THE UNIVERSITY OF MANITOBA

A COMPARISON OF THE BIOLOGICAL CHARACTERISTICS OF *TRICHINELLA SPIRALIS* VAR. *PSEUDOSPIRALIS* BETWEEN MICE AND BIRD HOSTS.

by

CHRISTIANE MICHELE BOBER

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

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ABSTRACT

Seven biological characteristics of the intestinal and muscle stages of T.spiralis var.pseudospiralis in Swiss Webster mice were compared with the same stages in five bird hosts: Japanese quail, Leghorn chicken, herring-gull, ring-billed gull, and Franklin's gull.

Position of the adult worms in the intestine remained anterior during the first week p.i. and did not differ significantly at day 5 p.i. between mice and bird hosts. Percentage recovery of adult worms was low in quail compared to mice. Female/male sex ratio was higher in birds. Inversion of the sex ratio occurred in 10 days in mice and 20 days in quail. Female worms recovered from gulls started to release larvae in vitro 1 day earlier (day 4 p.i.) than worms recovered from mice and quail (day 5 p.i.). The mean number of larvae released in vitro by adult females recovered from mice and birds during the day of peak larval production was similar. Duration of the intestinal stage was prolonged in birds (at least 60 days in quail) compared to mice (11 days).

Muscle invasion was less intensive in birds compared to mice (except for Franklin's gulls). RCI-values were a stable characteristic for the host. The high percentage of infective muscle larvae in the legs of four species of birds suggested the presence of an organotropism of the nematode for the leg muscle in birds. No virulence of the parasite was noted for doses up to 10,000 larvae/host.

A definite pattern of host influence on morphology of adult and larval worms could not be discerned. Adult male worms recovered from mice and quail were most dissimilar, and adult females recovered from mice and Franklin's gulls were most similar.

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INTRODUCTION

Trichinella pseudospiralis Garkavi, 1972, (Nematoda: Trichinellidae), was first isolated from an American raccoon, Procyon lotor L., in Dagestan, North Caucasus, U.S.S.R. . The original description was based on the absence of cysts in the muscle stage and the smaller dimensions of the infective larvae, compared to T.spiralis (Owen, 1835).

Trichinella taxonomy has and will probably be confusing for many years. Trichinella spiralis (Owen, 1835), (temperate form), T.nativa Britov and Boev, 1972, (arctic form), T.nelsoni Britov and Boev, 1972, (tropical form), and T.pseudospiralis, were elevated by some authors to separate species according to genetic isolation, geographic distribution and adaptability to individual hosts (Britov, 1971a, 1971b; Britov and Boev, 1972; Boev et al., 1979). T.pseudospiralis was considered to be different to T.spiralis and accepted as a species, based on the following biomorphological characteristics: smaller dimensions of the infective larvae, different shape and number of stichocytes of the adult worms, different molting and maturation times of adults and muscle larvae, ability to complete the life cycle in birds and the absence of

encapsulation around the larvae (Bessonov et al. 1975). Also, reproductive isolation between T.spiralis and T.pseudospiralis was demonstrated by Bessonov et al. (1975). T.pseudospiralis is closely related to T.spiralis, but does not differ from it serologically (Bessonov et al.,1976 ; Britov, 1977). All newly erected species were synonymized by Madsen (1976) and T.pseudospiralis was considered as a variety i.e., T.spiralis var. pseudospiralis by Machnicka (1979), Belosevic and Dick (1980), Chadee and Dick (1982), since they considered that there was insufficient evidence to raise it to species status.

Natural infections of "Trichinella-like" worms were previously described in meat-eating birds and in mammals (see Appendix I).

Laboratory animals - mouse, rat, Syrian hamster, guinea pig, rabbit, domestic animals, pig, cat, and monkeys - were shown to be susceptible to experimental infections (Garkavi, 1974, 1976; Miroshnichenko, 1976; Pereverseva et al., 1974). More than 14 species of birds representing several orders were experimentally infected with T.s. var.pseudospiralis (Britov, 1974 ; Tomasovicova, 1975; Tomasovicova and Gorkova, 1976; Geller and Malykhina, 1976; Miroshnichenko, 1976, 1978; Garkavi, 1976; Tomasovicova and Hovorka, 1982).

My objectives were: (1) To compare biological characteristics of T.s. var.pseudospiralis during its intestinal and muscle stages with those in Swiss Webster mice and in five bird hosts, i.e. Japanese quail, Coturnix coturnix japonica Temminck and Schlegel, 1849, chicken, Gallus gallus L., herring gull, Larus argentatus Pontoppidan, 1763, ring-billed gull, Larus delawarensis Ord, 1815, and Franklin's gull , Larus pipixcan Wagler, 1831

(2) To examine whether the host influenced the morphology of the nematode at the adult or the larval stage.

MATERIALS AND METHODS

I. Parasites

The strain of Trichinella spiralis var. pseudospiralis used in these experiments was supplied by Dr. G. Faubert (Mac Donald College, McGill University). The isolate was maintained in Swiss Webster mice (CRL: COBS CFW, Charles River Breeding Laboratories, Wilmington, Massachussetts, U.S.A.), by regular transfers for nine passages in this laboratory.

II. Maintenance of Experimental Animals

All experimental animals were maintained in the animal holding facilities (A.H.F.) of the Zoology Department, University of Manitoba, and kept under the following standard conditions: humidity 50 ± 10%, temperature 21 ± 2 °C and a diurnal cycle of 15 hours light and 9 hours dark.

Mice: The mice used in this study were 50 to 60 day old outbred Swiss Webster mice weighing 30 ± 10 g and given Purina mouse chow (Ralston Purina Co., Canada) and water ad libitum.

Quail: Japanese quail, C. c. japonica, were obtained from the Department of Animal Science of the University of Saskatchewan and maintained in A.H.F. for 12 to 18 generations. One week old and 2-3 months old adult quail were kept under standard conditions. Commercial chick starter (Feed-Rite, chick starter medicated with 0.0125% Amprolium, manufactured by Feed-Rite Ltd., Winnipeg, Canada) and water were given ad libitum.

Chickens: Young male white Leghorn chickens, G. gallus L., 2-3 days of age, were supplied by the veterinary services of the University of Manitoba and kept under conditions similar to the Japanese quail.

Gulls: Three species of gulls were used as hosts for T.s. var.pseudospiralis: herring gulls, L. argentatus, ring-billed gulls, L. delawarensis, and Franklin's gulls, L. pipixcan. Eggs were collected

from three different gull colonies in Manitoba, Canada: herring gull eggs came from East Shoal Lake, ring-billed gull eggs from Dog Lake, and Franklin's gull eggs from Oak-Hammock Marsh. These birds were taken under a Federal Permit issued to Dr. R. Evans, University of Manitoba.

Collected eggs were incubated in a forced-air incubator at 39 $\pm$ 1 °C and 65 $\pm$ 5% relative humidity for 2-3 weeks. After hatching all birds were leg banded, the band numbers and date of birth were recorded. Young gulls were fed previously frozen ocean perch fillets and beef liver, four times daily. At the age of 2 weeks, following a calcium and vitamin D deficiency (rickets), a protein and calcium supplement (2 ml of Geviral® and Similac® or Isomil®) was administered once a day by gastric intubation. Chick grit (Poultry Feed Distributor) and egg shells also complemented the diet. Gulls were infected with T.s. var.pseudospiralis, at the age of 2 months, and thereafter fed dehydrated cat food (Meow Mix, Purina) and given water ad libitum. Once a week ocean perch fillets or beef liver was added to the diet. Gulls were maintained in cages that appeared to cause no stress.

III. Biological Characterization of T. spiralis var. pseudospiralis

T.s. var.pseudospiralis may be separated from T.spiralis by its unencapsulated muscle larvae and ability to complete the life cycle in birds. In this study, the biological characteristics of T.s. var.pseudospiralis in five species of birds, C. c. japonica, and G. gallus, (Galliformes) or L. argentatus, L. delawarensis, and L. pipixcan, (Laridae) was compared to those obtained in Swiss Webster mice.

The relationship between adult worm numbers during the intestinal stage, the number of muscle larvae recovered in the muscle stage, and the number of larvae shed in vitro by adult worms was investigated.

In the course of the invasion, the following aspects of the intestinal stage were examined: (1) Distribution of the adult worms in the small intestine of the host (as defined by Dick and Silver, 1980). The median for each population of Trichinella was determined as that point of the intestine where 50% of the worms were anterior and 50% of the worms were posterior. The median values were averaged and a standard deviation was determined. It is assumed that worms in each 5.0% (1/20th) of the intestine were distributed evenly. Throughout this study the averaged median value will be referred to as position. (2) Percentage of invading larvae establishing themselves, (3) Female to male ratios, and (4) Duration of the intestinal phase.

The reproductive capacity index or RCI-value, defines as the number of muscle larvae recovered, divided by the number of larvae injected (Dick and Belosevic, 1978). This RCI-value was used to assess the level of infectivity of T.spiralis for different hosts. The RCI-value was determined at day 40 p.i., unless otherwise specified.

In vitro larval release (IVLR) was defined as the mean number of newborn larvae shed in vitro by adult female worms per 24 hour period.

Infection procedure.

Oral inoculation: Procedures for isolation of larvae from mice were as follows: infective muscle larvae were obtained day 40 p.i. by artificial digestion of infected CRL: COBS CFW (SW) mice. Infected mice were sacrificed by cervical dislocation, weighed, skinned and eviscerated. Each carcass was then put through a meat grinder and the ground tissue was suspended in a 1% solution of pepsin-HCl in tap water, [ratio of meat (g) to pepsin-HCl solution (ml) was 1:7], for 1 hour at 37 °C, with intermittent shaking. Digested material was placed in a Baermann apparatus consisting of a funnel with cheese cloth fixed on the large opening, and a lamp placed above the funnel providing the heat source. Larvae were allowed to accumulate for 30-45 minutes. The bottom 15 ml of digest was collected in a graduated centrifuge tube and allowed to settle for 5 minutes, and then the supernatant was discarded. Larvae

were resuspended in a 0.16% solution of agar in 0.85% saline, and mixed by aspirating and flushing several times with a Pasteur pipette. Dilutions were performed until the desired concentration of larvae/ml was obtained, usually 0.1 ml for each infection dose. Worms were counted at 16X with the aid of a dissecting microscope (Wild M3, Leitz, Canada), from four 0.1 ml aliquots, spread on the four separate grids of the counting slide devise. When large variations between counts occurred, four additional counts were done.

Oral infections were slightly different for mice and birds. Mice were orally infected, after a brief ether anaesthesia, by gastric intubation with a dose of 400 larvae/mouse. Birds were held firmly and inoculated with a dose of 1000 larvae/bird by placing a tube into the oesophagus.

Surgical inoculation: T.s. var.pseudospiralis muscle larvae were surgically transplanted in the duodenum, jejunum or ileum of Swiss Webster mice and the position of the adult worms in the host's intestine recorded day 5 p.i. and compared to the position obtained day 5 p.i. after oral inoculation of the parasite.

Recipient mice were briefly anaesthetized with ether, followed by an intraperitoneal injection of 0.15 ml/10 g body weight of a sodium pentobarbital solution (Nembutal, Abbot, Montréal, CANADA) in a 1:10 dilution of sterile distilled water, with a 25-gauge, 1.6 cm (5/8 inch) needle. The abdomen was swabbed with 70% ethanol. The experiment was performed under laminar air-flow sterile hood (Envirco) using instru-

ments disinfected in an alcohol bath (70% ethanol). An incision 1 cm long was made through the skin and the body wall to the left of the linea alba and slightly anterior to the groin area. The appropriate portion of the small intestine, the duodenum, jejunum or ileum was lifted gently up and out through the incision with the aid of a curved blunt surgical probe. The portion of the intestine to be injected was rested on the shaft of the probe during the injection. Four hundred larvae, suspended in 0.1 ml of 0.85% NaCl and 0.16% agar were injected with a 1 ml syringe fitted with a 25-gauge, 3.8 cm (1.5 inch) needle. The needle was held perpendicular to the longitudinal axis of the small intestine. After injection the syringe and needle were flushed to determine if any larvae remained. The site of injection was marked with black thread knotted in the mesentery. The abdominal muscle and skin were sutured with catgut chromic 3/0 USP (B.Braun Melsungen A.G., West Germany).

Intestinal stage of the parasite in mice and birds.

Mice: Ninety, 50-60 day old CRL: COBS CFW (SW) mice were infected orally with 400 larvae each. The small intestines of three male and three female mice were examined each day to study the intestinal longevity of the parasite (Table I). Worm position on day 5 p.i. was also studied in thirty orally-infected and eighteen surgically-infected mice.

Table I. Study of biological characteristics of T. spiralis var. pseudospiralis in various hosts. Infection dose 400 larvae/mouse and 1000 larvae/bird. Note: Days 2→11 : small intestines were examined each day from day 2 until day 11.

	HOSTS						
	Crl: COBS CFW (SW) mice (50 - 60 days)	Adult ♂ Japanese quail (2 - 3 mos.)	Young ♂ and ♀ Japanese quail (1 wk.)	Leghorn chicken	Ring-billed gull (2 mos.)	Herring gull (2 mos.)	Franklin's Gull (2 mos.)
Infection dose (larvae recovered from mice)	400 l/m	1000 l/b	1000 l/b	1000 l/b	1000 l/b	1000 l/b	1000 l/b
INTESTINAL STAGE	Days 2 ⟶ 11	Days 3 ⟶ 10,12,14, 16, 20, 24, 40, 60, 160	–	–	Days 3 ⟶ 10, 12, 14, 16	Days 3⟶20	Days 3⟶ 8, 12,14,20
Distribution and longevity							
No. animals/day	6	3			1	1	1
	n = 30 day 5 (oral infection)				n = 5 day 7	n = 3 day 5	n = 3 day 7
	n = 18 day 5 (surgical infection)					n = 5 day 7	
MUSCLE STAGE							
RCI-values day 40 post-infection							
No.	76	5	10	1	5	6	5
• increasing infection doses - No. larvae inject/animal	100,400,800,1000,2000, 2500,3000,3500,4000, 5000,7500,10000,20000	1000,5000,10000					
• increasing time post-infection (days)	40	40-60-160-250	40-80	40	40	40	40
• transfers	8 generations in mice	8 generations in quail					
IN VITRO LARVAL RELEASE (days)	2 ⟶ 11	3⟶10,12,14,16,20, 24,40,60,160	–	–	3⟶10,12,14 16,40	3⟶20,40	3⟶8,12,14,20 40

Infected mice were sacrificed and the entire small intestine removed. The small intestine, under uniform tension, was transferred to a biased grid (Brambell 1965), pinned, and then cut into 20 equal segments. Each segment was placed in a 15 ml vial containing 10 ml of 0.85% saline solution. Vials were then refrigerated overnight to allow the breakdown of the intestinal mucosa and facilitate the counting and sexing of the worms. After refrigeration, contents of each vial were emptied into a Petri dish, each intestinal segment slit lengthwise and the mucosa scraped from the muscularis. Numbers of male and female worms were recorded in each mouse for each segment and worm position, percentage recovery and sex ratio determined each day, from day 1 p.i. until such time that no adult worms were found for two consecutive days. Distribution of T.s. var.pseudospiralis was studied day 5 p.i. in each of the orally or surgically infected mice. Contents of the stomach and caecum were also checked for presence of parasites.

Birds: The intestinal stage of T.s. var.pseudospiralis was studied in adult (2-3 months old males) and young (1 week old males and females) Japanese quail. Thirty-six male adult quail were orally infected with 1000 larvae/bird, and each day three birds were sacrificed, starting from day 3 to day 8 p.i. and every second day from day 8 to day 16 p.i.; birds were also examined on day 20 and day 24 p.i. (Table I). Presence of adult worms in the small intestine was checked on day 40, 60 and 160 p.i., when RCI-values were determined. The intestinal distribution of T.s. var.pseudospiralis in one week old Japanese quail, orally infected with 1000 larvae/bird was studied on day 7 and day 9 p.i. (three birds each day).

Eighteen ring-billed gulls, 21 herring gulls and 16 Franklin's gulls were orally infected at the age of 2 months with 1000 T.s. var.pseudospiralis infective muscle larvae obtained day 40 p.i. from artificial digestion of infected CRL: COBS CFW (SW) mice. Worm position in the intestine was studied each day, starting from day 3 to day 12 p.i., and every second day from day 12 until day 20 p.i. . One gull of each species was sacrificed per day, except for day 7, when five ring-billed gulls, five herring gulls and three Franklin's gulls were examined (Table I).

Quail and gulls were sacrificed by asphyxiation with CO_2 , and the intestinal tract from gizzard to anus was removed. The small intestines of quail and gulls were divided into 20 equal segments using the method described for the mouse intestine. Each segment was deposited in a small Petri dish containing 37 °C saline, slit longitudinally and the mucosa scraped from the muscularis. The scraped intestines were incubated for at least 1 hour at 37 °C in an environmental chamber, to allow worms to free themselves from mucous and other intestinal debris prior to counting and sexing. Female worms were isolated and placed in a Petri dish containing saline at 37 °C before being washed and inoculated into culture medium (see section on in vitro larval release, p.17). The gizzard, caecum and large intestine were also examined for presence of worms.

The procedure used to study the distribution of T.s. var.pseudospiralis in the small intestine of gulls was different from that used for quail because partially digested food in the intestine of

gulls obscured worms, thus affecting accuracy of worm counts. Therefore each scraped segment was placed individually in a Baermann apparatus and adult worms were counted and sexed. The same birds were used to study the longevity and in vitro release of newborn larvae by female worms.

Muscle stage of the parasite in mice and birds.

The effect of infection dose, age of the infection, and age of the host on RCI-values were studied on the following hosts.

Mice: Eight to 12 male and female CRL: COBS CFW (SW) mice were orally infected with 400 larvae/mouse for each passage of the parasite. The RCI and larvae/gram values were determined for each infected mouse at day 40 p.i. for generations nine to sixteen .

The pathogenicity of T.s. var.pseudospiralis in Swiss Webster mice was studied after infecting eight to ten mice per infection group with 13 dosages (Table I). Mortalities were recorded daily for the first 20 days p.i. and the infection dose responsible for the death of 50% of the host population recorded (LD_{50}).

Quail: The infectivity index of T.s. var.pseudospiralis was determined in three Japanese quail, orally infected with 1000 larvae each, following each transfer of the parasite for generations two to nine (Table I).

Infective T.s. var.pseudospiralis muscle larvae from quail were obtained by artificial gastric digestion, using the procedures for isolating muscle larvae, (outlined in section on inoculation, p.8). Quail were examined for muscle larvae each time , i.e. day 40, 60, 160 and 250 p.i., the presence of adult worms in the intestine was also checked, using the method described for the collection of the adult worms from quail and gull intestine (see p.13), but without sectioning the intestine in 20 segments. Quail were sacrificed by asphyxiation with CO_2 , weighed, skinned and eviscerated. Pieces of breast and wing muscle were removed and compressed between two glass slides and examined for worms. The carcass was divided into several parts: neck, left and right breast, two wings, two legs, head, and remainder of the body. Each carcass or part of the body was then put individually through a meat grinder and the ground tissue suspended in a 1% pepsin-HCl solution for 1 hour at 37 °C with intermittent shaking [Ratio of meat (g) to pepsin-HCl solution (ml) was 1:7]. Various pepsin concentrations (1/2% and 1%), HCl concentrations (1/2% and 1%), duration of digestion (1 to 2 hours) and time in the Baermann apparatus (1/2 to 2 hours) did not influence the number of larvae recovered.

The survival rate of T.s. var pseudospiralis muscle larvae in adult and juvenile Japanese quail was studied by measuring the RCI-values on day 40, 60, 160 and 250 p.i. in adult quail and day 40 and 80 p.i. in young quail, all infected with 1000 larvae each. The influence of infective dose on the RCI-values was studied day 40 p.i. in adult Japanese quail infected with 1000, 5000, and 10,000 larvae per bird. Influence of the sex of the host on RCI values was studied in young male and female quail at day 80 p.i. .

Chickens: Reproductive capacity index of T.s. var.pseudospiralis was determined day 40 p.i. in one young male white Leghorn chicken.

Gulls: The RCI-value of T.s. var.pseudospiralis, as well as the distribution of muscle larvae were determined on day 40 p.i. in six 2 months old herring gulls, five ring-billed gulls and five Franklin's gulls. The technique for recovery of the parasite was similar to that described for quail. The influence of the host on the RCI-values was investigated. Larvae recovered from those gulls where a high RCI-value was found were transferred into Swiss Webster mice and RCI-values determined after each of four passages into mice. The infection dose was 400 larvae/mouse. Larvae obtained from herring gulls were also transferred into two golden hamsters, Mesocricetus auratus Waterhouse, 1839, with an infection dose of 800 larvae/hamster.

In vitro larval release (IVLR).

Mice: The mean number of larvae released in vitro by adult female worms/24 hours was determined from days 1 to 10 p.i. . Mice infected orally with a dose of 400 larvae/mouse were sacrificed and the entire small intestine removed from the abdominal cavity and deposited in a large Petri dish containing 0.85% NaCl at 37 °C. The intestine was slit longitudinally and the mucosa scraped from the muscularis. The mixture was placed in a Baermann apparatus and adult worms allowed to settle for 1 to 2 hours. The bottom 10 ml was collected, and male and female worms sexed and separated. Female worms were washed once in 37 °C saline and twice in 37 °C α- MEM Eagle, Earle's Base (modified) tissue culture medium and 10% fetal calf serum (by volume). Female worms were then deposited in 2 ml plastic cone bottom vials containing 1 ml of tissue culture medium. This operation was performed under a sterile hood. Vials were capped and stored at 37 °C in the dark in an environmental chamber for 24 hours. At the end of incubation, the supernatant was removed from each vial, and the number of newborn larvae counted. When number of larvae released was too high for accurate determination of numbers, the remaining content of the vial was placed on a counting grid and larvae counted (at 16 X).

Birds: The 24-hour rate of larval production by female T.s. var.pseudospiralis in birds was determined in vitro using a similar experimental procedure to the one used for females recovered from mice. The times p.i. that females were tested are outlined in Table I.

IV. Host Influence on Morphology of T. spiralis var. pseudospiralis

Measurements of adults and muscle larvae of T.s. var.pseudospiralis were assessed to determine if the host species affected the size of worms. In addition, variation in size of muscle larvae was studied in Swiss Webster mice, in relation to elapsed time after infection.

Collection of worms.

Adult worms used for morphology studies were collected from mice and birds day 8 p.i. . Day 8 p.i. was chosen because recovery rate was low in birds and maximum worms were recovered that day. As small numbers of female worms were recovered from the bird host, these female worms were used for both morphological studies and for in vitro larval production. Females were fixed 24 hours later, after being incubated in culture medium, except in the case of worms recovered from mice, when half of the female worm population recovered was fixed day 8 p.i. without being tested for IVLR. Male adult worms were also collected day 8, according to the method previously described for the retrieval of the parasites (see p.12).

Male and female muscle larvae were obtained from mice, quail and gulls by artificial digestion of the muscles, according to the technique described for oral inoculation (see p.8).

Fixation technique.

The standard nematological technique for relaxation of worms by applying gentle heat was not successful with all individuals, as some larvae remained coiled even after death. Adult and larvae were fixed in 70% ethanol for 1 to 8 months, then left for 2 days in an oven at 56 °C in a 50% glycerol-lactophenol solution for clearing. As the internal anatomy of larvae could not be seen clearly, due to the presence of a thick cuticle, cotton blue (0.01%) was added to the lactophenol and the slides heated for 2 to 3 minutes at 65 to 70 °C. Worms were mounted on glass slides in glycerol and the cover slip ringed with nail polish.

Measurements.

Worms were selected at random with a sample size of 50 chosen in most cases. Insufficient female worms were recovered from ring-billed gulls, and few male adult worms were recovered from all hosts, so sample sizes in these instances were smaller. Measurements of adult worms included: total length, length of the oesophagus measured from the tip of the head to the stichosome (including the buccal capsule), and width at the oesophago-intestinal junction. Measurements of adult female worms

included: total length, length of the oesophagus, width at the oesophago-intestinal junction and distance of the vulva from the anterior end.

Measurements of the larvae included: total length, length of the genital anlage, length of the rectum, and width at the oesophago-intestinal junction. Male and female larvae were selected at random and the criteria described by Kozek (1975) used to sex infective larvae. Male larvae were characterized by: (1) a crossing of the intestine over the gonad from the ventral to the dorsal side, (2) the blunt shape of the anterior pole of the gonad, and (3) the length of the rectum being about 50 μm.

Female larvae were characterized by: (1) the intestine always being along the dorsal side, (2) the sharp point of the anterior pole of the gonad, and (3) the length of the rectum being about 25 μm in length (Belosevic and Dick, 1979).

Worms were measured on a T.V. monitor (Conrac, model RNCA 9, Covina California, U.S.A.), connected to a Sony video camera (black and white) and a Zeiss photomicroscope II. A micrometer scale was used to convert measurements into millimeters.

V. Statistics

The mean for each parameter of experimental hosts was averaged and a standard deviation determined. The averaged median values for intestinal distribution were compared by a Student's t-test. A 0.05 probability level was considered significant. Student's t-test was also used to compare RCI- and IVLR values.

In the morphology studies, Behrens-Fisher's test was used to compare two samples where unequal variances were determined by Bartlett's test (Sokal and Rohlf, 1969). A 0.05 probability level was considered significant and paired tests were done on five variables for male worms, seven variables for female adult worms and six variables for larvae. Measurements of adult worms recovered from ring-billed gulls were not used for statistical analysis, due to the small sample size.

RESULTS

I. Biological Characteristics of the Parasite in Mice

Intestinal stage.

Position of T.s. var. pseudospiralis in the small intestine: Intestinal position of T.s. var.pseudospiralis, (see definition of the median, p.7) was determined each day from day 1 to day 10 p.i. (Table II). A lower median value indicated a more anterior location in the intestine and a higher median value indicated a more posterior location (Silver et al., 1980). Adult worms were mainly located in the anterior half of the small intestine of mice during the first week p.i. . Worm position varied little between day 1 and day 7 p.i. (21.9 $\pm$ 5.6) to (28.6 $\pm$ 13.8). Worms shifted posteriorly at day 8 p.i., (44.8 $\pm$ 12.4, Table II). Position of worms after oral or surgical infection did not differ significantly ($P>0.05$): the median position at day 5 p.i. after oral

infection of T.s. var.pseudospiralis was 18.9 ± 4.3, and it was 12.7 ± 4.7 after surgical inoculation of the larvae in the duodenum (Table III). When worms were injected in the jejunum and the ileum, worm position was more posterior, 49.7 ± 29.9 and 80.9 ± 6.1 respectively, suggesting that the worms remained at their point of injection and did not migrate to their normal location in the intestine, i.e. the duodenum. No worms were found in the stomach or caecum.

Percentage recovery: The mean percentage recovery of adult T.s. var.pseudospiralis from the intestine of orally inoculated mice was 18.1 ± 9.1% during the first 10 days of infection, with a maximum at day 3 p.i. of 27.5 ± 5.5% (Table II). The average percentage of larvae recovered on day 5 p.i. after oral inoculation was 33.4 ± 9.1 and was not significantly different ($P>0.05$) from percentage obtained after surgical injection of larvae in the duodenum. Worms started to leave the intestine after day 7 p.i., indicated by a decrease in the percentage of worms recovered.

Sex ratio: The average female/male sex ratio of adult worms in the small intestine of mice was stable (1.7 ± 0.51), until day 8 p.i.; thereafter it was reversed as fewer female worms were found in the intestine. The sex ratio was 3.13 on day 5 p.i. after oral inoculation and varied between 3.19 (duodenum), 4.6 (jejunum) and 3.73 (ileum) after surgical inoculation (Table III). The sex ratio was 0.61 at day 10 p.i., the last day that females were recovered in the intestine.

Longevity of adult parasites in the small intestine of mice: A rapid loss of worms occurred between day 8 and day 12 p.i. . No female worms

TABLE II. Intestinal distribution of T. spiralis var. pseudospiralis in orally infected Crl: COBS CFW (SW) mice.

Days postinfection	No. mice infected	Worm position ± S.D.	% recovery ± S.D.	♀/♂ Sex ratio
1	6	21.9 ± 5.6	16.2 ± 8.4	
2	6	17.3 ± 3.3	25.3 ± 5.5	2.00
3	6	23.3 ± 5.2	27.5 ± 5.5	2.08
4	6	18.9 ± 4.4	24.6 ± 6.6	1.99
5	6	14.3 ± 3.2	24.6 ± 4.9	2.19
6	6	17.0 ± 3.2	24.6 ± 4.9	2.03
7	6	28.6 ± 13.8	20.5 ± 2.2	2.14
8	6	44.8 ± 12.4	9.5 ± 3.6	1.76
9	6	43.3 ± 12.8	4.9 ± 4.5	1.37
10	6	54.6 ± 19.5	2.9 ± 2.6	0.61
11	6	*	*	*
12	6	0	0	0

* one male worm was recovered.

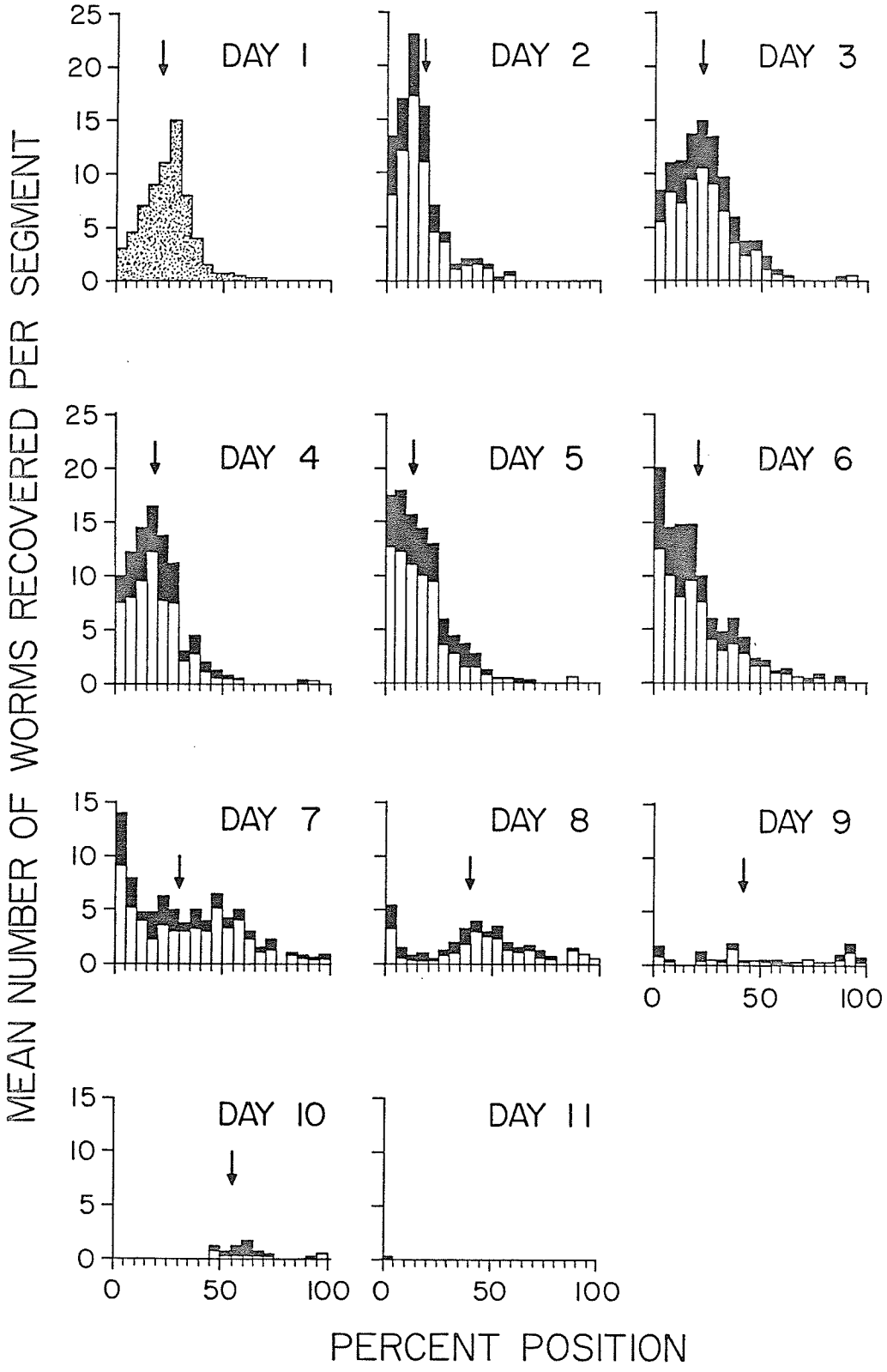
TABLE III. Intestinal distribution of T. spiralis var. pseudospiralis in Crl: COBS CFW Swiss Webster mice on day 5 postinfection. Surgical and oral infections.

	Portion intestine inject.	No. animals	Inject. position (%)*	Recovery position ± S.D.	% recovery ± S.D.	Sex ratio ♀/♂
SURGICAL INFECTION	Duodenum	11	7.5	12.7 ± 4.7	26.4 ± 7.5	3.19
	Jejunum	2	47.5	35.4 ± 3.3	23.8 ± 9.9	5.78
		1	62.5	78.4	29.5	3.37
	Ileum	1	87.5	83.8	46.8	3.67
		3	92.5	79.9 ± 7.0	22 ± 0.9	3.78
ORAL INFECTION		36		18.9 ± 4.3	33.3 ± 9.1	3.13

* Worms were injected in the same segment (i.e., 5%) of the intestine.

were recovered from the mouse intestine after day 10 p.i. (Figure 1). Only one male worm was recovered in one of the six mice examined on day 11 p.i., while on day 12 and 13 postinfection the intestines were free of worms.

Figure 1: The distribution of T. spiralis var. pseudospiralis in the small intestine of mice (six mice were examined each day). Note: worms were sexed after day 1. Shaded areas are males, unshaded areas are females, and arrows point to median position of population.


MEAN NUMBER OF WORMS RECOVERED PER SEGMENT
DAY 1
DAY 2
DAY 3
DAY 4
DAY 5
DAY 6
DAY 7
DAY 8
DAY 9
DAY 10
DAY 11
0
5
10
15
20
25
0
50
100
PERCENT POSITION

Muscle stage.

Passage experiments: The RCI-values for each transfer of T.s. var.pseudospiralis in Swiss Webster mice were determined and mean RCI-value for eight generations was 27.7 ± 12.2 (Table IV). The mean number of larvae/gram for total body weight for eight generations was 388.3 ± 163.9 (Table IV). No significant differences between RCI-values of male and of female mice and between larvae/gram in male and in female mice were noted, therefore data from both sexes were pooled.

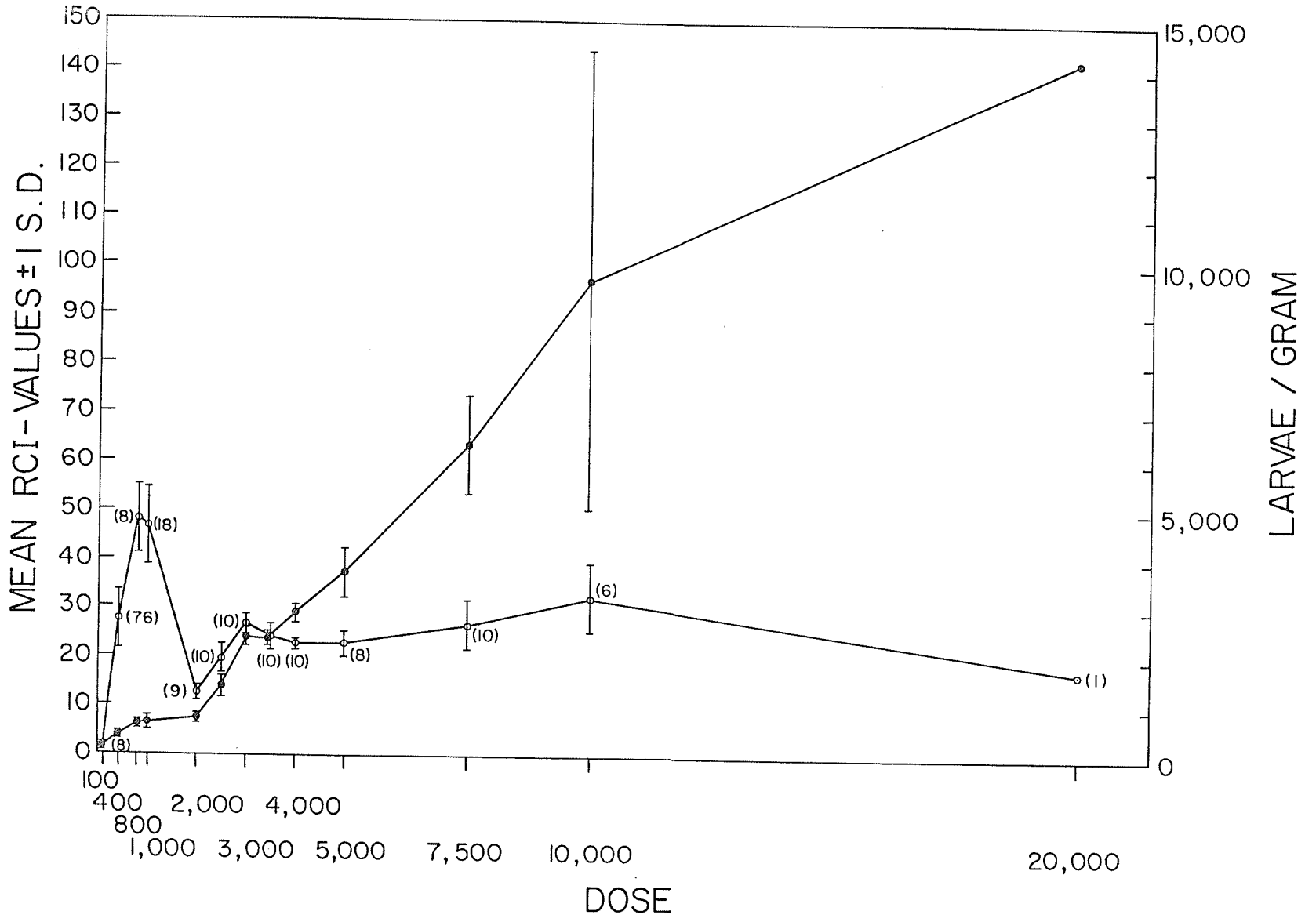
Pathogenicity: The effects of increasing doses of T.s. var.pseudospiralis larvae on the RCI-values and larvae/gram in male and female mice are presented in Figure 2. Both sexes were pooled since larvae/gram in male and female worms were not significantly different ($P>0.05$). RCI-values increased for each higher concentration inoculum until RCI-values reached a value of 26.2 ± 4.4 at an infection dose of 3,000 larvae/mouse. Thereafter, an increase in the inoculum did not result in a corresponding increase in the RCI-values. The larvae/gram in host tissue increased as the infection dose increased. High RCI-values obtained for infection doses of 800 and 1,000 larvae/mouse were unexpected. The experiment was repeated with a different group of experimental animals and a different source of inoculum (1,000 larvae)

TABLE IV. RCI-values and muscle larvae/g of body weight, for seven passages of T. spiralis var. pseudospiralis in Crl: COBS CFW Swiss Webster mice (day 40 postinfection). Sexes pooled, as there was no significant difference between male and female mice.

GENERATION OF PARASITE	9	10	11	12	13	14	15	16	$\bar{X}$ of 8 generations
NO. MICE	8	8	10	10	10	8	12	10	76
RCI-values	23.3	18.5	16.2	24.8	31.2	27.3	36.5	25.5	27.7
$\bar{X} \pm$ S.D.	$\pm$1.9	$\pm$5.1	$\pm$1.9	$\pm$4.3	$\pm$16.7	$\pm$14.2	$\pm$7.6	$\pm$12.7	$\pm$12.2
Larvae/g	567.0	499.2	221.8	320.7	435.9	368.5	429.6	291.5	388.3
$\bar{X} \pm$ S.D.	$\pm$67.6	$\pm$151.2	$\pm$37.9	$\pm$28.2	$\pm$212.8	$\pm$195.3	$\pm$108.8	$\pm$133.2	$\pm$163.9

and again a high RCI-value was produced. Four out of the ten inoculated mice were dead on the sixth day p.i. with a dose of 10,000 larvae/mouse. At the infection dose of 20,000 larvae/mouse, two mice out of ten died on the third day p.i. and five mice died on the fourth day p.i. . Therefore, the LD_{50} dose is somewhere between 10,000 and 20,000 larvae/mouse.

Figure 2: Influence of dose of the parasite on RCI-values and larvae/g recovered from mice muscle Note: closed circles represent larvae/gram, and open circles represent RCI-values. (n) = number of mice infected.


MEAN RCI-VALUES ± 1 S.D.
LARVAE / GRAM
DOSE
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
15,000
10,000
5,000
0
100
400
800
1,000
2,000
3,000
4,000
5,000
7,500
10,000
20,000
(8)
(76)
(8)
(18)
(9)
(10)
(10)
(10)
(10)
(8)
(10)
(6)
(1)

In vitro release of newborn larvae.

Larval production by individual females per 24 hours *in vitro* is seen in Table V. Females started to release larvae on day 5 p.i., and peak larval production occurred on day 8 p.i., with a mean number of larvae released/female worm of 26.9 $\pm$ 9.7. Female worms released larvae from day 5 p.i. until day 11, when the last worm was found in the intestine.

Table V. *In vitro* larval release by *T. spiralis* var. *pseudospiralis* females recovered from mice and birds. Infection dose 400 larvae/mouse and 1000 larvae/bird. Note: n=number of female worms examined. Maximum number of larvae recovered is underlined.

Days postinfection	MICE		BIRDS							
			Japanese quail		Ring-billed gulls		Herring gulls		Franklin's gulls	
	n	$\bar{X} \pm$ S.D.	n	$\bar{X} \pm$ S.D.	n	$\bar{X} \pm$ S.D.	n	$\bar{X} \pm$ S.D.	n	$\bar{X} \pm$ S.D.
1	50	0	–	–	–	–	–	–	–	–
2	50	0	–	–	–	–	–	–	–	–
3	50	0	50	0	50	0	50	0	50	0
4	50	0	50	0	50	3.4 ± 3.7	50	1.7 ± 2.9	50	0.4 ± 4.4
5	50	16.6 ± 8.1	50	14.6 ± 7.6	26	19.4 ± 7.5	150	16.9 ± 9.5	50	15.5 ± 5.9
6	50	23.1 ± 8.8	50	10.7 ± 7.8	50	13.4 ± 6.4	81	10.6 ± 9.2	50	15.3 ± 6.6
7	50	22.1 ± 6.3	23	16.6 ± 10.6	78	15.3 ± 8.8	79	12.6 ± 9.9	50	12.6 ± 4.4
8	50	26.9 ± 9.8	24	9.4 ± 8.4	50	8.7 ± 6.9	25	12.2 ± 11.2	46	13.7 ± 11.8
9	50	8.5 ± 7.5	–	–	50	14.9 ± 7.6	10	12.1 ± 12.1	–	–
10	50	12.7 ± 6.9	11	9.0 ± 7.6	50	12.8 ± 9.7	21	12.8 ± 14.4	–	–
11	–	–	–	–	–	–	50	24.5 ± 10.2	–	–
12	–	–	10	8.5 ± 4.2	50	16.1 ± 10.6	50	22.2 ± 12.7	50	21.3 ± 9.4
13	–	–	–	–	–	–	–	–	–	–
14	–	–	25	7.3 ± 5.6	50	8.3 ± 10.2	12	12.8 ± 12.9	27	13.5 ± 7.6
15	–	–	–	–	–	–	–	–	–	–
16	–	–	24	9.8 ± 7.4	10	2.1 ± 3.7	–	–	–	–
18	–	–	–	–	–	–	–	–	–	–
20	–	–	–	–	–	–	–	–	–	–
24	–	–	–	–	–	–	–	–	11	13.0 ± 6.9
40	–	–	5	1.8 ± 2.16	–	–	–	–	–	–
60	–	–	2	0	–	–	–	–	–	–
160	–	–	0	–	–	–	–	–	–	–

II. Biological Characteristics of the Parasite in a Variety of Bird Hosts

Intestinal stage.

Position of the worms in the small intestine: The position of T.s. var.pseudospiralis remained anterior (about 15%) in the quail and gull small intestine for the first week after infection. The average position in the quail intestine was 15.3 ± 2.6. Position of worms at day 7 p.i. in quail was 14.9 ± 9.1 and not significantly different from position in ring-billed gulls (17.9 ± 5.8), herring gulls (14.7 ± 3.4), and Franklin's gulls (14.8 ± 9.4), with $P>0.05$ (Tables VI and VII). Worms started to shift posteriorly on day 12 in gulls and on day 24 in quail. Worm position was posterior on day 20 p.i. in herring gulls (75%) and Franklin's gulls (56.5%). No worms were found in the gizzard or the caecum.

Percentage recovery: The percentage recovery in Japanese quail for the first 24 days of infection was 3.4 ± 3.9% (Table VI). A maximum of 12.6 ± 11.3% was recovered on day 5 p.i., while all other days p.i. had a percentage take of 7% or less (Table VI). Recovery of adult worms from the gull small intestine was low and variable: a maximum of 13.6% was

TABLE VI. Intestinal distribution of T. spiralis var. pseudospiralis from orally infected Japanese quail.

Days postinfection	No. birds infected	Worm position ± S.D.	% recovery ± S.D.	♀/♂ Sex ratio
3	3	13.4 ± 6.1	5.0 ± 4.7	2.51
4	3	16.5 ± 3.4	7.1 ± 3.5	4.92
5	3	18.5 ± 4.2	12.6 ± 11.3	2.90
6	3	13.5 ± 2.6	7.5 ± 5.2	3.26
7	3	14.9 ± 9.1	1.4 ± 1.3	3.78
8	3	13.8 ± 9.5	1.9 ± 1.7	3.07
10	3	17.1 ± 8.9	0.6 ± 0.3	4.67
12	3	17.5 ± 2.5	0.5 ± 0.6	14.00
14	3	17.6 ± 10.5	1.6 ± 2.4	4.44
16	3	10.2 + 5.4	1.3 ± 0.5	7.00
20	3	12.5 ± 3.5	0.2 ± 0.1	0.50
24	3	35.0 ± 14.1	0.3 ± 0.1	0.67

TABLE VII. Intestinal distribution of T. spiralis var. pseudospiralis in three species of gulls. R.B. (ring-billed gulls); H.G. (herring gulls); F.G. (Franklin's gulls). Note: a = 3 expt. hosts; b = 5 expt. hosts; otherwise only one bird used/observation.

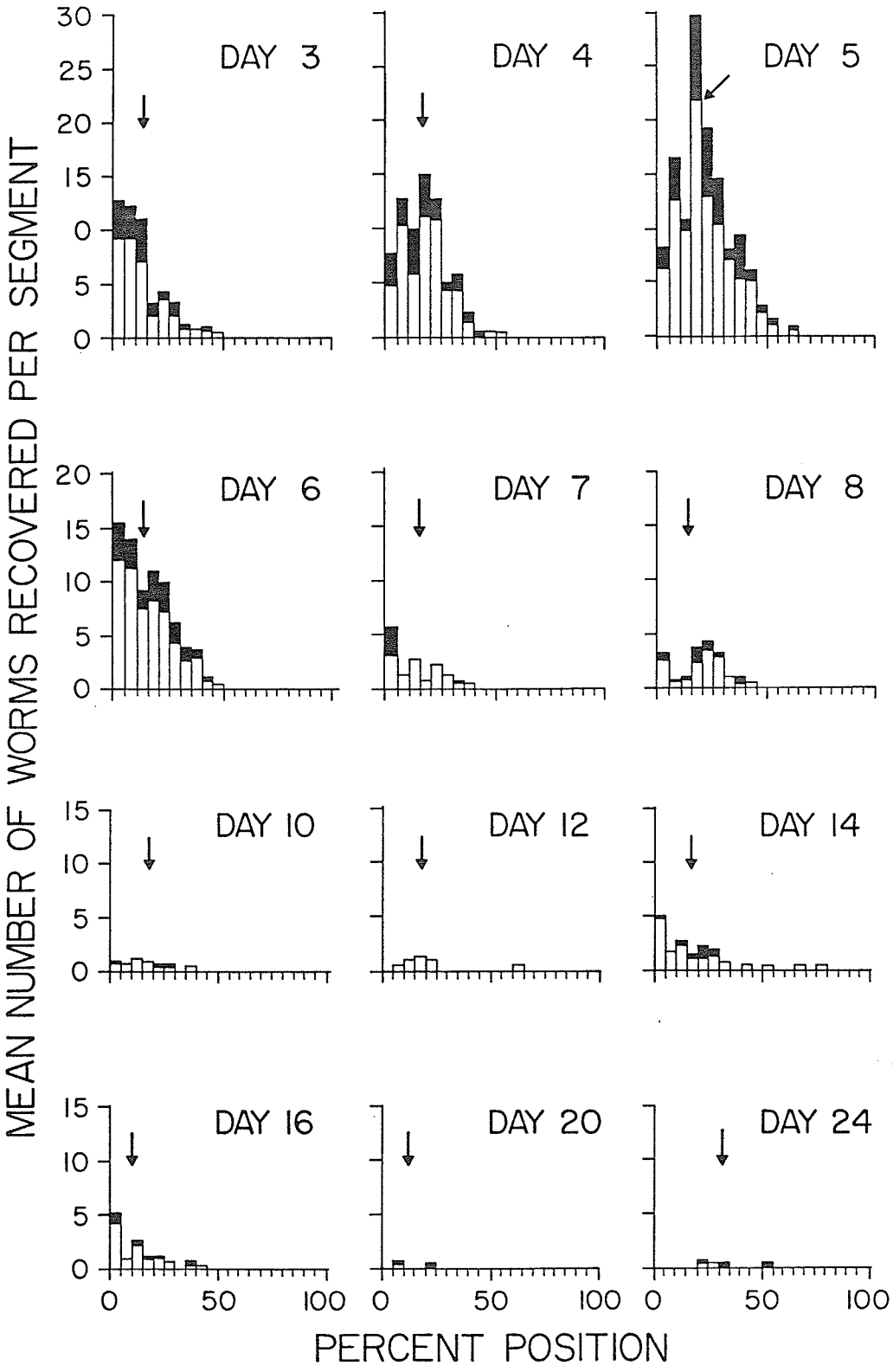
Days postinfection	Worm position ± S.D.			% recovery ± S.D.			♀/♂ Sex ratio		
	R.B.	H.G.	F.G.	R.B.	H.G.	F.G.	R.B.	H.G.	F.G.
3	26.8	26.6	15.8	2.9	13.4	44.6	6.25	3.47	2.58
4	10.5	14.4	21.4	7.7	16.4	32.9	6.70	2.64	1.56
5	16.9	20.2[a]	20.4	2.9	17.6[a]	22.5	13.50	3.34[a]	2.77
	—	(±5.5)	—	—	(±9.4)	—	—	—	—
6	18.8	24.8	15.0	11.8	12.6	12.8	9.00	4.12	1.98
7	17.9[b]	14.7[b]	14.8[a]	10.9[b]	4.3[b]	2.0[a]	3.41[b]	3.34[b]	9.00[a]
	(±5.8)	(±3.4)	(±9.4)	(±16.8)	(±1.7)	(±0.3)	—	—	—
8	37.3	6.8	15.4	6.6	2.8	6.3	7.25	8.33	2.70
9	18.1	6.7	—	13.6	1.0	—	3.69	10.00	—
10	17.2	27.0	—	10.0	2.0	—	50.00	22.00	—
11	—	7.7	—	—	9.0	—	—	3.28	—
12	47.9	50.9	24.7	8.2	8.5	22.9	3.10	2.54	2.27
14	28	4.7	7.3	6.4	1.3	4.2	31.00	12.00	1.80
16	11.3	87.5	—	1.1	0.5	—	10.00	4.00	—
18	—	86.3	—	—	0.5	—	—	5.00	—
20	—	75.0	59.5	—	0.6	1.9	—	2.00	28.00

found on day 9 p.i. in the ring-billed gull, 17.6 ± 9.4% on day 5 p.i. in the herring gull and 44.6% on day 3 p.i. for the Franklin's gull (Table VII).

Sex ratio: The average female/male ratio of adult worms in the small intestine of quail was 2.51 on day 3 p.i., versus 0.67 on day 24 p.i. . The sex ratio in gulls was extremely variable and sometimes reached values as high as 14.0 (in quail, on day 12 p.i.).

Longevity of adult parasites in the small intestine of birds: A rapid loss of adult worms occurred in the intestines after day 6 in quail (Figure 3) and day 12 in gulls (Table VII). Gravid females were found in quail and gull intestines on each of the 20 days of observation. Seven males and five fecund females were recovered on day 40 p.i. when the intestines of 12 adult quail were examined and one male and two females were recovered on day 60 p.i. from all three birds. No adult worms were found on day 160 p.i. from the intestine of adult quail. Gull intestines were checked on day 40 p.i. for presence of adult worms, but none were found.

Figure 3: The distribution of T. spiralis var. pseudospiralis in the small intestine of Japanese quail (three birds were examined each day). Note: shaded areas are males, unshaded areas are females, and arrows point to median position of population.


DAY 3
DAY 4
DAY 5
DAY 6
DAY 7
DAY 8
DAY 10
DAY 12
DAY 14
DAY 16
DAY 20
DAY 24
MEAN NUMBER OF WORMS RECOVERED PER SEGMENT
PERCENT POSITION
0
50
100

Muscle stage.

Passage experiments: RCI-values and larvae/gram after each transfer of the eight generations of T.s. var.pseudospiralis in Japanese quail are presented in Table VIII. RCI-values increased from generation two to nine from 1.8 to 7.6 ± 4.6, respectively. The average RCI-value for quail over eight generations was 4.2 ± 3.3 and the larvae/gram was 41.5 ± 30.8 respectively.

RCI-values recovered from ring-billed gulls and herring gulls were 11.1 ± 8.2 and 11.9 ± 11.7, respectively, whereas RCI-values recovered from Franklin's gulls were 27.2 ± 17.9 (Table IX). The number of muscle larvae/gram in Franklin's gulls (120.1 ± 84.9) was four times the amount of larvae/gram from quail muscle (41.5 ± 30.8). The number of larvae/gram from ring-billed gulls was 27.8 ± 17.7 and 13.7 ± 12.3 from herring gull (Table IX).

One chicken was examined on day 40 p.i. for presence of muscle larvae. The RCI-value was 1.5, and 2.4 larvae/gram of body weight were recovered.

Larvae obtained from gulls with particularly high RCI-values were transferred into Swiss Webster mice. The RCI-values doubled or tripled after the first passage of the parasite from gull to mice, except when

TABLE VIII. RCI-values and muscle larvae/g of body weight, for seven passages of T. spiralis var. pseudospiralis in adult male Japanese quail (day 40 postinfection).

Generation of parasite	2	3	4	5	6	7	8	9	$\bar{X}$ of eight generations
No. birds	2*	2*	3	2	3	2	3	3	20
Infect. dose larvae/bird	2000	2000	1000	1000	1000	1000	1000	1000	
RCI-value	1.8	2.6	2.7	3.9	4.1	8.1	1.4	7.6	4.2
$\bar{X} \pm$ S.D.			±0.4	±2.5	±1.8	±4.9	±0.9	±4.6	±3.3
larvae/g	29.6	41.6	25.4	37.8	40.9	78.4	11.9	69.1	41.5
$\bar{X} \pm$ S.D.			±2.4	±23.8	±21.0	±46.2	±6.7	±43.8	30.8

* quail were pooled and digested by group of two birds.

TABLE IX. RCI-values and T. spiralis var. pseudospiralis muscle larvae/g of body weight for three species of gulls (day 40 postinfection).

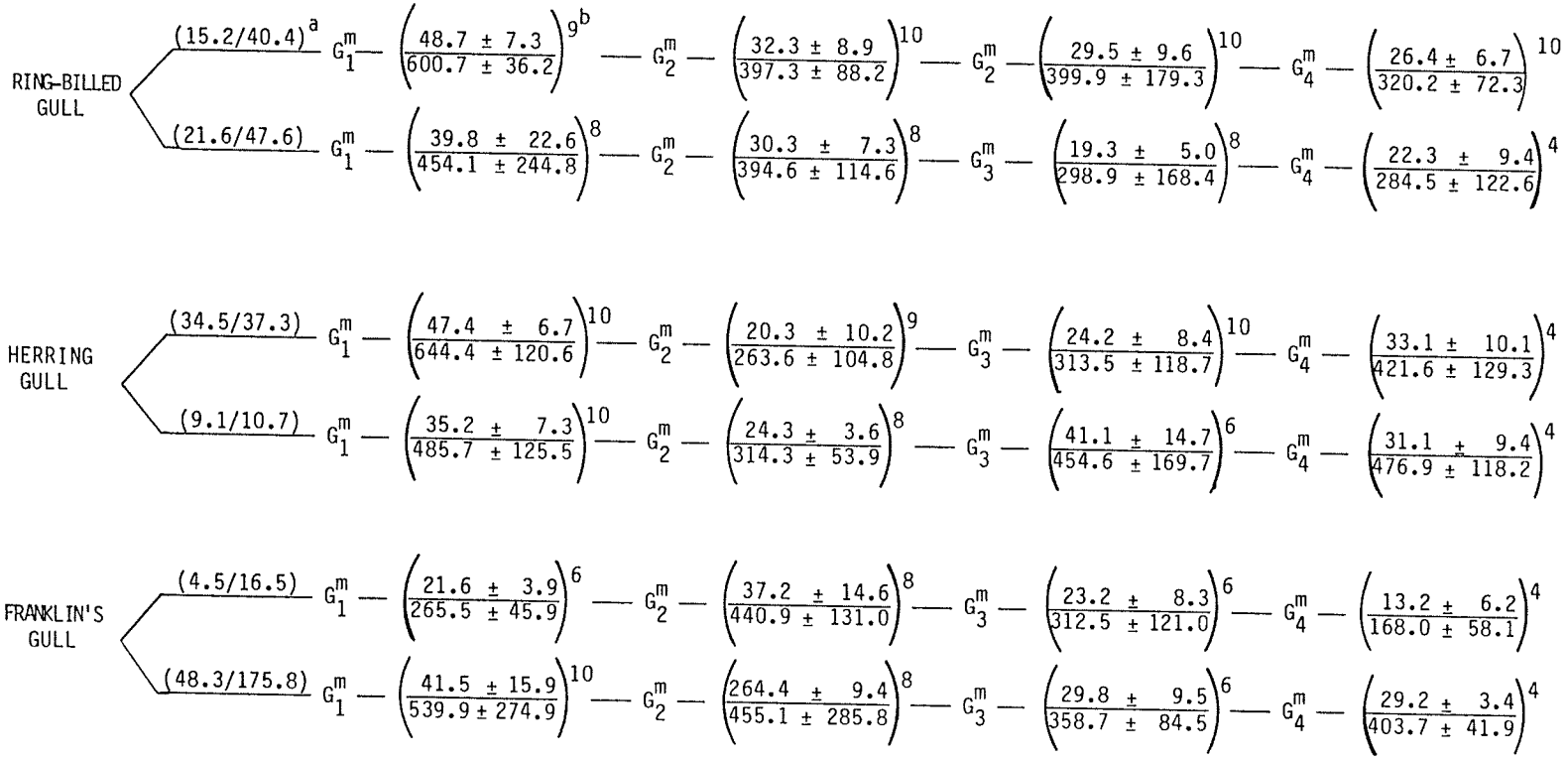
HOST	Ring-billed gull	Herring gull	Franklin's gull
No. birds	5	6	5
RCI-value	1.4	3.1	39.8
/bird	15.2	34.5	28.1
	12.9	14.3	16.1
	21.6	4.5	48.3
	4.5	9.1	3.5
		6.3	
$\bar{X}$ RCI-value	11.1	11.9	27.2
± S.D.	± 8.2	±11.7	±17.9
larvae/g	40.4	3.4	227.6
	30.3	37.3	123.3
	47.6	15.9	50.4
	16.5	5.9	175.8
	4.1	10.7	23.5
		8.6	
$\bar{X}$ larvae/g	27.8	13.7	120.1
± S.D.	±17.7	±12.3	±84.9

RCI-values from the gull were already high (Figure 4). RCI-value in one ring-billed gull was 15.2, but after the first transfer into mice RCI-values reached 48.7 $\pm$ 7.3. After four transfers in mice, the RCI-value was 26.4 $\pm$ 6.7, a value close to the value obtained during generation transfers in mice. High RCI-values (248.9 $\pm$ 15.8) were obtained from two golden hamsters infected with larvae recovered from a herring gull.

Figure 4: RCI-values and larvae/g after transfer of the parasite from gulls to mice for four generations (infection dose, 400 larvae/mouse). Note: a= RCI-value from gull;b= sample size; m= mouse; G= generation of parasite in mouse; (RCI-value $\pm$ S.D./larvae per gram $\pm$ S.D.).

HOST

GENERATIONS (G), RCI-VALUES AND LARVAE/GRAM



Distribution of muscle larvae in four species of birds: Distribution of muscle larvae in quail, ring-billed gull, herring gull and Franklin's gull are illustrated in Table X. Legs were the most heavily parasitized part of the body (25.0 - 38.8%) in all four species of birds studied. Breast muscles were also infected in higher proportion.

Influence of infection dose on RCI-values and number of muscle larvae recovered in adult Japanese quail on day 40 p.i.: RCI-values were not significantly different ($P>0.05$) for infection doses of 1000, 5000 and 10,000 larvae: the RCI-values were respectively 4.1 ± 4.1, 1.8 ± 2.1 and 2.6 ± 3.3. RCI-values obtained after seven transfers in quail were 4.2 ± 3.3., and not significantly different from a direct infection with parasites recovered from mice (4.1 ± 4.1).

Influence of the age of the host:One week old quail were less susceptible to the parasite than older quail. RCI-values were low (0.8 ± 0.8) at days 40 and 80 p.i. (1.3 ± 1.6) (Table XI).

Influence of the age of the infection: Muscle larvae were still present in adult quail on day 250 p.i., but the RCI-value was very low (0.4). RCI-values in adult quail appeared to decrease as a function of time (Table XI). In contrast RCI-values in young quail increased from day 40 to day 80 p.i. (Table XI).

Influence of the sex of the quail on RCI-values: Female young quail were more susceptible than young males to T.s. var.pseudospiralis, as indicated by higher RCI- values (1.5 ± 2.2 for females compared to 1.0 ± 0.6 for males).

TABLE X. Distribution of T. spiralis var. pseudospiralis larvae in the muscles of birds.

Muscle region	JAPANESE QUAIL		RING-BILLED GULLS		HERRING GULLS		FRANKLIN'S GULLS	
	% total larvae/ region	No. larvae /g	% total larvae/ region	No. larvae /g	% total larvae/ region	No. larvae /g	% total larvae/ region	No. larvae /g
Neck	9.45	63.7	5.08	24.1	17.09	20.7	6.02	28.3
Left breast muscle	11.98	28.5	21.74	42.8	6.71	8.3	11.56	28.0
Right breast muscle	15.81	33.7	16.23	49.0	8.94	10.8	11.23	28.2
Wings	9.39	38.0	8.39	12.3	9.16	5.1	11.08	15.0
Legs	31.87	43.0	30.46	55.2	25.03	14.9	38.79	73.8
Head	0.99	4.4	3.97	6.7	8.94	7.2	9.24	20.3
Remainder of carcasse*	20.51	21.8	14.13	16.8	24.13	8.3	12.08	15.5

* skeleton plus attached muscle, wall of abdomen and thoracic cavity.

TABLE XI. Influence of dose and age of infection on RCI-values in adult (2-3 months old) and young (1 week old) Japanese quail.
Infection from Crl: CQBS CFW (SW) mice.
Note: n = number of birds.

	Days postinfection	n	RCI-values ± S.D.	No. larvae/g ± S.D.
Adult quail	40	5 ♂	4.1 ± 4.1	44.3 ± 48.2
	60	3 ♂	1.9 ± 1.9	17.4 ± 17.0
	160	2 ♂	0.8 ± 0.5	28.5 ± 25.1
	250	1 ♂	0.4	4.3
Young quail	40	10 ♂+ ♀	0.8 ± 0.8	7.4 ± 7.4
	80	11 ♂+ ♀	1.3 ± 1.6	8.1 ± 13.1

In vitro larval release.

Female worms isolated from the quail intestine between days 5 and 40 p.i. produced larvae *in vitro* (Table V). No larvae were produced by females recovered from quail on day 60 p.i. . Larval production by females recovered from quail and assayed *in vitro* started on day 5 and peaked on day 7 p.i. (16.6 $\pm$ 10.6), as presented in Table V. Larval production by females recovered from gulls and assayed *in vitro* peaked on day 5 p.i. for females recovered from ring-billed gulls (19.4 $\pm$ 7.5), day 11 p.i. for females recovered from herring gulls (24.5 $\pm$ 10.2), and day 12 for females recovered from Franklin's gulls (21.3 $\pm$ 9.4, see Table V). High numbers of larvae were produced during 4 days by female worms recovered from quail, whereas high larval production lasted at least 2 weeks for females recovered from gulls. However, due to mortality of ring-billed and Franklin's gulls , *in vitro* release of newborn larvae could not be determined every day.

III. Host Influence on the Morphology of Adult and Larval T. spiralis var.pseudospiralis

Measurements of male and female adult T.s. var.pseudospiralis, recovered day 8 p.i. from mice, quail, ring-billed gulls, herring gulls and Franklin's gulls are presented in Tables XII and XIII. Male adult worms recovered from Crl: COBS CFW (SW) mice measured 1.11 ± 0.1 mm in length and 0.027 ± 0.003 mm in width. The oesophagus measured from the tip of the head to the stichosome (including the buccal capsule), was 0.21 ± 0.025 mm long. Female adult worms recovered from Swiss Webster mice measured 2.01 ± 0.22 mm in length and 0.032 ± 0.004 mm in width. The oesophagus was 0.24 ± 0.023 mm long and the vulva was situated 0.48 ± 0.041 mm from the anterior end. Those measurements were compared to measurements of Garkavi, 1972; Geller et al., 1977; Shaikenov, 1980.

Measurements of male and female larvae on day 40 p.i. are presented in Tables XIV and XV. Male larvae recovered from the muscle of Swiss Webster mice on day 40 p.i. measured 0.81 ± 0.034 mm in length and 0.029 ± 0.0003 mm in width. Length of the genital anlage was 0.21 ± 0.021 mm and length of the rectum was 0.042 ± 0.007. Female larvae recovered from Swiss Webster mice on day 40 p.i. measured 0.85 ± 0.047 mm in length and 0.027 ± 0.008 mm in width. Length of the genital anlage was 0.24 ± 0.026 mm and length of the rectum was 0.023 ± 0.005. Ratios between organs were calculated for adults and larvae. Ratios for adult worms: body length/body width, body length/length of the oesopha-

TABLE XII. Morphological measurements (in mm) and ratios of T. spiralis var. pseudospiralis adult male worms in several hosts, compared to measurements of Garkavi, 1972; Geller et al, 1977; Shaikenov, 1980. * = range; n.sp. = not specified.

Authority	Host	day	Body length ± S.D.	Body width ± S.D.	Length oes. ± S.D.	Body length/ width ± S.D.	Body length/ length oes. ± S.D.
THIS STUDY	Quail	8	0.96 ± 0.12 (0.72 - 1.16)*	0.026 ± 0.002 (0.002 - 0.003)	0.23 ± 0.039 (0.16 ± 0.29)	36.9 ± 5.3 (26.8 - 52.5)	4.3 ± 0.4 (3.5 - 4.9)
	Ring-billed gull	8	0.58 ± 0.025 (0.55 - 0.59)	0.029 ± 0.002 (0.027 - 0.032)	0.12 ± 0.006 (0.11 - 0.12)	19.9 ± 2.6 (17.2 - 22.4)	4.9 ± 0.1 (4.9 ± 5.0)
	Herring gull	8	0.97 ± 0.086 (0.89 - 1.06)	0.029 ± 0.002 (0.027 - 0.032)	0.19 ± 0.009 (0.19 - 0.21)	33.5 ± 5.9 (27.7 - 39.6)	4.9 ± 0.2 (4.6 - 5.0)
	Franklin's gull	8	0.89 ± 0.473 (0.55 - 1.2)	0.027	0.25 ± 0.059 0.204 - 0.287	33.1 ± 17.7 (20.6 - 45.6)	3.5 ± 1.1 (2.7 - 4.2)
Bober, 1982	Mouse	8	1.11 ± 0.1 (0.89 - 1.27)	0.027 ± 0.003 (0.024 - 0.035)	0.21 ± 0.025 (0.19 - 0.27)	40.8 ± 4.4 (33.3 - 52.4)	5.4 ± 0.5 (4.1 - 6.6)
Garkavi, 1972	Mouse	n.sp.	(0.62 - 0.9)	(0.027 - 0.035)	—	—	—
Geller, et al. 1977	Mouse	n.sp.	1.1	0.04	0.07	27.5	7.1
Shaikenov, 1980	Mouse	7	0.92	—	0.15	—	—
			(0.87 - 1.03)	(0.025 - 0.032)	(0.14 - 0.17)	—	—

TABLE XIII. Morphological measurements (in mm) and ratios of T. spiralis var. pseudospiralis adult female worms in several hosts, compared to measurements of Garkavi, 1972; Geller et al., 1977; Shaikenov, 1980. * = range; n.sp. = not specified.

Authority	Host	day	Body length ± S.D.	Body width ± S.D.	Length oes. ± S.D.	Dist. vulva ant. end ± S.D.	Body length/ width ± S.D.	Body length/ length oes. ± S.D.	Body length/dist. vulva ant. end ± S.D.
THIS STUDY	Quail	8	1.63 ± 0.20 (0.99 - 2.15)*	0.029 ± 0.004 (0.024 - 0.037)	0.24 ± 0.034 (0.17 ± 0.30)	0.42 ± 0.051 0.33 ± 0.57	54.9 ± 7.5 (41.1 - 72.2)	7.0 ± 0.9 (5.4 - 9.6)	3.9 ± 0.6 (2.8 - 5.7)
	Ring-billed gull	8	1.31 ± 0.033 (1.27 - 1.33	0.037 ± 0.005 (0.032 - 0.043)	0.17 ± 0.016 (0.16 - 0.19)	0.32 ± 0.016 (0.31 - 0.34)	35.2 ± 4.5 (30.6 - 39.5)	7.4 ± 0.5 (6.9 - 7.9)	4.1 ± 0.3 (3.9 - 4.4)
	Herring gull	8	1.56 ± 0.26 (0.81 - 1.92)	0.031 ± 0.004 (0.027 - 0.043)	0.20 ± 0.039 (0.12 - 0.29)	0.35 ± 0.065 (0.21 - 0.44)	49.6 ± 7.3 (30.4 - 67.2)	7.9 ± 1.6 (4.3 - 12.9)	4.6 ± 1.1 (2.4 - 8.9)
	Franklin's gull	8	1.96 ± 0.35 (1.09 - 2.51)	0.034 ± 0.005 (0.027 - 0.045)	0.24 ± 0.049 (0.17 - 0.37)	0.47 ± 0.085 (0.34 - 0.83)	58.2 ± 12.5 (3.38 - 87.0)	8.4 ± 1.6 (3.9 - 11.8)	4.3 ± 0.6 (2.7 - 5.3)
Bober, 1982	Mouse	8	2.01 ± 0.22 (1.32 - 2.59)	0.032 ± 0.004 (0.024 - 0.045)	0.24 ± 0.023 (0.19 - 0.31)	0.48 ± 0.041 (0.36 - 0.56)	64.2 ± 8.6 (47.7 - 83.8)	8.4 ± 1.2 (6.4 - 12.6)	4.2 ± 0.6 (2.9 - 6.3)
Garkavi, 1972	Mouse	n.sp.	(1.26 - 2.10)	(0.029 - 0.035)	–	(0.35 - 0.40)	–	–	–
Geller, et al. 1977	Mouse	n.sp.	2.05	0.05	0.08	0.56	41	6.9	3.4
Shaikenov, 1980	Mouse	7	1.51	–	0.17	–	–	–	–
			(1.33 - 2.04)	(0.035 - 0.038)	(0.14 - 0.18)	–	–	–	–

gus, and body length/distance of the vulva to the anterior end are given in Tables XII and XIII. Ratios for male and female larvae: body length/body width, and body length/length of genital anlage are given in Tables XIV and XV.

Paired Student's t-tests (Behrens-Fisher's solution) for five variables measured in male adult worms and seven variables for female adult worms are presented in Table XVI. Measurements obtained for larvae and for the ratios are presented in Table XVII. Worm size varied between hosts, and this was particularly noticeable with female adult worms (Table XVI). Adult males and females and larvae recovered from Franklin's gulls were most similar to those of mice (Tables XVI and XVII). Morphology of adult males was in general less affected by the host, but measurements were most dissimilar between mouse and quail hosts. No definite pattern as to host influence on the morphology of muscle larvae was noted, as measurements involved varied between hosts.

TABLE XIV. Morphological measurements (in mm) and ratios of T. spiralis var. pseudospiralis male larvae in several hosts, compared to measurements of Garkavi, 1972; Geller et al., 1977; Shaikenov, 1980. * = range; n.sp. = not specified.

Authority	Host	day	Body length ± S.D.	Body width ± S.D.	Length genital anlage ± S.D.	Length rectum ± S.D.	Body length/ width ± S.D.	Body length/ lenth genital anlage ± S.D.
T H I S	Quail	40	0.80 ± 0.084 (0.69 - 1.21)*	0.029 ± 0.003 (0.021 - 0.032)	0.22 ± 0.026 (0.16 - 0.27)	0.038 ± 0.004 (0.031 - 0.045)	28.7 ± 4.2 (22.3 - 40.5)	3.6 ± 0.5 (3.1 - 5.2)
	Quail	80	0.75 ± 0.056 (0.64 ± 0.89)	0.028 ± 0.003 (0.021 - 0.037)	0.20 ± 0.026 0.16 - 0.28	0.041 - 0.005 0.031 - 0.052	26.9 ± 3.2 (20.0 - 40.8)	3.8 ± 0.4 (2.9 - 4.9)
	Quail	160	0.78 ± 0.092 (0.64 - 1.19)	0.029 ± 0.004 (0.026 - 0.042)	0.21 ± 0.029 (0.15 - 0.30)	0.04 ± 0.004 (0.033 - 0.049)	27.2 ± 3.0 (21.1 - 37.2)	3.8 ± 0.5 (2.7 - 5.2)
	Quail	250	0.71 ± 0.048 (0.59 - 0.84)	0.029 ± 0.002 (0.026 - 0.032)	0.18 ± 0.022 (0.11 - 0.22)	0.039 ± 0.004 (0.031 - 0.054)	24.6 ± 2.7 (20.0 - 30.6)	3.9 ± 0.5 (3.2 - 6.0)
S	Chick	40	0.77 ± 0.088 (0.57 - 1.02)	0.027 ± 0.003 (0.016 - 0.032)	0.21 ± 0.037 (0.16 - 0.29)	0.037 ± 0.006 (0.018 - 0.054)	29.1 ± 5.5 (21.2 - 50.7)	3.6 ± 0.5 (2.4 - 4.6)
T U	Franklin's gull	40	0.81 ± 0.054 (0.73 - 0.98)	0.028 ± 0.002 (0.026 - 0.032)	0.21 ± 0.023 (0.17 - 0.26)	0.041 ± 0.005 (0.031 - 0.054)	28.6 ± 3.3 (22.8 - 36.6)	3.9 ± 0.4 (3.1 - 4.8)
D	Herring gull	40	0.71 ± 0.062 (0.059 - 0.88)	0.027 ± 0.02 (0.021 - 0.037)	0.18 ± 0.021 (0.14 - 0.23)	0.037 ± 0.004 (0.031 - 0.044)	25.8 ± 3.4 (18.4 - 37.8)	3.9 ± 0.3 (3.3 - 4.6)
Y	Ring-billed gull$_1$	40	0.71 ± 0.055 (0.059 - 0.88)	0.028 ± 0.003 (0.026 - 0.042)	0.19 ± 0.039 (0.10 - 0.28)	0.038 ± 0.004 (0.024 - 0.047)	25.2 ± 3.2 (15.9 - 31.4)	3.7 ± 0.5 (2.5 - 5.1)
	Ring-billed gull$_2$	40	0.81 ± 0.046 (0.71 - 0.89)	0.036 ± 0.052 (0.021 - 0.039)	0.18 ± 0.031 (0.037 - 0.23)	0.047 ± 0.027 (0.021 - 0.23)	28.5 ± 4.8 (20.9 - 37.0)	4.7 ± 2.4 (3.5 - 2.1)
	Ring-billed gull$_2$ -> mouse	40	0.77 ± 0.046 (0.66 - 0.26)	0.026 ± 0.003 (0.02 - 0.04)	0.21 ± 0.022 (0.015 - 0.26)	0.043 ± 0.004 0.035 - 0.054	28.9 ± 3.5 (21.0 - 37.0)	3.7 ± 0.4 (2.9 - 4.9)
Bober, 1982	Mouse	40	0.81 ± 0.034 (0.73 - 0.88)	0.029 ± 0.003 (0.021 - 0.037)	0.21 ± 0.021 (0.16 - 0.25)	0.042 ± 0.007 (0.029 - 0.06)	28.6 ± 3.2 (21.3 - 38.3)	3.9 ± 0.4 (3.2 - 4.9)
Bober, 1982	Mouse	160	0.75 ± 0.054 (0.64 - 0.87)	0.028 ± 0.002 (0.021 - 0.032)	0.18 ± 0.02 (0.14 - 0.23)	0.04 ± 0.004 (0.031 - 0.05)	26.8 ± 3.3 20.5 - 40.0	4.1 ± 0.5 (3.2 - 5.5)
Garkavi, 1972	Mouse	n.sp.	(0.65 - 0.85)	(0.03 - 0.04)	–	–	–	–
Geller, et al., 1977	Mouse	80	0.73	0.034	0.022	0.048	21.3	3.3
Shaikenov, 1980	Mouse	35	0.65 (0.64 - 0.71)	– (0.029 - 0.032)	0.20 (0.19 - 0.22)	0.029 (0.022 - 0.032)	–	–

TABLE XV. Morphological measurements (in mm) and ratios of T. spiralis var. pseudospiralis female larvae in several hosts, compared to measurements of Garkavi, 1972; Geller et al., 1977; Shaikenov, 1980. * = range; n.sp. = not specified.

Authority	Host	day	Body length ± S.D.	Body width ± S.D.	Length genital anlage ± S.D.	Length rectum ± S.D.	Body length/ width ± S.D.	Body length/ lenth genital anlage ± S.D.
	Quail	40	0.87 ± 0.089 (0.77 - 1.32)*	0.028 ± 0.003 (0.021 - 0.032)	0.24 ± 0.032 (0.17 - 0.30)	0.023 ± 0.005 0.012 - 0.043	31.5 ± 4.2 (24.7 - 49.2)	3.6 ± 0.5 (2.9 - 5.4)
T H	Quail	80	0.75 ± 0.061 (0.58 - 1.04)	0.026 ± 0.002 (0.021 - 0.032)	0.22 ± 0.023 (0.15 - 0.28)	0.023 ± 0.006 (0.010 - 0.039)	28.7 ± 3.5 (21.8 - 39.0)	3.4 ± 0.4 (2.7 - 4.8)
I S	Quail	160	0.84 ± 0.058 (0.76 - 1.00)	0.027 ± 0.003 (0.021 - 0.037)	0.23 ± 0.031 (0.14 - 0.29)	0.021 ± 0.004 0.012 - 0.029	30.4 ± 3.1 (23.3 - 38.0)	3.6 ± 0.5 (2.8 - 5.7)
	Quail	250	0.75 ± 0.054 (0.65 - 0.88)	0.028 ± 0.03 (0.027 - 0.037)	0.21 ± 0.024 (0.15 - 0.26)	0.22 ± 0.004 (0.010 - 0.039)	26.4 ± 2.9 (19.7 - 32.6)	3.5 ± 0.3 (2.9 - 4.6)
	Chick	40	0.84 ± 0.065 (0.72 - 1.99)	0.026 ± 0.002 (0.021 - 0.032)	0.24 ± 0.031 (0.17 - 0.31)	0.024 ± 0.005 0.012 - 0.037	32.6 ± 3.9 (26.2 - 46.0)	3.6 ± 0.4 (2.9 - 4.9)
S T	Franklin's gull	40	0.86 ± 0.054 (0.77 - 0.98)	0.027 ± 0.002 (0.021 - 0.032)	0.24 ± 0.027 (0.17 - 0.29)	0.023 ± 0.003 0.010 - 0.033	31.4 ± 2.9 (26.2 - 41.0)	3.6± 0.4 (3.0 - 5.0)
U D	Herring gull	40	0.82 ± 0.034 (0.76 - 0.93)	0.027 ± 0.002 (0.021 - 0.032)	0.24 ± 0.029 (0.17 - 0.32)	0.020 ± 0.003 (0.014 - 0.025)	30.5 ± 3.1 (25.0 - 39.5)	3.4 ± 0.4 2.6 - 4.5
Y	Ring-billed gull$_1$	40	0.75 ± 0.049 (0.62 - 0.87)	0.029 ± 0.004 (0.021 - 0.042)	0.22 ± 0.022 (0.18 - 0.28)	0.021 ± 0.004 (0.012 - 0.027)	26.3 ± 3.3 (17.8 - 33.3)	3.4 ± 0.4 2.6 - 4.1
	Ring-billed gull$_2$	40	0.85 ± 0.052 (0.73 - 0.94)	0.027 ± 0.003 (0.021 - 0.037)	0.22 ± 0.023 (0.17 - 0.27)	0.021 ± 0.003 (0.012 - 0.029)	31.0 ± 3.3 (23.7 - 40.8)	3.8 ± 0.4 (3.2 - 5.0)
	Ring-billed gull$_2$ -> mouse	40	0.81 ± 0.46 (0.66 - 0.90)	0.025 ± 0.002 (0.021 - 0.032)	0.24 ± 0.021 (0.19 - 0.28)	0.022 ± 0.002 (0.016 - 0.027)	31.9 ± 3.9 (24.5 - 41.0)	3.4 ± 0.3 (2.9 - 4.5)
Bober, 1982	Mouse	40	0.85 ± 0.047 (0.75 - 0.95)	0.027 ± 0.008 (0.021 - 0.085)	0.24 ± 0.026 (0.18 - 0.28)	0.023 ± 0.005 (0.014 - 0.035)	31.7 ± 4.8 (11.0 - 41.3)	3.6 ± 0.4 (3.1 - 4.9)
Bober, 1982	Mouse	160	0.82 ± 0.049 (0.64 ± 0.95)	0.027 ± 0.002 (0.021 - 0.032)	0.22 ± 0.034 (0.14 - 0.28)	0.023 ± 0.004 (0.017 - 0.039)	30.7 ± 3.5 (25.0 - 43.3)	3.8 ± 0.6 (3.1 - 6.0)
Garkavi, 1972	Mouse	n.sp.	(0.65 - 0.85)	(0.03 - 0.04)	—	—	—	—
Geller, et al., 1977	Mouse	80	0.83	0.035	0.18	0.028	23.6	4.5
Shaikenov, 1980	Mouse	35	0.69 (0.62 - 0.77)	— 0.027 - 0.032	0.21 (0.19 - 0.23)	0.017 (0.016 - 0.021)	—	—

TABLE XVI. Paired Students' t-tests to compare the morphology of T. spiralis var. pseudospiralis adult worm between different mice and bird hosts (day 8 postinfection). Letters indicate that the variable is not significantly different between those two hosts. ($P > 0.05$). L = length; W = width; OESL = length of the oesophagus; DVA = distance of the vulva to the auterior end; L/W = body length/body width; L/OESL = body length/length of the oesophagus; L/DVA = body length/ distance of the vulva to the anterior end.

HOSTS	Mouse	Quail	Franklin's gull	Herring gull
Mouse		OESL	L OESL DVA L/OESL L/DVA	W L/DVA
Quail			OESL L/W	L
Franklin's gull	L W OESL L/OESL L/W	L W OESL L/OESL L/W		L/OESL L/DVA
Herring gull	W OESL L/OESL	L OESL L/W	L W OESL L/OESL L/W	

♂ ♀

▬ at least 5 variables were not significantly different.

TABLE XVII. Paired Students' t-tests to compare the morphology of T. spiralis var. pseudospiralis larvae between different mice and bird hosts. Letters indicate that the variable is not significantly different between those two hosts. ($P > 0.05$). L = length; W = width; LGA = length of the genital anlage; LR = length of the rectum; L/W = length/width; L/LGA = body length/length of the genital anlage. Hosts - M_{40} = mouse day 40; M_{160} = mouse day 160; Q_{40} = Quail day 40 . . .; F.G. = Franklin's gull; H.G. - Herring gull; $R.B._1$ = generation1 of the parasite in Ring-billed gulls.

— 6 variables not significantly ≠ P (> 0.05)

Hosts	M_{40}	M_{160}	Q_{40}	Q_{80}	Q_{160}	Q_{250}	$Chick_{40}$	F.G.	H.G.	$R.B._1$	$R.B._2$	$R.B._2$-> mouse ♀
M_{40}		LR L/W W	L L/W W L/LGA LGA LR	LR W	L L/W W L/LGA LGA	LR L/LGA W	L L/W W L/LGA LGA LR	L L/W W L/LGA LGA LR	L/W W L/LGA	W	L L/W W	LR L/W W LGA
M_{160}	LR W		LR L/W L/LGA	LR W LGA	L L/W W L/LGA	LGA LR	L LR	LR L/W W L/LGA	L L/W W	LGA	LGA L/W W L/LGA LR	L L/W LR
Q_{40}	L L/W W	W LR		LR	LGA L/W W L/LGA	LR L/LGA W	LGA L/W LR L/LGA	L L/W W L/LGA LGA LR	LGA L/W W	W	L L/W W L/LGA LR	LGA L/W LR
Q_{80}	W L/LGA LGA LR	L L/W W	W		LR	L LGA LR	LR L/LGA W	LR	L L/W W L/LGA	L L/LGA LGA	LGA LR	LR L/LGA W
Q_{160}	L W LGA LR	W L/W LR	L L/W W L/LGA LR	W L/W LGA L/LGA LR		LR L/LGA W	L L/LGA	L L/W W L/LGA LGA	L L/W W LGA	LGA W	L L/W W L/LGA LGA LR	LGA LR
Q_{250}	W L/LGA LR	W LGA LR	W LR	LR	W L/LGA LR		LR L/LGA	LR L/LGA	LR L/LGA	L L/W W LR	LR	LR
$Chick_{40}$	LGA L/W	L	L L/W LGA L/LGA LR	L W	L L/W LGA L/LGA	LR		L L/W LGA L/LGA LR	L L/LGA LGA		L	LGA L/W W LR
F.G.	L L/W W L/LGA LGA LR	W LR	L L/W W	W L/LGA LGA LR	L L/LGA W LGA LR	W L/LGA	LGA L/W		LGA L/W W	W	L L/W W	LGA L/W LR
H.G.	W L/LGA	W L/W LGA	W	W L/W L/LGA	W L/LGA	L L/LGA LGA	W	W L/LGA		LR L/LGA	W L/W LR	L L/W LGA L/LGA
$R.B._1$	W L/LGA	W LGA LR	W L/LGA LR	W L/LGA LGA	W L/LGA LR	L L/W W L/LGA LGA LR	L/LGA	W L/LGA	L L/W W L/LGA LGA LR		LGA W LR	L/LGA
$R.B._2$	L L/W W LR	W L/W LGA LR	L L/W W	LR L/W W	L L/W W LR	LGA W LR	W L/W	L L/W W LR	LGA W	LGA W LR		LR L/W
$R.B._2$ -> mouse	LGA L/W LR		L/W L/LGA	L L/LGA W LR	L L/LGA LGA		L L/W W L/LGA LGA	LGA L/W LR	W	L/LGA	W L/W LR	

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DISCUSSION

The biological characteristics are well known for their variation in T.spiralis, and these differences are related to the genetic strain of parasite and the influence of the host (Kozar and Kozar, 1965; Concannon and Ritterson, 1965; Pawlowski and Rauhut, 1971; Rauhut, 1978; Belosevic and Dick, 1980; Chadee and Dick, 1982). Although T.s. var.pseudospiralis is being increasingly used as a parasite model system, little information exists on its phenotypic variability. Studies of T. spiralis var.pseudospiralis in mice, also carried out by Garkavi (1974), Pereverseva et al. (1974), Penkova (1975, 1976), Bessonov et al. (1976), Geller and Malykhina (1976), Przyjalkowski (1978), Kramar et al. (1981), were used to establish baseline data in the present study, as mice are the animals against which all others are compared.

The most commonly used biological characteristics of T.s. var.pseudospiralis are: percentage recovery, longevity of the worms during the intestinal phase, and number of larvae/gram recovered during the muscle phase. In this study additional biological characteristics were studied, i.e. distribution of the worms during the intestinal

phase, *in vitro* larval release by adult female worms, reproductive capacity indices (RCI-values) and virulence of the parasite.

I. Mice

Pereverseva *et al.* (1974), Penkova (1975), and Kramar *et al.* (1981) found an anterior distribution of adult *T.s.* var.*pseudospiralis* in C_{57} Br mice and in CD-1 Swiss white mice. Four factors are known to affect *Trichinella* distribution in the mouse intestine, namely, age of infection, host sex (Kramar *et al.* 1981) and strain of host (Chadee and Dick, 1982). In general, *T.spiralis* and *T.s.* var.*pseudospiralis* are found in the anterior half of the intestine (Pereverseva *et al.*, 1974; Penkova, 1975). *T.s.* var.*pseudospiralis* shifted posteriorly 7 days p.i. as noted also by Kramar *et al.* (1981) for *T.s.* var.*pseudospiralis* and by Larsh and Hendricks (1949), Podhajecky (1962) and Campbell (1967) for *T.spiralis*. This 'self cure' has been hypothesized to be related to expulsion mechanisms and immunocompetence of the host.

Results from surgical inoculation by Dick and Silver (1980) confirmed that *T.s.* var.*pseudospiralis* behaved similarly to *T.spiralis* in the mouse intestine, and worms remained near their injection point, as shown in rats. Worm position was related to inoculation point, rather than region of the intestine as worms did not migrate back to their normal location.

The sex ratio of T.s. var.pseudospiralis in mice was similar to that of T.spiralis in mice (Pereverseva et al., 1974; Penkova, 1975). Inversion of sex ratios during the course of the infection indicated that females were lost more rapidly than males in later stages of the intestinal phase. These results corroborate observations of Rappaport (1943) and Martinez Fernandez et al. (1981) on T.spiralis in mice and rats intestine, but differed from the findings of Belosevic and Dick (1979), who noted that the expulsion of male and female worms occurred at the same rate.

Percentage recovery in different strains of mice varies. Britov (1974) recovered 7.6% of the worms in the intestine (value calculated from his data) in white mice (strain undisclosed) on day 6 p.i., while Pereverseva et al., (1974) recovered 46.4% in albino mice on day 7 p.i. . Recovery herein was 20.5% in Crl: COBS CFW (SW) mice on day 7 p.i., but one cannot generalize on the significance of this observation without considerable more comparative data on the interaction of the mouse and other host strains on recovery rate.

The time necessary for females to reach sexual maturity; i.e larval release is quite variable and depends on strain of parasite, and/or host. T.spiralis females are known to produce larvae as early as day 5 p.i. in C_{57} Br mice (Penkova, 1975), while T.s. var.pseudospiralis in the same host reaches larval production by day 7 (Penkova, 1975). In this study females removed from Swiss Webster mice at day 5 p.i. were first to produce larvae in vitro. Despommier (cited by Kramar et al., 1981) observed a reduction in fecundity of adult T.s. var.pseudospiralis

compared to T.spiralis in CFW white mice. Kramar et al. (1981) observed the same reduction in fecundity in CD-1 mice, but Garkavi (1974) in white mice, and Przyjalkowski (1978) in C3H white mice found that a greater number of larvae were released by T.s. var.pseudospiralis. Kramar et al. (1981) noted that worms isolated from female mice deposited significantly fewer migratory larvae in vitro than did those isolated from male mice. Also the age of the host influenced the number of larvae released, as adult female worms isolated from 10-12 week old mice deposited fewer larvae than adults isolated from young mice (6-10 weeks old). Female worms deposited greater numbers of larvae at 42 °C than above or below this temperature (Kramar et al., 1981). In this study, data extrapolated from in vitro larval release experiments, showed that each female produced a total of 110 larvae. This is a much lower value than the 2532 larvae released/female from studies of a simple male X female infection in C_{57} Br mice reported by Penkova (1975).

Longevity of the intestinal stage in mice was the most variable characteristic. Duration of the intestinal stage of T.s. var.pseudospiralis is generally shorter than T.spiralis in mice. Pereverseva et al.(1974) and Penkova (1975) reported that adult T.s. var.pseudospiralis remained between 15 and 21 days, respectively in mice intestine. In this study adult T.s. var.pseudospiralis persisted for 11 days only in one intestine

of Swiss Webster mice.

Muscle invasion by T.s. var.pseudospiralis in mammals is known to be prolonged but much less intensive than T.spiralis (Pawlowski and Ruitenberg, 1978 ;Kocieka et al., 1981). The RCI-value calculated in white mice infected with 2,000 larvae/mouse was 33.8 (Garkavi, 1974) and Tomasovicova (1975) found mice more sensitive to T.s. var.pseudospiralis (135 larvae/gram) than T. spiralis (20-26 larvae/gram). During seven passages of the parasite through Swiss Webster mice, RCI-values stabilized at about 28 or 390 larvae/gram of host. Parasites are found highly infectious to golden hamsters as 1,800 larvae/gram were recovered from hamsters infected with larvae obtained from gulls. Garkavi (1980) also noted the high infectivity of T.s. var.pseudospiralis for Syrian hamsters (213 larvae/gram).

This is the first evaluation known to us of virulence of T.s. var.pseudospiralis in mice. Garkavi infected rats with high doses of the parasite 1,000, 3,000 and 10,000 larvae but none died. Virulence of T.s. var.pseudospiralis was low since an infection dose greater than 10,000 larvae was necessary to reach an LD_{50} in Swiss Webster mice. Considerable variations in the proportion of larvae recovered to larvae fed were encountered in all the larval dose groups. A positive correlation exists between dosage and larvae recovered at low dosages (Rappaport, 1943). An increase in infection dose above an infection dose of 3,000 larvae per mouse (Figure 2) did not affect RCI-values. It appears that females release an optimum number of larvae and that conditions such as crowding have no effect on IVLR. Since the death of the mice at day 4 p.i. occurred before production of larvae by female worms, mortality does not appear to be related to inflammation of the

small intestine caused by the release of newborn larvae, as Larsh and Race (1975) reported. Movement of adult worms plus a strong non specific inflammatory response to the worms are probably important in death of the host. Enteritis was reported to occur on day 3 p.i. in mice infected with T. spiralis var.pseudospiralis (Kramar et al., 1981). Macroscopic examination of the host revealed no intestinal pathology in this study. Kozar and Kozar (1963) suggested that pathogenicity was not directly related to the reproductive capacity, and Belosevic and Dick (1979) showed that virulence was both host and dose dependant. Kozar and Kozar (1965) compared the Kenya strain to two Polish strains of Trichinella and concluded that the lower pathogenicity of T.spiralis was related to its lower infectivity. The distribution of the parasite number per host was thought to be an important factor in the percentage recovery (Keymer, 1982). Existence of gut carrying capacity, a density-dependent parasite population (Keymer, 1982), or intra-specific competition for finite resources such as space or nutrients and immunological response of the host (Anderson, 1978) could be mechanisms explaining the high dose of larvae necessary to kill the host.

II. Birds

It is well known that T.s. var.pseudospiralis infects birds, and an extensive range of birds have been experimentally infected. Britov (1974), Miroshnichenko (1976), Geller and Malykhina (1976), infected hens, ducks, magpies, sparrows, starlings, partridges, owls, kites, and herons. Experimentally infected hens were studied by Tomasovicova (1975). Tomasovicova and Govorka (1976), and Tomasovicova and Hovorka

(1982) listed the following wild hosts; pheasant, pigeon, crow, buzzard, wild ducks. Biological characteristics previously studied include longevity, percentage recovery in the intestinal phase and number of larvae/gram during the muscle phase. This study added the following variables: distribution of the intestinal stage, sex ratio, number of larvae released *in vitro* by female worms and RCI-values.

Tomasovicova and Hovorka (1982) observed 100% susceptibility in Columbiformes, 88% in Passeriformes and 60% in Galliformes, while water fowl were successfully infected only 20% of the cases. Calero *et al.* (1978) were unable to infect mice from a naturally infected buzzard. Levine (cited by Calero *et al.*, 1978) suggested that raptors are sensitive to trichinellosis, therefore a high percentage of mortality occurs. Wheeldon *et al.* (1982) reported severe muscle lesions from a naturally infected hawk, and suggested that the infection inhibited flight. In this study susceptibility of both Galliformes and Laridae was almost 100%, as only one quail out of 60 and one gull out of 62 were not infected. A 17% death rate in Franklin's gulls may be related to the virulence of the parasite, but many replications are needed to confirm this.

Duration of the intestinal phase of *T.s.* var.*pseudospiralis* was 32 days as Miroshnichenko (1976) noted in intestines of owl, starling, and other birds. In this study, sexually mature worms were found in the quail intestine on days 40 and 60 p.i., but only on day 40 postinfection were females still able to produce larvae *in vitro*. No worms were found in the quail intestine on day 160 p.i. . Penkova (1976) suggested that

the prolonged existence of the parasite within the host was due to the ability of the parasite to inhibit the immune response of the host. Tomasovicova (1975) found three migratory larvae on day 4 p.i. in the blood and the muscles of an infected chicken. Miroshnichenko (1976) observed T.s. var.pseudospiralis larvae in bird muscles on day 19 p.i. . In the present study female worms recovered from quail released larvae as early as day 5 p.i., while females recovered from gulls produced larvae by day 4 p.i. . One, 2 and 88 muscle larvae were recovered on days 8, 16 and 20 p.i. respectively (two quail examined each day).

Miroshnichenko (1976) observed that young birds (2-3 weeks old) were more heavily infected than adult birds. In some cases numbers of larvae increased up to 3-4 months and numerous larvae were found to atrophy after 5-6 months. Invasive larvae were still found in the muscle of infected birds after 18 months (Miroshnichenko, 1976). In the present study young birds were less susceptible than older birds. An increase from 40 to 80 days occurred in young quail while in two months old quail RCI-values decreased over time from 4.1 to 0.4 eight months after infection. Larvae recovered eight months after infection were still infective to mice.

Tomasovicova (1975) infected chickens and RCI-values calculated from her data vary between 0.12 and 7.31. The RCI-value for the chicken infected in the present study was 1.5. Increases in RCI-values noted after seven passages in Japanese quail may be due to the adaptation of the parasite to the bird host as more muscle larvae were found in the leg muscles of all four bird species, and is similar to observations of

Nemeseri (1968) on T. spiralis infections in chickens. T.pseudospiralis seems to have a different organotropism in birds than in mammals, where larvae are known to develop in striated muscles, more particularly the most active ones [pectoral, tongue, and diaphragm, Chandler et al, (1974)]. All T. s. var.pseudospiralis recovered from the muscles of birds were infective to Swiss Webster mice. Considerable individual variation was observed in RCI-values for all bird hosts, with greatest variation occurring in gulls. This high degree of variability noted for each host species is probably related to the outbred nature of the gulls since eggs were collected from natural populations. High infection doses (10,000 larvae/bird) did not kill the quail. It was of interest to note that RCI-values obtained after seven transfers in quail or after the first transfer from mice to quail were similar (4.2 and 4.1). Therefore the RCI-values appear to be host-dependent.

Some similarities were noted for all bird hosts studied: 1) The position was anterior and not significantly different for all bird hosts. 2) The same number of larvae were released in vitro by adult female worms recovered from quail and gulls. 3) The parasite had a similar pattern of larval production in all three species of gulls as females started releasing larvae on the same day, peak larval production was similar and RCI-values were identical in herring and ring-billed gulls.

When RCI-values of T.s. var.pseudospiralis recovered in bird hosts were compared much variability was noted. RCI-values from Galliformes were low, at 4.2 and 1.5 for quail and chicken, respectively, whereas

RCI-values recovered for Laridae were high at 11, 11 and 27 for herring, ring-billed and Franklin's gulls, respectively. Several factors could possibly be responsible for these differences in susceptibility to infection: 1) The structural differences of the gizzard of Galliformes to that of Laridae might affect the level of infection. Mechanical destruction of the larvae during their passage in the gizzard of Galliformes, as Augustine (1933) and Matoff (1938) suggested, might explain the low percentage recovery of the parasite. In order to check this hypothesis the droppings of T.s. var.pseudospiralis infected Japanese quail were checked on day one p.i. and a few damaged larvae were found. Meat-eating birds (Laridae) by contrast, have a thin soft muscular stomach wall but grit is absent (Biedermann, 1911). Therefore larvae are less likely to be destroyed. The destructive action of the gizzard in Galliformes could be checked by surgically inoculating larvae directly into the quail's intestine or by inoculating larvae by the anal route. 2) Differences in the intestinal environment ,i.e. variation in ph, enzymes, composition of the intestinal flora or other physiological factors might affect the establishment of worms. The composition of the intestinal flora might affect the duration of the parasite in the intestine, as Przyjalkowski (1978) showed that more T.s. var.pseudospiralis were found in conventional than in germ free mice. 3) The shape of the villi and length of the intestine might affect parasite establishment in the intestine. 4) The immunological response of the host could influence the length of the intestinal stage of the worms. It was suggested that increased peristalsis and metabolism of birds may influence establishment of the parasite in the

intestine of birds (Trommer, 1970). This is questionable considering the high level of infectivity obtained in Franklin's gulls.

Differences in other biological characteristics between bird hosts were also noted. Adult female worms started to release larvae one day earlier in gulls than in quail. Sex ratios were higher in gulls as fewer males were recovered, particularly in the case of ring-billed gulls in which no inversion of sex ratio occurred. Percentage recovery was lower in quail and chick. A posterior shift of the worms occurred earlier in quail (day 12 p.i.) than gulls (day 16 postinfection) and is probably related to intestinal pathology induced by the presence of the worms. Longevity seems to be related to degree of inflammation induced by the parasite in the intestine. Larsh and Race (1975) suggested that inflammation of the intestine and expulsion of the worms are related, with the elimination of worms occurring during the period just after the peak inflammation response. Worms were recovered from the quail intestine 60 days after infection. Penkova (1976) suggested that the prolonged existence of the parasite within the host was due to the ability of Trichinella to inhibit the protective mechanism of the host. The above assumptions and the fact that worms were more rapidly expelled from the intestine of mice suggest that mice have a stronger immunity towards this stage than birds. RCI-values were low in Galliformes compared to Laridae.

III. Comparison between Mice and Bird Hosts

T.s. var.pseudospiralis biological characteristics vary between hosts but this is expected of a parasite which is able to infect both birds and mammals. Four similarities in the biology of the parasite in birds and mice are noted. This is remarkable considering the variation in intestinal environments, food habits and immunocompetence of these hosts. Position was anterior and not significantly different on day 5 p.i. in mice and bird hosts. Inversion in sex ratio between the first day and later stages of the intestinal stage of the parasite was noted in quail and mice . The mean number of larvae released in vitro by adult female worms on day of peak production was identical for all hosts, suggesting that females released a relatively fixed number of larvae regardless of the host. RCI-values were similar for Crl: COBS CFW (SW) mice and Franklin's gulls. Five differences in biological characteristics of the parasite in different hosts were noted. Percentage recovery was very low in quail and high in Franklin's gulls. Worms started to shift posterioly on different days p.i., i.e. mice (day 8), quail (day 12) and gulls (day 16). Peak larval production by females in vitro varied between day 7 (quail), day 8 (mice) and day 12 (gulls). Total larvae produced/female was higher in birds than in mice but RCI-values were lower in all birds with the exception of Franklin's gulls. Duration of the intestinal phase was shorter in mice (11 days) than in quail (at least 60 days). Some conflicting information must also be noted: percentage recoveries in bird hosts were lower in quail than in gulls; first day of larval production by females from quail was

identical to these from mice but different from females recovered from gulls.

Considerable controversy surrounds the influence of the host on morphology of Trichinella both larvae and adults. Other morphometric studies showed a difference in size of worms according to the strain of parasite. Studies by Britov (1973) showed a difference in total length of adults between T.spiralis (north temperate form) and arctic isolate of Trichinella. Belosevic and Dick (1979) noted a difference of male and female infective larvae between Trichinella sp. and T. spiralis, but a convergence in size of isolates occurred after prolonged passages of the parasite in mice. In general measurements agreed with those of Garkavi (1972), Geller et al.(1977), and Shaikenov (1980) in mice (Tables XII to XV), but the ranges in length obtained from Swiss Webster mice were slightly greater in this study. Measurements of the oesophagus in this study were not comparable to those of Geller et al.(1977), as it was not clear whether or not the buccal capsule was included in their measurements. The similarities in measurements suggest that the host had little effect on the morphology of the nematode. When all variables were compared, body length and ratios were the characteristics that showed most consistent differences and were statistically different (male adult worms recovered from quail and mice). In general, female adults and larvae were longer than the males, but it was difficult to discern a pattern that related to host group, (i.e., mammals versus birds), as to the most important morphological characters affected by the host. Some tentative correlation could be made: female adult worms

recovered from mice and Franklin's gulls had five identical characters, more particularly total length, length of the oesophagus, distance of the vulva to the anterior end and the ratios of body length/length of the oesophagus and body length/distance of the vulva to the anterior end of the worm, which could be interpreted as an indication of similar sized uteri. Sukhdeo and Meerovitch (1980) suggested that the number of larvae of Trichinella released by females was related to the size of the uterus, and that uterine length and infectivity were directly correlated. Ratios, distance of the vulva to anterior end/body length were compared, and the smallest ratios were found for worms recovered from mice and Franklin's gull host, they were not significantly different and from this one might conclude that size of females was correlated with RCI-values. Male adult worms were most dissimilar between mice and quail, while the morphology of male worms recovered from other hosts was little affected.

Morphology of male and female larvae recovered from the various hosts was not affected in any definite pattern. Differences in measurements may be explained by a variation in age of the worms, since the infections were initiated via the oral route with muscle larvae. Larvae deposited in the stomach were released into the duodenum gradually, and establish themselves at different times. Since larvae are released by Trichinella females for 6 days only in mice and for at least 35 days in quail, a lack of synchrony in the development of the worms from the same population might also account for the variation in measurements encountered, and more particularly for worms recovered from birds. High variation in length measurements were most evident for larvae recovered from quail and chicken on day 40 p.i. .

The importance of molting larvae in our understanding of host influence on Trichinella was examined, particularly as a loose cuticle (two layers in one instance) was noted from several specimens recovered on day 40 p.i. from quail and chick. Berntzen (1965) found "evidences of two molts in trypsin digest of muscle", but Richels (1955), Wu (1955), and Ali Khan (1966), concluded that the sheaths were simply an artifact occuring during the pepsin digestion. Since the second cuticle was observed from fixed specimens, worms from the digest were examined prior to fixation but no second cuticle or sheath was seen. Therefore the presence of loose cuticle in some worms was attributed to the procedure rather than to a molt.

In conclusion, it is difficult to clearly state that host species influenced the morphology of T.s. var.pseudospiralis. There is some circumstancial evidence to suggest that size of females may be correlated with RCI-values and that size of the uterus may be indicative of total larval production/female.

IV. Theoretical Considerations on the Life Cycle of Trichinella

Ten years after the original description of Garkavi (1972) the status of T.s. var.pseudospiralis as a variety, subspecies or species is still being debated. The parent species, T.spiralis, clearly has evolved into an ubiquitous, highly adaptable and phenotypically variable species of wild carnivores, domestic and wild pigs and rodents. Some authors

suggest that it evolved as a parasite of carnivorous animals of northern regions (Cameron, 1956). A major unresolved problem in the evolution of the species is how birds became involved as a host. This is further confused by the fact that T.s. var.pseudospiralis considered as a species by some workers, interbreeds with a number of natural isolates of T.spiralis (Dick and Chadee, 1982). A number of questions need to be addressed. Is the bird- Trichinella relationship an ancient one that evolved independently of the mammalian cycle, or is it a recent acquisition in which birds are largely a carrier host that can be accidentaly parasitized if the host-parasite equilibrium, particularly during the intestinal stage is disturbed?

If T.s. var.pseudospiralis had an ancient and viable relationship with birds we would expect to find it relatively frequently in nature with a well developed host-parasite equilibrium. In fact, few cases of natural infections have been reported ,even though workers are actively looking for it. Experimental studies by Britov (1974) and observations of natural infections (Wheeldon et al., 1982) indicate that the parasite is not as well adapted to birds as previously believed. On the other hand, Shaikenov (1980) suggested that birds are obligatory hosts for the parasite. The present study clearly showed that, while the parasite stayed in the intestine of birds for a longer period of time than in mice, it had difficulty reaching the bird muscle. Furthermore, no evidence suggests that T.s. var.pseudospiralis induced any intestinal pathology in quail. Yet, it is well known that the bird intestine does react to antigenic stimuli induced by other species of intestinal nematodes (Wehr, 1939). Birds have some degree of natural immunity to

the disseminating and developing larvae of Trichinella and is supported by the work of Stankiewicz and Jeska (1979) who found intense precipitin deposits on T.s. var.pseudospiralis larvae incubated in the serum of normal uninfected chicken. This is particularly surprising considering the short duration of the parasite in the mouse intestine, its higher infectivity and low virulence in mice. The parasite would seem, from these observations, to be better adapted to mice and other mammal hosts, such as hamsters. The ease with which the parasite can switch from mammal or bird hosts, and its success in mammalian hosts, makes it difficult to accept the idea of a species that evolved independently of mammalian species.

If we accept the hypothesis that birds can play a role in spreading the parasite, we may be observing a form that was normally restricted to the intestine of birds. Support for this hypothesis includes low recovery and longevity in the intestine indicating a strong natural immunity to Trichinella. Trichinella can invade birds muscle only rarely and when conditions are suitable. Suitable conditions include a decrease in the immune resistance of the host induced by starvation or disease, and the presence of optimal conditions for the parasite, such as limited destruction of the worms in a gizzard and a suitable intestinal environment generated by a diet of meat in certain meat or carrion-feeding birds. Otherwise, transmission could have occurred via the faeces and through insects acting as paratenic hosts (Merkushev, 1960). Several authors suggested that birds play a part in the epidemiology of Trichinella as adult worms can survive for several days in the faeces and still be infective (Abs and Schmidt, 1954; Merkushev,

1960). Merkushev (1960) successfully infected mice with droppings from birds containing T.spiralis adults and Tomasovicova (1981) found that T.spiralis survived in fish faeces and in water for some time.

The strong immunogenic intestinal response of mammals could be, in whole or part, responsible for the short duration of the intestinal phase, and the synchronized development of the parasite in the intestine. Consequently, larvae are produced in mammals prior to the development of a strong humoral response and are able to reach the muscle, survive, and induce capsule formation. The ability of Trichinella to induce capsule formation by the host immune system becomes an important consideration. Some evidence exists that the capsule, normally induced by T.spiralis in mammalian hosts, was not formed when this parasite was transferred into birds. Augustine (1933) and Matoff (1938) observed the presence of few lifeless larvae in the muscle of T.spiralis infected chicken , and Merkushev (1954-1960) and Berezantsev (1963-1964) both cited in Berezantsev and Ananiev (1966), observed the presence of a few Trichinella larvae but "without the typical capsule" in the muscle of infected birds. Nemeseri (1968) found that chickens fed on a meat diet or starved harboured Trichinella larvae in their muscle, indicating that both immune response and diet may be important. Perhaps, birds are largely accidental hosts and only when conditions are suitable, i.e. diet of meat or carrion, lowered resistance, and less mechanical damage by the intestinal tract do sufficient numbers of larvae reach the muscle to be readily detectable. It is also interesting to speculate that Trichinella produced in the intestine of birds eating meat or carrion are less immunogenic and therefore more larvae

are able to reach the muscle stage. There is some evidence from other studies that host diet modifies some species of parasite sufficiently for them to be considered different strains (Bryant, 1982). Larvae observed in bird muscle are smaller and the structure of the stichosome could be interpreted as similar to an incomplete or "immature" stichosome of T.spiralis. It is well known that much of the antigenic properties of Trichinella resides in the stichosome (Despommier, 1972). Perhaps these larvae are not as strongly immunogenic as the more mature larvae of T.spiralis from pig and cannot induce the host's immune response to produce formation of a capsule.

T. spiralis has an extraordinary ability to adapt to a diverse range of hosts. Other studies and observations from this work confirm this adaptibility. Host species plays a role in establishing the "normal" phenotypic variability of the species T.spiralis and for the variety T.s. var.pseudospiralis. The role of birds and Trichinella as an ancient relationship unique to T.s. var.pseudospiralis is challenged, especially as the parasite appears to be better adapted to mammals. A more likely hypothesis,is that birds are a relatively recent acquisition in the life cycle and transmission of Trichinella, and that a new host-parasite equilibrium and perhaps even a new species may evolve. The relative rareness with which this bird-parasite relationship is observed raises doubts on its success. Perhaps, more realistically we have merely observed an extension of the repertoire of adaptive strategies Trichinella has developed to make it a most successful parasite, as far as known host species are concerned.

LITERATURE CITED

ABS, O., AND H.W. SCHMIDT. 1954. Wie infizieren sich arctische Tiere mit Trichinen. Z. Inner. Med. 9: 758-760.

ALI KHAN, Z. 1966. The post-embryonic development of Trichinella spiralis with special reference to ecdysis. J. Parasitol. 52: 248-259.

ANDERSON, R.M. 1978. The regulation of host population growth by parasitic species. Parasitology 76: 119-157.

AUGUSTINE, D.L. 1933. Experimental trichinosis in chicks. Science 78: 2035-2036.

BELOSEVIC, M., AND T.A. DICK. 1979. Trichinella spiralis: Comparison of stages in host intestine with those of an arctic Trichinella sp.. Exp. Parasitol. 48: 432-446.

BELOSEVIC, M., AND T.A. DICK. 1980. Trichinella spiralis: Comparison with an Arctic isolate. J. Parasitol. 49: 266-276.

BEREZANTSEV, Y.A., AND G.I. ANANIEV. 1966. Experimental trichinellosis in birds. In: Mater. Nauchn. Konf. Vses. Obshch. Gelmint., Moskva, pp. 42-47 (in Russian).

BERNTZEN, A.K. 1965. Comparative growth and development of Trichinella spiralis in vitro and in vivo with a redescription of the life cycle. Exp. Parasitol. 16: 74-106.

BESSONOV, A.S., R.A. PENKOVA, AND A.V. USPENSKII. 1975. On the independence of Trichinella species. Wiad. Parazytol. 21: 561-565.

BESSONOV,A.S., R.A. PENKOVA, AND U.P. GUMENSHCHIKOVA. 1976. Trichinella pseudospiralis Garkavi 1972: Morphological and biological characteristics and host specificity. In: Trichinellosis, C.W. Kim and Z.S. Pawlowski (Eds.) University Press, New England U.S.A. pp.73-93.

BIEDERMAN, W. 1911. Die Aufnahme Verarbeitung und Assimilation der Nahrung. In:Handbuch der Vergleichenden Physiologie. Hans Winterstein Iena Verlag von Gustav Fisher.

BOEV, S.N., B. SHAIKENOV, AND L.A. SOKOLOVA. 1979. Disputed questions of the taxonomy of Trichinella. Parazitologia 13: 144-149 (in Russian).

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

BRITOV, V.A. 1971a. Results of crossing Trichinella from wild animals with those from pigs. Doklady Vsesoyuznoi Akademii Sel' Skokhozyaistuennykh Nauk 2: 40-41 (in Russian).

BRITOV, V.A. 1971b. Biological methods for the determination of varieties of Trichinella spiralis Owen, 1835. Wiadom. Parazytol. 17: 477-480 (in Russian).

BRITOV, V.A. 1973. Population variability of Trichinella. Novoe o Stroneii Organov i Tkaneei Zhivotnykh. Ul'yanovk, CCCP 36-37 (in Russian).

BRITOV, V.A. 1974. On the sensibility of birds to trichinellosis. Borbas Boleznyami Zhivotnykh Na Dalnem Vostoke Blagovesh Chensk 48-49 (in Russian).

BRITOV, V.A. 1977. On the technique of crossing of Trichinella. Parasitologia 11: 460-461.

BRITOV, V.A., AND S.N. BOEV. 1972. Taxonomic rank of various strains of Trichinella and their circulation in nature. Vest. Akad. Nauk. Kazakhskoi 28(4): 27-32 (in Russian).

BRYANT, C. 1982. Strain variation of energy metabolism in helminths - a biochemist's nightmare. Annual General and Scientific Meeting. Austral. Soc. Parasitol.

CALERO, R., F. MARTINEZ, S. HERNANDEZ, AND I. ACOSTA. 1978. Parasitization of Buteo buteo (Aves: Accipitridae) by Trichinella sp. in the Zoological Park of Jerez de la Frontera. Rev. Iber. Parasitol. 38: 135-138 (in Spanish).

CAMERON, T.W.M. 1956. Parasits and parasitism. Wiley, New York xix + 322 pp.

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

CHADEE, K.C. AND T.A. DICK. 1982. Biological characteristics and host influence on a geographical isolate of Trichinella (Wolwerine: 55° 00'N, 100°00'W, 1979). J. Parasitol. 68: 451-456.

CHANDLER, A.C., J.E. ALICATA, AND M.B. CHITWOOD. 1974. II. Parasites of vertebrates. In: An introduction to nematology (J.R. Christie, ed.), Part II, Section II, Chapter VI. Monumental Printing Co., Baltimore, Maryland pp. 267-301.

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

DESPOMMIER, D.D. 1972. The stichocytes of Trichinella spiralis during the morphogenesis in the small intestine of the rat. In Trichinellosis, C.W. KIM (Ed.) Intext Educational Publishers New York. pp. 239-254.

DICK, T.A. AND M.BELOSEVIC. 1978. Observations on a Trichinella spiralis isolate from a polar bear. J. Parasitol. 64: 1143-1145.

DICK, T.A., AND K. CHADEE. 1982. Interbreeding and gene flow in the genus Trichinella (in press).

DICK, T.A., AND B.B. SILVER. 1980. Intestinal distribution of Trichinella spiralis in rats. J. Parasitol. 66: 472-477.

GARKAVI, B.L. 1972. Trichinella species recovered from wild carnivores. Veterinaryia (Moscow) 49: 90-91 (in Russian).

GARKAVI, B.L. 1974. Potential hosts of Trichinella pseudospiralis. Parazitologiya 8: 489-493 (in Russian).

GARKAVI, B.L. 1976. Susceptibility of laboratory animals to Trichinella from infection from both natural and associated with man in nothern Caucasus. Parazitologia 10: 154-157 (in Russian).

GARKAVI, B.L. 1980. Susceptibility of the Syrian hamster to Trichinella pseudospiralis. Third Nat. Conference of Parasitology Albera, Bulgaria pp.124-125 (in Russian).

GELLER, E.R. AND A.N. MALYKHINA. 1976. On the interspecific relations between Trichinella pseudospiralis Garkavi,1972, and T.spiralis Owen, 1835. Materials of the 2nd All-Union Conference on the Problem of Trichinellosis in Man and Animals, pp.80-82 (in Russian).

GELLER, E.R., A.N. MALYKHINA, L.N. SILAKOVA, AND E.V. TIMONOV. 1977. Morphological and physiological criteria of taxonomic independance of Trichinella pseudospiralis Garkavi, 1972. Parazitologiya (Leningrad) 11: 113-118.

KEYMER A. 1982. Density-dependent mechanisms in the regulation of intestinal helminth populations. Parasitology 84: 573-587.

KOCIEKA, W., F. VAN KNAPEN, AND E.J. RUITENBERG. 1981. Trichinella pseudospiralis and T.spiralis in monkeys. I. Parasitological aspects. In: Trichinellosis, C.W. KIM, E.J. RUITENBERG AND J.S. TEPPEMA (Eds.) Reedbooks Ltd. Surrey, England, pp. 199-203.

KOZAR, Z. AND M. KOZAR. 1963. The course of experimental trichinellosis in mice. Methods of infection and influence of various invasive doses on the mortality and weight of animals. Wiad. Parazytol. 9: 403-418.

KOZAR, Z., AND M. KOZAR. 1965. A comparison of the infectivity and pathogenicity of Trichinella spiralis strains from Poland and Kenya. J. Helminthol. 39: 19-34.

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

KRAMAR, M. ,G.L. STEWART, AND L. CHARNIGA. 1981. A comparative study of Trichinella spiralis (Owen,1835) and Trichinella pseudospiralis (Garkavi, 1972). J. Parasitol. 67: 911-916.

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

LARSH, J.E., AND G.J. RACE. 1975. Allergic inflammation as a hypothesis for the expulsion of worms from tissue: a review. Exp. Parasitol. 37: 251-266.

MACHNICKA, B. 1979. The problem of geographical strain of Trichinella spiralis and their biological properties. Wiad. Parazytol. 25: 197-205 (in Polish).

MADSEN, H. 1976. 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 Parasitol. Pol. 24: 143-158.

MARTINEZ FERNANDEZ, A.R., M.L. SANMARTIN DURAN, M.G. ORTEGA AND T. GARATE. 1981. Cross immunity levels of sibling species of Trichinella. In: Trichinellosis, C.W. KIM, E.J. RUITENBERG and J.S. TEPPEMA (Eds.) Reedbooks Ltd. Surrey, England, pp. 129-133.

MATOFF, K. 1938. Zur Frage der Muskeltrichinellose beim Geflugel. Z. Infektkr. Haustiere 54: 116-134.

MERKUSHEV, A.V. 1960. Birds, as possible carriers of trichinellae. Wiadom. Parazytol. 6: 318-320 (in Russian).

MIROSHNICHENKO, L.S. 1976. Sensibility of birds to trichinellosis. Mater. dokl. kII Vses. konf. po probl. trikhinelleza cheloveka i zhivotnykh., Vilnius, pp.86-90 (in Russian).

MIROSHNICHENKO, L.S. 1978 . Pathology of skeletal muscle in birds infected with Trichinella pseudospiralis. Wiad. Parazytol. 24(1): 91-96 (in Russian).

NEMESERI, L. 1968. Invasionsversuche beim Geflugel mit Trichinella spiralis. Helminthologia. Bratislava 8-9: 417-420.

NIPHADKAR, S.M. 1973. Trichinella spiralis (Owen, 1935) in Bandicota bengalensis (Gray) in Bombay. Curr. Sci. 42: 135-136.

PAWLOWSKI, Z. AND W. RAUHUT. 1971. Comparative observations on three local strains of Trichinella spiralis. Wiad. Parazytol. 17: 481-486.

PAWLOWSKI, Z.S., AND E.J. RUITENBERG. 1978. Is Trichinella pseudospiralis likely to be a human pathogen? Lancet, 1 (8078), 1357.

PENKOVA, R.A. 1975. Morphological, biological and serological peculiarities of Trichinella species and their significance in the epizootiology of swine trichinellosis. Dissertation, Moscow (in Russian).

PENKOVA, R.A. 1976. Morphological and biological peculiarities of Trichinella pseudospiralis. Materials of the 2nd All-Union Conference on the Problem of Trichinellosis in Man and Animals. pp.90-92 (in Russian).

PEREVERSEVA, E.V., N.N. OSERETSKOVSKAYA, AND N.L. VERETENNIKOVA. 1974. The development of Trichinella larvae isolated from raccoon muscles in albino mice. Wiad. Parazytol. 20: 67-80 (in Russian).

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

PRZYJALKOWSKI, Z. 1978. Effect of intestinal flora on the course of infection and haematological changes in germfree and conventional mice exposed to Trichinella pseudospiralis (Garkavi, 1972). Bull. Acad. Polon. Sci. 24: 331-336.

RAPPAPORT, I. 1943. 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. Am. J. Trop. Med. 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. Wiad. Paraztol. 24: 67-90 (in Polish).

RAUSCH, R., B.B. BABERO, R.V. RAUSCH, AND E.L. SCHILLER. 1956. Studies on the helminth fauna of Alaska. XXVII. The occurence of larvae of Trichinella spiralis in Alaskan mammals. J. Parasitol. 42: 259-271.

RICHELS, I. 1955. Histopathologische Studien zu den Problemen der Zellconstanz: Untersuchungen zur microscopischen Anatomie im Lebenzyclus von Trichinella spiralis. Zbl. Bakt. I, Orig. 163, 46.

SHAIKENOV, B. 1980. Spontaneous infection of birds with Trichinella pseudospiralis Garkavi, 1972. Folia Parasitol. 27: 227-230.

SILVER, B.B., T.A. DICK, AND H.E. WELCH. 1980. Concurrent infections of Hymenolepis diminuta and Trichinella spiralis in the rat intestine. J. Parasitol. 66: 786-791.

SOKAL, R.R., AND F.J. ROHLF. 1969. Biometry. W.H. Freeman and Co., San Francisco.

STANKIEWICZ, M. AND E.L. JESKA. 1979. Cuticular interactions of Trichinella var.pseudospiralis with normal chicken sera. Bull. Acad. Polon. Sci. (Sciences Biol.) 27: 349-352.

SUKHDEO, M.V.K., AND E. MEEROVITCH. 1980. A biological explanation for the differences in infectivity of geographical isolates of Trichinella. Can. J. Zool. 58: 1227-1231.

TOMASOVICOVA, O. 1975. Poultry. A new host of Trichinella pseudospiralis (Garkavi, 1972). Biologia (Bratislava) 30: 821-826.

TOMASOVICOVA, O. 1981. The role of fresh-water fish in transfer and maintenance of Trichinellae under natural conditions. Biologia (Bratislava), Ser. B. Zoologia 36: 115-125.

TOMASOVICOVA, O., AND Y. GOVORKA. 1976. Sensibility of various ecological groups of birds to T.pseudospiralis Garkavi, 1972. Sb. tezisov 3go Mezhdunar. symp. Gelmintolog. Instituta SAV, Vysoke Tatry, CCCP: 209 (in Russian).

TOMASOVICOVA, O., AND J. HOVORKA. 1982. On the susceptibility of birds to Trichinella pseudospiralis Garkavi, 1972. Biologia 37(2): 169-173.

TROMMER, J. 1970. Fleischfressende Vogel als UberTrager von Trichinen. Prakt. Tierartz 51: 46-47.

WEHR, E.E. 1939. Studies on the development of the pigeon capillarid Capillaria columbae. U.S.D.A. Tech. Bull. N.679: 1-9. 1 1

WHEELDON, E.B., T.A. DICK, AND T.A. SCHULZ. (1982). First report of Trichinella spiralis var.pseudospiralis in North America. J. Am. Vet. Assoc.(in press).

WU, L.Y. 1955. The development of the stichosome and associated sructures in Trichinella spiralis. Can. J. Zool. 33: 440-446.

ZIMMERMAN, W.J., E.D. HUBBARD, L.H. SCHWARTE, AND H.E. BIESTER. 1962. Trichinella spiralis in Iowa wild life during the years 1953 to 1961. J. Parasitol. 48: 429-432.

Appendix I

Known species of wild animals infected by T. s. var. pseudospiralis

Common name	Location	Scientific name	Taxonomic authority	Authority for infection
pomarine jaeger*	Alaska	Stercorarius pomarinus	Temminck, 1815	Rausch et al., 1956
great horned owl*	Iowa	Bubo virginianus	(Gmelin, 1788)	Zimmermann et al., 1962
American raccoon	U.S.S.R.	Procyon lotor	L.	Garkavi, 1972
Indian plague rat	India	Bandicota bengalensis	Gray and Hardwicke, 1833	Niphadkar, 1973
buzzard	Spain	Buteo buteo	L.	Calero et al., 1978
rook	U.S.S.R.	Corvus frugilegus	L.	Shaikenov, 1980
Cooper's hawk	California	Accipiter cooperi	Bonaparte, 1828	Wheeldon et al., 1982

* Birds found infected with 'Trichinella-like' worms.

APPENDIX II

Detailed taxonomic history of *Trichinella*[1]

Phylum	Nematoda	
Class	Adenophora (Aphasmidea)	
Order	Trichurata	
Family	Trichinellidae	Ward, 1907
Syn.	Trichinidae	Cobbold, 1879
Genus	*Trichinella*	Railliet, 1895
Syn.	*Trichina*	Owen, 1835
Type of genus:	*T. spiralis*	(Owen, 1835)
Syns.	*T. canis*	Kraemer, 1853
	T. circumflexa	Polonio, 1860
	T. pseudalius	Dengler, 1863
	Pseudalius trichina	Davaine, 1863
Other species:	*T. nativa*	Britov and Boev, 1972
	T. nelsoni	Britov and Boev, 1972
	T. pseudospiralis	Garkavi, 1972

[1] YAMAGUTI, S. 1961. Systema Helminthum. *The nematodes of vertebrates*. Volume III. Part 1. Interscience Publishers INC., New York. Interscience Publishers Ltd., London.