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Trichinella spiralis: size of F_1 generations produced in mice by transplanted females or from infections with known numbers of male and female larvae*

by

Wieslaw J. Kozek**

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Abstract: To determine the reproductive potential of adult *Trichinella spiralis* males and females, Swiss mice were inoculated orally with known numbers of male and female larvae. Five or 6 weeks after inoculations the mice were killed and the larvae present in the diaphragm and in the pepsin digests of the carcass were counted. Diaphragms of all infected mice contained larvae. Inocula consisting of 1 male and 1 female produced from 274 to 1,094 larvae; 1 female and 10 males from 433 to 2,158 larvae; 1 male and 10 females from 1,240 to 3,162 larvae. Forty-eight-hour-old *T. spiralis* females transplanted into normal mice, one female per mouse, produced from 19 to 283 larvae; transplanted 96-hour-old females produced from 265 to 863 larvae. Up to three inseminated females were recovered on the 6th day of infection from the intestine of mice inoculated with 10 female and 1 male larvae. These results indicate that a male can produce sufficient sperm to fertilize approximately 3,200 oöcytes, or more, and can inseminate more than one female; a female can produce approximately 2,200 oöcytes, or more. Furthermore, they suggest that multiple inseminations may occur during the course of infection. Presence or absence of larvae in the diaphragm of a mouse can be used as an absolute criterion to confirm or rule out infection with *T. spiralis*.

Trichinellosis is one of the few helminthic infections during which both the parent and the F_1 generation of parasites inflict specific, although different, pathological insults on the host. Moreover, if present in sufficient numbers, either generation can kill the host. Thus, determination of the number of larvae that a female *T. spiralis* can produce during a course of infection and of other factors

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** Department of Immunology and Medical Microbiology, University of Florida, Gainesville, Florida 32601 and Department of Microbiology, University of Chicago, Chicago, Illinois 60637. Present address: California Primate Research Center, University of California, Davis, California 95616, U.S.A.

which determine larviposition, e.g. number of matings, would not only elucidate some basic biological aspects of this parasite, but also have some practical applications, since the degree of muscle damage is directly proportional to the number of larvae produced. In addition, in many immunological studies the number of intestine-infecting larvae (IIL) recovered from the muscles after the challenge inoculations is commonly used as an index of immunity. According to the published reports summarized in Table 1, the number of larvae produce by female *T. spiralis* varies from less than 100 to more than 2,000, but we have no indication what proportion of the total reproductive potential these larval yields represent. The purpose of this investigation was: (1) to determine whether the reproductive potential of *T. spiralis* adults could be estimated by comparing the larval yield obtained from mice inoculated with known numbers of sexed IIL; (2) to compare the larval yield of 48- and 96-hour-old females transplanted into clean recipients; and (3) to determine whether 1 male could inseminate more than 1 female.

MATERIAL AND METHODS

Recipient hosts in these experiments were either Swiss or CD 20-25 gm female mice (Charles River, Wilmington, Mass.) maintained in facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The capsules containing the IIL were individually excised from the diaphragm of a rat harboring a 6-month-old infection, placed in a drop of saline on a microscope slide, covered with a cover-slip, and sexed according to the criteria described in a separate report (**Kozek,** 1975). The light pressure of the coverslip slightly flattened the capsules and facilitated sexing of the encapsulated larvae. Free IIL, obtained by digesting large pieces of infected mouse diaphragms in 1% HCl-1% pepsin solution, were sexed in the same manner but without the use of the coverslip. The inoculum was prepared by grouping the desired number of sexed larvae in a clean, saline-containing petri dish. The larvae were inoculated into ether-anaesthetized mice according to the technique described by **Chirasak** (1971), except that a new plastic tube and a new syringe were used for each inoculation to insure that only the prescribed inoculum was given.

The mice in groups Ia, IIa, and III received encapsulated IIL, those in Ib and IIb received free IIL. Each mouse in group Ia and Ib was inoculated with 1 male and 1 female larvae, in IIa and IIb with 1 male and 10 females, in III with 10 males and 1 female.

The *T. spiralis* females to be transplanted were obtained from 200 gm female Sprague Dawley rats inoculated by stomach intubation with approximately 4,000 IIL. At 48 and 96 hours post inoculation, respectively, 1 rat was killed, its small intestine excised, washed briefly in warm tap water to remove blood and cut open in warm saline solution. Emerging females were transferred, by means of a Pasteur pipette, onto a microscope slide and examined through a compound microscope for the presence of sperm in the seminal receptacle and/or fertilized eggs in the lower portion of the uterus (cf. Figs. 28 and 29, **Kozek,** 1971). Inseminated females were introduced into the duodenum of the recipient mice essentially by the same technique used to introduce IILs into the duodenum of rats (**Kozek,** 1971), except that the females were sucked up from the microscope slide into a Pasteur pipette and expelled into the intestine through a very small incision in the duodenal wall.

The pipette was then examined through a dissecting microscope to ascertain that the female had been expelled.

The mice were sacrificed 5 to 6 weeks after inoculation. The diaphragm of each mouse was excised, cut into 5 or 6 pieces which were pressed between two microscope slides, examined under a dissecting microscope, and the number of encapsulated larvae present counted. Each carcass was skinned, and eviscerated; the muscles and bones were ground in 1% pepsin-1% HCl solution in a Waring blender for approximately 5 minutes and digested separately overnight at 37 C. The digests of mice from Groups Ia, IIa, and III were stirred slightly the next day to insure sedimentation of all larvae. After approximately 6 hours, the top half of the liquid was siphoned off and replaced with an equal volume of 20% formalin. At a later date the larvae were counted in the whole volume of each preserved digest.

The digests of mice from Groups Ib, IIb, IV and V were examined within 24 hours after the animals were killed. The total number of larvae in each carcass was obtained by counting the larvae in 3 or 4 5-ml samples of the digest and extrapolating to the total volume. In Group Ib, carcasses were not digested if larvae were not present in the diaphragm.

To determine whether one male could inseminate more than 1 female, six mice were inoculated with 1 male and 10 female IIL. Five days after inoculation all mice were killed, their small intestine excised, rinsed briefly in tap water, placed in saline, and cut open. The petri dishes were covered and warmed underneath a desk lamp for 3-4 hours. The adults that emerged from each intestine were pooled in separate containers. The mucosa of each intestine was then carefully scraped with a bent dissecting needle and examined through a dissecting microscope; adults recovered from the scrapings were added to their respective groups. The adults were sexed and the uterus of the females examined for the presence of larvae, sperm, or unfertilized oöcytes.

RESULTS

Tables 2 and 3 list the total number of larvae recovered from each mouse in Groups I through V.

Inocula consisting of 1 male and 1 female produced from 274 to 1,094 larvae; 1 male and 10 females from 1,240 to 3,162 larvae; and 1 female and 10 males from 433 to 2,158 larvae (Table 2).

Transplanted 48-hour-old females yielded from 19 to 283 larvae, 96-hour-old females from 265 to 863 larvae (Table 3).

Each mouse which harbored IILs had a small number of larvae in the diaphragm, easily detectable by microscopic examination. Conversely, if the examined diaphragm contained no IILs, none were recovered from the rest of that carcass.

As shown in Table 4, only females were recovered from the intestine of each mouse 5 days after inoculation. The number recovered ranged from 1 to 6 per mouse. One mouse (No. 4) harbored only non-inseminated females, 3 of the 6 females from mouse No. 1 were inseminated, and the other 3 mice harbored only inseminated females.

TABLE 1

Progeny recovered by other investigators from experimental hosts inoculated with known numbers and/or sex of T. spiralis *larvae*

	Doerr & Menzi (1933)	Matoff & Wapzarowa (1937)	Thomas (1965)	Campbell & Yakstis (1969)	Chirasak (1971)*			Nolf (1937)	Edney, *et al.* (1953)	
Host	Rat	Mouse	Mouse	Mouse	Mouse			Rat	Rat	Rat
Inoculum	2 larvae	2 larvae	1 ♂ : 1 ♀	2 larvae	1 ♀ : 1 ♂	1 ♀ : 2 ♂	1 ♂ : 2 ♀	Trans-planted 1 adult ♀	Transplanted 1 5-day-old ♀	
Animal No.	Number of *T. spiralis* larvae recovered.									
1	51	2	91	Range	628	228	638	14	345	247
2		230	625	from	962	528	1.022	1,112	70	641
3		242	91	1,496 ± 339	666	438	630		462	370
4		315	98	to	753	684	1,082		205	431
5		259	107	2,297 ± 463	770	362	464		923	386
6		479	108	(Group	1,432	976	742		884	271
7		494	109	mean ±	999	487	1,249		5	230
8		507	215	standard	546	598	868		1	378
9			289	error of	672	283	620		5	490
10			336	mean)	401	1,017	2,187		330	364
11			443		1,431	615	666		462	
12			625		185	1,598	1,063		101	
13					1,659	604	241			
14					960	1,352	1,614			
15					1,373	1,119	730			
16						1,320	1,409			
Total No. infected	1/10	8/23	12/20	15/110	26/32	30/31	26/31	2/4	12/26	10/47

* Only the range limits were given for all groups examined. Transcribed data is limited to the groups examined on and after 28 days of infection.

TABLE 2

Number of Trichinella spiralis *larvae recovered from mice inoculated with either 1 male and 1 female, 10 male and 1 female, or 1 male and 10 female Intestine-Infecting larvae (IIL)*

Group	Mouse No.	No. of larvae in diaphragm	Total No. of larvae*
Ia	1	43	442
	2	83	641
(1 ♂ : 1 ♀)	3	16	274
	4	0	0
	5	27	327
Ib	1	0	–
	2	7	347
(1 ♂ : 1 ♀)	3	0	–
	4	0	–
	5	0	–
	6	38	1,048
	7	59	1,069
	8	0	–
	9	0	–
	10	54	1,094
	11	0	–
IIa	1	126	2,353
	2	0	0
(1 ♂ : 10 ♀)	3	199	2,754
	4	240	3,162
	5	0	0
IIb	1	37	1,447
	2	101	2,521
(1 ♂ : 10 ♀)	3	135	2,265
	4	50	1,240
	5	156	1,716
	6	0	0
III	1	36	776
	2	78	2,158
(10 ♂ : 1 ♀)	3	23	433
	4	96	(Lost)
	5	0	0
	6	72	962

* Digests not examined because diaphragms contained no larvae.

TABLE 3

Number of larvae produced by 48- and 96-hr-old Trichinella spiralis *females transplanted into mice*

Group	Mouse No.	No. of larvae in diaphragm	Total No. of larvae
IV[a] (48-hr-old females)	1	0	0
	2	0	0
	3	17	283
	4	0	0
	5	0	0
	6	0	0
	7	3	19
	8	5	104
	9	0	0
	10	30	185
	11	22	229
V (96-hr-old females)	1	14	536
	2	25	305
	3	29	779
	4	19	265
	5	0	0
	6	19	289
	7	12	612
	8	28	863
	9	0	0
	10	0	0

TABLE 4

T. spiralis *adults recovered from intestine of mice 5 days after inoculation with 10 female and 1 male IIL*

Mouse No.	Total No. recovered	
	Males	Females
1	0	6 (3)
2	0	1 (1)
3	0	2 (2)
4	0	4 (0)
5	0	2 (2)

( ) Number of inseminated females.

DISCUSSION

The results of these investigations indicate that a *T. spiralis* female can produce at least 2,200 oöcytes, the male can produce sufficient sperm to fertilize at least 3,100 oöcytes, and that 1 male can inseminate more than 1 female during the course of an infection. It should be emphasized, however, that these estimates should not be interpreted as the *absolute* reproductive potential of each sex. The larvae recovered and counted in these experiments were the ones which successfully matured in the muscles. Obviously not all muscle-infecting larvae (MILs) which reach muscles survive to maturity and, furthermore, some MILs are lost in organs devoid of striated muscle, e.g. brain. Thus an unknown number of larvae of each generation is lost and this number cannot be determined by any reasonably practical experimental method.

The 1:10 male: female or female: male ratios were used because, at the time these experiments were conducted, it was supposed that 10 females would suffice to exhaust the sperm supply of 1 male, and that 10 males would provide sufficient sperm to fertilize all the oöcytes produce by 1 female, assuming that most of the larvae in the inoculum would survive and mate. As seen in Table 4, relatively few of the 10 females could be found at 6 days post inoculation. It appears that a more accurate estimate of the absolute reproductive potential would have been obtained if extremely skewed sex ratios, e.g. 1:100 or 1:200, were used in the inocula. However, these investigations were discontinued before any such experiments could be conducted.

The variable number of larvae recovered from mice in Groups I through V compares well with the range of yields reported by other investigators (Table 1). It is noteworthy, however, that the smallest numbers of larvae were produced by the 48-hr-old transplanted females. Since it has been well documented (**Shanta** and **Meerovitch,** 1967; **Thomas,** 1965; and **Wu** and **Kingscote,** 1957) that in mice *T. spiralis* females are inseminated approximately 36 hours after inoculation, the transplantation of the females was purposefully delayed in this study by 12 hours to insure that most females would have ample time to be inseminated. Since, in all probability, the 48-hr-old females were inseminated only once, it seems reasonable to assume that the larval yields obtained in Group IV represent the product of one insemination. It thus appears that after one insemination a female *T. spiralis* produces approximately 300 larvae.

Most of the females transplanted at 96 hours after inoculation produced more larvae than those transplanted at 48 hours, suggesting that some of the former might have been inseminated more than once. Three of the 7 females in Group V produced approximately 300 larvae (265, 289, 305), suggesting 1 insemination, but 2 produced approximately 600 larvae (536, 612), suggesting 2 inseminations, and 2 produced approximately 900 larvae (779 and 863), suggesting 3 inseminations. A similar pattern was observed in other groups. Three females in Groups Ia and Ib appeared to have been inseminated once (274, 327, 347), 2 inseminated twice (442, 641), and 3 inseminated 4 times (1,048, 1,069, 1,094). As the mice in these groups harbored only 1 male and 1 female *T. spiralis,* multiple matings presumably occurred during the period of infection.

Since the mice in Group IIa and IIb received 10 females, it could not be determined whether all females were inseminated once, or whether some were inseminated several times. Nevertheless, the data suggest that 4, 5, and 6 inseminations occurred (respectively: 1,240, 1,447, and 1,716 larvae) among the

worms in each of 3 mice, 8 inseminations occurred among the worms in each of the other 3 mice (2,353, 2,521, 2,265), and 9 (2,754) and 11 (3,162) inseminations occurred, respectively, among the worms in each of the remaining 2 mice.

Results of Group III also suggest that *T. spiralis* females were inseminated more than one time, but it could not be determined whether one or several males inseminated the single female in each mouse.

Edney *et al.* (1953) proposed the concept of a "variable productive potential factor" in *T. spiralis* to explain the great extremes in the numbers of larvae recovered from experimental host animals. The results of the studies communicated in this report suggest that the variable factor which determines the number of progeny produce by each female is insemination, rather than some intrinsic mechanism within each female. This interpretation could explain the different larval yields obtained from each group of mice in the present study, as well as the yields obtained by other workers (Table 1).

The low numbers or complete absence of the larvae in some of the mice in Groups IV and V could be attributed to many factors, among which one could include incomplete insemination, damage to the worm during transplantation, the inability of some transplanted worms to establish themselves in the intestine, or other unknown causes. In addition to these factors, it is possible that in some mice inoculated with larvae of both sexes (Groups Ia through III) the larvae of the opposite sex failed either to establish itself or to locate its partners, thus precluding the possibility of mating.

The presence or absence of larvae in the diaphragm of mice appeared to be a valid criterion of the presence or absence of a patent infection. This criterion could be exploited to monitor unisexual infections in mice, since contamination of the inoculum with even one larva of the opposite sex could result in sufficient progeny to render the contamination detectable by a quick examination of the diaphragm.

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RESUMEN

Para determinar el potencial reproductivo de los machos y hembras adultos de *Trichinella spiralis* se inoculó oralmente números conocidos de larvas de ambos sexos en ratones suizos. Cinco o 6 semanas después de la inoculación se sacrificó a los ratones y se contó las larvas presentes en los diafragmas y en los digeridos pépticos de otras partes de los cadáveres. Los diafragmas de todos los ratones infectados contenían larvas. Los inóculos de 1 macho y 1 hembra produjeron de 274 a 1.094 larvas; los de 1 hembra y 10 machos, de 433 a 2.158 larvas; los de 1 macho y 10 hembras, de 1.240 a 3.162 larvas. Hembras de *T. spiralis* de 48 horas de edad, trasnplantadas una en cada ratón normal, produjeron de 19 a 283 larvas; las de 96 horas de edad produjeron de 265 a 863 larvas. Se recobró hasta 3 hembras inseminadas al sexto día de la infección, del intestino de los ratones inoculados con 10 larvas hembra y 1 macho. Estos resultados indican que un macho puede producir

suficiente esperma para fertilizar aproximadamente 3.200 o más oocitos y que puede inseminar a más de una hembra; una hembra puede producir aproximadamente 2.200 o más oocitos. Además, puede haber inseminaciones múltiples durante el curso de la infección. La presencia o ausencia de larvas en el diafragma de un ratón puede usarse como criterio absoluto para confirmar o rechazar la infección con *T. spiralis.*

LITERATURE CITED

Campbell, W. C., & J. J. Yakstis
1969. Mating success and fecundity of pairs of *Trichinella* larvae administered to mice. *Wiad. Parazytol.*, 15: 526-532.

Chirasak, K.
1971. Output of larvae and life span of *Trichinella spiralis* in relation to worm burden and superinfection in the mouse. *Parasitology*, 57: 289-297.

Doerr, R., & E. Menzi
1933. Studien über den Mechanismus der Trichinelleninfektion. VIII. Mitteilung: Vergleichende Unterscuchungen über die Empfänglichkeit der Ratte und des Meerschweinchens für die Infektion per os. *Centralblatt für Bakteriologic Originale*, 128: 177-188.

Edney, J. M., F. Arbogast, & J. Stepp
1953. Productivity in gravid *Trichinella spiralis* (Owen, 1835) transplanted into laboratory rats. *Tennessee Acad. Sci*, 28: 62-68.

Kozek, W. J.
1971. The molting pattern in *Trichinella spiralis.* I. A. light microscope study. *J. Parasit.*, 57: 1015-1028.

Kozek, W. J.
1975. *Trichinella spiralis:* morphoiogical characteristics of male and female intestine-infecting larvae. *Exp. Parasitol.* (in press).

Matoff, K., & M. Wapzarowa
1937. Wieviel Jungtrichinellen Kann eine weibliche Darmtrichinelle gebären? *Z. InfektKr. Haustiere*, 51: 89-98.

Nolf, L. O.
1937. The transplantation of gravid *Trichinella spiralis. Parasitology.*, 23: 574 (Abstr.)

Shanta, C. S., & E. Meerovitch
1967. The life cycle of *Trichinella spiralis.* I. the intestinal phase of development. *J. Zool*, 45: 1255-1260.

Thomas, H.
1965. Beitrage zur Biologie und mikroskopischen Anatomic von *Trichinella spiralis* Owen, 1835. *Z. Tropenmed. Parasit*, 16: 148-180.

Wu, L. -Y., & A. A. Kingscote.
1957. Studies on *Trichinella spiralis.* II. Times of final moult, spermatozoa formation, ovulation and insemination. *Canad. Zool*, 35: 207-211.