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PRODUCTIVITY IN GRAVID TRICHINELLA SPIRALIS (OWEN, 1835) TRANSPLANTED INTO LABORATORY RATS

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INTRODUCTION

Numerous attempts have been made to determine how many larvae may be produced by *Trichinella spiralis*. This has been impossible to determine accurately, because there are so many variable factors involved. Some attempts were made by McCoy (1931) who fed known numbers of larvae to laboratory rats. He estimated the number of larvae produced by each female *Trichinella* in the rats to be, on the average, about 200 in light infections and about 400 in heavy infections. Edney (1934) fed known numbers of larvae to laboratory rats. Later, he recovered the larvae thus obtained from his infected rats and determined the larva-larva ratio. There appeared to be no constant factor of productivity by female worms in these experiments.

Roth (1938) also did some work with larva-larva ratio using guinea pigs as the experimental animals. But the guinea pig is an abnormal host. The larvae administered in his experiments were obtained from the muscles of guinea pigs or rabbits infected at least eight weeks previously. The sexes of the larvae were determined by a method described by Bugge (1934). When a single female larvae and two male larvae were administered, they produced a total infection, which he estimated at approximately 2,100 larvae. He concluded from the experiments that the ratio of larvae encysted in the muscles of guinea pigs per female ingested varies as a rule from about 1,000 to about 2,500; numbers between 1,500 and 2,000 being most frequently found.

Rappaport (1943) experimented with three strains of *T. spiralis* to determine larva-larva ratio. Mice infected with small doses of *T. spiralis* showed considerable variations in the average proportions of larvae recovered to larvae fed. The results were much more consistent when doses bordered on the lethal.

Numerous factors influence the production of larvae. The age of the rats used has been found to have an effect on the size of the larva-larva ratio (Marchant, 1939). He said that as the rat grows older, it becomes more refractive to *T. spiralis* infections. His work was done with groups of rats ranging in age from three to thirty-six weeks, respectively.

Nolf and Zaiman (1941) used rats of three different age groups in their experiments to study the effect of age on *T. spiralis* in the host intestine. The first group was made up of rats ranging in age from three to twelve months; the second group was five-week old

rats and the third group consisted of unweaned rats from eight to fifteen days old. Each of the rats in the three groups received 2,000 *T. spiralis* larvae per ounce of gross weight. Recovery of worms was made between 36 and 86 hours after the infection. The lowest average number of worms recovered was from unweaned rats. The next highest number was recovered from the three to twelve month old rats. The highest average recovery was from the five week old rats. This work agrees in part with that done by Marchant (1939).

Riedel (1948) and Larsh and Hendricks (1949) reported the absence of age resistance in mice to *T. spiralis*. Riedel (1949) made a further study of the problem of age resistance, allowing a 15-day period of parasitism instead of a 6-day period, and found that young mice (26-32 days of age) harbored more adult *T. spiralis* than old mice (age 157-171 days).

McCoy (1932) said that a heavy infection breaks down the resistance in the rat, which will ordinarily limit the duration of the adult worms in the intestine to two weeks; thus this may increase the number of larvae produced by the females. It was also found (McCoy, 1931) that rats which had been previously infected by this parasite, withstood more than twice the dose of larvae which would kill the majority of a control group.

Edney (1934), in his experiments for the purpose of finding the larva-larva ratio, fed the rats in his experiments from 20 to 9,000 larvae, respectively. After an appropriate period the rats were killed, digested and the larvae counted. He found in his experiments that in rats fed up to 320 larvae, the larva-larva ratio increased; but above this point it declined. This would seem to indicate that the size of the infection played a part in larval production. This is contrary to the statement of McCoy (1932) who said that heavy infections break down the resistance in rats, thus increasing the larval production.

Individual differences in the rats apparently are responsible for some variations in the production of larvae by adult worms. The size of the rat seems to play a part, since the larger the rat the better its chances for infection and survival. The age of the rat would influence size; so, this would appear to be an important factor. Certain other unknown factors may influence the production of larvae by the adult worm.

The first and only attempt made to determine the number of larvae produced by a single gravid transplanted *T. spiralis* was made by Nolf (1937). He did four such experiments using one rat per experiment. Two rats died from post-operative septicemia. The others were autopsied six weeks later and their carcasses digested. The larvae recovered were counted directly. One rat had 1,112 larvae, while the other one contained only 14 larvae. This work involved transplanting gravid worms from one host to another. The ages of these worms and host were not stated.

Until the work done by Nolf (1937), no work had been done on the transplanting of gravid *T. spiralis*. This was the reason for doing the present problem, the purpose of which was to determine, if possible, whether there is a more or less constant number of larvae

produced by a single female *T. spiralis* under certain controlled conditions.

Materials and Methods

For these experiments albino and hooded rats were used. These varied in age from weanlings to adults of twelve months, respectively. The weights in the entire group varied from 71 to 253 grams, respectively. The rats were given a diet of Purina Dog Chow, which is considered a balanced diet for these animals. Rats used for transplant purposes were starved for twenty-four hours before and after the transplants were made. This was done in order to reduce the intestinal content so as to reduce the possibility of peritonitis following the operation. Also, by doing this, the adult worms have a greater chance of penetrating into the intestinal mucosa and become established in the host.

TABLE 1. *Larval Production in Rats Given One Gravid Trichinella spiralis by Transplantation. Experiment 1*

Rat No.	Sex	Initial Gross Weight in Grams	Final Gross Weight in Grams	Skinned Carcass Weight in Grams	Number of Larvae Recovered
1	M	71	219	138	345
2	F	85	172	92	70
3	F	87	183	100	462
4	F	96	145	88	205
5	M	100	124	80	923
6	F	100	217	157	884
7	F	104	125	77	5
8	M	132	148	106	1
9	F	160	200	128	5
10	F	167	193	126	330
11	F	172	174	116	462
12	F	214	129	103	101
Average		124	154.75	109.25	316.1

Material for transplanting was obtained by feeding encysted larvae in infected meat to a stock rat five days before the transplant was to be done. At this time the worm is sexually mature but has not started larval production. At the end of five days the stock rat was killed, the small intestine removed and placed in normal saline warmed to body temperature. The intestine was divided into anterior and posterior portions. The intestinal contents were removed into a dish by gently pulling the intestine between the fingers. Then a piece of the intestine was cut lengthwise and placed in another dish of warmed normal saline. This was found to be an effective method of getting large numbers of adults to emerge from the intestinal tissues (Christenson, 1927). After this had been done, the rat to be used for the transplant attempt was etherized, and kept under the anaesthetic during the entire course of the operation. By using small animal

clippers, the rat was shaved on the abdomen and flanks. The abdomen was painted with merthyolate, and surgical technique followed thereafter. Two types of incisions were done; in some rats a flank incision was made, while in others an abdominal incision was used. The abdominal incision was found to be more successful from the standpoint of locating the anterior portion of the intestine. An incision about one inch long was made with a Bard-Parker #3 lancing scalpel and the anterior portion of the intestine withdrawn from the coelome and kept moist with warm normal saline applied with a pipette. It is necessary for two people to perform this operation, one person acting as the anaesthetist while the other is the surgeon making the transplant.

With this technique, the shock to small rats is more severe than in larger animals; however, there is little loss of blood. Anaesthetic ether was used throughout the entire series of operations.

Adult worms were removed from the stock solution by using a binocular dissection microscope and micropipette. One adult female was placed in normal saline in a watch glass kept at body temperature. The worm was picked up in about one cubic centimeter of saline by using a small syringe and injected into the anterior end of the host intestine with a 16-gauge needle. After the injection, the syringe was filled with saline and rinsed into a watch glass. This was done in order to determine if the worm was left in the syringe or placed in the host intestine.

TABLE 2. *Larval Production in Rats Given One Gravid Trichinella spiralis by Transplantation. Experiment 2*

Rat No.	Sex	Initial Gross Weight in Grams	Final Gross Weight in Grams	Skinned Carcass Weight in Grams	Number of Larvae Recovered
1.....	F	197	301	178	247
2.....	F	211	344	197	641
3.....	F	222	317	180	370
4.....	F	153	242	114	431
5.....	M	167	252	131	386
6.....	M	189	257	118	271
7.....	M	167	207	129	230
8.....	M	248	190	111	378
9.....	M	253	178	113	490
10.....	M	251	183	117	364
Average.....	.....	205.8	247.1	138.8	380.8

After the transplant was made, the intestine was painted with antiseptic and replaced in the rat by using the blunt end of a pair of tweezers. The open wound was painted with antiseptic solution and sutures were made both on the muscle layers and the skin with black sterilized sewing thread. The closed incision was swabbed with merthyolate and the rat put into a cage to regain consciousness.

After thirty-five days or longer, the experimental rats were killed

and eviscerated. The carcasses were ground in a food chopper and this chopped material was digested artificially, according to standard procedure, to excyst the larvae.

Direct counting was done with a binocular dissection microscope. A clean glass plate, three inches by four inches in size, was ruled into small squares for convenience and accuracy in counting. The worms to be counted were pipetted onto the slide and counted by using a Veeder counter for accuracy.

Discussion

Presumably, all the transplanted worms lived and became established in the host intestine. But this assumption was incorrect because 14 out of 26 rats surviving the operation in the first experiment did not become infected. And in the second experiment 47 rats survived the operation, but only 10 became infected. We offer no explanation as to why part of the rats did not become infected because there are many possible reasons for the failure. Daily fecal examinations to determine if any of the transplanted worms passed from the intestine were impractical. It is assumed that all the transplants which became established in the host intestine, produced the maximum possible number of larvae.

There is a very great range in the weight of the rats used for these experiments. This is shown in Table 1 where the initial weight ranges from 71 grams to 214 grams. Further study of these data indicates that there is no correlation between the number of larvae produced by each female worm and the size of the rat. As noted in Table 1, rat no. 1, which had an initial weight of 71 grams, had a total of 345 larvae, while in the same experiment, a rat with an initial weight of 100 grams produced a total of 943 larvae. And in another rat with an initial weight of 100 grams, 884 larvae were recovered. We are unable to explain the small numbers of larvae recovered from three rats in the group. But, we do not believe it was due to faulty technique because the same procedure was used throughout the experiments.

The age of a normal rat plays a part in the size of the animal, an older rat weighing more than a young one. Marchant (1939) said that as a rat grows older it becomes more refractive to *T. spiralis*. Nolf and Zaiman (1941) used three different age groups in a study of the effect of age. They found that five-week-old rats harbored more larvae than unweaned, or three to twelve-month-old rats. Riedel (1949) made a study of the problem of age resistance in mice and found that young mice harbored more adult *T. spiralis* than old mice and might be expected to harbor more larvae. This is contrary to what we found to be true in rats. Data in our experiments appears to verify this concept. For example, in rats 9 and 10, Table 2, there was 2 grams difference in weight at the time of infection, yet there was a difference of 134 larvae recovered from them. And again a young rat weighing 71 grams when infected yielded 345 larvae as compared to 490 recovered from a rat weighing

253 grams when infected. The data would seem to indicate that larval production is not a function of the size of the rat.

The strain of rat played no part in the intensity of infection as both hooded and albino rats were used and there was no noticeable difference in the number of larvae produced in these cases.

The sex of the rat played no part in the infection as is indicated by comparable numbers of larvae present in rats of both sexes. Data in Table 1 shows this to be true. A young albino female with an initial weight of 167 grams had a total larvae count of 330, while a young hooded male with an initial weight of 71 grams had a total worm count of 345.

The productive potential of female *T. spiralis* apparently varies with the individual worm. The length of time that the worm lives in the intestine would seem to have an effect on this potential. The female begins to produce larvae six to seven days after the infection is established. The adult female worm may persist in the intestine for as long as four or five weeks in heavy infections in rats and may produce larvae for this length of time, according to McCoy (1931, 1932a). Gursch (1949) has done the most comprehensive and convincing study yet made on the problem of adult longevity in the rat. If his conclusions are correct, presumably more larvae will be produced in heavy infections than in lighter ones.

We believe the data in our experiments warrants the conclusion that the number of larvae produced by a single gravid worm is a function independent of size, age, sex, and strain of the host rat. And we also conclude that the productive potential factor alone is sufficient to explain the great extremes in the numbers of larvae recovered from experimental host animals. Consequently, we propose the concept of a *variable productive potential factor* in *T. spiralis* because we believe the evidence supports this novel idea.

Summary

1. Successful transplants of single gravid *T. spiralis* were made in 22 rats.

2. The average number of larvae produced per adult was 345.4.

3. The age, size, sex, and strain of host did not appear to influence productivity in the parasites.

4. Difference in numbers of larvae recovered may be due to a variable productive potential factor in the adult worms.

Literature Cited

Bugge, B. 1934. Trichinen in Dorm. *Arch of Wissensck.* Tierkielk.

Christenson, R. O. 1927. The sex ratio of adult trichinae. *Science,* 66:259.

Edney, James Marion. 1934. "On the biology of *Trichinella spiralis.*" Unpublished Master's thesis, State University of Iowa, Iowa City, Iowa.

Gursch, Otto F. 1949. Intestinal phase of *Trichinella spiralis* (Owen, 1835) Ralliet, 1895. *Jour. Parasit.,* 24:225-231.

Larsh, John E., and James R. Hendricks. 1949. The probable explanation for the difference in the localization of adult *Trichinella spiralis* in young and old mice. *Jour. Parasit.,* 35(1):101-108.

McCoy, O. R. 1932. Size of infection as an influence on the persistence of adult Trichinae in rats. *Science,* 75(1944):364-365.

Marchant, E. H. 1939. Effect of age of rat on the size of *Trichinella* infection. *Jour. Parasit.*, 25(6):23.

Nolf, L. O. 1937. The transplantation of gravid *Trichinella spiralis*. *Jour. Parasit.*, 23:574.

Nolf, L. O., and H. Zaiman. The effect of host age on the number of *Trichinella spiralis* recovered from rats during the early period of infection. *Jour. Parasit.*, 27(6):24.

Rappaport, Irving. 1934. A comparison of three strains of *Trichinella spiralis*. *Amer. Jour. Trop. Med.*, 23:343-350.

Riedel, Bernard B. 1948. Age resistance of mice to the nematode *Trichinella spiralis*. *Trans. Amer. Microsc. Soc.*, 67(3):268-271.

Riedel, Bernard B. 1950. Further studies on the effect of age of mice upon adult *Trichinella spiralis*. *Jour. Parasit.*, 36(1):27-28.

Roth, Hans. 1938. Experimental studies on the course of *Trichina* infection in guinea pigs. i. The minimum dose of Trichinae larvae required to produce infestation of the muscles; with an account of the potential productiveness of female *Trichina*. *Amer. Jour. Hyg.*, 28(1):85-103.