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Variable Infectivity of Human-Derived *Giardia lamblia* Cysts for Mongolian Gerbils (*Meriones unguiculatus*)

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To determine whether gerbils can be used as a suitable animal model for giardiasis, we attempted to infect Mongolian gerbils with cysts of *Giardia lamblia* isolated from the stools of 10 humans with symptomatic and asymptomatic giardiasis. We obtained 100% infection with one isolate (CDC:0284:1), as evidenced by the presence of numerous trophozoites in the intestines of the gerbil and cysts in the feces. Cysts from four patients were not infective, while cysts from the other five patients produced infections in 11 to 75% of the animals. On the basis of these and other experiments, we concluded that (i) only certain isolates of human *G. lamblia* infect gerbils, colonize the intestine, and complete their life cycle by undergoing differentation into cysts; (ii) the infection could last for about 39 days, but the animals excreted maximum numbers of cysts on about day 13 postinfection; (iii) the pattern of cyst excretion was irregular, and some gerbils, like humans, excreted cysts intermittently; (iv) the minimum number of cysts needed to establish an infection in 50% of the gerbils was 100; and (v) only certain strains retained the ability to infect gerbils even after repeated animal passage.

Giardia lamblia, worldwide in its distribution, is the most frequently identified intestinal protozoan parasite in public health laboratories in the United States (2). Giardiasis is endemic in various parts of the United States, and the prevalence rate in adults can be as high as 24% (5). During the past 15 years in the United States, many outbreaks of waterborne giardiasis, affecting thousands of people, have been described (4). Person-to-person transmission of the parasite also has been frequently described, most commonly in day care centers (9). Because of the increased interest in this parasite in recent years, several investigators have attempted to establish an animal model for this disease. Of the various animals used as hosts for *G. lamblia*, including rats (3, 12), suckling and adult mice (7, 13), dogs (6), rabbits (11), Mongolian gerbils (1), and cats (8), consistently good results have been obtained only with suckling mice and gerbils. We therefore elected to use gerbils for our studies because of their larger size, easier handling and manipulation, and increased output of cysts. The purpose of this study was to determine (i) whether cysts obtained from patients with either symptomatic or asymptomatic giardiasis can establish infection by colonizing gerbil intestines and complete their life cycle to produce viable cysts; (ii) the duration of infection and the excretion pattern of cysts; and (iii) the minimum number of cysts necessary to establish infection. The ability to passage the same strain continuously in gerbils was also studied.

MATERIALS AND METHODS

Six- to eight-week-old male Mongolian gerbils (*Meriones unguiculatus*), weighing 40 to 50 g, were obtained from Tumblebrook Farm (West Brookfield, Mass.). After 3 days of acclimatization, all animals were given 20.0 mg of metronidazole per day orally for 3 days as described by Belosevic et al. (1). We determined that the gerbils were free of *Giardia* infection by microscopically examining wet mounts of fresh stools collected on three consecutive days, starting on day 7 after metronidazole treatment. Periodically, randomly selected treated and untreated gerbils were killed, the small intestines were slit open longitudinally, the entire surface was scraped, and the intestinal contents were suspended in 10 ml of phosphate-buffered saline, pH 7.2, and examined for *Giardia* trophozoites. The stool specimens were also examined for cysts. We found that all (>50) of the randomly selected untreated animals were giardia free, so treatment with metronidazole was discontinued.

Experimental protocols. (i) Sources, collection, and purification of cysts. *G. lamblia* cysts were obtained from stool samples of 10 patients (7 symptomatic, 3 asymptomatic) at the Veterans Administration Medical Center and Grady Memorial Hospital in Atlanta. The stool samples were mixed with 0.85% sterile saline (saline) and filtered through three layers of cheesecloth, and the filtrate was centrifuged at 250 × *g* for 10 min in a refrigerated centrifuge (Damon CRU model 5000; ICR, Needham Heights, Mass.) at 4°C. The supernatant was aspirated, and the sediment (ca. 2 to 3 ml) was suspended in 50 ml of saline and centrifuged as described above. After one additional saline wash, the sediment was suspended in 5 ml of saline, layered over 15 ml of 0.8 M sucrose solution in a 50-ml conical centrifuge tube, and centrifuged at 1,565 × *g* for 15 min. The top layer (ca. 0.5 to 0.6 ml) was carefully removed with a sterile Pasteur pipette and transferred to a 50-ml centrifuge tube, and saline was added to 50 ml, mixed well, and centrifuged for 10 min at 250 × *g*. The supernatant was aspirated, and the sediment was mixed well with 50 ml of saline and counted in a hemacytometer. This suspension was then centrifuged again, and the pellet containing the cysts was stored in saline at 4°C. Cysts of *G. lamblia* from infected gerbils were handled similarly. Gerbils were inoculated orally with a known number of cysts suspended in 0.1 ml of saline. They were housed two or three animals per cage and fed ad libitum on Centers for Disease Control mouse diet wafer (Zeigler Bros. Inc., Gardners, Pa.). Fecal pellets were collected from each cage, beginning on day 1 postinfection (p.i.). Fresh stools were microscopically examined by direct wet mount for *G. lamblia* cysts, and if cysts were detected, the animals were individually placed in separate cages.

(ii) Quantification of cysts excreted by gerbils. We collected all stool pellets from individually housed gerbils over a 24-h

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period. To prevent the stool from drying out during this period, 4-mm-thick absorbent cotton pads were thoroughly wetted with tap water and placed at the bottom of each cage. Stool pellets trapped on this pad were scraped from the surface of the pad and transferred to a 50-ml centrifuge tube containing 30 ml of saline and were either kept for 2 h at 4°C or vortexed for 2 min immediately. Cysts were concentrated on 0.8 M sucrose and counted as described above.

Duration of infection and excretion pattern of cysts. Each of five gerbils sequestered in individual cages was infected with 5,000 cysts derived from donor 4 after eight passages in gerbils. The entire stool sample from each animal was collected daily from days 1 to 29 p.i. The stool samples were weighed individually and examined microscopically for cysts, and the cysts were concentrated on sucrose as described above. All animals were killed on day 29 p.i., and the intestinal contents were examined for trophozoites as described above.

Minimum number of cysts necessary to cause infection. To determine the fewest number of cysts that would cause infection to the gerbils, we divided the animals into several groups, and each group was given a known number (0, 10 to 15, 40 to 50, 100, or 1,000 to 50,000) of 3- to 7-day-old cysts. Cysts from different passages of donor 4 were used, and all animals were killed between days 6 and 15 p.i. Because these experiments were designed to determine the infectivity of cysts, no efforts were made to identify or isolate cysts in the gerbil stools. Infectivity was determined by the presence of trophozoites in the intestines.

Effect of aging on infectivity of cysts. To study the effect of aging on the infectivity of cysts, we kept sucrose-concentrated cysts from donor 4 in saline at 4°C for up to 69 days. Cysts were tested for infectivity twice during this period, after 34 and 69 days of storage. Twelve gerbils received 100,000 34-day-old cysts each, and their stools were checked for cysts from days 8 to 33 p.i. In another experiment, four gerbils each received 100,000 cysts stored at 4°C for 69 days. These animals were killed 6 days later, and their intestinal contents were examined for trophozoites of *G. lamblia*.

RESULTS

Infection of gerbils with 10 different isolates of *G. lamblia*. Of the 10 human isolates tested for infectivity in gerbils, only 1, derived from donor 4, an asymptomatic person, infected all 12 animals, completed its life cycle, and produced cysts (Table 1). The duodenum and the proximal region of the ileum were covered with trophozoites. *Trichomonas* spp. were also seen, rarely in the duodenum but in moderate-to-large numbers in the ileum. The distal region of the ileum also had *Cercomonas* sp. and *Entamoeba muris*.

Of the nine other isolates fed to gerbils, cysts from donors 1 and 6 (both asymptomatic) and 8 and 10 (both symptomatic) produced no infection since neither trophozoites in the duodenum nor cysts in the feces were seen.

Cysts from donor 2 produced infection in 9 (75%) of 12 animals when infected with relatively young (6- to 8-h-old) cysts (10,000 per gerbil), as reflected by moderate numbers of trophozoites in the small intestines. However, only 4 (33%) of the 12 animals excreted cysts, and the numbers of cysts were low. Cysts isolated from the same donor, after he had undergone a course of therapy, were fed to 12 gerbils, but the inoculum size was increased to 50,000 cysts per gerbil. Only 4 (33%) of the 12 animals were infected, as

TABLE 1. Infectivity of *G. lamblia* cysts from 10 human donors for Mongolian gerbils

Donor no. (sex)[a]	Cyst age (days)	Inoculum (10^3)	No. of gerbils inoculated	No. positive for trophozoites at necropsy (days p.i.)	No. of gerbils that excreted cysts
1 (M)	18	10	5	0 (14)	0
2 (M)	0.25–0.33	10	12	9 (9–17)	4
	10	50	12	4 (14–17)	0
3 (F)	2	100	9	1 (12–19)	1
4 (M)	14	100	12	12 (12)	12
	34	100	12	8 (18–35)	12
	69	100	4	0 (6)	Not done
5[b]	3	100	8	3 (18–38)	2
6 (F)	10	100	2	0 (15)	0
7 (F)	4	100	9	0 (13)	7 (Rare to few cysts from days 7–13 p.i.)
		10	10	0 (13)	6 (Rare to few cysts on days 7–13 p.i.)
	18	250	10	1 (10–13)	1
8 (M)	14	49	1	0 (19)	0
9 (F)	0.25	100	9	2[c] (17)	6 (Few cysts on days 7–11 p.i.)
10 (M)	9	100	6	0 (22)	0

[a] M, Male; F, female. Donor 4 provided strain CDC:0284:1.
[b] Pool of three patients.
[c] Only five of nine were necropsied.

evidenced by moderate numbers of trophozoites seen in the intestines. None of the 12 animals, however, excreted cysts, as evidenced by daily stool microscopy for 17 days.

None of the 19 animals infected with relatively young (4-day-old) cysts from donor 7 were found to have trophozoites in the duodenum on day 13 p.i. However, 13 (68%) of 19 animals excreted cysts (rare to few) from days 7 to 13 p.i. Only 1 of 10 gerbils became infected when a large number (250,000) of stored (18 days at 4°C) cysts from the same donor were fed to the animals. This gerbil excreted rare cysts. Feces excreted on days 7 to 13 by this gerbil were pooled and concentrated on sucrose and used to reinfect two gerbils (40,000 per animal). These gerbils excreted rare cysts on days 7, 12, and 13 p.i.

Cysts (6 h old) from donor 9 were fed to nine gerbils (100,000 per gerbil). Only two of five gerbils killed 17 days p.i. had trophozoites in their guts. The other four gerbils were not examined. Six gerbils, however, excreted cysts. These cysts were pooled and used to reinfect nine other gerbils (100,000 per gerbil). Only five of nine animals were positive for trophozoites in their duodena and excreted no or rare cysts in their feces on days 5 to 8 p.i.

Even though some of the animals inoculated with cysts from donors 2, 3, 5, 7, and 9 excreted cysts, the number of cysts excreted was so small that it was decided to end further experimentation with these isolates.

Cysts excreted by gerbils infected with cysts from donor 4 were processed for further experimentation. Irrespective of the number of cysts (3,000 to 100,000) injected, 85 of 86 gerbils became infected and began to excrete cysts from day 5 p.i. until the day when they were killed (days 12 to 33 p.i.). Only 1 of 18 animals that received an inoculum of 3,000 cysts (from passage 2) did not become infected (Table 2).

TABLE 2. Serial passaging of *G. lamblia* cysts from donor 4 in Mongolian gerbils

Source of cysts and inoculum (10^3)	No. of gerbils inoculated	No. positive for trophozoites at necropsy (days p.i.)	No. of gerbils that excreted cysts	Cyst passage no.
Human donor 4				
100	6	6 (12)	6	1
10	6	6 (12)	6	
Passage 1				
100	6	5 (21)	5	2
50	6	6 (21)	6	
5	3	3 (24)	3	
Passage 2				
3	18	17 (19)	17	3
9	3	3 (19)	3	
Passage 3				
4	4	4 (13)	4	4
6	4	3[a] (7)	ND[b]	
Passage 4, 4.5	14	13 (28)	14	5
Passage 5, 4.3	4	3 (33)	4	6
Passage 6, 5	6	2 (32)	6	7
Passage 7, 5	6	ND	6	8

[a] One of four died before necropsy.
[b] ND, Not done.

TABLE 3. Infectivity of *G. lamblia* cysts serially passaged in Mongolian gerbils

Expt no. and inoculum	Passage no.	No. of gerbils inoculated	No. positive for trophozoites at necropsy (days p.i.)
1			
100	1	2	2 (6)
1,000	1	2	2 (6)
10,000	1	2	2 (6)
50,000	1	2	2 (6)
0		3	0 (6)
2			
15	2	4	0 (7)
45	2	4	0 (7)
100	2	4	0 (7)
0		3	0 (7)
3			
10	3	4	0 (10)
100	3	4	3 (10)
0		3	0 (10)
4			
10	7	4	0 (6)
50	7	4	2 (6)
100	7	4	2 (6)
0		3	0 (6)
5			
40	8	8	0 (7)
0		2	0 (7)
6			
100	9	6	4 (11)
0		3	0 (15)
7			
50	10	10	0 (7)
0		2	0 (7)
8			
100	12	6	4 (13)
0		2	0 (13)
9			
10	15	8	0 (12)
0		3	0 (12)
10			
10	16	8	0 (13)
0		2	0 (13)

The results of the experiments to determine the minimum number of cysts necessary to cause infection (Table 3) can be summarized as follows. All 6 of the gerbils inoculated with 1,000 or more cysts each were positive for *G. lamblia* trophozoites; 15 (58%) of 26 gerbils inoculated with 100 cysts had trophozoites in their intestine; 2 (8%) of 26 gerbils inoculated with 40 to 50 cysts were positive, and none of 28 gerbils inoculated with 10 to 15 cysts were positive at necropsy. None of 26 uninoculated animals were positive for *G. lamblia* trophozoites.

The duration of infection was at least 25 days, as evidenced by cyst excretion. The pattern of cyst excretion was very irregular; for example, animal 4 started excreting cysts on day 5 p.i., animals 1 and 2 started on day 6 p.i., and animals 5 and 3 began to excrete cysts on days 7 and 10 p.i.,

respectively. Gerbils 1 through 4 excreted peak numbers of cysts on day 13 p.i., and the maximum number of cysts excreted varied from 5×10^6 to 15.4×10^6. Gerbil 5 was unique in that it was negative for cysts longer (10 days) than it was positive for cysts (8 days) (Fig. 1). All animals stopped excreting cysts from days 25 to 29, when they were necropsied. The weight of the gerbil stool pellets varied from animal to animal, and the average weight was calculated to be 3.1 g per gerbil for a 24-h period. The total cyst output during peak cyst excretion ranged from 1.6×10^6 to 5×10^6/g of feces. All animals were still positive for trophozoites at necropsy at 29 days p.i.

The results of the experiments using isolate 4 to study the effect of aging on the infectivity of cysts revealed that the cysts could be stored in the cold for up to 34 days with no loss of infectivity. Stools collected from 12 gerbils that each received 100,000 34-day-old cysts were positive for cysts from days 8 to 18 p.i. Three animals were killed on day 18; all were positive for *G. lamblia* trophozoites. The feces of the other nine gerbils were examined for cysts periodically, and all were positive for cysts up to day 33 p.i. These gerbils were killed on day 35, when six of nine were positive for trophozoites of *G. lamblia* in their duodena. When 100,000 cysts stored at 4°C for 69 days were injected into each of four gerbils, no evidence of infection could be found when the animals were killed and examined 6 days later.

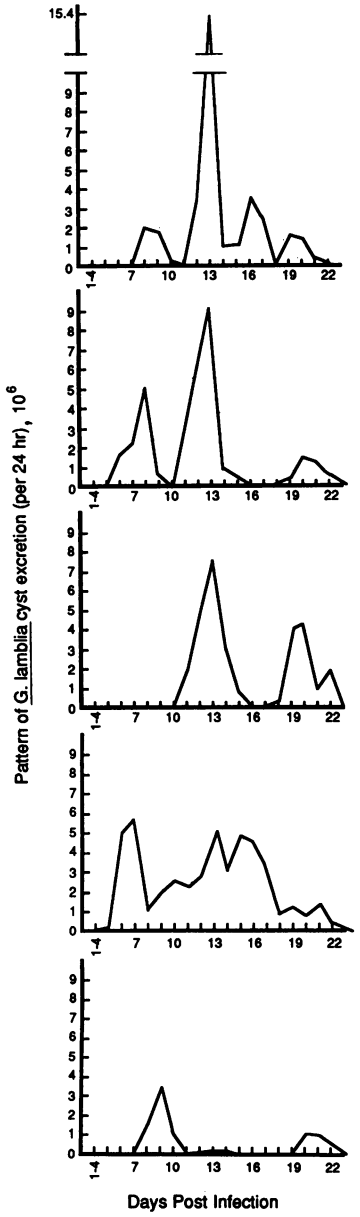

FIG. 1. Pattern of *G. lamblia* cyst excretion by infected Mongolian gerbils.

DISCUSSION

These experiments showed that (i) only certain isolates of human *G. lamblia* had the ability to infect gerbils, colonize gerbil intestines, and complete their life cycle by undergoing differentiation into cysts; (ii) the duration of infection could be as long as 33 days, but excretion of cysts reached peak levels on about day 13 p.i.; (iii) the minimum number of cysts needed to establish an infection (i.e., presence of trophozoites in the intestine) in 50% of the gerbils was 100; (iv) the pattern of cyst excretion was irregular, and some gerbils, like humans, excreted cysts intermittently; and (v) only certain strains retained the ability to infect gerbils even after repeated passaging.

Belosevic et al. (1) inoculated each of five gerbils with 10^5 cysts harvested from each of five patients from India, the Cayman Islands, Canada, and Saudi Arabia and obtained infections, as evidenced by high trophozoite counts in the intestines and cysts in the feces. They reported that cysts were excreted intermittently, and the number of cysts excreted ranged from 0 to 5×10^3, with a mean of 8.8×10^2 cysts per gerbil per 2-h period.

The results of our study clearly indicate differences between isolates of *G. lamblia* in their innate ability to infect gerbils, and only certain strains retained this infectivity even after continuous serial passaging. As of September 1987, this strain (CDC:0284:1) had been passaged in gerbils 62 times.

We have shown that gerbils inoculated with 5,000 cysts begin excreting cysts on day 5 p.i. and continue excreting cysts at least until day 22 p.i. The total number of cysts excreted by these gerbils was much higher than that recorded by Belosevic et al. (1). The large difference noted between the two studies is probably due to the fact that Belosevic et al. obtained their figures on the basis of 2-h excretion. It was evident to us from initial observations that some gerbils excreted no cysts at all during several 2-h periods. Hence, we opted to count the number of cysts excreted on the basis of a 24-h collection with a view to obtaining the true picture of the cyst excretion pattern.

According to Rendtorff and Holt (10), human volunteers (65%) became infected with *G. lamblia* when they drank cold aerated tap water seeded with 100 *Giardia* cysts. Also, cysts stored in cold temperatures retained their infectivity for at least 16 days. The infected patients excreted cysts intermittently. Our results also indicate that a minimum of 100 cysts is necessary to establish infection at least 50% of the time. Rarely, as few as 45 to 50 cysts may cause infection.

All available data on the ability of physical and chemical agents to inactivate cysts were based on in vitro excystation, which is not only imprecise but also unreliable. By using the CDC:0284:1 strain, which can produce 100% infection in gerbils, the capacity of physical and chemical agents to inactivate cysts can be established precisely, so clear-cut public health policies can be formulated. Experiments to delineate the activity of chlorine and temperature on the

infectivity of *Giardia* cysts are under way in our laboratories.

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