen with human hosts, if available, would be more applicable for research studies in which animal infectivity is used to determine cyst viability.

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