Survival of *C. immitis* In Passage Through the Digestive Tract of Mice

By Robert Lubarsky, M.A.,
and Orda A. Plunkett, Ph.D.

COCCIDIOIDOMYCOSIS, or valley fever, is a deep seated mycotic infection caused by the phycomycete *Coccidioides immitis*. Although found in many areas, coccidioidomycosis is endemic to large areas of southwestern United States, especially the San Joaquin Valley of southern California (*1*).

In 1942, Emmons (*2, 3*) suggested that certain wild rodents, especially pocket mice, may act as reservoirs of the parasites through infection. Since there have been no published reports that *C. immitis* is destroyed, either in the vegetative arthrospore stage or in the parasitic spherule stage, by passage through the digestive tract of animals, the possibility that animals ingesting viable spores may act as disseminators of *C. immitis* by means of fecal droppings has been overlooked.

The experiments reported here show that *C. immitis* arthrospores and spherules survive intestinal passage through rodents.

Mr. Lubarsky is a teaching assistant and Dr. Plunkett is a professor, department of botany (mycology), at the University of California. Dr. Plunkett is also a consultant on fungus diseases at the Los Angeles County General Hospital. *This paper was presented in June 1953 before the Southern California Branch, Society of American Bacteriologists, American Association for the Advancement of Science, at Santa Barbara, Calif.*

First Experiment

It was found that *C. immitis* could be recovered from feces directly from the intestinal tract of mice infected by means of intraperitoneal inoculation. Such fecal infections were found to be due to ulceration of the intestinal wall by needle puncture, with resulting drainage of material containing spherules directly into the intestinal tract. Since these viable spores had not been subjected to the digestive juices of the stomach, it was decided to test their ability to withstand digestion by feeding arthrospores and spherules in drinking water to healthy mice in such a way that external contamination of the fecal matter would not be possible.

A screwcapped 25 x 200 mm. test tube was used to hold the contaminated water. A hole drilled into the top of the cap was large enough for the mice to drink by lapping at the hole, but small enough to prevent any dripping when the tube was inverted. The tube was then clamped to a wooden platform and inserted inside the glass animal cage. The platform protected and prevented the tube from being jarred by any movements of the mice (see illustration).

Two mice in separate cages were fed dry mouse food, but given no water for 24 hours prior to being exposed to contaminated water. The cages were cleaned and sterilized, and fresh sawdust was placed in them just prior to exposure.

A very heavy aqueous suspension of *C. immitis* arthrospores and mycelia was prepared, and each drinking tube was filled with 10 ml. of this suspension. The tubes were inverted over paper towels saturated with formal acetic alcohol to catch any of the contaminated water forced out by the inversion. The tubes were wiped dry and carefully inserted into the animal jars, avoiding any cause for dripping. The mice were allowed access to this water for a period of 3 hours, and then the contaminated water and containers were removed and replaced with uncontaminated water.

Twenty-four hours later a composite sample of feces was taken from each cage. The sample consisted of 10 pieces of feces taken at random from the cages. Each sample was then macerated in 5 ml. of sterile distilled water and a loopful of the mixture was streaked on a plate of the cycloheximide medium developed by Georg, Ajello, and Gordon in 1951 (*4*). Ten plates were made of each fecal sample.

A second group of composite samples was taken 12 hours after the first samples and given the same treatment. This experiment was repeated three times within 1 week using the same animals, allowing a 1-day interval between tests. At the end of 3 weeks the mice were killed and autopsied.

Results

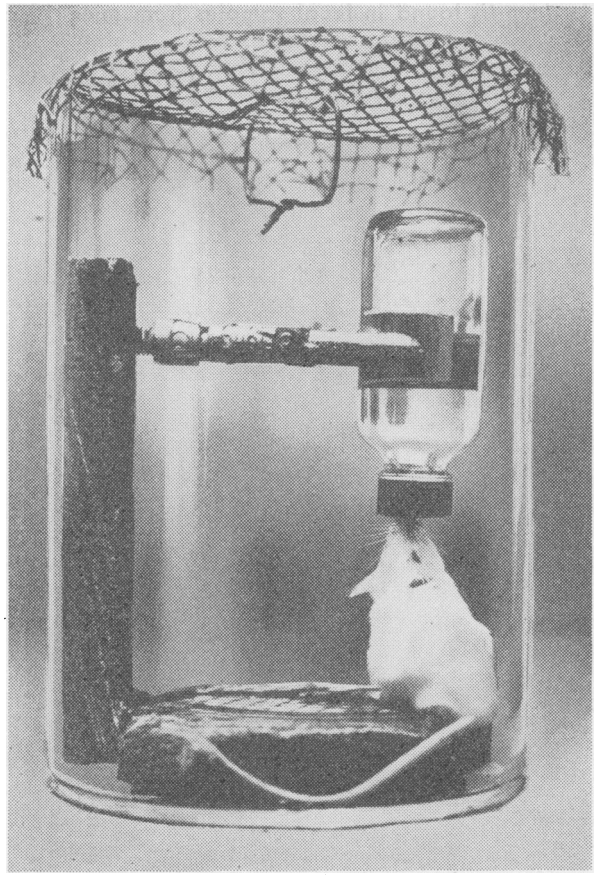
Plating of all the 24-hour samples yielded positive growth of *C. immitis*, confirmed microscopically. Isolation of *C. immitis* was negative on all samples taken after 36 hours. Autopsy of the mice showed no visual signs of infection; smear slides and cultures proved to be negative.

Second Experiment

A similar experiment was conducted using six mice. In the first series the mice were fed suspensions containing spherules obtained from 2 mice that had been inoculated intraperitoneally with 1 ml. of a heavy aqueous suspension of arthrospores and mycelia.

At the end of 6 days, both mice showed signs of intense illness. The first mouse was then killed and autopsy revealed a heavy infection due to *C. immitis*, which was later isolated on cycloheximide medium and also through the germination of spherules on broth culture slides. The liver, diaphragm, lungs, and spleen of this mouse were then macerated in 60 ml. of sterile distilled water, and 10 ml. of this macerated suspension was placed in each drinking tube for each mouse. The feces of each mouse were collected individually for a 24-hour period.

Two days later, the second infected mouse was killed and the experiment was repeated using the same tissues in a macerated suspension. The feces were again collected for a 24-hour period. Two days later, the experiment



Apparatus for delivery of contaminated drinking water

reverted to the use of *C. immitis* arthrospores and mycelia.

The experiment using the arthrospores was repeated every 2 days for 2 weeks, and then halted. Samples of feces were taken from each mouse every 24 hours. Subsamples consisting of 10 pieces of feces were macerated in sterile distilled water and plated on cycloheximide medium. Five plates were made from each subsample, 6 samples from each 24-hour run, or a total of 30 plates for each run.

One week later, one of the mice was killed and examined for evidence of infection with *C. immitis*. Two weeks after this, another of the mice was killed and examined for infection with *C. immitis*. Neither mouse showed any sign of infection with *C. immitis* and isolation of the organism was not possible.

Results

The feeding of *C. immitis* spherules resulted in positive isolation of *C. immitis* from the

C. immitis found in fecal samples from mice 12 and 24 hours after ingesting arthrospores and spherules

Animal No.	Arthrospore exposure [1]									Spherule exposure [1]								
	Sample 1			Sample 2			Sample 3			Sample 4			Sample 5			Sample 6		
	S	S	C	S	S	C	S	S	C	S	S	C	S	S	C	S	S	C
	12	24	24	12	24	24	12	24	24	12	24	24	12	24	24	12	24	24
	Laboratory mice																	
1	+2	+2	+3	+2	+2	+1	+3	+2	+3	+2	+3	+2	+3	+2	+3	+3	+2	+3
	−1	−1	0	−1	−1	−2	0	−1	0	−1	0	−1	0	−1	0	0	−1	0
2	+2	+2	+3	+2	+3	+3	+3	+3	+2	+3	+2	+3	+2	+2	+3	+3	+2	+3
	−1	−1	0	−1	0	0	0	0	−1	0	−1	0	−1	−1	0	0	−1	0
3	+2	+1	+3	+2	+2	+3	+2	+3	+2	+3	+3	+3	+3	+3	+3	+3	+3	+2
	−1	−2	0	−1	−1	0	−1	0	−1	0	0	0	0	0	0	0	0	−1
4	+2	+1	+3	+2	+3	+2	+2	+2	+3	+2	+1	+3	+3	+3	+3	+2	+2	+3
	−1	−2	0	−1	0	−1	−1	−1	0	−1	−2	0	0	0	0	−1	−1	0
5	+1	+2	+3	+2	+1	+3	+1	+2	+3	+1	+3	+2	+1	+2	+3	+3	+3	+2
	−2	−1	0	−1	−2	0	−2	−1	0	−2	0	−1	−2	−1	0	0	0	−1
6	+1	+1	+3	+2	+3	+2	+3	+2	+1	+2	+3	+2	+3	+2	+3	+2	+2	+3
	−2	−2	0	−1	0	−1	0	−1	−2	−1	0	−1	0	−1	0	−1	−1	0
7	+2	+1	+3	+2	+1	+1	+1	+2	+3	+2	+2	+3	+2	+3	+2	+3	+3	+3
	−1	−2	0	−1	−2	−2	−2	−1	0	−1	−1	0	−1	0	−1	0	0	0
8	+2	+2	+3	+2	+2	+3	+2	+3	+3	+3	+2	+3	+2	+1	+2	+2	+2	+3
	−1	−1	0	−1	−1	0	−1	0	0	0	−1	0	−1	−2	−1	−1	−1	0
9	+1	+1	+3	+2	+3	+3	+2	+1	+3	+3	+1	+2	+3	+1	+3	+3	+3	+3
	−2	−2	0	−1	0	0	−1	−2	0	0	−2	−1	0	−2	0	0	0	0
10	+2	+1	+3	+1	+1	+2	+3	+2	+2	+3	+1	+3	+3	+2	+3	+2	+3	+3
	−1	−2	0	−2	−2	−1	0	−1	−1	0	−2	0	0	−1	0	−1	0	0
11	+1	+2	+3	+2	+3	+3	+1	+2	+3	+2	+3	+3	+2	+3	+3	+3	+2	+2
	−2	−1	0	−1	0	0	−2	−1	0	−1	0	0	−1	0	0	0	−1	−1
12	+1	+1	+3	+2	+2	+3	+3	+2	+3	+3	+2	+1	+2	+3	+3	+3	+3	+3
	−2	−2	0	−1	−1	0	0	−1	0	0	−1	−2	−1	0	0	0	0	0
	Wild mice																	
1	+3	+2	+3	+3	+2	+2	+3	+1	+3	+2	+1	+3	+3	+2	+2	+3	+1	+3
	0	−1	0	0	−1	−1	0	−2	0	−1	−2	0	0	−1	−1	0	−2	0
2	+3	+2	+3	+3	+2	+3	+3	+2	+3	+3	+2	+2	+3	+1	+3	+3	+1	+3
	0	−1	0	0	−1	0	0	−1	0	0	−1	−1	0	−2	0	0	−2	0
3	+3	+2	+3	+2	+2	+2	+3	+2	+3	+3	+2	+2	+3	+1	+3	+3	+1	+3
	0	−1	0	−1	−1	−1	0	−1	0	0	−1	−1	0	−2	0	0	−2	0
4	+3	+2	+3	+3	+2	+3	+3	+1	+2	+3	+2	+3	+3	+1	+3	+3	+1	+3
	0	−1	0	0	−1	0	0	−2	−1	0	−1	0	0	−2	0	0	−2	0

[1] S=single sample; C=composite sample. The numbers 12 and 24 equal the time in hours after exposure that samples were taken.

+=positive; −=negative.

fecal matter of all of the mice. On both spherule tests, all plates yielded positive growth of *C. immitis*. Upon reversion to the use of *C. immitis* arthrospores, the result of the first test yielded 25 positive plates and 5 negative plates. The five negative plates all came from the same mouse. The next test yielded the same results, except that the negative plates came from a different mouse. During the remainder of the experiment, all of the plates yielded positive growth of *C. immitis*.

Third Experiment

The following experiment was designed to include individual fecal samples taken directly from the mice to eliminate any chance of contamination of the feces due to the mice dripping contaminated water from their mouths after drinking. Sixteen glass animal cages and drinking bottles were prepared as in the preceding experiments. Into these were placed 12 white mice and 4 wild deer mice (*Peromyscus* sp.) These animals were fed, but kept from water for 24 hours. Each drinking tube was filled with 10 ml. of a heavy aqueous suspension of coccidioidal arthrospores, and the mice were allowed access to this water for 3 hours. The bottles were then withdrawn and no other water given to the mice.

Twelve hours after exposure each mouse was forced to emit a single piece of feces. Twelve hours after this sample was taken, each mouse was forced to emit another single piece of feces. At this time, a composite sample of the cage droppings was taken, the cage was cleaned and sterilized, and the mice again subjected to arthrospore contaminated water. This was repeated for 3 days. Samples were taken 12 and 24 hours after exposure.

On the fourth, fifth, and sixth days, the mice were given water containing the macerated tissues, lungs, liver, diaphragm, and spleen of mice which had been infected with *C. immitis*. The presence of spherules in the water was confirmed by microscopic examination, and also by re-inoculation into other mice and by re-isolation of the vegetative state of *C. immitis*. The mice were subjected to the same type of treatment given to them when the arthrospores were used.

All fecal samples were plated on cycloheximide medium as soon as they were taken from the animals; each sample was plated on three different plates.

Results

C. immitis was isolated on at least one plate from each fecal sample at all times. A total of 648 plates was made from the fecal samples of the experimental animals, of which 504, or 77.7 percent, were positive for *C. immitis*. A total of 216 plates was made for the fecal samples of the wild rodents, of which 174, or 80.5 percent, were positive (see table).

Conclusions

1. *C. immitis* arthrospores, when fed to mice, survive passage through the digestive tract and, in most cases, can be isolated from the feces.
2. *C. immitis* was recovered from the feces of mice fed suspensions of spherules and endospores.
3. Constant feeding of *C. immitis*, either as arthrospores or spherules, fails to produce any coccidioidal infection in mice.
4. There is a possibility that *C. immitis* may be disseminated in soils by fecal droppings of predatory animals who have fed on infected rodents. Since *C. immitis* passes through the intestinal tract of rodents unharmed, it is reasonable to assume that it passes through the intestinal tract of predatory animals in a viable condition.

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