THE UNIVERSITY OF MANITOBA

BIOLOGICAL CHARACTERIZATION OF *TRICHINELLA SPIRALIS* (Owen, 1835) AND AN ARCTIC ISOLATE OF GENUS *TRICHINELLA* Railliet, 1895

BY

MIODRAG BELOSEVIC

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

WINNIPEG, MANITOBA

OCTOBER 1979

BIOLOGICAL CHARACTERIZATION OF *TRICHINELLA SPIRALIS* (Owen, 1835) AND AN ARCTIC ISOLATE OF GENUS *TRICHINELLA* Railliet, 1895

BY

MIODRAG BELOSEVIC

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

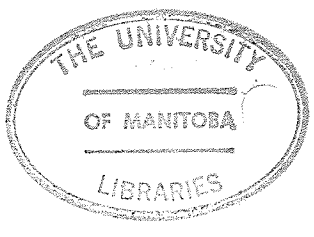


TABLE OF CONTENTS

ABSTRACT

Biological characteristics for the intestinal and muscle phases of *Trichinella spiralis* and an arctic isolate of genus *Trichinella* (TC-isolate) were compared.

The parasites differed in pathogenicity, reproductive capacity in laboratory rodents, distribution and persistence of adults in the small intestine, *in vitro* release of newborn larvae and survival of muscle larvae in mice. Multiple pair and transplant interbreeding experiments indicated that *T. spiralis* and TC-isolate are reproductively compatible, but single pair interbreeding experiments did not indicate reproductive compatibility. *In vitro* behaviour experiments showed high cross-specificity in chemical attractants of the two parasites. Adult worms of *T. spiralis* and TC-isolate were morphologically indistinguishable and did not differ in size. Infective larvae of *T. spiralis* and TC-isolate differed morphometrically, but a convergence in size of worms was observed after prolonged passages of the parasites in mice. It is proposed that TC-isolate is a physiological variant of *T. spiralis* and not a distinct species.

ACKNOWLEDGEMENTS

I would like to thank Dr. T.A. Dick who generously gave his guidance, patience, interest and financial support. I would also like to thank Drs. G. Lubinsky, H.E. Welch and T. Galloway for reviewing this thesis and suggesting changes. I am indebted to N. Hoffman and R. Hancox who took care of experimental animals and to D. Hill for careful typing of this thesis. Last but not least, I thank my wife Priscilla for her patience, understanding, and moral support.

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Trichinella spiralis (Owen, 1835) has remained for many years the only species in the family *Trichinellidae*, Ward 1907. In recent years, several different strains or varieties of this parasite have been studied and are characterized by having low infectivity in laboratory rodents (Read and Schiller 1969; Nelson et al. 1961; Britov 1969; Oseretskovskaya et al. 1969; Dick and Belosevic 1978). Other workers (Britov 1971a, 1971b; Britov and Boev 1972; Boev 1975) have elevated varieties of *Trichinella* to species: *T. nativa* (arctic form); *T. nelsoni* (tropical form) and *T. spiralis* (north-temperate form). Their reasons were based on reproductive isolation, geographical location and limited biomorphological differences. Reproductive isolation of *T. nativa* and *T. spiralis* was reported by Komandarev et al. (1975) and Sukhdeo and Meerovitch (1978), but Bessonov et al. (1975) demonstrated reproductive compatibility between these two "species" and concluded that *T. nativa* was not an independent species.

The description of another species, *T. pseudospiralis*, Garkavi 1972, was based on the absence of a cyst in the muscle stage and dimensions of infective larvae. From their work on *T. pseudospiralis* Pereverseva et al. (1974) concluded it was too early to elevate *T. pseudospiralis*, "native Dagestan strain" to the species level. Bessonov et al. (1975)

demonstrated reproductive isolation between T. pseudospiralis and T. spiralis and concluded that biomorphological differences and reproductive isolation of T. pseudospiralis made it distinct from T. spiralis. To further complicate the designation of species in the genus Trichinella, Madsen (1975) synonymized all newly erected species stating that criteria used in assigning varieties to species level were insufficient and that further morphological, physiological, genetic and biochemical characteristics needed to be defined.

The objectives of this study were: (1) To determine and compare biological characteristics for the intestinal phase of Trichinella spiralis (north-temperate form) and Trichinella sp. isolated from a polar bear killed near Churchill, Manitoba, Canada (designated as TC-isolate); (2) to determine and compare biological characteristics of T. spiralis and TC-isolate for the muscle phase of Trichinella life cycle; (3) to examine the in vitro chemical attraction in genus Trichinella; (4) to establish if T. spiralis and TC-isolate interbreed; (5) to determine an overall comparative index for Trichinella isolates; (6) to re-evaluate the origin and evolution of Trichinella species.

CHAPTER I

COMPARISON OF THE INTESTINAL PHASE OF *T. SPIRALIS* AND TC-ISOLATE

INTRODUCTION

The infectivity of parasites in experimental hosts was used as a comparative characteristic when new isolates or species of Trichinella were evaluated (Nelson et al. 1961; Kozar and Kozar 1965; Arakawa and Todd 1971; Sukhdeo and Meerovitch 1978). Some authors have examined the morphology of larvae and/or adults (Garkavi 1972; Britov 1973; Sukhdeo and Meerovitch 1978), whereas other studies focussed on biochemical comparison of the isolates (Romanova et al. 1971; Belozerov 1976). Although Rappaport (1943b) and Pawlowski and Rauhut (1971) compared the intestinal phase of local T. spiralis strains (north-temperate forms), biological characteristics for the intestinal phase of arctic isolates of Trichinella have not been studied.

In this chapter comparative studies on the intestinal phase of T. spiralis and TC-isolate are reported. The biological characteristics examined are: (1) Reproductive capacity indices (infectivity), as used by Bessonov et al. (1975), for 15 passages of TC-isolate and 11 passages of T. spiralis in mice; (2) pathogenicity of T. spiralis and TC-isolate in different hosts; (3) intestinal distribution; (4) longevity of intestinal phase; (5) in vitro release of newborn larvae; (6) morphological measurements of adult T. spiralis and TC-isolate

MATERIALS AND METHODS

Parasites

Trichinella spiralis was obtained from the University of Toronto where it has been maintained in mice for numerous generations. Subsequently, the parasite was maintained in our animal holding facilities for 5 years in Sprague-Dawley rats [Bbl: (SD), Biobreeding Laboratories, Ottawa, Canada]. For this study the parasite was passaged in outbred Swiss Webster mice.

TC-isolate was recovered from a frozen diaphragm of a female polar bear killed near Churchill, Manitoba in 1975, and maintained in outbred Swiss Webster mice.

Maintenance of Experimental Animals

All experimental animals used in this study were kept under the following conditions: humidity 55 ± 5%, temperature 21 ± 2 C; diurnal cycle of 15 hours (light) and 9 hours (dark); food and water *ad libitum*.

Passage Experiments

T. spiralis and the TC-isolate were maintained in the laboratory under identical experimental conditions. Forty days post infection larvae from preceeding passage were isolated for experimental re-infection. Fifteen and eleven passages for the TC-isolate and *T. spiralis* respectively, were performed using 50-60 day old outbred Swiss Webster mice [Crl: COBS CFW (SW), Charles River Breeding Laboratories,

Wilmington, Mass. U.S.A.]. Another outbred strain of Swiss Webster mice, bred at Animal Holding Facilities, Department of Zoology, University of Manitoba [AHF: (SW)] was used and although slight differences between biological characteristics of TC-isolate and T. spiralis were noted in these two outbred strains of mice they were not significantly different. Only the infection dose causing 50% mortality differed between the AHF: (SW) and the Crl: COBS CFW (SW) mice and is presented in this chapter.

The infection dose of 400 larvae/mouse and determination of reproductive capacity was carried out as follows;

Infected animals were killed by cervical dislocation, weighed, skinned and eviscerated. A piece of diaphragm of each mouse was compressed and examined for worms. Five mice of the same sex were combined and ground in a meat grinder, and then placed in a flask containing a 1% pepsin-HCl solution for 1 hour at 37 C with stirring (ratio of meat (gms) to pepsin-HCl solution (mls) was 1:7). Digested material was placed in a Baermann apparatus and larvae allowed to accumulate for 30-45 minutes. Larvae were collected in a graduated centrifuge tube from the bottom 15 mls of digest, suspended in 0.16% agar and 0.85% saline at 37 C and stirred several times to ensure even distribution of worms. Each of ten 0.1 ml aliquots were removed, evenly spread on counting grids and the worms counted with the aid of a

dissecting microscope. If counts varied by more than 10%, four additional counts were done. Number of larvae/ml and total number of larvae in suspension was determined. Four hundred larvae were administered by gastric intubation to etherized mice for all experiments.

Pathogenicity Experiments

The degree of pathogenicity during the intestinal phase of T. spiralis and the TC-isolate was determined using mortality of hosts as an index. The pathogenicity indices were determined for Crl: COBS CFW (SW), the AHF: (SW) and Microtus pennsylvanicus (Ord). Due to absence of adult worms from the small intestine of these hosts after day 20 post infection, deaths were recorded during the first 20 days after infection.

A preliminary experiment to approximate the infection dose resulting in 50% mortality used 60, 50-60 day old AHF: (SW) mice infected with 1000 larvae; 30 with TC-isolate and 30 with T. spiralis. Experimental animals were examined daily until day 20 post infection, mortalities recorded and the small intestine checked for intensity of infection and gross pathology.

A second experiment to determine more precisely the dose causing 50% mortality used 200, 50-60 day old Crl: COBS CFW (SW) mice; 100 infected with doses ranging from 700 to 2000 larvae of the TC-isolate and 100 infected with doses ranging from 800 to 2400 larvae of T. spiralis. Mortalities were recorded daily for 20 days and the small intestine examined for intensity of infection and gross pathology.

A third experiment to evaluate susceptibility of natural populations of mice and voles (F_1 generation in the laboratory) to TC-isolate and T. spiralis was undertaken. Each group of host-species was divided equally and infected with TC-isolate and T. spiralis. Hosts used and numbers were: Microtus pennsylvanicus (28); Clethrionomys gapperi (Vigors) (12) and Peromyscus maniculatus (Wagner) (12). Mortalities of hosts were recorded daily for 20 days and small intestine examined for intensity of infection and gross pathology.

Distribution of Adult Parasites in the Small Intestine

The population distribution in the small intestine and changes in population distribution during passages of T. spiralis and TC-isolate were studied in 50-60 day old Crl: COBS CFW (SW) mice. Mice were infected by gastric intubation with 400 larvae each of T. spiralis or TC-isolate. At day 5 post infection, mice were killed by cervical dislocation and the small intestine removed and placed on a graduated dissecting board (Brambell 1965). The intestine was cut in 20 equal segments and each segment placed in a vial partially filled with 0.85% saline solution. Vials containing these segments were refrigerated for 24 hours to facilitate breakdown of the intestinal mucosa. After refrigeration, contents of each vial were emptied into a petri dish, each intestinal segment slit longitudinally,

and the mucosa lining vigorously scraped and stirred. Number of worms per segment was established with the aid of a dissecting microscope.

The median for each population of Trichinella in the small intestine was determined as follows; that point of the intestine where 50% of the worms were anterior and 50% of the worms were posterior. The median values from a group of animals were averaged and a standard deviation determined. It was assumed that worms in each 5% (1/20th of the intestine) were evenly distributed. Throughout this chapter the averaged median value will be referred to as position.

Longevity of Adult Parasites in the Small Intestine

The longevity of TC-isolate and T. spiralis in the small intestine was studied using 70, 50-60 day old female Crl: COBS CFW (SW) mice infected by gastric intubation with 400 larvae each of TC-isolate or T. spiralis. From day 1 post infection and every second day thereafter for 20 days, the population distribution for both parasites was determined (see section on distribution of worms in intestine). Numbers of male and female parasites were recorded so that sex ratio, rate of establishment and rate of expulsion of male and female worms could be determined.

In vitro Release of Newborn Larvae

The 24 hour rate of larval production by females during the intestinal phase was determined for both T. spiralis and the TC-isolate. Adult females were isolated from Crl: COBS CFW (SW) mice infected by gastric intubation with 400 larvae. From day 3 post infection, through and including day 17, mice were killed by cervical dislocation, and the anterior half of the small intestines removed and placed in 37 C Tyrode's solution. The intestine was slit longitudinally, cut in 2 cm pieces and the mucosa lining vigorously scraped and stirred. The mixture was placed in a Baermann apparatus and the worms collected for 1-2 hours, following which the bottom 10 mls of Tyrode's containing adult worms were removed and placed in a petri dish. Worms were washed twice in 37 C Tyrode's, males and females separated and passed through four washes of α-MEM Eagle, Earle's Base (modified) tissue culture medium and 10% fetal calf serum (by volume). Worms treated in this manner were free of intestinal debris. Each female was placed in 2 ml plastic (cone-bottom) vial containing 1 ml of tissue culture medium, and the vials capped and stored at 37 C in an environmental chamber for 24 hours. At the end of incubation, the top 0.75 mls of medium was removed from each vial and the remaining 0.25 mls was placed on counting grids. All newborn larvae in the sample were counted using a dissecting microscope.

Since maximum larval production occurred on day 7 post infection for both T. spiralis and TC-isolate, additional data on larval production from 300 females of each were determined.

Morphological Measurements of Adult Parasites

Adult T. spiralis and TC-isolate were obtained at day 7 post infection from Crl: COBS CFW (SW) mice infected by gastric intubation with 400 larvae. Variability in worm size was pronounced and a comparison of large and small worms from the same population revealed significant differences ($p < 0.05$) in length. Consequently, for our morphological studies all worms to be measured were randomly selected. Specimens were fixed in hot 70% alcohol and cleared in lactophenol/glycerol (1:1) at 57 C. Individual worms were mounted on slides in glycerol, projected with Leitz Micro-promar slide projector and traced. A micrometer scale was projected onto each diagram to determine its magnification. Length and width of T. spiralis and TC-isolate males and females and length of uterus was measured using drafting dividers. The volume of worms was estimated using the method described by Despommier et al. (1975) for muscle larvae which were considered to be a composite of a cylinder and cone. This assumption allowed use of an empirical formula for determination of worm volume.

Student's t-test was used for statistical analysis and Bartlett's test was applied to check for homogeneity of variances; 0.05 probability level was considered significant.

RESULTS AND DISCUSSION

Passage Experiments

A common biological characteristic used to compare varieties of Trichinella is infectivity of parasites expressed as an index of reproductive capacity. Most researchers agree that reproductive capacities of new geographical isolates of Trichinella from Arctic and Africa are lower compared to T. spiralis reproductive capacity (Nelson and Mukundi 1963; Sukhdeo and Meerovitch 1978; Dick and Belosevic 1978). However, the indices of reproductive capacity reported, particularly for arctic isolates of Trichinella are not consistent (Dick and Belosevic 1978). Arakawa and Todd (1971) reported that infectivity of a polar bear isolate of Trichinella changed after four passages through white mice reaching an index value of 143.8. Bessonov et al. (1975) stated that after 6 passages through pigs and 5 passages through cats and rats, the reproductive capacity of T. nativa equalled that of T. spiralis. In contrast, Sukhdeo and Meerovitch (1978) reported that prolonged passages through various hosts (more than 9 years) did not effect the infectivity of an arctic isolate and suggested that degree of infectivity is a genetically fixed character of the parasite.

I tested the adaptation of T. spiralis and the TC-isolate by numerous 40 day passages through the same

species and strain of host, thus eliminating the possibility of different hosts influencing the reproductive capacities of the parasites (Tables I and II).

After 15 passages through mice, the TC-isolate had a slightly lower reproductive capacity index at passage 15 than at passage 1 (Table II). Initially, worms in passages 4, 5, and 6 of the TC-isolate showed an increase in reproductive capacity that corresponds to an increase in reproductive capacity of T. nativa as reported by Bessonov et al. (1975). From passage 6 to 15 the reproductive capacity of the TC-isolate decreased reaching an index value of 41 (Table II). The mean index of reproductive capacity for 15 passages of TC-isolate through mice, I = 69 $\pm$ 29, was higher than the value reported for an arctic isolate by Sukhdeo and Meerovitch (1978) in inbred A/J mice. This may be due to different strains of mice used, age of the arctic isolate and effects of passaging of the isolate through different species of hosts.

In eleven passages through mice, T. spiralis indices of reproductive capacity were consistently higher for each passage compared to TC-isolate (Tables I and II). An initial increase in reproductive capacity (to passage 6), and a subsequent decrease was also observed for T. spiralis. The mean index of reproductive capacity for 11 passages of T. spiralis through mice, I = 155 $\pm$ 31, was 2.2 times higher compared to the TC-isolate mean index of reproductive capacity (Tables I and II). Mean number of larvae recovered

and number of larvae per gm of muscle were significantly higher for T. spiralis than for the TC-isolate (Tables I and II). My observations indicate that low reproductive capacity in laboratory rodents is a stable biological characteristic of the TC-isolate. Lower reproductive capacity of the TC-isolate in Crl: COBS CFW (SW) mice and other laboratory rodents (see Chapter II, p. 44), suggests that these animals are not optimal hosts for this parasite. The opposite is true for T. spiralis which was maintained under laboratory conditions for years and is well adapted to laboratory rodents as shown by its high reproductive capacity in mice.

An initial increase in the reproductive capacity of both T. spiralis and the TC-isolate is difficult to explain since Kozar and Kozar (1963) showed that rapid passages of Trichinella increased its antigenicity. The increase in reproductive capacity cannot be related to the specific groups of experimental mice because T. spiralis passage experiments were started 160 days later than the TC-isolate passages. Perhaps, in early passages, our strains of Trichinella were adjusting to the experimental hosts and thereafter the antigenicity increased with a concomitant decline in reproductive capacity.

TABLE I: Reproductive capacity of *T. spiralis* through eleven passages in outbred Swiss Webster mice [Crl: COBS CFW (SW)]

Passage number	No. of mice	Mean no. of larvae recovered per mouse	Mean index of reproductive capacity/mouse	Mean larvae per gm of muscle
1	20	64,000	160	2121
2	20	48,900	122	1401
3	20	75,900	190	2618
4	20	71,900	180	2318
5	20	80,500	201	2439
6	19	56,700	142	1829
7	20	49,600	124	1505
8	10	54,500	136	1730
9	10	53,700	134	1760
10	10	54,800	137	1712
11	10	55,100	138	1579
	179	61,868 ± 10,900	155 ± 31	1901 ± 403

TABLE II: Reproductive capacity of TC-isolate through fifteen passages in outbred Swiss Webster mice [Crl: COBS CFW (SW)]

Passage number	No. of mice	Mean no. of larvae recovered per mouse	Mean index of reproductive capacity/mouse	Mean larvae per gm of muscle
1	2*	2,400	58	-
2	2	21,000	52	709
3	20	28,000	70	848
4	20	34,800	87	1010
5	20	40,300	101	1198
6	20	38,600	97	1063
7	20	29,400	74	743
8	10	28,800	72	741
9	10	25,400	63	753
10	10	19,900	50	548
11	10	19,600	49	523
12	10	18,900	47	516
13	10	16,300	41	440
14	10	20,100	50	560
15	10	16,300	41	480
	184	27,581 ± 7,700	69 ± 29	724 ± 235

* Infected with 41 larvae each

The reproductive capacity of the TC-isolate can be used to separate it from T. spiralis because continuous rapid passaging of the TC-isolate reported here indicated that this capacity is a genetically stable character of the parasite.

Pathogenicity Experiments

Results showing the TC-isolate to be more pathogenic than T. spiralis were not expected. Kozar and Kozar (1965) reported that the Kenya strain of Trichinella was significantly less pathogenic to mice and rats than the two Polish strains. They stated that lower pathogenicity of the Kenya strain was related to its lower infectivity. Rappaport (1943a) was unable to show differences in pathogenicity between three American strains of Trichinella, stating that differential responses of the hosts may have influenced the results.

TC-isolate was significantly more pathogenic to mice than T. spiralis during the intestinal phase of parasitism (Figs. 1 and 2). Fifty percent mortality of Crl: COBS CFW (SW) mice occurred at a lower infection dose of the TC-isolate (1700 larvae/mouse) than of T. spiralis (2300 larvae/mouse). The majority of mice died during the first 10 days post-infection, a period corresponding to maximum larval release (Table V). Of 30 outbred AHF: (SW) (Fig. 3) that were infected with 1000 TC-isolate larvae, 18 died by day 11 post infection compared to one of 30 that were infected with 1000 T. spiralis larvae, indicating that T. spiralis is considerably less pathogenic to this strain of mice. The

degree of pathogenicity of the TC-isolate is host dependent since the infection dose causing 50% mortality in Crl: COBS CFW (SW) mice was twice as high compared to the infection dose that produced 50% mortality in AHF: (SW) mice (Figs. 2 and 3). Further, our results indicate that reproductive capacity is not directly related to the degree of pathogenicity as suggested by Kozar and Kozar (1965), since TC-isolate caused higher mortality in mice but its reproductive capacity was lower (Tables I and II).

To determine if there were differences in pathogenicity of the TC-isolate and T. spiralis between laboratory and wild populations of rodents (F_1 generation in the laboratory), three species of rodents (M. pennsylvanicus, C. gapperi and P. maniculatus) were infected with the parasites. Due to low numbers of experimental animals I used a single infection dose of 400 larvae/animal. During the first 20 days post infection none of C. gapperi and P. maniculatus infected with the TC-isolate and T. spiralis died. However, 50% mortality of M. pennsylvanicus infected with TC-isolate occurred by day 11 post infection (Fig. 3). No M. pennsylvanicus infected with T. spiralis died during first 20 days post infection, indicating that the TC-isolate is more pathogenic to this host than T. spiralis.

The reproductive capacities of TC-isolate and T. spiralis were similar in M. pennsylvanicus (see Chapter II, p. 44)

Fig. 1. Mortality of Crl: COBS CFW (SW) mice infected with Trichinella spiralis during first 20 days post infection.
ID = infection dose/mouse.
( ) = number of mice that died.
↓ = 50% mortality.

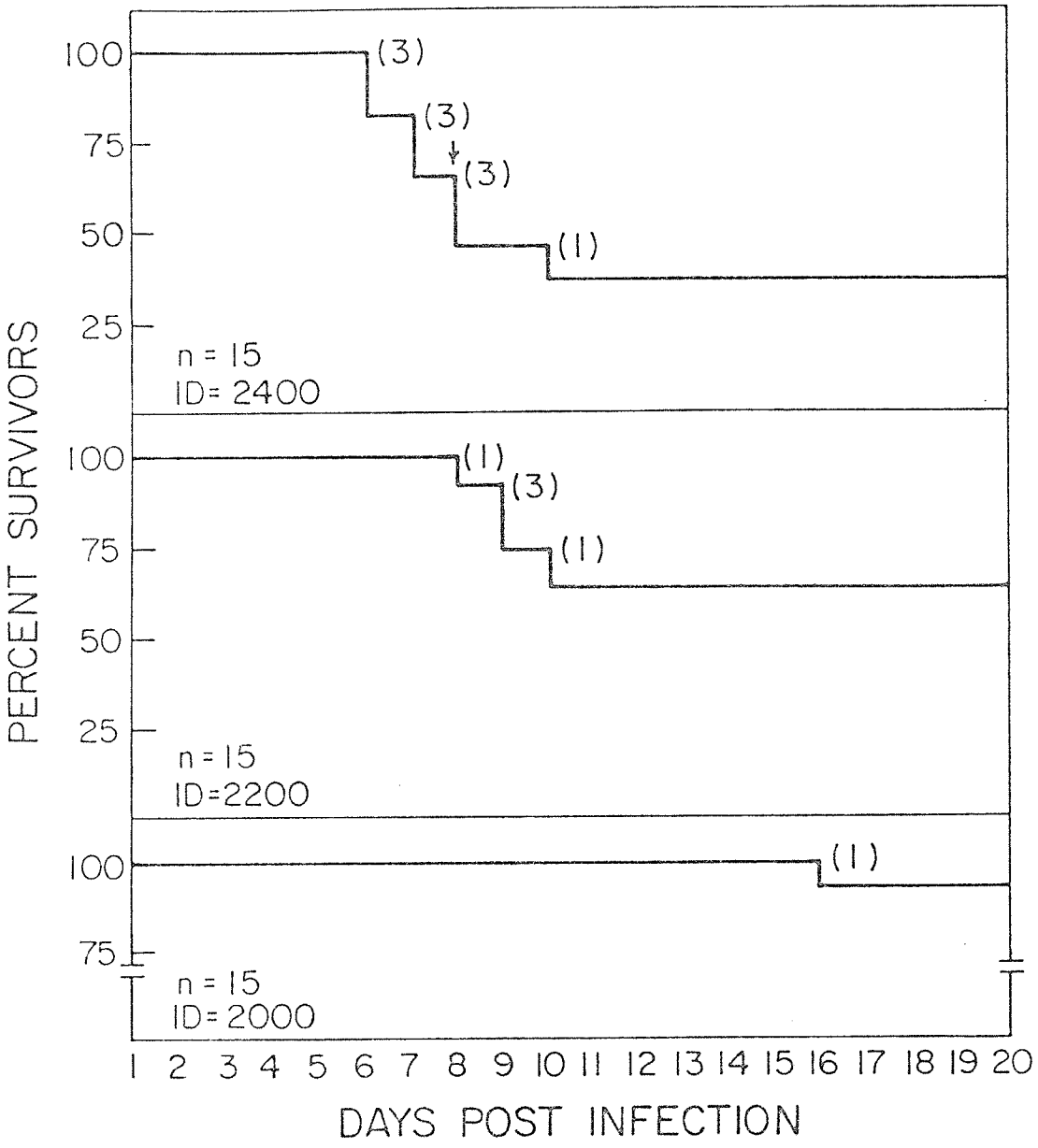
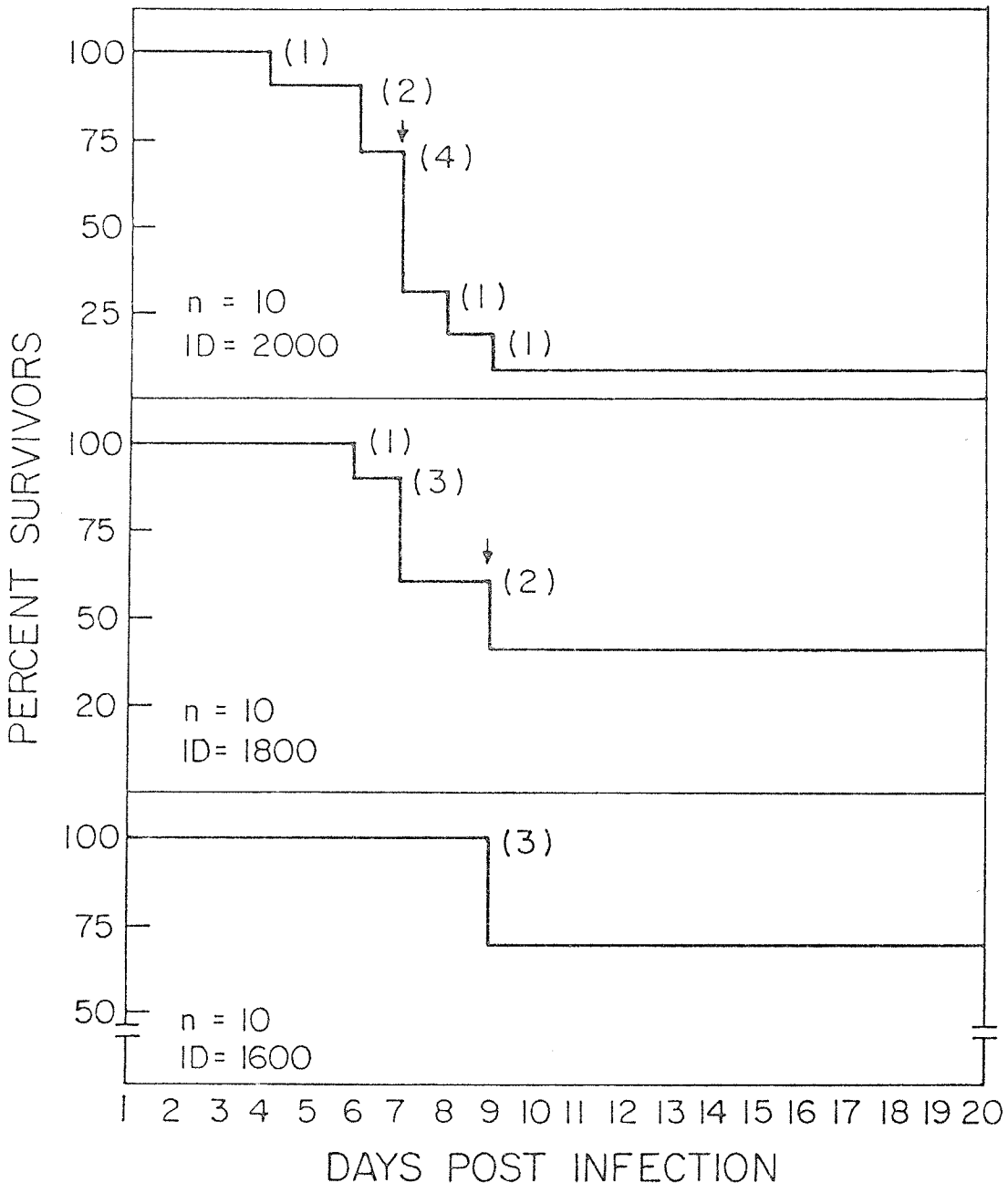

PERCENT SURVIVORS
100
75
50
25
(3)
(3)
(3)
(1)
n = 15
ID= 2400
100
75
50
25
(1)
(3)
(1)
n = 15
ID=2200
100
75
(1)
n = 15
ID= 2000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
DAYS POST INFECTION

Fig. 2. Mortality of Crl: COBS CFW (SW) mice infected with TC-isolate during first 20 days post infection. ID = infection dose/mouse. ( ) = number of mice that died. ↓ = 50% mortality.


PERCENT SURVIVORS
100
75
50
25
(1)
(2)
(4)
(1)
(1)
n = 10
ID = 2000
100
75
50
20
(1)
(3)
(2)
n = 10
ID= 1800
100
75
50
(3)
n = 10
ID= 1600
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
DAYS POST INFECTION

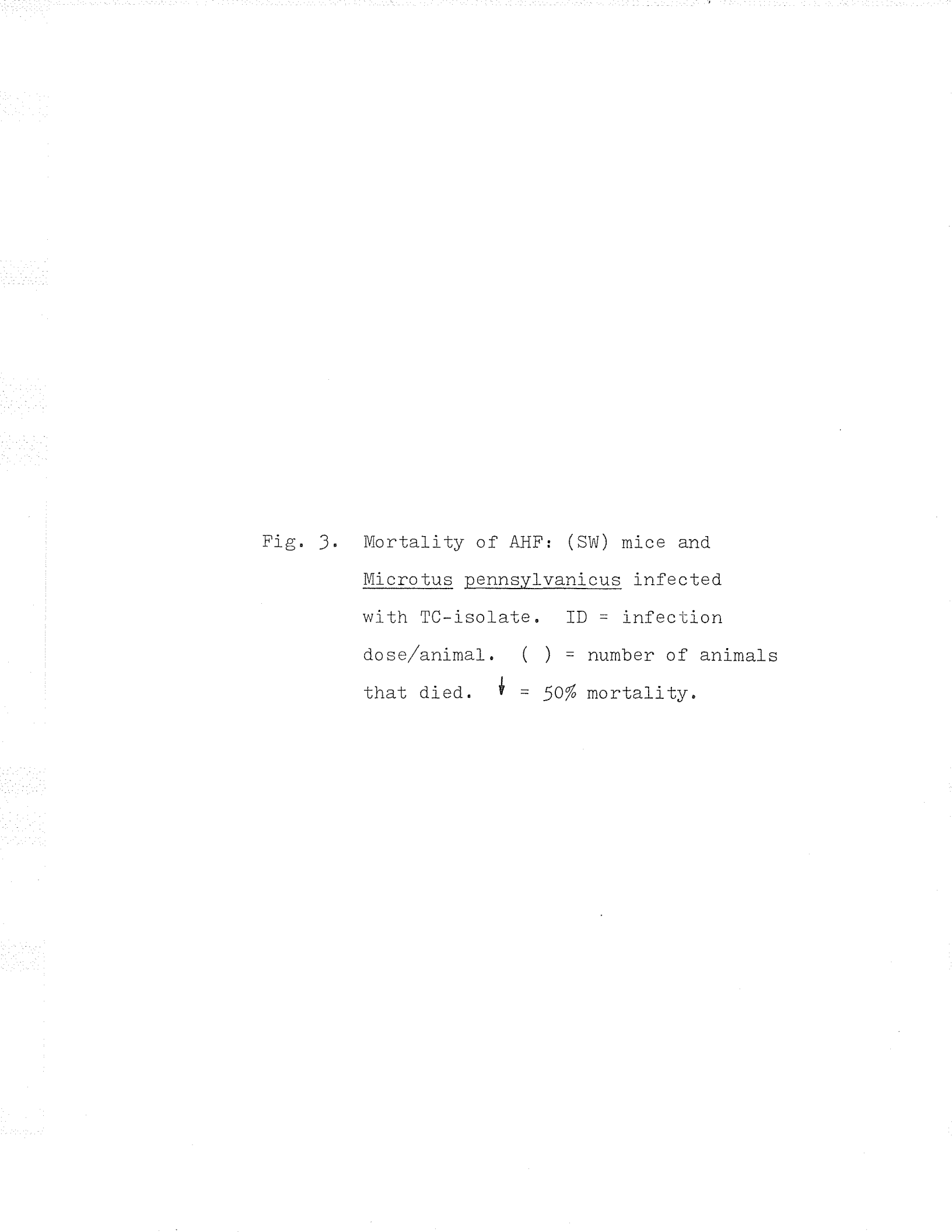
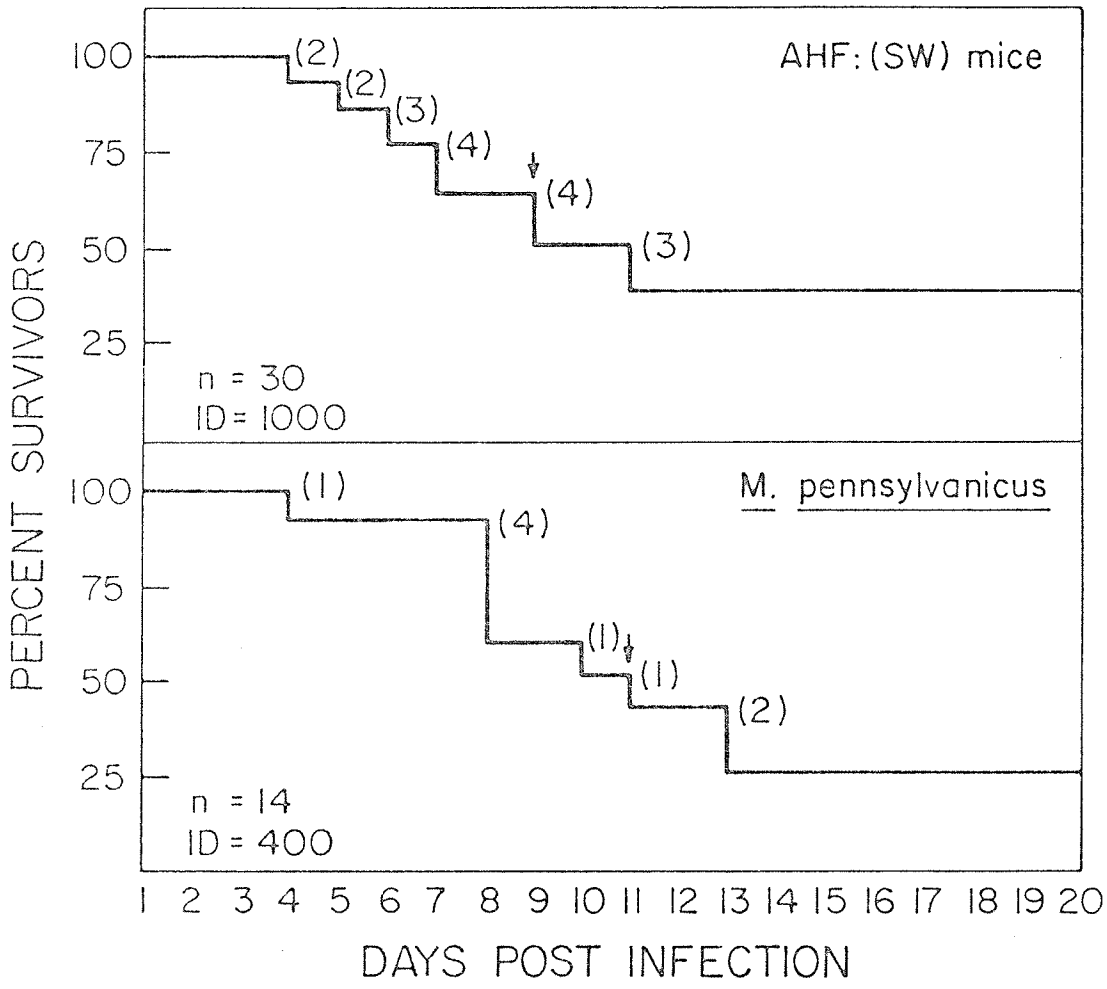


Fig. 3. Mortality of AHF: (SW) mice and *Microtus pennsylvanicus* infected with TC-isolate. ID = infection dose/animal. ( ) = number of animals that died. ↓ = 50% mortality.


AHF: (SW) mice
100
75
50
25
(2)
(2)
(3)
(4)
(4)
(3)
n = 30
ID = 1000
M. pennsylvanicus
(1)
(4)
(1)
(1)
(2)
n = 14
ID = 400
PERCENT SURVIVORS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
DAYS POST INFECTION

but the pathogenicity of TC-isolate was higher in this host. This supports my findings for Crl: COBS CFW (SW) mice where the reproductive capacity of the TC-isolate was inversely related to degree of pathogenicity during the intestinal phase of parasitism.

Death of hosts may be due to factors other than number of larvae released since TC-isolate females in vitro released significantly lower numbers of larvae compared to T. spiralis (Table V). Furthermore, mice infected with TC-isolate started to die at day 4 post infection, prior to larval release (Table V).

Differences in pathogenicity of the TC-isolate and T. spiralis may be due to higher antigenicity of the TC-isolate, and/or relationship of adults and newborn larvae to the intestinal wall of the host. Intestines of hosts infected with the TC-isolate had more haemorrhaging and mucus present compared to the intestines of hosts infected with T. spiralis. Migration pattern of newborn larvae and degree of penetration and movement of the TC-isolate adults and larvae in the mucosa are factors that may account for higher pathogenicity of this parasite to laboratory mice and wild rodents.

Distribution of Adult Parasites in the Small Intestine

The intestinal distribution of T. spiralis has been reported for mice (Larsh and Hendricks 1949; Podhajecky 1962;

Campbell 1967), rats, (Gursch 1949; Pawlowski and Rauhut 1961), hamsters, (Boyd and Huston 1954; Concannon and Ritterson 1965), guinea pigs (Roth 1938) and chicks (Marty 1966). It is accepted that most adult Trichinella are located in the anterior half of the small intestine in mice, rats and hamsters and the posterior half of the small intestine in guinea pigs and chicks.

Results on the intestinal distribution of the TC-isolate and T. spiralis confirm the findings of Campbell (1967) and others, in that most adult worms were situated in the anterior half of the small intestine, 94% and 88.9% for T. spiralis and the TC-isolate, respectively (Tables III and IV). The mean number of adults recovered from the small intestine at day 5 post infection was significantly higher for T. spiralis compared to the TC-isolate, indicating that T. spiralis adult worms establish better in the small intestine of mice (Tables III and IV). Position of the TC-isolate adults in the small intestine ($P = 23.49 \pm 3.23$) was significantly different from position of T. spiralis adult worms ($P = 17.00 \pm 3.33$), indicating that the TC-isolate adult worms range over a greater area of the small intestine (Tables III and IV). Position of worms for each passage was consistently more posterior for the TC-isolate than T. spiralis suggesting that the population distribution is a stable character for T. spiralis and the TC-isolate (Tables III and IV).

TABLE III: Recovery and distribution of T. spiralis (day 5) from small intestine through eleven passages in outbred Swiss Webster mice [Crl: COBS CFW (SW)]

Passage number	No. mice	Mean no. adults recovered ± SD	Position of worms ± SD	Per cent take
1	10	136 ± 21	13.45 ± 3.35	34
2	10	200 ± 44	14.85 ± 4.49	50
3	10	153 ± 52	16.68 ± 4.51	38
4	8	229 ± 50	19.11 ± 3.46	57
5	8	187 ± 32	19.38 ± 2.21	47
6	8	257 ± 65	18.10 ± 4.86	64
7	10	199 ± 43	19.18 ± 3.32	50
8	7	223 ± 23	16.28 ± 2.82	56
9	8	183 ± 13	15.54 ± 2.26	46
10	8	251 ± 34	17.99 ± 2.00	63
11	8	182 ± 36	17.36 ± 2.72	46
	95	197 ± 38	17.00 ± 3.33	49

TABLE IV: Recovery and distribution of TC-isolate (day 5) from small intestine through thirteen passages in outbred Swiss Webster mice [Crl: COBS CFW (SW)]

Passage number	No. mice	Mean no.adults recovered ± SD	Position of worms ± SD	Per cent take
4	20	140 ± 54	23.07 ± 4.39	35
5	18	127 ± 47	23.92 ± 2.89	32
6	20	181 ± 46	21.72 ± 4.46	45
7	20	145 ± 39	24.78 ± 3.95	36
8	10	138 ± 24	26.39 ± 4.39	35
9	10	155 ± 36	25.33 ± 2.28	39
10	8	208 ± 43	21.42 ± 2.28	52
11	10	166 ± 39	26.75 ± 2.89	42
12	10	153 ± 11	20.75 ± 0.91	38
13	8	203 ± 32	23.49 ± 3.19	51
14	8	219 ± 47	21.38 ± 1.92	55
15	8	227 ± 32	22.91 ± 1.96	57
16	8	171 ± 49	23.04 ± 2.90	43
	158	164 ± 40	23.49 ± 3.23	41

Intestinal distribution of Trichinella isolates can be used as a characteristic in defining new isolates, providing the genetic stability of the character is tested by numerous passages through the same species and strain of host, and the small intestine is subdivided into adequate number of subunits to allow for accurate determination of the position of worms in the intestine.

Longevity of Adult Parasites in the Small Intestine

One of the most variable characteristics of T. spiralis infections is the longevity of adult worms in the intestines of various hosts. The longevity of T. spiralis is reported to be from 10 days in dogs (Matoff 1937) to 16 weeks in humans (Carter 1949). Variability of results reported in the literature, particularly for mice (16-38 days), is dependent on the following conditions: the experimental method, host and strain of the parasite (Kozar and Kozar 1965).

Pawlowski and Rauhut (1971) showed differences in the dynamics of the intestinal phase of "old" and freshly isolated strains of Trichinella from Poland, indicating that strain differences of the parasite may account for variability in results obtained. Rauhut (1978) reported that different strains of animal host (in this case mice) affected the persistence of the parasite in the gut. Similar results were obtained by Concannon and Ritterson (1965) for Chinese and Golden hamsters, where Chinese

hamsters expelled worms at a faster rate than Golden hamsters.

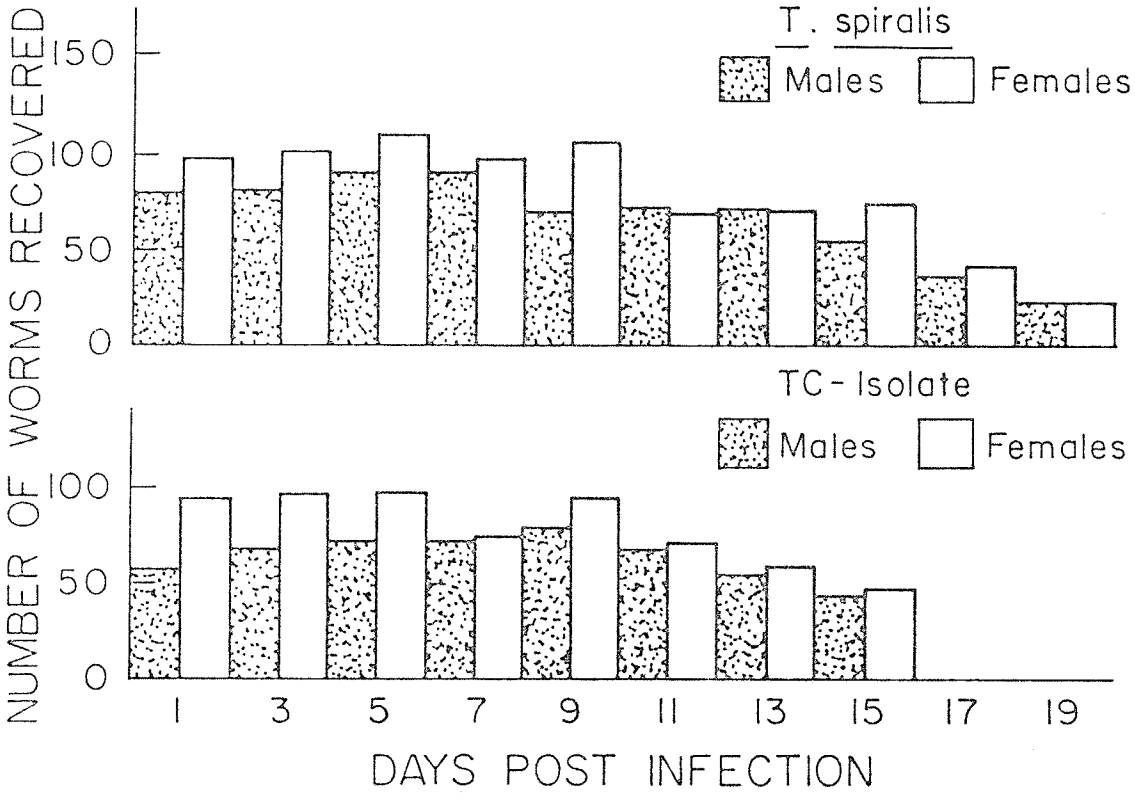
The mice used in longevity experiments were of the same age, sex (females) and strain, and the experimental method has been employed in our laboratory for years. I found differences in longevity of the intestinal phase of the TC-isolate and T. spiralis. T. spiralis adult worms persisted in the small intestine for 20 days, whereas the TC-isolate adults were not found in the small intestine past day 15. The sex ratios (female:male) were 1.17 and 1.19 for T. spiralis and TC-isolate, respectively. Male and female worms were expelled from the small intestine at the same rate since the sex ratio did not significantly change during the intestinal phase (Fig. 4). My findings differ from those of Rappaport (1943b) who found a shift in female to male ratio in later stages of the intestinal phase, indicating that females of Trichinella are expelled faster than males.

In vitro Release of Newborn Larvae

In vitro release of newborn larvae by adult females of Trichinella has been used to assess levels of immunity in trichinellosis (Despommier et al. 1977), but not to compare geographical varieties of Trichinella.

Results in Table V show that in vitro release of newborn larvae by individual females of T. spiralis and

Fig. 4. Longevity of male and female Trichinella spiralis and TC-isolate in the small intestine of Crl: COBS CFW (SW) mice.


T. spiralis
Males
Females
TC - Isolate
Males
Females
NUMBER OF WORMS RECOVERED
150
100
50
0
100
50
0
1
3
5
7
9
11
13
15
17
19
DAYS POST INFECTION

TC-isolate is continuous during the intestinal phase. The majority of larvae were released between day 5 and 8 post infection, 75% and 62% of total fecundity for T. spiralis and TC-isolate, respectively. From day 9 post infection until the end of intestinal phase, T. spiralis females released significantly higher numbers of larvae for each day post infection compared to TC-isolate females (Table V). At day 7 post infection, T. spiralis females released 56 ± 15 larvae/24 hours (range 22-103) compared to TC-isolate females that released 21 ± 7 larvae/24 hours (range 0-39). The average fecundity of a single female for the entire intestinal phase was 3 times higher for T. spiralis (335 larvae/female) than TC-isolate (114 larvae/female).

Of interest to me was a greater variation in larval production per female and lower average fecundity than previously reported (Despommier et al. 1977). Differences may be due to strain of parasite, host or incubation medium used. It is evident from in vitro fecundity and morphological measurements of adults (see section on morphology) that I was dealing with populations of Trichinella with a greater range of normal variation.

Morphological Measurements of Adult Parasites

Results indicate that T. spiralis and TC-isolate are morphologically indistinguishable (Table VI), and differ from work reported by Sukhdeo and Meerovitch (1978) who

TABLE V: The release of newborn larvae by individual females of T. spiralis and TC-isolate during the intestinal phase.

Days post infection	No. females examined in each group	Mean no. larvae released per 24 hr/female ± SD	
		T. spiralis	TC-isolate
3	25	0.0	0.0
4	25	0.0	0.0
5	25	44.68 ± 7.72	22.16 ± 4.91
6	25	52.56 ± 12.36	21.16 ± 4.34
7	325	56.44 ± 14.82 ++	20.66 ± 6.50+++
8	25	55.52 ± 10.80	14.64 ± 2.97
9	25	15.64 ± 4.18	4.16 ± 3.98
10	25	11.92 ± 2.02	2.04 ± 2.49
11	25	15.28 ± 2.67	6.16 ± 4.87
12	25	13.24 ± 5.39	9.48 ± 4.06
13	25	9.40 ± 4.13	3.04 ± 2.51
14	25	22.20 ± 4.85	6.40 ± 2.39
15	25	12.44 ± 3.78	4.18 ± 1.66+
16	25	12.36 ± 2.94	-
17	25	13.56 ± 4.61	-

\+ Calculations based on 11 females

++ Range for T. spiralis = 22 - 103

+++ Range for TC-isolate = 0 - 39

showed significant differences in size of adult worms between north-temperate and arctic isolates of Trichinella. Analysis of measurements of small and large worms from a single host infection with 400 larvae of either TC-isolate or T. spiralis gave statistically significant results ($p < 0.05$), indicating that there is considerable size variability within a population. Furthermore, measurements of adults from the fourth and 15th passage of TC-isolate and fourth and 11th passage of T. spiralis did not show significant differences, suggesting that adult worm size was not influenced by passaging.

Sukhdeo and Meerovitch (1978) suggested that number of larvae produced by females of three Trichinella isolates was dependent on uterus size since uterine lengths and infectivities were directly correlated. I did not find significant differences in uterine lengths of T. spiralis and TC-isolate (Table VI) indicating that uterus size and reproductive capacities of T. spiralis and TC-isolate are not directly correlated. Consequently, the use of morphological measurements of adults to compare isolates of Trichinella should be viewed with caution.

TABLE VI: Morphological measurements (mm) of T. spiralis (passage 11) and TC-isolate (passage 15) adults recovered from mice 7 days post infection[1]

	T. spiralis		TC-isolate	
	Males	Females	Males	Females
N	30	30	30	30
Total Length	1.21 ± 0.10	2.19 ± 0.24	1.22 ± 0.09	2.12 ± 0.26
Width	0.033 ± 0.003	0.038 ± 0.003	0.029 ± 0.004	0.038 ± 0.005
Volume	$8.81 \times 10^{-4} \pm 1.05 \times 10^{-4}$	$1.98 \times 10^{-3} \pm 0.45 \times 10^{-3}$	$7.8 \times 10^{-4} \pm 1.22 \times 10^{-4}$	$1.88 \times 10^{-3} \pm 0.32 \times 10^{-3}$
Length of Uterus	--	1.36 ± 0.20	--	1.26 ± 0.19

[1] Males and females from passage 4 for both TC-isolate and T. spiralis were measured and did not differ significantly in size.

CONCLUSIONS

The TC-isolate and T. spiralis differ in pathogenicity, reproductive capacity, distribution and persistence of adults in the small intestine, and fecundity of females in vitro. Reproductive capacity and intestinal distribution appear to be genetically stable characters of T. spiralis and TC-isolate, since no major change in characters was observed after numerous passages through the same host. Lower reproductive capacity of the TC-isolate is the result of a shorter intestinal phase, decreased rate of establishment and lower fecundity of females. These parameters are influenced by the host immune system (Despommier et al. 1977), and suggest that mice are not optimal hosts for the TC-isolate. A higher mortality of mice infected with the TC-isolate may be due to its higher antigenicity compared to T. spiralis and could explain faster expulsion and lower fecundity of TC-isolate females.

On the bases of my studies a comparative index for the intestinal phase of T. spiralis and any isolate of Trichinella can be determined within 7 days of receiving an isolate. A basic assumption is that an accepted laboratory-maintained T. spiralis is used as a standard.

The comparative index for the intestinal phase (C_i) is:

$$C_i^{*} = \frac{N_1 \cdot S_1 \cdot F_1}{N_2 \cdot S_2 \cdot F_2}$$

* 1 refers to T. spiralis (lab form)

2 refers to Trichinella isolate.

N_2 and N_1 represent the number of worms recovered from the small intestine for the isolate and T. spiralis; S_2 and S_1 are the sex ratios of the isolate and T. spiralis, and F_2 and F_1 represent the average fecundity of individual females of the isolate and T. spiralis.

$C_i = 1$ indicates that the isolate is identical to the standard and C_i greater than 1 indicates that the isolate is different, therefore, the higher C_i the greater the difference. As mentioned above the reproductive capacities of Trichinella isolates are influenced by various factors, consequently, a C_i value for Trichinella isolates should fall within the range of the ratios of their reproductive capacities, providing the rate of survival and encystment of migrating larvae is similar for the two parasites. Our value of C_i (3.47) falls within the range (2.6 - 3.64) of the ratios of reproductive capacities of the parasites and clearly shows that T. spiralis and TC-isolate differ in their biological characteristics during the intestinal phase of the life cycle.

To test the usefulness of a comparative index for the intestinal phase and to determine a significantly different lower limit, somewhat greater than 1, other isolates of Trichinella should be compared to a standardized laboratory T. spiralis.

CHAPTER II

COMPARISON OF THE MUSCLE PHASE OF *T. SPIRALIS* AND TC-ISOLATE

INTRODUCTION

Comparative studies of *Trichinella* geographical isolates have revealed differences in biological characteristics, but the taxonomic rank of isolates is still not established. Most work to date emphasizes characteristics of the muscle phase such as differences in size (Britov 1973; Britov 1974; Sukhdeo and Meerovitch 1978), levels of infectivity in laboratory animals only (Arakawa and Todd 1971; Pereverseva et al. 1974; Sukhdeo and Meerovitch 1978) interbreeding or lack thereof (Britov 1971a; Britov et al. 1971; Komandarev et al. 1975; Bessonov et al. 1975) and provides only limited information on biochemical differences between the varieties or species. Of considerable interest, but largely overlooked in these studies, are the age of the isolate (the time from original isolation of the *Trichinella* strain) and the number of passages in experimental animals. Furthermore, lack of concurrent comparative data on *T. spiralis* (north-temperate), a form well adapted to laboratory rodents, makes comparisons difficult.

In this chapter comparative studies on the muscle phase of *T. spiralis* (north-temperate) and TC-isolate are reported. Biological characteristics examined are: (1) Reproductive capacity indices (RCI) for *T. spiralis* and TC-isolate in laboratory and wild rodents; (2) survival of infective (muscle) larvae of *T. spiralis* and TC-isolate

in male and female mice; (3) interbreeding of T. spiralis and TC-isolate; (4) morphological measurements of the infective larvae.

MATERIALS AND METHODS

Reproductive Capacity in Rodents

The reproductive capacity indices (RCI) of T. spiralis and TC-isolate were determined for two strains of outbred Swiss Webster mice [50-60 day old Crl: COBS CFW (SW) and AHF: (SW)], one strain of inbred mice (50-60 day old SEC-J, Jackson Memorial Laboratories, Bar Harbour, Maine, U.S.A.), Sprague-Dawley rats [Bbl: (SD), Biobreeding Laboratories, Ottawa, Canada], and three species of wild rodents Microtus pennsylvanicus (Ord), Clethrionomys gapperi (Vigors) and Peromyscus maniculatus (Wagner).

Maintenance of the parasites, infection procedure and determination of RCI values were previously described (see Chapter I, p. 5). Mice and wild rodents were infected by gastric intubation with 400 larvae of T. spiralis or TC-isolate and rats with 2000 larvae each (Table I). Infected animals were killed at day 40 post infection and the numbers of muscle larvae determined by standardized techniques (see Chapter I, p. 6).

Survival of Muscle Larvae in Mice

Survival rate of T. spiralis and TC-isolate muscle larvae was studied in male and female AHF: (SW) mice. Determination of the survival rate assumed that the reproductive capacities of T. spiralis and TC-isolate reached a maximum at day 40 post infection and that no encysted larvae died prior to that day. Differences in the number of larvae recovered from mice at day 40 post infection and from experimental mice

killed between day 50 and 520 post infection, represent the number of worms that died. The number of larvae was determined by standardized techniques (see Chapter I, p. 6).

Interbreeding Experiments

Single and multiple pair interbreeding experiments of T. spiralis and TC-isolate were performed using 50-60 day old Crl: COBS CFW (SW) mice. Isolated larvae were washed twice in Tyrode's solution (37 C) and sexed according to the following criteria. Males: distance of 60-70 μm from posterior pole of the gonad to posterior end of worm; rectum length of about 50 μm; blunt anterior pole of gonad; intestine crossing over the gonad from ventral (convex) to dorsal (concave) surface; intestinal bulb ventral to the gonad. Females: distance of 30-40 μm from the posterior pole of gonad to posterior end of worm; rectum length of about 25-30 μm; pointed anterior pole of gonad; intestine always on the dorsal side; intestinal bulb dorsal to the gonad.

Mice were anesthetized by intraperitoneal injection of Sodium Pentobarbital solution (3.3 mg/mouse). The abdominal wall was cut along linea alba and the duodenal portion of the small intestine elevated for injection with a surgical hook. Single and multiple pairs of sexed larvae were suspended in a syringe containing 0.05 ml of Tyrode's solution and injected directly into the lumen of the duodenum. The muscle layer of the body wall was sutured with Catgut Chromic

(Naila Bayern, West Germany) and the skin closed with black braided 00-silk (Ethicon Inc., Somerville, N.Y., U.S.A.).

Sexing accuracy of infective larvae (Table II) and inter and intraisolate mating ability (Tables III and IV) were determined.

In addition, preliminary experiments were done using sexed muscle larvae injected into duodenum and collected by thermal migration 20 hours post infection (prior to final moult). This allowed two screenings of males and females; as larvae and juvenile males and females. Various intra and interisolate male and female combinations (1 ♂:1 ♀, 5 ♂:1 ♀, 10 ♂:1 ♀, 20 ♂:1 ♀, 5 ♂:5 ♀, 10 ♂:5 ♀, 10 ♂:10 ♀) were transplanted by duodenal injection to mice. The animals were checked for the presence of gravid females in the intestine (day 10 post infection) or larvae in the muscles (30 days post infection).

Morphological Measurements of Infective Larvae

Infective larvae of T. spiralis and TC-isolate were obtained at day 40 post infection from Crl: COBS CFW (SW) mice infected by gastric intubation with 400 larvae. Fixation, clearing and measuring of the infective larvae was described in Chapter I, p. 11. Measurements were; total length, width, length of gonad, intestine, stichosome and rectum, and the distance from posterior pole of gonad to posterior end of worm (post-gonad distance).

Preliminary measurements indicated differences in size of "early" TC-isolate males and females (generations 1-5) and "late" TC-isolate males and females (generations 6-12). Specimens of each isolate were divided into four groups; "early" and "late" TC-isolate males and females and "early" and "late" T. spiralis males and females (Tables V and VI). Two hundred worms (100 males and 100 females) from each group were measured.

Statistics

Reproductive capacity indices (RCI) were subjected to Student's t-test and Bartlett's test for homogeneity of variances. Other data used Biomedical Computer Programs (BMDP): Data on survival rate of infective larvae were subjected to multiple linear regression (P1R) and one-way analysis of variance and covariance (P1V); morphological measurements of infective larvae were analysed by P1V and stepwise discriminant analysis (P7M).

RESULTS

Reproductive Capacity in Rodents

Reproductive capacity indices (RCI) of T. spiralis and TC-isolate in laboratory and wild rodents are presented in Table I. It shows that all laboratory rodents were significantly less susceptible to the TC-isolate than to T. spiralis. The RCI for TC-isolate in Sprague-Dawley rats was 28 times lower than that for T. spiralis. There were no significant differences in susceptibility of two strains of Swiss Webster mice to either T. spiralis or TC-isolate. The RCI values of T. spiralis and TC-isolate were significantly lower ($p < 0.05$) in SEC-J mice than in Swiss Webster mice. The susceptibility of wild rodent populations (F_1 generation in laboratory) to T. spiralis was significantly lower than that of laboratory rodents, but the susceptibility of wild and laboratory rodents to the TC-isolate was similar.

The RCI for T. spiralis in M. pennsylvanicus (78 ± 19) was not significantly different from the TC-isolate index (64 ± 24) in the same host. Similar results were obtained for C. gapperi and P. maniculatus. M. pennsylvanicus was more susceptible to T. spiralis compared to C. gapperi and P. maniculatus, whereas the RCI values for TC-isolate were similar in M. pennsylvanicus and C. gapperi and lower in P. maniculatus (Table I).

Table I: Reproductive capacity of *Trichinella spiralis* and TC-isolate in laboratory and wild rodents. N = number of mice.

Host	*T. spiralis*		TC-isolate	
	N	Mean index reproductive capacity/animal ± SD	N	Mean index reproductive capacity/animal ± SD
Crl: COBS CFW (SW) mice (outbred)	179	155 ± 31	184	69 ± 29
AHF: (SW) mice (outbred)	55	140 ± 23	75	55 ± 24
SEC-J mice (inbred)	40	94 ± 10	40	29 ± 7
Sprague-Dawley rats	50	204 ± 24	52	7 ± 3
M. pennsylvanicus	14	78 ± 19	5	64 ± 24
C. gapperi	6	33 ± 14	6	52 ± 11
P. maniculatus	6	33 ± 7	6	19 ± 9

Survival of Muscle Larvae in Mice

The survival of T. spiralis and TC-isolate larvae in male and female AHF: (SW) mice is shown in Figure 1. Multiple linear regression between time and per cent of larvae recovered yielded highly significant R^2 coefficients indicating a linear relationship between the variables (Fig. 1). Survival of both T. spiralis and TC-isolate larvae was significantly higher ($p < 0.01$) in female mice compared to male mice (Figs. 1a and 1b). Furthermore, percent survival of larvae in male and female mice infected with the TC-isolate was significantly lower ($p < 0.01$) compared to the survival of T. spiralis larvae (Figs. 1c and 1d).

One-way analysis of variance and covariance indicated significant differences when y-intercepts of regression lines were compared and a non-significant difference in slopes of linear regressions.

By day 470 post infection all of the TC-isolate muscle larvae in male mice were dead compared to 75% in female mice. In contrast 85% and 63% of T. spiralis muscle larvae were dead for the same time period in male and female mice, respectively.

Interbreeding Experiments

The presence or absence of worms during the intestinal and muscle phases of the life cycle evaluated the accuracy of the sexing method for muscle larvae (Table II). It showed that sexes were determined accurately and that fewer

Figure 1 (a) Regression between time and per cent of muscle larvae of Trichinella spiralis recovered in male and female outbred Swiss Webster mice [AHF: (SW)]

(b) Regression between time and per cent of muscle larvae of TC-isolate recovered in male and female outbred Swiss Webster mice [AHF: (SW)]

(c) Regression between time and per cent of muscle larvae of Trichinella spiralis recovered and TC-isolate in male outbred Swiss Webster mice [AHF: (SW)]

(d) Regression between time and per cent of muscle larvae of Trichinella spiralis recovered and TC-isolate in female outbred Swiss Webster mice [AHF: (SW)]

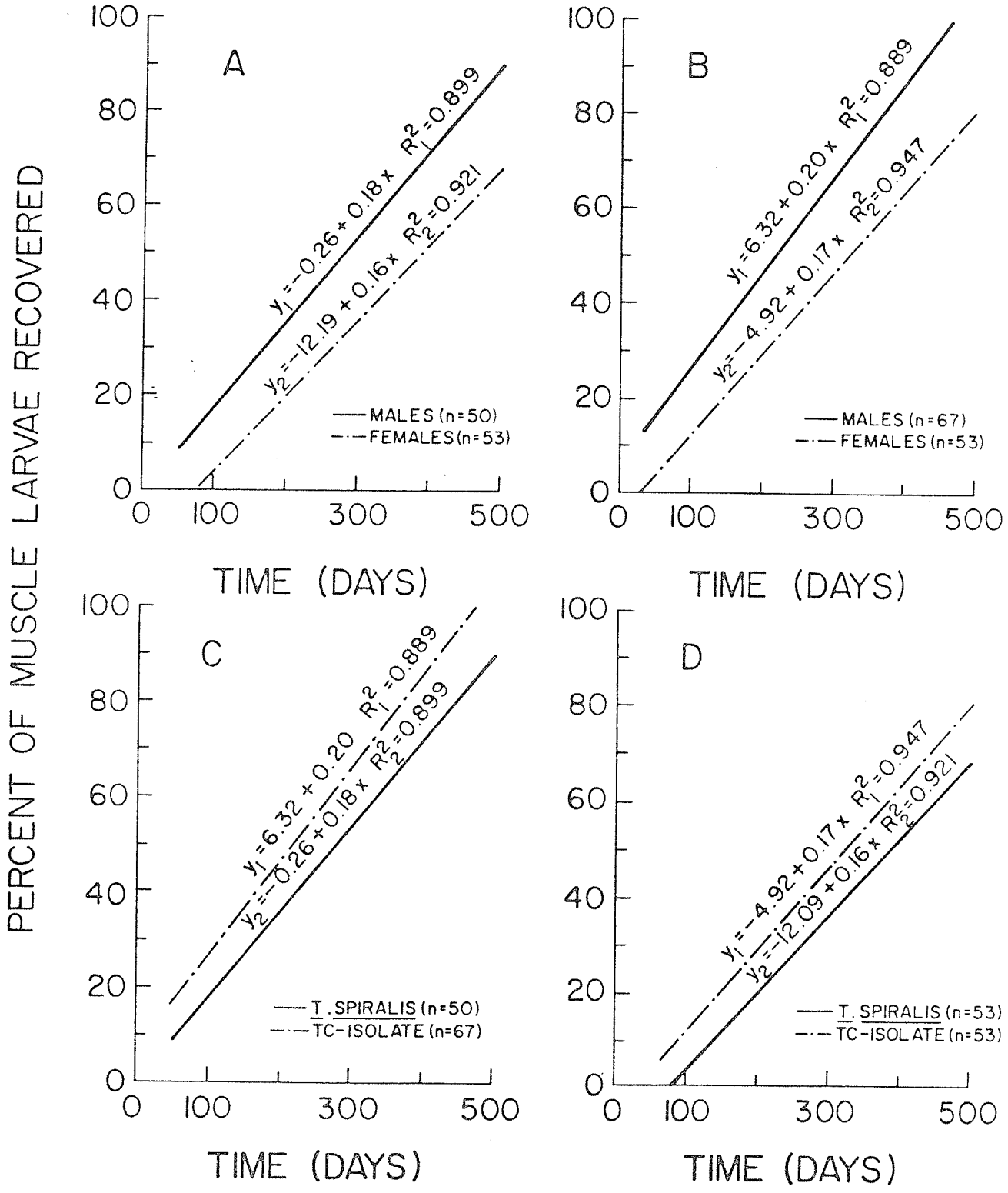

PERCENT OF MUSCLE LARVAE RECOVERED
A
$y_1 = -0.26 + 0.18x$ $R_1^2 = 0.899$
$y_2 = -12.19 + 0.16x$ $R_2^2 = 0.921$
MALES (n=50)
FEMALES (n=53)
TIME (DAYS)
B
$y_1 = 6.32 + 0.20x$ $R_1^2 = 0.889$
$y_2 = -4.92 + 0.17x$ $R_2^2 = 0.947$
MALES (n=67)
FEMALES (n=53)
TIME (DAYS)
C
$y_1 = 6.32 + 0.20$ $R_1^2 = 0.889$
$y_2 = -0.26 + 0.18x$ $R_2^2 = 0.899$
T. SPIRALIS (n=50)
TC-ISOLATE (n=67)
TIME (DAYS)
D
$y_1 = -4.92 + 0.17x$ $R_1^2 = 0.947$
$y_2 = -12.09 + 0.16x$ $R_2^2 = 0.921$
T. SPIRALIS (n=53)
TC-ISOLATE (n=53)
TIME (DAYS)

Table II: Controls for sexing accuracy of *Trichinella spiralis* and TC-isolate muscle larvae in Crl: COBS CFW (SW) mice. I = mice killed at day 5 post infection and intestines examined for presence of adult worms. II = mice killed at day 40 post infection and checked for presence of worms in muscles. (10, ♂) (10, ♀) = number of worms/mouse inoculated for I and II and sex of worms. N = number of mice.

Inoculum	Control I			Control II	
	N	No. mice infected	No. worms recovered (sex)	N	No. mice infected
TC-isolate (10, ♂)	11	5	14 (♂)	7	-
TC-isolate (10, ♀)	13	9	58 (♀)	7	-
T. spiralis (10, ♂)	12	8	31 (♂)	7	-
T. spiralis (10, ♀)	11	8	56 (♀)	7	-

Table III: Single pair intra and interbreeding of *Trichinella spiralis* and TC-isolate in Crl: COBS CFW (SW) mice. (1, ♂) (1, ♀) = number of worms/mouse inoculated and sex of worms. (F_1) *T. spiralis* and (F_1) TC-isolate refers to progeny of original cross. N = number of mice.

Cross	N	No.mice infected at day 40 post infection	% positive breeding trials
TC-isolate (1, ♂) x *T. spiralis* (1, ♀)	66	0.0	0.0
T. spiralis (1, ♂) x TC-isolate (1, ♀)	61	0.0	0.0
T. spiralis (1, ♂) x *T. spiralis* (1, ♀)	26	11	42
TC-isolate (1, ♂) x TC-isolate (1, ♀)	23	9	39
(F_1) *T. spiralis* (1, ♂) x (F_1) *T. spiralis* (1, ♀)	4	2	(50)
(F_1) TC-isolate (1, ♂) x (F_1) TC-isolate (1, ♀)	4	1	(25)

Table IV: Multiple pair intra and interbreeding of *Trichinella spiralis* and TC-isolate in Crl: COBS CFW (SW) mice. (5, ♂) (5, ♀) = number of worms/mouse inoculated and sex of worms. Hybrid = F_1 progeny of original cross. N = number of mice.

Cross	N	No.mice infected at day 40 post infection	% positive breeding trials
TC-isolate (5, ♂) x *T. spiralis* (5, ♀)	15	12	80
T. spiralis (5, ♂) x TC-isolate (5, ♀)	14	5	36
T. spiralis (5, ♂) x *T. spiralis* (5, ♀)	10	9	90
TC-isolate (5, ♂) x TC-isolate (5, ♀)	10	10	100
Hybrid (5, ♂) x Hybrid (5, ♀)	2	1	(50)
Hybrid (5, ♂) x *T. spiralis* (5, ♀)	2	2	(100)
Hybrid (5, ♂) x TC-isolate (5, ♀)	2	1	(50)
T. spiralis (5, ♂) x Hybrid (5, ♀)	2	1	(50)
TC-isolate (5, ♂) x Hybrid (5, ♀)	2	2	(100)

adult males of both T. spiralis and TC-isolate were established in the small intestine compared to adult females.

Single pair interbreeding experiments of T. spiralis and TC-isolate are presented in Table III. Mating between T. spiralis and TC-isolate did not occur in 127 breeding trials. Intrabreeding trials showed 42% and 39% mating success for T. spiralis and TC-isolate, respectively, and the progeny were viable and infective (Table III).

Multiple pair interbreeding results of T. spiralis and TC-isolate are presented in Table IV, and show reproductive compatibility between T. spiralis and TC-isolate. Mating success of TC-isolate males and T. spiralis females was higher (80%) compared to the mating success of T. spiralis males and TC-isolate females (36%). Mating between T. spiralis males and females was 90% and between TC-isolate males and females 100% (Table IV). Hybrid to hybrid and backcross breeding trials produced viable and infective progeny (Table IV).

Preliminary results of transplant experiments showed reproductive compatibility between T. spiralis and TC-isolate, and indicated that 20 hour old Trichinella were able to re-establish in the small intestine and develop normally after transplantation.

Morphological Measurements of Infective Larvae

Morphological measurements of T. spiralis and TC-isolate male and female infective larvae are presented in Tables V

and VI. One-way analysis of variance and covariance (BMDP1V) of total length measurements between groups indicated that "early" TC-isolate males and females were significantly shorter ($p < 0.01$) compared to "early" and "late" T. spiralis and "late" TC-isolate worms. There were no significant differences in total length of either male or female infective larvae between "early" T. spiralis and "late" TC-isolate worms. Total length of "early" and "late" T. spiralis worms was not significantly different, but, "late" TC-isolate worms were significantly shorter ($p < 0.01$) than "late" T. spiralis worms.

The analysis of other measurements of male infective larvae (Table V) between groups yielded the following results (at 0.01 level of significance): "early" and "late" TC-isolate differed in width, length of stichosome, rectum and post-gonad distance; "early" TC-isolate and "late" T. spiralis differed in width, length of gonad, stichosome, rectum and post-gonad distance; "early" and "late" T. spiralis differed in length of rectum and post-gonad distance; "early" T. spiralis and "late" TC-isolate differed in length of rectum and post-gonad distance; "early" TC-isolate and "early" T. spiralis differed in width, length of gonad, stichosome, rectum and post-gonad distance; and "late" TC-isolate and "late" T. spiralis differed in width and length of stichosome.

Table V: Morphological measurements of male muscle larvae of *Trichinella spiralis* and TC-isolate. N = number of worms.

	Early TC-isolate	Late TC-isolate	Early *T. spiralis*	Late *T. spiralis*
N	100	100	100	100
Overall Length ± SD	0.857 ± 0.064	0.974 ± 0.065	1.009 ± 0.071	1.010 ± 0.061
Width ± SD	0.033 ± 0.005	0.029 ± 0.002	0.029 ± 0.002	0.029 ± 0.002
Length of gonad ± SD	0.262 ± 0.029	0.272 ± 0.032	0.259 ± 0.037	0.258 ± 0.032
Length of intestine ± SD	0.307 ± 0.028	0.328 ± 0.031	0.319 ± 0.041	0.325 ± 0.036
Length of stichosome ± SD	0.547 ± 0.052	0.637 ± 0.068	0.683 ± 0.070	0.683 ± 0.061
Length of rectum ± SD	0.048 ± 0.007	0.053 ± 0.008	0.056 ± 0.005	0.054 ± 0.007
Post-gonad distance ± SD	0.054 ± 0.007	0.061 ± 0.008	0.063 ± 0.005	0.064 ± 0.006

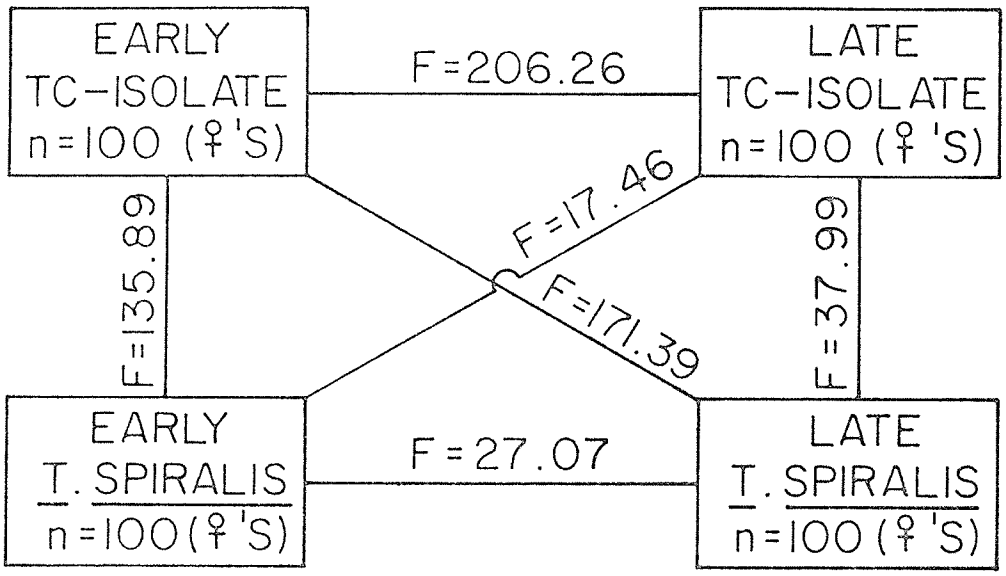
Table VI: Morphological measurements of female muscle larvae of *Trichinella spiralis* and TC-isolate. N = number of worms.

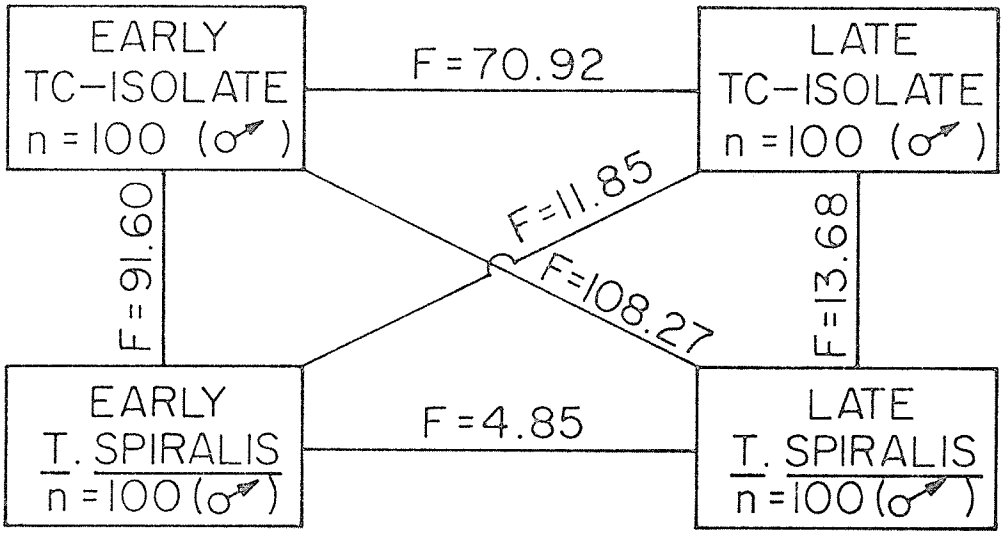
	Early TC-isolate	Late TC-isolate	Early *T. spiralis*	Late *T. spiralis*
N	100	100	100	100
Overall Length ± SD	0.876 ± 0.064	0.999 ± 0.060	1.030 ± 0.053	1.043 ± 0.048
Width ± SD	0.029 ± 0.003	0.029 ± 0.003	0.029 ± 0.003	0.032 ± 0.003
Length of gonad ± SD	0.281 ± 0.035	0.299 ± 0.041	0.291 ± 0.030	0.309 ± 0.033
Length of intestine ± SD	0.299 ± 0.033	0.321 ± 0.039	0.316 ± 0.027	0.339 ± 0.034
Length of stichosome ± SD	0.579 ± 0.054	0.681 ± 0.061	0.712 ± 0.047	0.700 ± 0.051
Length of rectum ± SD	0.021 ± 0.003	0.023 ± 0.003	0.025 ± 0.004	0.026 ± 0.003
Post-gonad distance ± SD	0.027 ± 0.004	0.030 ± 0.004	0.033 ± 0.005	0.035 ± 0.004

The analysis of other measurements of female infective larvae (Table VI) between groups yielded the following results (at 0.01 level of significance): "early" and "late" TC-isolate differed in length of stichosome, rectum and post-gonad distance; "early" TC-isolate and "late" T. spiralis differed in length of intestine, stichosome, rectum and post-gonad distance; "early" and "late" T. spiralis differed in length of intestine, rectum and post-gonad distance; "early" T. spiralis and "late" TC-isolate differed in length of stichosome, rectum and post-gonad distance; "early" TC-isolate and "early" T. spiralis differed in width, length of gonad, stichosome, rectum and post-gonad distance; and "late" TC-isolate and "late" T. spiralis differed in length of rectum and post-gonad distance.

Results of multivariate analysis (BMDP7M) using all variables listed in Tables V and VI are presented in Figure 2. Although significance between paired comparisons can not be assessed in this multivariate analysis (Subramaniam, pers. comm.), distances between pairs, shown by F values for Mahalanobis D^2, can be used to reveal morphometric trends (Fig. 2). The distance between "early" TC-isolate and "early" T. spiralis females was greater (F = 135.89) compared to the distance (F = 37.99) between "late" TC-isolate and "late" T. spiralis females, indicating a change in morphometric characters (Fig. 2). Furthermore, the distance between "early" and "late" TC-isolate females was large (F = 206.26) compared to the distance between "early" and "late" T. spiralis females (F = 27.07), indicating that the size change of

Figure 2 Multivariate discriminant analysis of morphological measurements of male and female muscle larvae of *Trichinella spiralis* and TC-isolate. F = statistic for Mahalanobis D^2 which represents the distance between two groups of data.


EARLY
TC-ISOLATE
n=100 (♀'S)
F=206.26
LATE
TC-ISOLATE
n=100 (♀'S)
F=135.89
F=17.46
F=171.39
F=37.99
EARLY
T. SPIRALIS
n=100(♀'S)
F=27.07
LATE
T. SPIRALIS
n=100(♀'S)


EARLY
TC-ISOLATE
n=100 (♂)
F=70.92
LATE
TC-ISOLATE
n=100 (♂)
F=91.60
F=11.85
F=108.27
F=13.68
EARLY
T. SPIRALIS
n=100(♂)
F=4.85
LATE
T. SPIRALIS
n=100(♂)

TC-isolate females was greater compared to T. spiralis females through passages in mice. Similar results were obtained for male muscle larvae of T. spiralis and TC-isolate (Fig. 2).

DISCUSSION

Reproductive Capacity in Rodents

Although reproductive capacity index (RCI) of the arctic isolate of *Trichinella* is lower in laboratory rodents compared to *T. spiralis* index (Dick and Belosevic 1978), and higher than *T. spiralis* in wild animals (Britov 1971b), no attempt has been made to compare RCI values of arctic isolates of *Trichinella* and *T. spiralis* in laboratory and wild rodents.

In my experiments the RCI values of *T. spiralis* and TC-isolate were established for two outbred and an inbred strain of mice, an outbred strain of rat and three species of wild rodents, belonging to three different genera. The RCI values of TC-isolate were significantly lower compared to *T. spiralis* indices in all laboratory rodents, but there were no significant differences between RCI values of *T. spiralis* and TC-isolate in wild rodents. Among laboratory rodents, Sprague-Dawley rats were least susceptible to the TC-isolate ($I = 7 \pm 3$), which agrees with the data of Bessonov et al. (1975) and Britov (1971b) who found that rats were highly refractory to their arctic isolates (*T. nativa*). In contrast, *T. spiralis* RCI was very high in Sprague-Dawley rats ($I = 204 \pm 24$), indicating that the parasite is well adapted to this host.

Although RCI values of *T. spiralis* and TC-isolate were higher in Crl: COBS CFW (SW) mice compared to AHF: (SW) mice the difference was not significant (Table I). The susceptibility

of SEC-J mice to either T. spiralis or TC-isolate was significantly lower compared to the susceptibilities of Crl: COBS CFW (SW) and AHF: (SW) mice, indicating that genetic differences of hosts may influence reproductive capacities of T. spiralis and TC-isolate. The RCI value for TC-isolate in SEC-J mice (29 ± 7) was similar to the RCI values for an arctic isolate in inbred A/J mice reported by Sukhdeo and Meerovitch (1978) and the index of a polar bear isolate (1^{st} passage) in white mice of unknown strain reported by Arakawa and Todd (1971).

The RCI values of T. spiralis in wild rodents were generally low compared to those of T. spiralis in laboratory rodents (Table I). In contrast, TC-isolate RCI values in wild rodents were similar to the TC-isolate indices in laboratory rodents (Table I). There were no significant differences in the RCI values of T. spiralis and TC-isolate in wild rodents, indicating that these differences are not consistent and are host dependent. Consequently, RCI values for Trichinella isolates should be determined for a variety of host species before an accurate assessment of reproductive capacity differences among Trichinella isolates can be established.

Survival of Muscle Larvae in Mice

The survival of Trichinella larvae in muscles of hosts has been reported to be from 30 days to 31 years (Gould 1970).

Britov (1975) reported that muscle larvae of T. nativa died in pigs between 90-130 days post infection, compared to T. spiralis muscle larvae which survived in the muscles of pigs for 2.75 years. Nelson and Forester (1962) reported that many muscle larvae of the East-African Trichinella strain died in rat muscles one month post infection.

Survival of T. spiralis muscle larvae in male and female mice was significantly higher ($p < 0.01$) compared to survival of TC-isolate muscle larvae. Higher death rate of TC-isolate muscle larvae may be due to a stronger host immune response brought about by greater antigenicity of this parasite (see Chapter I, p. 18).

Calcified cysts were first observed between 60-90 days post infection in male mice and between 90-120 days post infection in female mice. All TC-isolate muscle larvae in male mice died by day 470 post infection, compared to 85% death rate of T. spiralis muscle larvae for the same period, indicating that T. spiralis larvae survive longer in mice than TC-isolate larvae.

Survival of either T. spiralis or TC-isolate larvae in male mice was significantly lower ($p < 0.01$) compared to the survival of larvae in female mice, indicating that the immune response of male mice to Trichinella larvae and calcification of cysts is higher than in female mice. Von Brand et al. (1938) reported that administration of parathormone

and irradiated ergosterol caused more calcification of four month old Trichinella cysts in rats, suggesting hormonal control of the calcification process. The immune processes involved in elimination of muscle larvae are not directly associated with calcium deposition, rather, the calcification process appears to be a secondary host reaction to the parasite. Differences in calcium deposition between male and female mice is probably related to differences in calcium metabolism between sexes. One-way analysis of variance and covariance showed that y-intercepts of regression lines were significantly different ($p < 0.01$). This indicates that differences in survival are probably related to the level of immune elimination and calcification of the muscle larvae in male and female mice, and not to the rate at which the parasites were eliminated.

Interbreeding Experiments

Both reproductive isolation between T. spiralis and arctic isolates of Trichinella (Sukhdeo and Meerovitch 1978; Komandarev et al. 1975; Britov 1971a; Britov et al. 1971), and reproductive compatibility (Bessonov et al. 1975) have been reported. Inability of T. spiralis and arctic isolates of Trichinella to interbreed prompted Britov and Boev (1972) to elevate all Trichinella isolates recovered from wild animals north of 40^{th} parallel to species level (i.e. T. nativa). Since our TC-isolate was recovered from a polar bear north

of 40^{th} parallel, according to Britov and Boev (1972) it could be T. nativa.

Campbell and Yakstis (1969) reported that mating success of orally introduced single pairs of unsexed Trichinella larvae in mice was very low (19%) and that it partially depended on inability of males and females to find each other in the intestine. Consequently, mice in interbreeding experiments were infected by duodenal injection to ensure that larvae were in close proximity during establishment in the intestine. The probability of worms finding each other in the intestine was increased by performing 127 interisolate breeding trials.

Care was taken in sexing the infective larvae and for each interbreeding experiment sexing accuracy controls were established by monitoring both the intestinal and muscle phases of the life cycle (Table II). In using a combination of criteria for sexing of infective larvae described by Villella (1966) and Kozek (1975) it should be emphasized that both male and female Trichinella bend towards their dorsal side.

Forty percent of intrabreeding trials with single pairs were positive using duodenal injection, compared to 19% positive for unsexed larvae using the oral route (Campbell and Yakstis 1969). Thus duodenal injection is a better method to study breeding of Trichinella isolates.

Failure of T. spiralis and TC-isolate to interbreed in single pair experiments (Table III), suggested that the parasites were reproductively isolated. However, the results of in vitro behaviour studies indicated that TC-isolate and T. spiralis males were attracted to each others females and that there was an overall population aggregation pheromone important in maintaining the integrity of the population (see Chapter III, p. 81). Consequently, multiple pair interbreeding experiments were performed to increase the probability of worms finding each other in the intestine. Larger numbers of worms per breeding trial (Table IV) may have helped to concentrate the population thereby ensuring copulation.

Results of multiple pair interbreeding experiments indicated that T. spiralis and TC-isolate are reproductively compatible. Mating success of TC-isolate males and T. spiralis females was higher (80%) than that of T. spiralis males and TC-isolate females (36%), indicating greater reproductive compatibility between TC-isolate males and T. spiralis females. This is supported by results of in vitro behaviour experiments in which mean migration distance of TC-isolate males toward T. spiralis females was 7.82 mm, as compared to 4.01 mm of T. spiralis males toward TC-isolate females (see Chapter III, Table I, p. 76). Mating in intrabreeding trials was higher, 90% and 100% for T. spiralis and TC-isolate, respectively (Table IV). All hybrid to hybrid and backcross breeding trials produced

viable and infective progeny (Table IV).

Transplantation of 20 hour old juvenile males and females is additional evidence that TC-isolate and T. spiralis are reproductively compatible. This method permits the checking of initial sexing of larval males and females. Contrary to the opinion of Levin (1941), transplanted 20 hour old Trichinella juveniles of both sexes can re-establish in the small intestine, attain sexual maturity, copulate and become fecund.

Results show that single pair interbreeding of Trichinella is not a reliable method in assessing mating ability between geographical isolates of Trichinella because of a low probability of worms finding each other in the intestine. Multiple pair or transplant interbreeding experiments are much more reliable in assessing mating ability between the isolates, due to increased probability of worms finding each other in the intestine.

Morphological Measurements of Infective Larvae

Arctic isolates of Trichinella have been reported to be morphologically indistinguishable from T. spiralis (Madsen 1975), but some workers found differences in size of cysts, adults and infective larvae. Britov (1974) found that muscle cysts of T. spiralis and T. nativa differ in size, but Madsen (1975) stated that the size of Trichinella muscle

cysts is not a reliable comparative characteristic because cyst dimensions vary with time (age of infection) and host species. Sukhdeo and Meerovitch (1978) reported differences in total length of adults between T. spiralis (north-temperate form) and an arctic isolate of Trichinella, but I found (p. 30) that the total length of T. spiralis and TC-isolate adults were not significantly different.

Total length is most frequently used as a morphometric characteristic when infective larvae of Trichinella isolates are compared. Britov (1973) found differences in total length of Trichinella larvae isolated from different hosts.

Seven variables were used in order to determine if the infective larvae differed in size (Tables V and VI). A comparison of single morphometric variables between different groups (BMDP1V) indicated significant differences, but patterns of morphometric trends of different variables were inconsistent. For example, "early" TC-isolate worms(males or females), were significantly shorter compared to "late" TC-isolate worms, but the length of "early" and "late" T. spiralis larvae was not significantly different (Tables V and VI). However, "late" TC-isolate worms (males or females), although significantly shorter than "late" T. spiralis worms, were similar in total length to the "early" T. spiralis worms. In other words, a comparison of lengths showed that TC-isolate and T. spiralis are similar only if TC-isolate is passaged, but if both parasites are

passaged and lengths compared, a difference in length is still evident. Comparisons of other variables such as width, length of gonad (males) and length of intestine (females) indicated inconsistencies in the pattern of morphometric change.

Multivariate analysis was used to determine if a combination of all variables would show morphometric differences between the two parasites and if the degree of morphometric change within and between T. spiralis and TC-isolate could be evaluated.

The results show that T. spiralis and TC-isolate larvae (males or females) differ morphometrically. The distance (shown by F values for Mahalanobis D^2) between "early" TC-isolate and "early" T. spiralis female larvae was greater (F = 135.89) compared to the distance (F = 37.99) between "late" TC-isolate and "late" T. spiralis females (Fig. 2). Similarly, the distance between "early" TC-isolate and "early" T. spiralis male larvae was greater (F = 91.60) compared to the distance (F = 13.68) between "late" TC-isolate and "late" T. spiralis males (Fig. 2). This suggests convergence in morphometric characters of T. spiralis and TC-isolate infective larvae after prolonged passages of the parasites through mice. Furthermore, the distances between "early" and "late" TC-isolate groups (males and females) were greater compared to the distances between "early" and "late" T. spiralis groups, indicating that

morphometric characters of TC-isolate infective larvae changed more through passages in mice compared to T. spiralis larvae (Fig. 2).

A convergence in morphometric measurements of T. spiralis and TC-isolate infective larvae after prolonged passages of the parasites in laboratory rodents indicated that the size of worms changed with time.

Superficially, biological characteristics such as RCI values, survival of muscle larvae, and length of infective larvae for the TC-isolate appear to be similar to other reports on arctic isolates of Trichinella, but more detailed analysis clearly showed the importance of host species and number of passages to both the TC-isolate and T. spiralis. Any comparative morphometric study between infective larvae of Trichinella geographical isolates is possible only if complete histories of isolates are known and a laboratory maintained T. spiralis is used as a standard.

Although differences in RCI values and survival of muscle larvae were found between TC-isolates and T. spiralis (north-temperate), morphometric similarity and reproductive compatibility indicate that the TC-isolate is a physiological variant of T. spiralis and not a distinct species.

CHAPTER III

CHEMICAL ATTRACTION IN THE GENUS *TRICHINELLA*

INTRODUCTION

Chemical attraction or pheromone-induced behaviour has been reported for numerous nematodes but only a few zooparasitic nematodes have been studied: Trichinella spiralis (Owen, 1835) by Bonner and Etges 1967; Nippostrongylus brasiliensis (Travassos, 1914) by Bone and Shorey 1977; Ancylostoma caninum (Ercolani, 1859) by Roche 1966; Aspiculuris tetraptera (Nitzsch, 1821) by Anya 1976; and Camallanus sp. (Railliet et Henry, 1915) by Salm and Fried 1973. These studies with zooparasitic helminthes were concerned primarily with heterosexual and homosexual attraction within a single species. Studies with the free-living nematode Panagrellus redivivus (DeMan, 1913) by Blakanich and Samoiloff (1974), and plant parasitic nematode Heterodera sp. by Green and Plumb (1970), suggested cross-specificity of chemical attractant(s) between strains and species of nematodes.

Although the taxonomy of the genus Trichinella is in a state of flux with conflicting information on validity of species and assignment of new isolates to a species (Bessonov et al. 1975; Britov and Boev 1972), the cross-specificity of chemical attractants between species, strains and isolates of Trichinella was not studied. Assignment of isolates of Trichinella to a species is usually based on the ability or inability to interbreed. Differences in results from our laboratory and elsewhere (Bessonov et al. 1975; Britov and Boev 1972) suggest that factor(s) other than

copulation might be involved such as chemical attraction of one species or isolate to another.

In this chapter the experiments designed to determine if chemical attraction could be used to distinguish between T. spiralis, T. spiralis var. pseudospiralis (originally described as T. pseudospiralis Garkavi 1972) and TC-isolate are reported.

MATERIALS AND METHODS

Parasites

Trichinella spiralis and TC-isolate were passaged for seven and eleven generations, respectively, in AHF: (SW) mice prior to use in these experiments.

T. spiralis var. *pseudospiralis* was obtained from Dr. G. Faubert (MacDonald College, McGill University), where it was maintained in mice. This organism is considered to be a variant of *T. spiralis*, since no conclusive morphological or biochemical differences have been reported. However, certain characteristics are known and were verified in our laboratory [i.e. no cyst formation, the ability to infect birds (complete life cycle), and low infectivity in laboratory rodents]. The parasite was passaged for five generations in AHF: (SW) mice prior to use in the attraction experiments.

Chemical Attraction Experiments

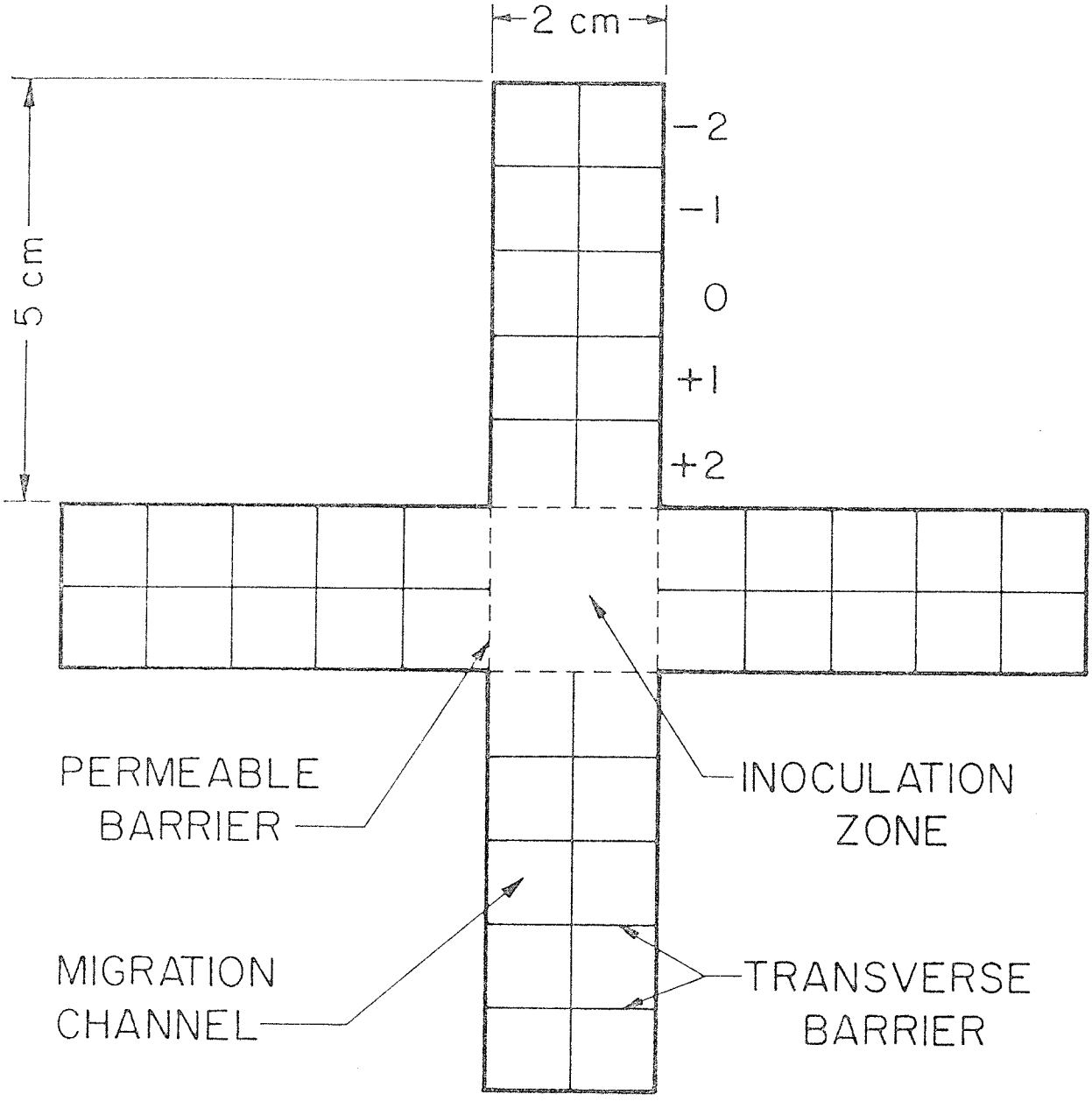
Chemical attraction of adult *T. spiralis*, *T. spiralis* var. *pseudospiralis* and TC-isolate were studied *in vitro*. The experimental migration apparatus consisted of 8 migration channels and a centrally located inoculation chamber, physically separated from channels with Whatman #1 filter paper (Fig. 1). Each migration channel was grooved at 1 cm intervals for the insertion of transverse barriers (glass cover slips). Prior to use, the apparatus was sterilized, and 12 mls of sterile 2% agar were poured into the chamber and channels and allowed to solidify,

forming a uniform layer approximately 2 mm thick. The inoculum chamber was lined (bottom and sides) with Whatman #1 filter paper and filled with 4.5 mls of sterile Tyrode's solution. Four mls of sterile Tyrode's were added to each migration channel. Before inoculating with either releasers or migrators, the apparatus was placed in a light-free environmental chamber held at 35-37 C and 100% relative humidity and allowed to equilibrate for one hour.

Adult male and female worms were collected 48-52 hours post-infection from Crl: COBS CFW (SW) mice infected by gastric intubation with 800-1600 larvae. Mice were killed by cervical dislocation, the upper half of intestine removed and placed in 37 C Tyrode's solution. Intestine was slit longitudinally and the mucosa lining vigorously scraped and stirred. This material was placed in a Baermann apparatus, the worms collected for $2\frac{1}{2}$ hours, and the bottom 10 mls of Tyrode's solution containing the worms removed and placed in a sterile petri dish. The parasites were washed twice in warm Tyrode's solution and males and females separated.

Preliminary experiments indicated that at least 6-7 hours were required for soluble chemicals to be emitted and diffuse through the barrier, and then elicit a response of the migrators. In a series of preliminary trials, the following combinations of releasers and migrators were examined: blanks, female to female, female to male, male to male, and male to female. In addition, trials with target

Fig. 1. The experimental behaviour apparatus


2 cm
5 cm
-2
-1
0
+1
+2
PERMEABLE BARRIER
INOCULATION ZONE
MIGRATION CHANNEL
TRANSVERSE BARRIER

doses of 100, 200, 300 and 400 adults were performed to determine the optimum response dose for the migrators. Males responded best to a target dose of between 300-400 females at a temperature of 35-37 C. Contrary to Bonner and Etges (1967), male worms of Trichinella were more strongly attracted to females (70-80% positive migration), as compared to a 50-55% migration of females towards males. Consequently, experiments were designed to use female worms as releasers and males as migrators. To differentiate between a population aggregation chemical attractant and a sexual attractant, both male and female migrators were used concurrently. In each experiment using the same releaser source, four migration channels contained males, and four females.

Four hundred females were placed in the inoculum chamber, inside the environmental chamber for 6-7 hours at 35-37 C, prior to inoculating each of the migration channels with 25 males or 25 females. Zone zero was separated by transverse barriers from the remainder of the channel during inoculation. After inoculation, the barriers were removed and the worms maintained in darkness at 35-37 C for 4-5 hours. At the end of this time, the transverse barriers were inserted into grooves, trapping the migrating worms in different zones of the migration channels. The apparatus was removed from the environmental chamber and placed over a diagram which duplicated the migration apparatus. With the aid of a dissecting microscope position of each migrator was plotted

and straight line migration distance of each migrator measured from zero line of the zone in a positive and negative direction. The mean value for all worms was considered to be the mean positive or negative distance travelled by migrators in one trial.

In the first group of experiments, attraction of males to their own females was examined for T. spiralis, T. spiralis var. pseudospiralis and TC-isolate, where females were releasers and males and females were migrators. Fifty-two replicates for each of T. spiralis, T. spiralis var. pseudospiralis and TC-isolate were performed. In the second group of experiments, the cross-specificity of chemical attractants between T. spiralis, T. spiralis var. pseudospiralis and TC-isolate was examined. In 36 trials, the females were releasers and males were migrators (Table I). In 36 trials, females were both releasers and migrators (Table II).

Results of all experiments were analyzed with the chi-square test. Student's t-test was used to compare mean positive and negative distances of migration of males and females between T. spiralis, TC-isolate and T. spiralis var. pseudospiralis, and f-test was applied to check for homogeneity of variances. The 0.05 probability level was considered significant.

RESULTS

Although the experiments for heterosexual and homosexual chemical attraction were performed concurrently, using the same female worms as releasers, for ease of analysis the results are presented in two sections.

Heterosexual Chemical Attraction

The results of heterosexual attraction are presented in Table I. Male worms of T. spiralis, T. spiralis var. pseudospiralis and TC-isolate were significantly ($p < 0.001$) attracted to their own females. The mean positive migration of males of T. spiralis var. pseudospiralis was significantly ($p < 0.05$) greater, than the TC-isolate and T. spiralis male migration. There was no significant difference between means of positive migration of T. spiralis and TC-isolate.

The males in all trials migrated "en masse" in the course of the first two hours, but dispersed more towards the end of the experiment. T. spiralis var. pseudospiralis males were more active than those of T. spiralis and TC-isolate, as evidenced by their undulatory movements and ability to aggregate.

The results of heterosexual chemical attraction between T. spiralis, T. spiralis var. pseudospiralis and TC-isolate are presented in Table I. The mean distance of migration shows that both TC-isolate and T. spiralis var. pseudospiralis males were attracted to females of T. spiralis, but

Table I. Heterosexual chemical attraction in the genus Trichinella

No. Trials	No. Trials with Positive Migration+	Releasers	Migrators	Mean Distance Migration (in mm) ±SD++	X^{2}+++
52	45	T. spiralis (♀)	T. spiralis (♂)	8.71 ± 3.77	7.89
52	48	TC-isolate (♀)	TC-isolate (♂)	8.33 ± 5.35	11.99
52	52	T. spiralis var pseudospiralis (♀)	T. spiralis var pseudospiralis (♂)	10.93 ± 3.04	15.17
12	9	T. spiralis (♀)	TC-isolate (♂)	7.82 ± 1.93	4.40
12	7	T. spiralis (♀)	T. spiralis var pseudospiralis (♂)	1.91 ± 2.77	1.55
12	9	TC-isolate (♀)	T. spiralis (♂)	4.01 ± 2.79	4.49
12	8	TC-isolate (♀)	T. spiralis var pseudospiralis (♂)	3.29 ± 2.49	1.01
12	8	T. spiralis var pseudospiralis (♀)	T. spiralis (♂)	2.27 ± 5.65	0.82
12	8	T. spiralis var pseudospiralis (♀)	TC-isolate (♂)	2.93 ± 3.04	2.81

+ Refers to net positive migration of 25 males per trial.

++ Refers to mean distance of migration toward releasers by 25 male migrators.

+++ All values greater than 3.84 are significant.

only the attraction of TC-isolate males to females of T. spiralis was significant ($p < 0.05$). The chemical attraction of TC-isolate males by T. spiralis females was not significantly different from male to female attraction within T. spiralis.

Substances released by TC-isolate females significantly attracted T. spiralis males, but T. spiralis var. pseudospiralis males were not significantly attracted to TC-isolate females ($p < 0.05$). The mean positive migration of T. spiralis and TC-isolate males toward the females of TC-isolate was not significantly different. T. spiralis var. pseudospiralis males migrated significantly less ($p < 0.05$), when compared to male migration within the TC-isolate.

T. spiralis and TC-isolate males were not significantly attracted by T. spiralis var. pseudospiralis females. However, both T. spiralis and TC-isolate males showed net positive migration toward the females of T. spiralis var. pseudospiralis, suggesting partial cross-specificity of the chemical attractant(s).

The number of trials with positive migration (Table I) indicate that T. spiralis var. pseudospiralis males were more consistent migrators toward their own females, when compared to T. spiralis and TC-isolate males. TC-isolate and T. spiralis males were attracted equally well by each others females, whereas T. spiralis var. pseudospiralis males were less attracted by either T. spiralis or TC-isolate females.

The number of trials with positive migration for T. spiralis and TC-isolate males toward T. spiralis var. pseudospiralis females was similar to the number of positive trials for T. spiralis and TC-isolate, but distance travelled was not significant (Table I).

Homosexual Chemical Attraction

The results of experiments on attraction of T. spiralis, TC-isolate and T. spiralis var. pseudospiralis are shown in Table II. Female worms of all three parasites were significantly ($p < 0.001$) repulsed by their own females. The mean negative migration of T. spiralis, T. spiralis var. pseudospiralis and TC-isolate females was not significantly different. Females of the three parasites were equally active, migrating "en masse" throughout the migration period.

The repulsion of TC-isolate females, when exposed to T. spiralis females was not significant ($p < 0.05$). However, T. spiralis var. pseudospiralis females were repulsed significantly ($p < 0.05$) by T. spiralis females. The net negative migration of T. spiralis var. pseudospiralis and TC-isolate females away from T. spiralis females was significantly different between the two groups of parasites.

Substance(s) released by TC-isolate females elicited a significant negative migration of T. spiralis var. pseudospiralis females, and a non-significant repulsion of T. spiralis females. Mean negative migration of T. spiralis var. pseudospiralis females, when exposed to

Table II. Homosexual chemical attraction in the genus Trichinella

No. Trials	No. Trials with Negative Migration[+]	Releasers	Migrators	Mean Distance Migration (in mm) ±SD[++]	X^2[+++]
52	49	T. spiralis (♀)	T. spiralis (♀)	-3.93 ± 6.17	8.51
52	45	TC-isolate (♀)	TC-isolate (♀)	-5.09 ± 7.49	8.19
52	50	T. spiralis var pseudospiralis (♀)	T. spiralis var pseudospiralis (♀)	-6.12 ± 4.36	9.58
12	6	T. spiralis (♀)	TC-isolate (♀)	-1.53 ± 3.04	0.08
12	9	T. spiralis (♀)	T. spiralis var pseudospiralis (♀)	-8.64 ± 5.72	4.84
12	7	TC-isolate (♀)	T. spiralis (♀)	-0.39 ± 3.15	0.12
12	11	TC-isolate (♀)	T. spiralis var pseudospiralis (♀)	-11.83 ± 3.69	11.75
12	9	T. spiralis var pseudospiralis (♀)	T. spiralis (♀)	-2.41 ± 5.47	1.42
12	8	T. spiralis var pseudospiralis (♀)	TC-isolate (♀)	-0.79 ± 4.51	0.40

[+] Refers to net negative migration of 25 females per trial.

[++] Refers to mean distance of migration away from releasers.

[+++] All values greater than 3.84 are significant.

TC-isolate females, was significantly greater ($p < 0.05$) than the mean negative migration of T. spiralis females exposed to TC-isolate females.

T. spiralis and TC-isolate females were not significantly repulsed by T. spiralis var. pseudospiralis females, suggesting low cross-specificity of the chemicals involved.

In homosexual experiments, T. spiralis var. pseudospiralis females were repulsed by their own females in more trials, than the T. spiralis and TC-isolate females. The repulsion of T. spiralis and TC-isolate females by each others females was similar, 7 of 12 and 6 of 12 trials, respectively. T. spiralis var. pseudospiralis females were repulsed more by T. spiralis and TC-isolate females, as indicated by higher number of trials with negative migration.

DISCUSSION

The existence of chemically mediated water soluble sex attractants in various nematodes is a recognized phenomenon (Anya 1976a). In recent years controversy has centered on whether the attraction of male and female worms is mutual or one-sided in favor of either male or females. Bone et al. (1977), suggested that the adult males of Nippostrongylus brasiliensis were more strongly attracted to females than females to males. Similar results were obtained by Anya (1976b), for an oxyuroid Aspiculuris tetraptera. Bonner and Etges (1967), reported that females of T. spiralis were more strongly attracted to males than males to females. This view is shared by Thorson (as reported by Bonner and Etges 1967) for N. brasiliensis.

Results of control experiments (blanks), showed a strong aggregation in the inoculation zone of both male and female migrators. I did not find a random migration in blank trials as reported for T. spiralis by Bonner and Etges (1967). Consequently, any movement by migrators, positive or negative, must overcome a natural tendency of worms to aggregate.

In preliminary attraction experiments, the males of Trichinella were more strongly attracted to females than females to males. The intensity of male to female attraction was 70-80%, as compared to 50-55% for female attraction to males. The results differ from those of Bonner and

Etges (1967), and may be related to age of worms used, temperature, light versus dark migration period and strain of parasite.

The results of heterosexual attraction in Trichinella indicate that T. spiralis var. pseudospiralis males respond better to chemical attractant(s) of their females, when compared to T. spiralis and TC-isolate males exposed to their females. TC-isolate and T. spiralis males responded equally well to the attractant(s) of their females. Higher net positive migration of T. spiralis var. pseudospiralis males could, in part, be attributed to greater activity of the worms when compared to males of T. spiralis and TC-isolate. It is also possible that chemical attractant(s) released by T. spiralis var. pseudospiralis females are more effective in attracting their own males. Finally, male to male short range migration inhibition (Bone et al. 1978), may be less effective in T. spiralis var. pseudospiralis, resulting in significantly higher net positive migration of T. spiralis var. pseudospiralis males.

The results of heterosexual attraction between T. spiralis, T. spiralis var. pseudospiralis and TC-isolate, suggest that T. spiralis var. pseudospiralis males were significantly less responsive to the female chemical attractant(s) of either T. spiralis or TC-isolate. The results also show a non-significant difference in response of T. spiralis and

TC-isolate males to each others females. Greater cross-specificity of chemical attractant(s) may account for similar responses of T. spiralis and TC-isolate males to each others females, whereas low cross-specificity of attractant(s) resulted in a weak response by T. spiralis var. pseudospiralis males to females of TC-isolate and T. spiralis.

The homosexual responses of male and female nematodes were reported by several researchers (Anya 1976a). Bone and Shorey (1977), working with N. brasiliensis reported that male and female worms were neither significantly attracted nor repulsed by chemical substances released by members of the same sex. Further, these authors demonstrated a high level male to male inhibition of movement toward target females, suggesting the presence of a male produced pheromone (Bone and Shorey 1977). Homosexual experiments with T. spiralis (Bonner and Etges 1967) showed a non-significant movement between females and a significant repulsion between males. In my experiments only a slight repulsion between male worms occurred. In female homosexual experiments a significant negative migration was observed (Table II). High pheromone source (Bone et al. 1978) and/or female to female close range inhibition of movement may have influenced the movement of migrators. Further, the repulsion of adult females may be a part of a mechanism by which mated females protect and define their niches. The

results indicate a significant repulsion of females by females, for all three nematodes studied. The mean negative migration within females of TC-isolate, T. spiralis and T. spiralis var. pseudospiralis, respectively, was not significantly different. T. spiralis var. pseudospiralis were repulsed significantly more by females of T. spiralis and TC-isolate, than were females of T. spiralis and TC-isolate by females of T. spiralis var. pseudospiralis. The female to female repulsion between T. spiralis and TC-isolate was similar, but females of T. spiralis were repulsed quantitatively less (-3.93 mm) by their own females compared to female-female repulsion within TC-isolate (-5.09 mm). This may be related to observations on the intestinal distribution and density of these nematodes in mice, where the population of the TC-isolate ranged over a greater portion of the intestine than for T. spiralis (see Capter I, p. 23).

The results indicate a greater cross-specificity of chemical attractant(s) between T. spiralis and TC-isolate than between T. spiralis var. pseudospiralis and either T. spiralis or TC-isolate. Therefore, I conclude that T. spiralis and the TC-isolate are more closely related taxa of the genus Trichinella. Differences in chemical attraction of T. spiralis var. pseudospiralis support the opinion of Garkavi (1972), that it is distinct from T. spiralis.

GENERAL CONCLUSIONS

From this study the following conclusions are made:

1. The intestinal phase

(a) T. spiralis and TC-isolate differed in pathogenicity, reproductive capacity, distribution and persistence of adults in the small intestine and fecundity of females in vitro.

(b) Reproductive capacity and intestinal distribution in laboratory rodents appear to be genetically stable characteristics of both nematodes since no major change in characteristics occurred in the course of the passages in mice.

(c) The pathogenicity of T. spiralis and TC-isolate during the intestinal phase is inversely proportional to the reproductive capacity of parasites.

(d) T. spiralis and TC-isolate adult males and females were morphologically indistinguishable and did not differ in size.

2. The muscle phase

(a) Reproductive capacity indices (RCI) of Trichinella isolates should be determined for laboratory reared and wild animals before an accurate assessment of differences in RCI values can be made.

(b) T. spiralis muscle larvae survived longer in mice compared to TC-isolate larvae. Survival of both T. spiralis and TC-isolate larvae was higher in female than in male mice.

(c) Infective larvae of T. spiralis and TC-isolate differed morphometrically. There was convergence in their morphometric characteristics with continuous passaging through mice, indicating that any morphometric comparison of Trichinella geographical isolates requires complete knowledge of an isolate's history.

3. In vitro chemical attraction

(a) Chemical attraction in Trichinella occurs at two levels; a population aggregation attraction important in maintaining the integrity of a population, and a sexual attraction.

(b) The degree of cross-specificity of chemical attractants between T. spiralis and TC-isolate indicated that they are closely related taxa of Trichinella. Low cross-specificity of chemical attractants between T. spiralis var. pseudospiralis and either T. spiralis or TC-isolate suggests a more distant relationship between this variant and other members of the genus Trichinella.

4. Interbreeding of T. spiralis and TC-isolate

(a) Single pair interbreeding experiments of T. spiralis and TC-isolate created the impression that they are reproductively isolated, but multiple pair and transplant interbreeding experiments showed reproductive compatibility of T. spiralis and TC-isolate.

(b) Inconsistencies in mating ability of T. spiralis and TC-isolate (single versus multiple pair and transplant interbreeding) are due to low probability of contact between individuals in the small intestine.

(c) Methods of assessing the mating ability between T. spiralis and Trichinella geographical isolates must include; (1) adequate controls for sexing accuracy of muscle larvae; (2) an adequate method of introduction (duodenal injection) of larvae to ensure copulation and higher numbers of breeding pairs.

5. Comparative Index

An overall comparative index (C) between T. spiralis and any Trichinella isolate is proposed and includes biological characteristics for both the intestinal and muscle phases of the life cycle. A basic assumption is that an accepted laboratory-maintained Trichinella is used as a standard.

$$\text{Overall comparative index (C)} = \frac{\text{Comparative index for the intestinal phase } (C_i) + \text{Comparative index for muscle phase } (C_m)}{2}$$

$$C^* = \frac{(N_1 \times S_1 \times F_1)}{(N_2 \times S_2 \times F_2)} + \frac{(I_1 \times M_1)}{(I_2 \times M_2)}$$

*1 refers to T. spiralis (standard)

2 refers to Trichinella isolate

N_2 and N_1 represent the number of worms recovered from the small intestine for the isolate and T. spiralis; S_2 and S_1 are the sex ratios of the isolate and T. spiralis in the intestine; F_2 and F_1 represent the average fecundity of individual females for the isolate and T. spiralis; I_2 and I_1 are mean reproductive capacity indices in different hosts; and M_2 and M_1 represent per cent of muscle larvae that survived one year post infection.

C = 1 indicated that the isolate is identical to the standard and C>1 indicates that the isolate is different, therefore, the higher C the greater the difference between biological characters. This index would allow for comparison of biological characteristics of T. spiralis and Trichinella isolates by taking into account the relationships between the characteristics and different phases of the life cycle.

Comparative index for the intestinal phase of T. spiralis and TC-isolate Ci = 3.47 was similar to comparative index for the muscle phase (C_m = 3.43) indicating that differences in biological characters of T. spiralis and TC-isolate are remarkably consistent for different phases of the life cycle.

In order to determine a significant lower limit for the comparative index, somewhat greater than 1, standard T. spiralis (north-temperate) from different laboratories and additional Trichinella geographical isolates need to be evaluated.

6. Origin and evolution

Mayr (1971) defined species as "groups of interbreeding natural populations that are reproductively isolated from other such groups". My results indicated that T. spiralis and TC-isolate are reproductively compatible, therefore, according to Mayr (1971) they are the same species.

Chebotarev (1969) stated that T. spiralis probably originated recently (during glacial times) from a capillarid ancestor. Chitwood and Chitwood (1951) indicated that transfer of Capillaria hepatica to new hosts (predation) is suggestive of a possible step in the evolution of the Trichinella life cycle. Recent origin of T. spiralis is supported by its uniqueness (only member of genus and family), wide range of hosts and high pathogenicity. The existence of individual strains or biological "races" of T. spiralis, which are according to Chebotarev (1969) "just emerging sister or daughter species" is further evidence that T. spiralis evolved recently.

Differences in biological characteristics of Trichinella isolates may have been caused by geographical isolation of the parasites and/or host species. Trichinella is circulated among carnivores by predation and cannibalism (natural or "sylvatic" cycle) and in pigs and rats (domestic or "synanthropic" cycle). Rausch et al. (1956) stated "the occurrence of this nematode in man, in

some of his domesticated animals, and in his commensals seems now to be entirely independent of the natural cycle occurring in wild mammals, and the latter exists independent of man's influence." The "sylvatic" cycle originated earlier than the "synanthropic" cycle (Cameron 1950) which suggests that TC-isolate is the bona fide species and T. spiralis (north-temperate) is a modified form of the same species. Maintenance of T. spiralis (north-temperate) in laboratory animals for prolonged periods may have modified biological characteristics of this parasite, since this form probably has a much more rapid turnover than "sylvatic" Trichinella. Thus it is conceivable that "synanthropic" Trichinella is in the process of evolving into a distinct species.

The taxonomic status of new geographical isolates of Trichinella is far from being established. A more critical approach to biological characterization including a detailed history of each isolate, additional biochemical and immunological studies, and standardized comparative indices are essential for understanding the taxonomy of the genus Trichinella.

REFERENCES

ANYA, A.O. 1976a. Physiological aspects of reproduction in nematodes. Advances in Parasitology 14, 267-351.

ANYA, A.O. 1976b. Studies on the reproductive physiology of nematodes: The phenomenon of sexual attraction and the origin of attractants in Aspiculuris tetraptera. International Journal of Parasitology 6, 173-177.

ARAKAWA, A., and TODD, A.C. 1971. Comparative development of temperate zones and arctic isolates of Trichinella spiralis in the white mouse. Journal of Parasitology 57, 526-530.

BELOZEROV, S.N. 1976. Serological identification of strains and species of Trichinella by IFAT. Moscow Veseoiuznyi Institut Gel'mintologii. Biulleten 18, 508. (Ru).

BESSONOV, A.S., PENKOVA, R.A. and USPENSKY, A.V. 1975. On the independence of Trichinella species. Wiadomosci Parazytologiczne 21, 561-575.

BLAKANICH, S., and SAMOILOFF, M.R. 1974. Development of nematode behaviour: sex attraction among different strains of the free-living Panagrellus redivivus. Canadian Journal of Zoology 52, 835-845.

BOEV, S.N. 1975. On the species composition of Trichinella in Kazakhstan. Meditsinskaya Parazytologiia 44, 220-222. (Ru).

BONE, L.W. and SHOREY, H.H. 1977. Interactive influences of male and female-produced pheromones on male attraction to female Nippostrongylus brasiliensis. Journal of Parasitology 52, 835-845.

BONE, L.W., SHOREY, H.H. and GASTON, L.K. 1977. Sexual attraction and pheromonal dosage response of Nippostrongylus brasiliensis. Journal of Parasitology 63, 364-367.

BONE, L.S., SHOREY, H.H. and GASTON, L.K. 1978. Nippostrongylus brasiliensis: Factors influencing movement of males toward a female pheromone. Experimental Parasitology 44, 100-108.

BONNER, T.P., and ETGES, F.J. 1967. Chemically mediated attraction in Trichinella spiralis. Experimental Parasitology 21, 53-60.

BOYD, E.M., and HUSTON, E.J. 1954. The distribution, longevity, and sex ratio of Trichinella spiralis in hamsters following an initial infection. Journal of Parasitology 40, 686-690.

BRAMBELL, M.R. 1965. The distribution of a primary infestation of Nippostrongylus brasiliensis in the small intestine of laboratory rats. Parasitology 55, 313-324.

BRAND, v. T., OTTO, G.F., and ABRAMS, E. 1938. Forced calcification with parathormone in experimental trichina (Trichinella spiralis) infections. American Journal of Hygiene 27, 461-470.

BRITOV, V.A. 1969. Some differences between natural and synanthropic strains of Trichinella. Wiadomosci Parazytologiczne 15, 555-560. (Ru).

BRITOV, V.A. 1971a. Results of crossing Trichinella from wild animals with those from pigs. Doklady Vsesuyoznoi Akademii Sel'skokhozyaistvennykh Nauk 2, 40-41. (Ru).

_____ 1971b. Biological methods of determining Trichinella spiralis (Owen, 1835) varieties. Wiadomosci Parazytologiczne 17, 477-480. (Ru).

_____ 1973. Population variability of Trichinella. Novoe o Strnoeii Organov i Tkanei Zhivotnykh. Ul'yanovsk, USSR 36-37. (Ru).

_____ 1974. Intraspecific variations of Trichinella. Proceedings IIIrd International Congress of Parasitology, Munich vol 2 Section B14(5), 660.

_____ 1975. The importance of the identification of Trichinella in the prophylaxis of trichinellosis. Voprosy Prirodnoi Ochagovosti Bolesnei. Alma-Ata 7, 109-112. (Ru).

BRITOV, V.A., and BOEV, S.N. 1972. Taxonomic rank of various strains of Trichinella and their circulation in nature. Vestnik Akademii Nauk Kazakhskoi SSR 28, 27-32. (Ru).

BRITOV, V.A., ERMOLIN, G.A., TARAKANOV, V.I., and NIKITINA, T.L. 1971. Genetic isolation of two variants of Trichinella. Meditsinskaya Parazytologia 40, 515-521. (Ru).

CAMERON, T.W.M. 1950. Parasitology and evolution. Transactions of Royal Society of Canada 44, 1-20.

CAMPBELL, W.C. 1967. Distribution of Trichinella spiralis in the small intestine of young mice. Journal of Parasitology 53, 395-397.

CAMPBELL, W.C., and YAKSTIS, J.J. 1969. Mating success and fecundity of pairs of Trichinella larvae administered to mice. Wiadomosci Parazytologiczne 15, 526-532.

CARTER, J.R. 1949. Plasma cell hyperplasia and hyperglobulinemia in trichinosis. The duration of larviposition. American Journal of Pathology 25, 309-323.

CHEBOTAREV, R.S. 1969. Origin and evolution of Trichinella spiralis. Wiadomosci Parazytologiczne 15, 513-519. (Ru).

CHITWOOD, B.G., and CHITWOOD, M.B. 1951. Introduction to nematology. University Park Press, Baltimore, Maryland pp. 334.

CONCANNON, I., and RITTERSON, A.L. 1965. Comparative recoveries of adult worms from Chinese and Golden hamsters fed known doses of Trichinella spiralis. Journal of Parasitology 51, 939-941.

DESPOMMIER, D.D., ARON, L., and TURGEON, L. 1975. Trichinella spiralis: Growth of the intracellular (muscle) larvae. Experimental Parasitology 37, 108-116.

DESPOMMIER, D.D., CAMPBELL, W.C., and BLAIR, L.S. 1977. The in vivo and in vitro analysis of immunity to Trichinella spiralis in mice and rats. Parasitology 74, 109-119.

DICK, T.A. and BELOSEVIC, M. 1978. Observations on a Trichinella spiralis isolate from a polar bear. Journal of Parasitology 64, 1143-1145.

GARKAVI, B.L. 1972. Species of Trichinella from wild carnivores. Veterinariya (Moscow) 49, 90-91. (Ru).

GOULD, S.E. 1970. Trichinosis in man and animals. Gould S.E. (Ed), Charles C. Thomas, Springfield, Illinois USA, pp. 540.

GREEN, C.D., and S.C. PLUMB. 1970. The interrelationships of some Heterodera spp. indicated by the specificity of the male attractants emitted by their females. Nematologica 16, 39-46.

GURSCH, O.F. 1949. Intestinal phase of Trichinella spiralis (Owen, 1835) Railliet, 1895. Journal of Parasitology 35, 19-26.

KOMANDAREV, S., BRITOV, V.A., and MIHOV, L. 1975. Identification of two Trichinella strains from Bulgaria. Doklady Bulgarska Akademiia na Naukite 28, 1541-1542.

KOZAR, Z., and KOZAR, M. 1963. The course of experimental trichinellosis in mice. Methods of infection and the influence of various invasive doses on the mortality and weight of animals. Wiadomosci Parazytologiczne 9, 403-418. (Po).

_____ 1965. A comparison of the infectivity and pathogenicity of Trichinella spiralis strains from Poland and Kenya. Journal of Helminthology 39, 19-34.

KOZEK, W.J. 1975. Trichinella spiralis: Morphological characteristics of male and female intestine-infecting larvae. Experimental Parasitology 37, 380-387.

LARSH, J.E., and HENDRICKS, I.R. 1949. The probable explanation for the difference in the localization of adult Trichinella spiralis in young and old mice. Journal of Parasitology 35, 101-106.

LEVIN, A.J. 1941. Recovery of Trichinella spiralis larvae in early stages of infection. Journal of Parasitology 27, 107-113.

MADSEN, H. 1975. The life cycle of Trichinella spiralis (Owen, 1835) Railliet, 1895 (Syns.: T. nativa Britov et Boev 1972, T. nelsoni Britov et Boev 1972, T. pseudospiralis Garkavi 1972), with remarks on epidemiology and a new diagram. Acta Parasitologica Polonica 24, 143-158.

MARTY, W.G. 1966. The intestinal phase of an experimental infection of chicks with Trichinella spiralis. Journal of Parasitology 52, 903-907.

MATOFF, K. 1937. Der Mechanismus der Altersimmunität des Hundes gegen die Trichinellen infection. Tieraerztliche Rundschau 43, 354-359, 369-373.

NELSON, G.S., RICKMAN, R., and PESTER, F.R.N. 1961. Feral trichinosis in Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene 55, 514-517.

NELSON, G.S., and FORESTER, A.T.T. 1962. Trichinosis in Kenya. Wiadomosci Parazytologiczne 8, 2-28.

NELSON, G.S., and MUKUNDI, I. 1963. A strain of Trichinella spiralis from Kenya of low infectivity to rats and domestic pigs. Journal of Helminthology 37, 329-338.

OSERETSKOVSKAYA, N.N., ROMANOVA, V.I., ALEKSEEVA, M.I. PEREVERSEVA, E.V., and USPENSKY, S.M. 1969. Physiological and biochemical characteristics of natural arctic, synanthropic North Caucasian and laboratory strains of Trichinella spiralis. Wiadomosci Parazytologiczne 15, 561-570. (Ru).

PAWLOWSKI, Z., and RAUHUT, W. 1971. Comparative observations on three local strains of Trichinella spiralis. Wiadomosci Parazytologiczne 17, 481-486.

PEREVERSEVA, E.V., OSERETSKOVSKAYA, N.N., and VERETENNIKOVA, N.L. 1974. The development of Trichinella larvae isolated from raccoon muscles in albino mice. Wiadomosci Parazytologiczne 20, 67-80. (Ru).

PODHAJECKY, K. 1962. Localization of intestinal Trichinella in the small intestine of mice in their intestinal phase. Wiadomosci Parazytologiczne 8, 633-636.

RAPPAPORT, I. 1943a. A comparison of three strains of Trichinella spiralis. I. Pathogenicity and extent of larval development in the musculature. American Journal of Tropical Medicine 23, 343-350.

RAPPAPORT, I. 1943b. A comparison of three strains of Trichinella spiralis. II. Longevity and sex ratio of adults in the intestine and rapidity of larval development in the musculature. American Journal of Tropical Medicine 23, 351-362.

RAUHUT, W. 1978. Comparative studies on the course of intestinal phase of Trichinella spiralis invasion in various species and strains of rodents. Wiadomosci Parazytologiczne 24, 67-90. (Po).

RAUSCH, R., BABERO, B.B., RAUSCH, R.V., and SCHILLER, E.L. 1956. Studies on the helminth fauna of Alaska XXVII. The occurrence of larvae of Trichinella spiralis in Alaskan mammals. Journal of Parasitology 42, 259-271.

READ, C.P., and SCHILLER, E.L. 1969. Infectivity of Trichinella from the temperate and arctic zones of North America. Journal of Parasitology 55, 72-73.

ROCHE, M. 1966. Influence of male and female Ancylostoma caninum on each others distribution in the intestine of the dog. Experimental Parasitology 19, 327-331.

ROMANOVA, V.I., OSERETSKOVSKAYA, N.N., ALEKSEEVA, M.I. and PEREVERSEVA, E.V. 1971. Protein composition of Trichinella larvae of natural Arctic, synanthropic Krasnodar and laboratory strains. Moscow Vsesoiuznyi Institut Gel'mintologii,Trudy 17, 37-38. (Ru).

ROTH, H. 1938. On the localization of adult Trichinae in the intestine. Journal of Parasitology 24, 225-231.

SALM, R.W. and FRIED, B. 1973. Heterosexual chemical attraction in Camallanus sp. (Nematoda) in the absence of worm-mediated tactile behaviour. Journal of Parasitology 59, 434-436.

SUKHDEO, M.V.K., and MEEROVITCH, E. 1978. Comparison of three geographical isolates of Trichinella. Canadian Journal of Zoology 55, 2060-2064.

VILLELLA, J.B. 1966. Morphological criteria for distinguishing the sex of Trichinella spiralis larvae from muscle. Journal of Parasitology 52, 908-910.

APPENDIX I

In this thesis, the word variant or variety is used and its' meaning is that of a geographical race. Similarly, geographical isolates of Trichinella also referred to geographical races of the parasite.

Britov 1969, 1971a, 1971b, Britov et al. 1971, used the term variety to denote geographical races of Trichinella. The term isolate has been used by Read and Schiller 1969 and Arakawa and Todd 1971, to denote geographical races of the parasite. Consequently, I felt justified to use both terms throughout the thesis.