EXPERIMENTAL TRICHINIASIS OF YOUNG WHITE MICE BY STOMACH INTUBATION

A Thesis

Presented to

The School of Graduate Studies

Drake University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts in Biology

by

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August 1969

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INTRODUCTION AND REVIEW OF THE LITERATURE

The clinical history of Trichiniasis has been thoroughly described by Belding (1942). Leuchart and Virchow were the first to make significant progress in unraveling specific questions about the helminth Trichinella spiralis. From the time they described the life cycle of this organism in the 1850's, as pointed out by Larsh (1963), it has remained one of the most intensively studied forms of life.

Pertinent work has been done in the area of host resistance. Riedel (1948) demonstrated that there seemed to be a natural resistance that increased with age. His work showed that mice that were from 34 to 42 days of age when infected, yielded more than twice as many muscle larvae as those infected at from 5 to 6 months of age. All age groups yielded nearly the same number of adult worms, the difference being in the number of larvae produced and their invasion of host muscle tissue.

Much of the current research is involved with resistance to repeated infections. Bass and Olson (1965), Ewert and Olson (1960), Mills and Kent (1965), Olson and Ewert (1961) and Olson and Hill (1966), have shown that it is difficult to demonstrate any great immunological reaction by the host toward T. spiralis. Most of these workers have dealt with crude extracts of adult worms and/or Excretion and Secretion antigens (ES antigens) in different age groups of mice.

Even though Silverman (1965) worked with rats, his findings are of interest. He found that T. spiralis larvae will mature and infect both the mother and her embryos if they are injected in utero during pregnancy. He also found that if the larvae are injected into the amniotic sac of each embryo, only the embryo is infected.

While Bass and Olson (1965), Berntzen (1965), Katz (1960), Khan (1966) and Larsh (1963) do not agree on the number of stages or on the time spent in each stage, they all have contributed greatly to the understanding of the life cycle of T. spiralis. The phases of this organism's life cycle that are of particular importance to this study are the reproductive, the muscle invasion by the larvae and the subsequent encapsulation. Most of these researchers agree that the freed larvae will mature in the gut of their hosts from within 1 to 2 days. Stefanski and Przyjalkowski (1965) demonstrated that some bacteria in the intestinal flora seem to inhibit ecdysis and adult development, while others seem to favor their maturation. There is also an indication that location and retention within the gut may have an influence on resistance and acceptance of the infective larvae as cited by Campbell (1967). New muscle larvae can be found as early as the sixth day post infection, as pointed out by Khan (1966). Encapsulation may occur by the seventeenth day post infection as reported by Larsh (1963), but reaches its peak by the nineteenth or twentieth day.

In terms of worm burdens, Bass and Olson (1965) determined that newborn mice seem to be more resistant to this infection than are adults. Silverman (1965) has shown that rat embryos *in utero* will accept challenging infections of *Trichinella spiralis*. Of the many questions still unanswered, one of particular interest here deals with host resistance. This study is an attempt to determine if there is a significant difference in the acceptance of infections of *T. spiralis* in differing age groups of young white mice.

MATERIALS AND METHODS

Eight adult female and four adult male white mice were obtained from the Small Animal Supply Company in Omaha, Nebraska. All of the mice arrived in Mequon, Wisconsin, August 7, 1967.

Cages were made out of styrene plastic utility boxes, measuring 12 1/2" x 10 1/2" x 6 3/4". Each cage was divided into two equal halves by cementing a piece of the same type of plastic through the middle of each box. The covers were made out of 1/4" x 1/4" hardware cloth. Food hoppers were placed in each lid so that the Purina Mouse Chow was available *ad libitum*. Water bottles were also placed in each cage so that water was available at all times. All mice were kept in a basement maintained at a temperature of approximately 74° Fahrenheit.

The stock female mice were bred and were separated from their cage mates shortly before delivery. The young were kept with their mothers until weaned, at which time the mothers were put back with the other adult females to be used again as breeding stock. Each of the young in a litter was numbered by cutting a particular toe off of a given paw. This was done so that each individual in every litter could be identified from the time of infection to the time of sacrifice.

The larvae were maintained in some stock mice; however, the carcasses of experimental mice were used more often. An acidified pepsin digest solution was made up by adding 3 gm pepsin, N.F. powder (Matheson, Coleman and Bell) and 2 ml of concentrated HCl to distilled water to make up a total volume of 200 ml. The viscerated carcasses of infected mice were ground in a Model 477 Series B Osterizer Blender for 20 seconds at a speed indicated as number 2. The carcasses were suspended in part of the digest fluid while being ground. This slurry was then maintained at a temperature of 37° Centigrade for from three to four hours in a Blue M "Budget" bacteriological incubator. The fluid was then filtered through several layers of sterile gauze to remove bone and other material fragments from the larvae. The dilution method employed by Riedel (1948) was used to separate other materials by allowing the worms to settle to the bottom upon standing. The supernatant was removed by decantation and the

remaining portion was diluted using warm tap water until the fluid became relatively clear. The larvae were counted on a pyrex spot plate and appropriate larvae added or removed so that there remained fifty larvae per depression. The volume of fluid containing the larvae varied from 0.1 ml to 0.01 ml according to the age of the recipients, the younger mice receiving the smaller volume. Larva that had been isolated for more than eighteen hours were not used because Mills and Kent (1965) reported they lost infectivity during incubation.

Since oral intubation worked well for Bass and Olson (1965), it was chosen for use in this study. A minimum of twenty experimental individuals in each of five age groups, varying in increments of five days, were infected by oral intubation of 40-50 larvae. A tuberculin syringe with a blunted 20 gauge needle was used to deposit the larvae in the stomachs of the twenty, fifteen and ten day old experimental groups. Because of their limited size, the five and one day old mice were intubated by using a drawn glass pipet. Within each litter one mouse was used as a viability control and another as a placebo control. The placebo control received the same volume of tap water as the experimentals, but void of larvae.

All experimental and control animals were kept for a minimum of thirty days post infection so that cyst walls might be well formed. Each individual was sacrificed by a

sharp blow to the back of the head and necropsied by the standard diaphragm press, similar to the procedure used by Riedel (1948). A Bioscope (Model No. 60) with a 5X lens was used to check each diaphragm for the approximate number of larval cysts. This number was recorded and the diaphragm was then placed with the others of the same age group. A 20 per cent formalin solution was used as a preservative.

DISCUSSION

It was found that not all larvae, in either the hypodermic needle or the glass pipet, were expelled when introducing them to the recipient hosts. The number remaining in the needle or pipet could be determined by flushing it and checking the wash water for larvae. From observation of this procedure it can be said that anywhere from 40 to 50 larvae were transferred to each host.

While fresh the diaphragms of all experimental and control animals were checked microscopically and pressed between two microscope slides. The total number of cysts were counted and rated as to a light, medium or heavy infection. A light infection was designated as having from 1 to 25 cysts, medium 26 to 50 cysts, and a heavy infection having 51 or more cysts per diaphragm. It should be noted that all control animals were negative for the presence of any cysts. The results of the cyst counts were recorded and tabulated in Table 1. It summarizes the comparison of the

intensity of infection among the various age groups of mice. It shows both the total number and percentages of diaphragms in each category. As an example, there were 27 experimental hosts in the 1 day old group. All proved to be positive, but not to the same degree. As can be seen 14 were heavily infected, 5 medium and 8 lightly infected. These numbers expressed as a per cent of the total 27 are: 52%, 18% and 30% respectively. It can also be seen, by following each category across the table, that there were 52% heavy in the 1 day, 74% in the 5 day, 25% in the 10 day, 73% in the 15 day and 62% heavy infections in the 20 day group. Each category can be analyzed for the number and percentage light, medium or heavy infections by this manner. The per cent in each category can also be seen graphically in Figure 1. It illustrates each of the three categories, (heavy, medium, light), as well as the total per cent positive in each group. It is interesting to see that in all groups except the 10 day the heavy infection category has the greatest percentage. It can be seen that the light infection category seems to decrease with age. The per cent positive shows little to no significant variance from group to group.

The data was also analyzed by the use of the Chi-Square test, as seen in Table 2. The one and five day old groups could not be analyzed by this method because they were all positive, thus they had no deviation. The ten, fifteen and twenty day old groups, showed Chi-Square values

TABLE 1. Intensity of Trichinization of various age groups of young white mice

	1 DAY OLD		5 DAY OLD		*10 DAY OLD		15 DAY OLD		**20 DAY OLD	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total number observed	27	100	23	100	24	100	33	100	29	100
Total number positive	27	100	23	100	21	88	29	88	26	90
Total number negative	0	0	0	0	3	12	4	12	3	10
HEAVY Trichinization (51 or more cysts per diaphragm)	14	52	17	74	6	25	24	73	18	62
MEDIUM Trichinization (26-50 cysts per diaphragm)	5	18	4	17	14	58	5	15	8	28
LIGHT Trichinization (1-25 cysts per diaphragm)	8	30	2	9	1	4	0	0	0	0

*One mouse died the day of infection, thus it was not included in the data analysis.
**One mouse died on the third day post infection, thus it was not included in the data analysis.

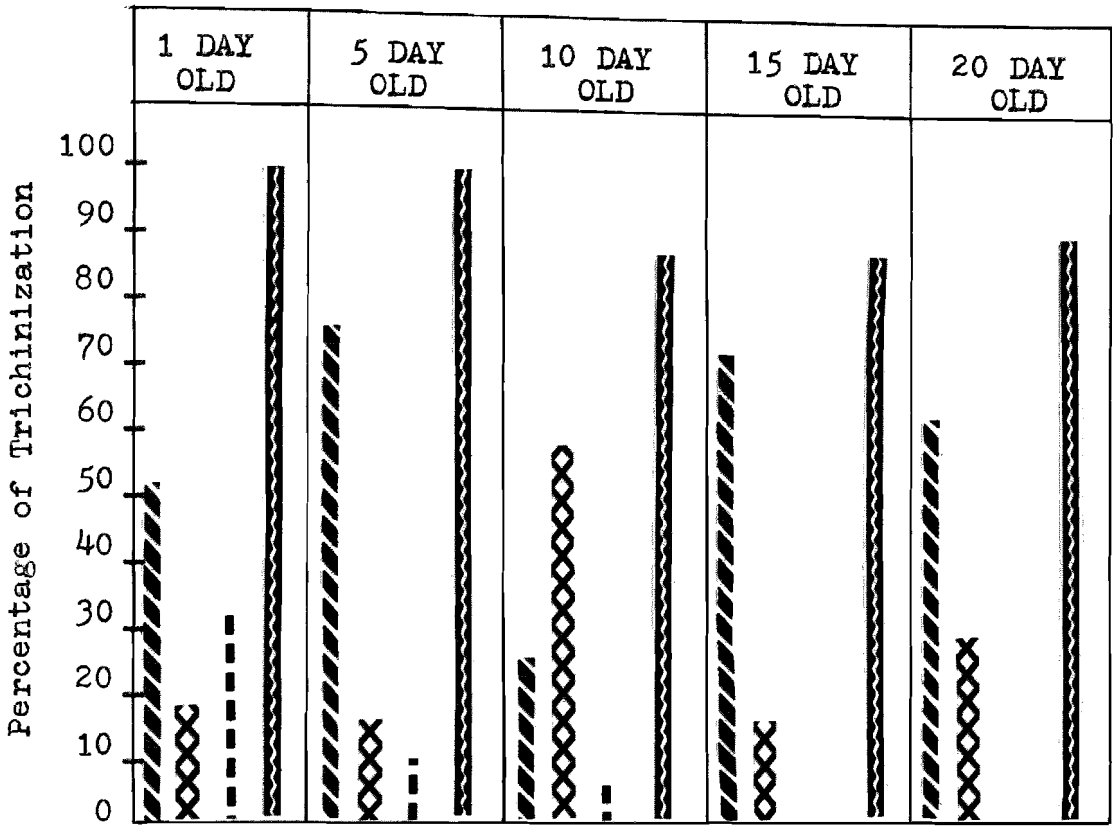

Figure 1. Intensity of Trichinization of various age groups of young white mice.

KEY: Per cent HEAVY Trichinization (51 or more cysts per diaphragm).

Per cent MEDIUM Trichinization (26-50 cysts per diaphragm).

Per cent LIGHT Trichinization (1-25 cysts per diaphragm).

Total per cent positive.

TABLE 2. The determination of Chi-Square test as an analysis of experimental Trichinization data

Experimental Groups	Deviation Squared $(X - m)^2$	$\frac{(X - m)^2}{m}$
1 DAY OLD	0	0.0
5 DAY OLD	0	0.0
10 DAY OLD	9	0.376
15 DAY OLD	16	0.486
20 DAY OLD	9	0.310

of 0.376, 0.486 and 0.310 respectively, as seen in the last column of Table 2. These values can be expressed as probability factors (p factors) of 0.6, 0.5 and 0.7. This means that the results of these three groups could be expected (on the whole) to be the same from 50 to 70 per cent of the time.

CONCLUSIONS

This study attempted to determine if there is a significant difference in the acceptance of infections of Trichinella spiralis in differing age groups of young white mice. The ages of the mice varied in five day increments so that some were one day old, five days old, ten days old, fifteen days old and twenty days old when infection was attempted.

It has been clearly shown in this investigation that there was no significant difference in the acceptance of infections of T. spiralis. It is likely that errors in technique contributed to the 10-12 per cent deviation from the 100 per cent level in the experimental mice ten days and older.

As a result of the diaphragms examined, it is recommended that a more precise technique be used in determining the number of larvae introduced and the subsequent invasion of host tissues. In this study only the diaphragm was examined. Katz (1960) however mentioned in his study that when a diaphragm press proved negative, he did a total digest and examined the fluids for the presence of larvae. Some of

those that seemed negative in this study may have shown that while the diaphragm was negative there was infective larvae elsewhere in the carcass of the host. It would be of value to use the total digest technique in order to obtain more precise data in terms of the total number of larvae accepted by each host. Other suggestions are to increase the number and age range of hosts sampled and to vary the number of larvae introduced into each host.

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