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PROCEEDINGS OF THE
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Physiology of the Body Wall Muscles in an Acanthocephalan[1]

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ABSTRACT: The circular and longitudinal muscles of the body wall of the acanthocephalan, *Macracanthorhynchus hirudinaceus*, were studied. Both circular and longitudinal muscles were found to be electrically inexcitable. Evidence was also obtained for a through conduction system located near the median-longitudinal lacunar canal. Isolated muscles could not be stimulated to contract, cut body wall fragments could be stimulated. Strength-duration, tension-time, and relaxation-time curves were obtained. On the basis of the difference in response to stimuli a separate activation system for each set of muscles is postulated. The cuticular layer of the body wall was not necessary for contraction; however, its presence did alter the tension-time and relaxation-time parameters.

Considerable variation occurs in the physiology of invertebrate muscles. For example, in crustaceans the speed of contraction and relaxation as well as the dependence upon facilitation are influenced by properties of both the muscle fibers and the excitatory motor axons. Thus, in muscles with multiple types of innervation, responses may be determined by the particular axon which is active. Among the parasitic worms, the unique muscular system of *Ascaris* (Rosenbluth, 1965) surprised most invertebrate physiologists when the resting potential of the muscle fibers was observed to decrease when Cl_0 was increased. In this species a hyperpolarizing phase of spikes was due to K-activation and pulses applied in one fiber were observed to spread to adjacent ones, indicating a high degree of electrical coupling (Grundfest, 1967). The unique features found in the physiology of *Ascaris* muscles seems to reflect the more general premise that the muscles of each organism have a rate of movement, an ability to develop tension, a degree of shortening, and such other features as reflect the animal's life habits. Since the habits and morphology of *Macracanthorhynchus hirudinaceus* are considerably different from those of other parasitic worms whose muscle physiology has been studied, a number of questions need to be answered. How does eutely affect the neuromuscular physiology of an organism which undergoes such extreme hypertrophy? What type of innervation do the muscles possess? How is the neuromuscular system organized? This study is an investigation of the physiology of the body wall musculature of *M. hirudinaceus* in an attempt to partially answer some of these questions.

Materials and Methods

Female specimens of *M. hirudinaceus* were obtained from swine small intestines at Hunter Packing Company, East St. Louis, Illinois, and placed in Dewar flasks along with minimal amounts of gut contents for transport to the laboratory. Afterwards, the worms were transferred at 8- to 12-hr intervals into a physiological saline solution (PSS) consisting of 30% seawater (artificial), 0.1 M glucose, and 0.1 M sucrose (Denbo, 1971). The PSS was used for the external medium in all preparations. Internal potentials were recorded by means of glass-capillary micropipettes (Transidyne) and amplified by a Mentor probe system. Potentials from metal (tungsten or stainless steel) electrodes were amplified by the same system used

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for the glass-capillary electrodes. The Mentor output was displayed on a Tektronix 502A oscilloscope and recorded on both an Ampex SP-300 AM/FM tape recorder and a Beckman Dynagraph recorder.

Because of the activity of the worm, the recording of potentials presented problems which were solved by a variety of methods. The easiest and longest term recordings of body wall muscle potentials were accomplished with metal electrodes. In an unrestrained worm, glass micropipette electrodes usually broke as a result of muscle contraction.

Several techniques were used to expose the muscle layers. One such technique used was to strip the cuticle from the body wall and expose the circular muscle layer. Another technique was to cut open the body wall in order to approach the longitudinal muscles from the inside. Finally, in order to record potentials from the internal muscles from the inside without extensive dissection, we resorted to a technique of inverting the worm. To do this, the most posterior end of the worm was cut, a long needle applied to the end of the apical sensory organ, and the body "rolled" down over the needle. The advantages of this method are apparent: (1) the muscles of the worm are maintained intact and recordings can be made with minimum difficulty; (2) movements of the worm are minimized and the proboscis receptacle is completely exposed.

For some experiments it was desirable to record potentials while heating one portion of the worm and cooling another portion. In these experiments a split chamber was used. The temperature of the ganglion area was maintained by focusing a tungsten lamp through a convex lens into the PSS. The temperature of the PSS was monitored by means of an epoxy-coated, copper-constantine thermocouple connected to a digital millivolt meter (Digitest 500). The amount of radiant energy transferred to the solution to maintain temperature was controlled by an iris diaphragm. When not recording, the bathing media temperature was maintained by a specially designed nichrome heating device, encapsulated in high-dielectric potting compound. For iontophoretic injection the cobalt chloride technique was used (Pittman et al., 1972). Muscle activity was monitored by means of Bionix isometric and isotonic transducers. Additional preparations referred to in this study are illustrated by Figure 1.

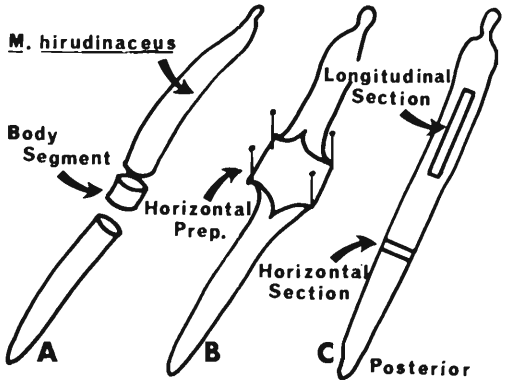

Figure 1. Diagrammatic sketch of preparations used in this study. Specimens used were approximately 36 cm long and approximately 8 mm in diameter at midpoint of body.

Results

Spontaneous contractions and potentials were recorded from entire worms. When recording spontaneous tension development in the tail and body wall potentials (Hightower et al., 1975) of an inverted worm (Fig. 2A), the peak tension reached 5 g with a rise time to peak tension of 7 to 10 sec. The associated potentials were recorded 18 cm anterior to the monitored tail, and in this case the tail contracture occurred 0.6 sec prior to the potentials. This same occurrence of the potentials after the initiation of the contracture occurred when a whole worm was induced to contract by stimulation (Fig. 2B) with a pulse of 6 v at 0.1 sec or when stimulated to contract by the application of a d-c level (head positive) of 2.0 v (2.5 ma) for 18 sec (Fig. 2C). With the application of a d-c potential to an entire worm, an interesting phenomenon occurred—there was an increase in tension development of the caudal area up to a clonuslike state and the potentials underwent what might be termed adaptation. Finally, when the stimulus was removed, the monitored area returned to its resting tension.

In order to gain access to the body wall muscles, inverted whole worms were used and stimuli applied to the area of the retinaculum

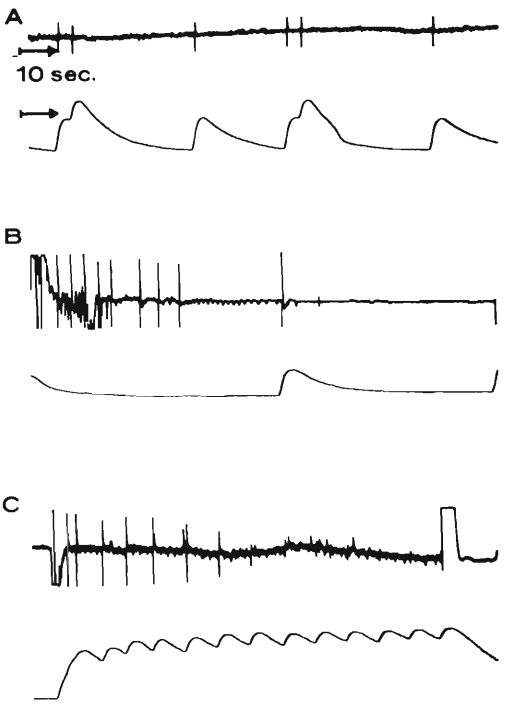

Figure 2. A. Bottom trace: Longitudinal contraction (spontaneous) of a posterior section of an inverted worm. Peak tension (PT) of 5 g and rise time to PT of 3 sec. Upper trace: Extracellular potentials recorded anterior to the tension-monitored portion of the worm. Contractions preceded the small potentials (3 mv for 200 msec) by nearly 0.6 sec. Occasionally a second smaller contraction occurred at the peak of one contraction. B. Induced burst of potentials with no immediate change in tension. Spontaneous contraction subsequently occurred and in association with a potential. C. Threshold stimulus applied for the duration of the record (2 v, 2.5 ma). Initial response accompanied by 4-mv potentials in a one-to-one correspondence. These gradually diminished in amplitude and finally disappeared. With stimulus *off, the resting* tension was resumed.

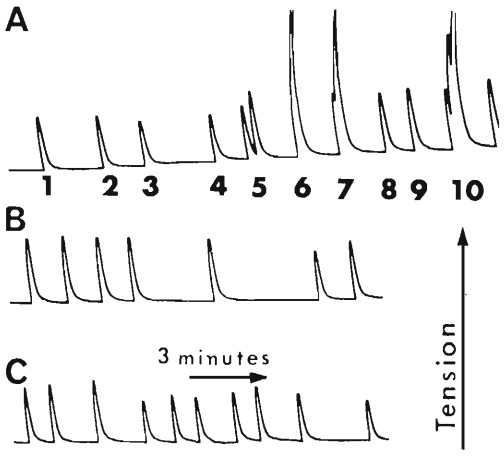

Figure 3. Anterior contractions (isometric) of a whole intact worm in response to stimuli delivered to tissue where retinacular muscle attaches to body wall. First four responses in A are due to 2-, 2-, 1.5-, and 0.8-ma (100 msec) stimuli. All other pulses are 2 ma and 100 msec in duration. Responses A5, A6, and A7 are due to a pair of pulses separated by 25-, 5-, and 10-sec, respectively. A8, A9, and A10 are due to a pair of stimuli with interval times of 0.1, 1.0, and 2.0 sec, respectively. In A10, stimuli consisted of three pulses, 15 sec apart with marked mechanical facilitation obvious. The first four responses observed in B were due to paired stimuli, each 1.0 sec apart, while the fifth was for a 0.1-sec interval. The second-from-last response was due to one pulse. C shows responses after 30 min of continual stimulation. Note the decline in magnitude of the responses.

(Fig. 3). The results were found to be consistent with the preceding experiments on intact worms. Using floating glass electrodes (Woodbury and Brady, 1956) and varying the site of recording of potentials and tension development, it was observed (Fig. 4) that potentials which followed contractions could be recorded from a variety of sites. In particular, in one case (Fig. 4C) the potentials recorded were not homogeneous, but the larger potentials had a faster repolarization than depolarization with rates of membrane change near 0.15 to 0.3 v/sec. Repolarization was observed to overshoot by a few mv the membrane potential where depolarization begins.

To investigate the various muscle responses without the influence of the cerebral ganglion we extirpated the proboscis of the worm. Local electrical stimulation of inverted, headless worms at anterior body wall sites where the head had been severed always resulted in contractions even for pulses which were very short in duration. A threshold curve for such a preparation (Fig. 5) revealed that the shortest

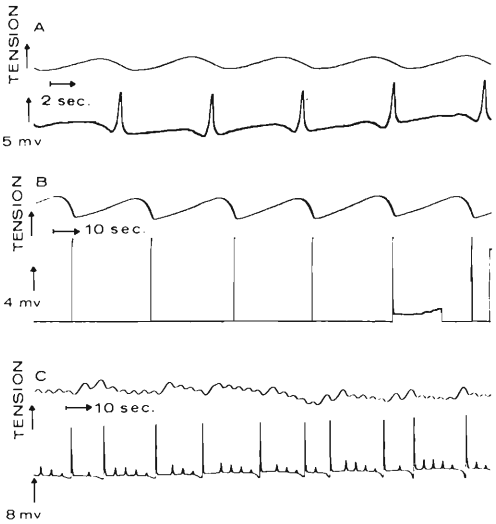

Figure 4. Body wall potentials (A2, B2, C2) in association with body activity (A1, B1, C1). A. Slow potentials (1 sec) from longitudinal musculature were obtained with glass-micropipette floating electrode. Upward deflections of potentials were depolarizing. Simultaneous muscle activity of same portion of worm is seen to be clonuslike smooth oscillations of tension changes, and in one-to-one correspondence with the potentials. B. Recording site on a different worm was slightly posterior to the portion of the worm monitored for tension changes. Potentials (12 mv and nearly 0.2 sec) obtained from the area of the circular muscles. Contractions were nearly 3-g increases in tension and occurred in a one-to-one correspondence with these potentials. C. Another worm was monitored for slightly irregular clonuslike activity, and was accompanied by large 15-mv potentials with small, slower potentials occurring regularly between.

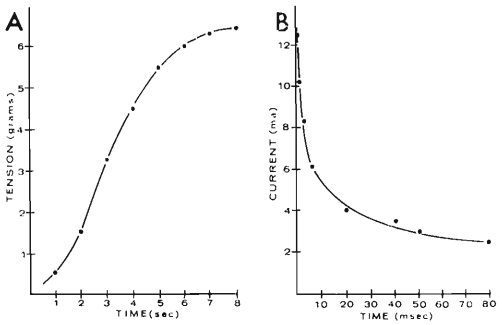

Figure 5. A. Graph showing rate of tension development of a 10-cm portion of body of a whole, intact worm. Average tension rate was typically 1g/sec up to peak tension development (4.3 g here) at 25 C. Shorter sections monitored developed tension with similar rates, although peak tension was less. B. Strength-duration curve obtained from stimulation of body wall tissue in the immediate area of the lateral posterior nerves using silver-silver chloride wires fashioned into hook electrodes. The lowest current effective for evoking contractions was 2.0 ma, while the shortest pulse effective was 0.9 msec at 12.5 ma. Each point in the graph corresponds to stimulus parameters associated with identical contractions elicited.

effective pulse was 0.9 msec. When partial transverse incisions were made across the dorsal side of the section being monitored, responses to stimulating pulses were not changed. Ventral cuts made 0.5 cm anterior to dorsal cut caused no change in the responses observed in each section. Short longitudinal incisions, however, did reduce the magnitude of response, but did not abolish it. Longer longitudinal incisions resulted in a much greater reduction in tension developed upon stimulation. Tension development by both apical area (anterior inch) and caudal area (posterior inch) of headless worms were monitored simultaneously, and stimuli were delivered to the head area with silver wire hook electrodes. With a 50-msec stimulus the anterior portion of the worm contracted after 0.3 sec and the posterior portion contracted after a delay of 0.4 sec (Fig. 6). Thus total time for stimulus to tail contraction was 0.7 sec. Since a delay of 0.4 sec (Fig. 6). Thus total time for stimulus to tail contraction was 0.7 sec. Since the worm was 14 cm long, effective conduction of transmission of stimulus down the length of the worm was 35 ± 5 cm/sec. The source of variance was attributable to inaccuracy of mechanical recording device from which times were measured. This average was the result of 10 determinations on the same worm. Each determination was separated by 2 min. Other worms used had calculated averages of 22, 33, 38, and 42 cm/sec. When the entire upper third of the worm was monitored for isometric contraction after stimulation with different trains of pulsed stimuli, the extent of the contraction was a function of the number and frequency of pulses (Fig. 7). Indeed, the results were much the same if the entire worm was used (Fig. 8).

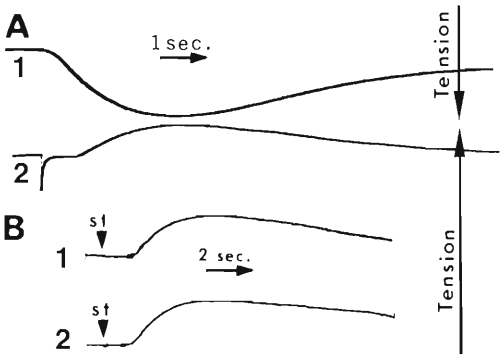

Figure 6. A-1 shows anterior inch of an intact worm shortening in response to a stimulus applied to anteriormost end of body. A-2 shows posterior inch of worm shortening 0.7 sec later. Stimulus artifact precedes the response by 0.3 sec. Notice the "apparent" velocity of excitation may be calculated from distance/time.

The preparation shown in Figure 1B allowed the recording of tension developed by circular muscles at the same time as the development of tension by the tail was measured. In this preparation stimulation along the longitudinal axis still evoked tension development by the posterior section of the worm; however, tension was not recorded from the central circular muscles. Nevertheless (in the same preparation) (Fig. 9), transverse stimulation across the body wall was capable of eliciting tension development in the circular muscles.

To investigate muscle responses to stimulation on a smaller scale, 5-cm segments of worms were positioned so that the anterior ends of the segments were connected to an isotonic lever to minimize inertial effects. Loads from 40 to 100 g were used. For 40 g, changes in length were consistently 6.4 mm for spontaneous contractions at 38 C. Contraction times as fast as 2.6 sec were recorded, giving a velocity of shortening of 2.4 mm/sec. Similarily, for 80-g loads velocities of 0.27 mm/sec were observed, while for 100 g 0.20 mm/sec was an average velocity of shortening. For unloaded segments, shortening was observed maximally at 10 mm/sec at 38 C. Since these loads were applied via a lever system, only a fraction (10%) of the gram weights added were acting on these sections.

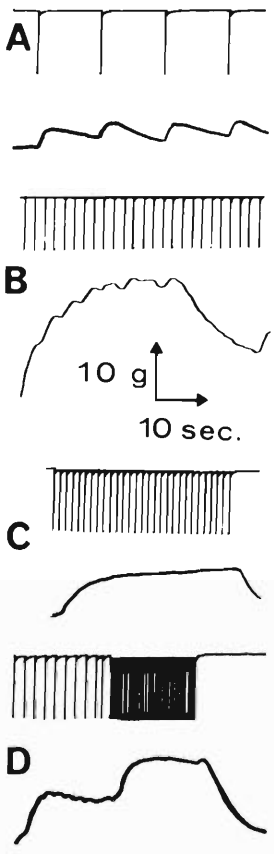

Figure 7. Isometric contractions of inverted worm (upper two-thirds) in response to stimulus trains of varying frequencies. All pulses were 50 msec. Frequencies are: A, 0.125 Hz; B, 0.5 Hz; C, 1.25 Hz; D, 0.5 and 0.25 Hz.

Similar preparations of body segments were employed for isometric monitoring (Fig. 1A). Resting tension of the segment was 3 g, therefore maximum changes in tension were 7.3 – 3.0 or 4.3 g average. The initial rates of contraction were plotted (Fig. 5) with an average rate of tension development of 1 g/sec for the first 7 sec of contraction—the time at which peak tension was reached. Half-relaxation times were variable, ranging from 48 to 60 sec, while total relaxation required nearly 8 min in some cases. It should be noted that after repetitive stimulation for 20 min, peak tension times increased from 7 to 14 sec. Shorter lengths

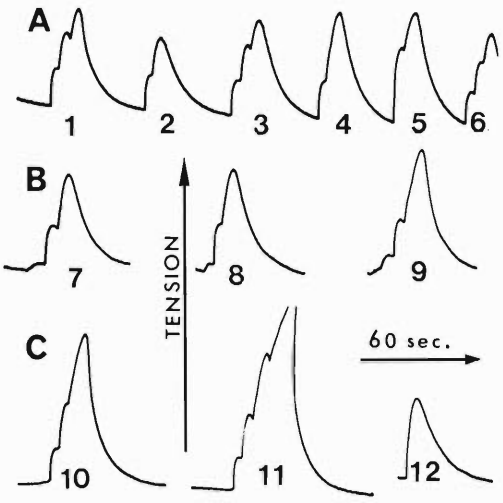

Figure 8. Summation of contractile responses (isometric) of whole worm with electrodes in body wall (dorsal, anterior surface). The numbered responses were due to stimuli (S) as follows: A1, 3 S, 3, 2, and 1 ma, 0.1 sec pulse duration (D) 3 sec Internal (I); A2, 2 S, 3 and 3 ma, 0.1 sec D 3 sec I; A3, 3 S, 3, 2, and 1 ma, 0.1 sec D 3 sec I; A4, 2 S, 3 and 5 ma, 0.1 sec D 3 sec I; A5, 2 S, 5 and 3 ma, 0.1 sec D 3 sec I; A6, 3 S, 4, 4, and 4 S, 0.1 sec D 3 sec I; B7, 3 S, 0.5, 2, and 3 ma, 0.1 sec D 3 sec I; B8, 3 S, 0.5, 3, and 4 ma, 0.1 sec D 3 sec I; B9, 3 S, 1.0, 3, and 5 ma, 0.1 sec D 3 sec I; C10, 3 S, 2.0, 3, and 5 ma, 0.1 sec D 0.1 sec I; C11, 4 S, 1.0, 2.0, 5.0, and 4.0 ma, 0.1 sec D 0.1 sec I; C12, 1 S, 6.0 ma, 0.1 sec I.

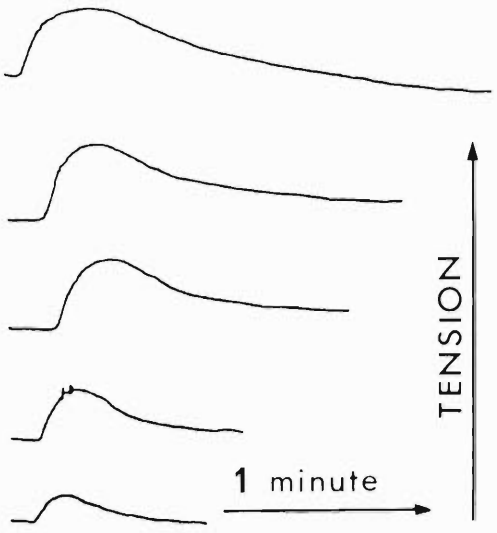

Figure 9. Isometric tension development of circular muscles (eight to 10 fibers) of a horizontal preparation. Maximum amplitude of tension occurred for a 10-v pulse, with each lower record showing the response to successively smaller pulse intensities.

of body wall segments gave proportionately smaller peak tension development. When body wall segments were given multiple stimuli, the maximum tension developed depended upon the stimulation parameters. Fusion frequencies characteristic of these preparations were from 1 to 3 Hz and initial rates of tension development for the first 10 sec were all approximately 1.7 g/sec. Inverted body wall segments, when stimulated with trains of 50-msec, 5-v pulses, exhibited contractions depending upon the number of stimuli in the train (Fig. 10). Tension development in the body wall segments could be accelerated if the outer body wall layers (cuticle and outer epidermis) were stripped away. These contractions attained maximum value in 5.5 sec. Similar rates could be observed for stretch-induced contractions (Fig. 11). Corresponding tension rates varied from 0.6 to 1.1 g/sec. Isometric tension development ranged from 5 to 20 sec and the peak tension developed was a function of the stimulating voltage. Contraction times at room temperature were: 19, 14, 14, 9, 6, and 7.0 sec. It is clear that the half-relaxation varied considerably (from 25 sec to 1.4 min) depending upon the magnitude of the tension developed. Contraction rates from spontaneous activity were effectively increased by nearly 8% in going from room temperature to 38 C. Contraction of longitudinal strips of musculature (Fig. 1C) with the cuticle and outer epidermis removed occurred with a time to peak tension of 12 sec. In addition, relaxation times were consistently long, with half-relaxation times taking an average of 36 sec (30 to 60). Circular muscle fibers were dissected away from the body wall and looped on hook electrodes which delivered stimuli; no stimulus intensity used would evoke posterior contraction or cause local contraction of the muscle. Results were the same when just the longitudinal muscles were isolated and stimulated.

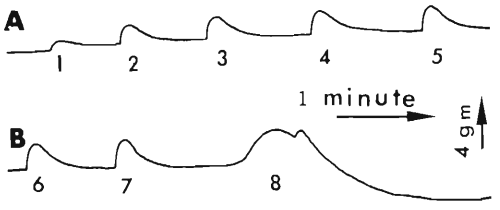

Figure 10. Graded isometric body contractions in response to stimulus trains of different numbers of pulses, separated by 50 msec. Stimuli delivered to inverted body wall fragment near the lateral nerves. The top trace shows the numbered responses—the number represents the number of pulses contained in the stimulus train.

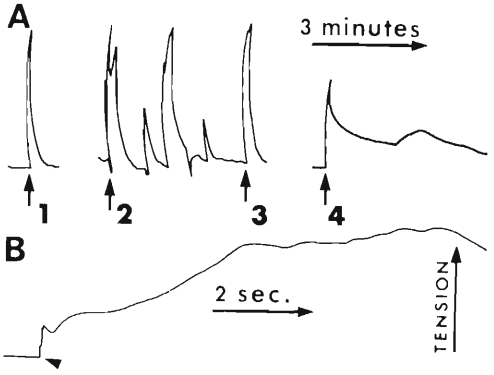

Figure 11. Contraction responses induced by stretch (some marked by arrows). In A-1 a small shoulder is visible during the initial contraction—this marks the end of the stretch. B at faster chart speed shows this initial increase in tension clearly. The contraction usually followed within 0.2 to 0.4 sec. A-2, 3 illustrates typical contractions induced by stretch, while the response in A-4 shows a passive response to stretch by an "old" worm (1 week after being brought to the laboratory).

Discussion

The fact that both circular and longitudinal muscles when dissected free from the body wall could not be stimulated with electrical pulses indicates that the body wall musculature of this acanthocephalan is electrically inexcitable. Our study has shown that upon stimulation of the anterior end, the stimulus was conducted in some fashion to the posterior end of the worm with an average velocity of 0.37 m/sec. This time lag from stimulation to contraction may include factors other than excitation of the muscles via conductive tissue with a characteristic propagation velocity of 0.37 m/sec. The time delay includes the time needed for muscle activation. Also, the time may have included a period necessary for the posterior end of the muscles to move as a consequence of shortening elsewhere—that is, mechanical reexcitation. Inasmuch as the musculature in body segments could be activated by electrical stimulation of whole worms, inverted worms, body wall segments, and body wall strips, it is concluded that there is an electrically excitable system which activated the muscles. Moreover, since the longitudinal muscles responded to pulsed stimuli while the circular muscles did not (preparation shown in Fig. 1B), we conclude that the systems which activate the two sets of muscles are separate. The fact that the circular muscles only responded to d-c stimuli which traversed the body wall while the longitudinal muscles responded to pulses in the anterior–posterior axis supports the argument for two separate activating systems. Tail contracture induced by anterior stimuli was not affected by horizontal cuts above the tail but was reduced appreciably by longitudinal cuts parallel to the site monitored. Thus a portion of the system which transmits information to the muscles is described as being more horizontal than longitudinal in nature. Nevertheless, since the response was reduced but not eliminated it is perhaps best characterized as being a plexus. Indeed, the most common experimental evidence of a plexus is the capability of excitation to pass around a variety of incisions or transections and through narrow bridges of tissue lacking in definitive nerves.

When tissue between monitoring and stimulating sites was severed at lateral positions, stimulation anterior to the cut no longer evoked the expected size of contraction. Small, graded contractions could be evoked, but at much higher stimulus intensities (at least 10 times higher). At these higher intensities even body wall fragments with a variety of lateral, dorsal, or ventral incisions were still able to contract. It is likely that such activity involved some other mechanism and was not due to stimulation of the through-conduction system in the body wall. In whole worms after severing

tissue containing the median-longitudinal lacunar-canal (MLLC) strength-duration curves could not be obtained. In terms of these stimulation experiments, it does seem likely a through-conduction system which parallels the MLLC is involved with conducting impulses to the plexus which activate the body wall muscles.

Our results with the recording of stimulus-induced tail contractions and potentials indicate that the potentials are usually correlated with contractions of the longitudinal muscles but never precede the contraction. Hence, it would seem that the potentials we record are not the causative factor in the contraction.

Contraction in this preparation was a function of pulse height, duration, and frequency. Maximum facilitation of the second contraction in a train was obtained when the second pulse was delivered at the peak of the first pulse action. After 30 min of continuous trains of stimulation the preparation showed a diminished response.

Our experiments in body wall segments denuded of cuticle demonstrated that the presence of the cuticular layer of the body wall dampened the contraction rate of the muscles and also tended to increase the relaxation time of the longitudinal muscles. The reason for the latter may have been simply due to a mechanical spring-type effect on the body wall, or the stripping of the cuticle may have caused a slight activation of the circular muscles which tended to stretch the longitudinal muscles, or it may have been a combination of both. We prefer the first or mechanical explanation. In any event these experiments led us to conclude that the system which conducts impulses to activate the musculature does not reside in or require the presence of the cuticular layer of the body wall. A discussion of mechanical properties is useful for comparative purposes, since many differences among muscles are associated with time relations, as indicated by Prosser and Brown (1961). Generally many mammalian and lower vertebrate muscles that have higher fusion frequencies are associated with shorter contraction and relaxation rates. A comparative table on muscle in Prosser and Brown's text (1961) indicates that a fusion frequency of 100 Hz for cat gastrocnemius is matched by a short contraction time of 0.039 sec; at the other extreme is the *Helix* tentacle retractor with a 0.3- to 1-Hz fusion frequency and a 2.5-sec contraction rate; and similarly, earthworm body wall longitudinal muscle strips develop smooth tentanus at 0.4 Hz. In *M. hirudinaceus*, overall body shortening due to longitudinal muscle contraction required 4 to 7 sec with a fusion frequency between 1 and 3 Hz. Contraction rates calculated from Koopowitz's data (1973) on the flatworm *Gyrocotyl* were approximately 0.18 g/sec with peak tension developed in 2 sec. His preparation consisted of a 3-cm longitudinal section of the body wall, and these relaxed with half-relaxation times of 5 sec. Although he referred to these movements as slow, acanthocephalan rates were even slower, but were capable of developing tension at a higher rate. However, without having such data on individual muscle fibers, little comparison can be made with respect to intrinsic muscle speed. Nevertheless, the results obtained here further characterize the tonic nature of these muscles. The circular muscles appear to be capable of developing tension at a higher rate than the longitudinals with corresponding smaller changes in length, as might be expected. There were also indications that with these muscles, velocity of shortening was some function of the load. This is not surprising since this seems to be a general observation on most muscles (Hill, 1970). The time for contraction to peak tension of the body wall muscles at room temperature is slower than that of most animal muscles exceeding even that of the lantern retractor muscle of the holothurian which has a contraction time of 4 sec (Prosser, 1967). However, the worm normally operates at core temperature in the domestic hog and *in situ* the time to contraction is approximately 2.0 sec which would make these muscles as fast or faster than several preparations including mammalian smooth intestinal muscle! Even at room temperature the potentials recorded from the acanthocephalan are faster than, but comparable to, potentials from intestinal smooth muscles of the cat (Kobayashi et al., 1966, Fig. 8). Our potentials are triphasic and are therefore indicative of a conducted potential. It is perhaps fitting that the muscle which is most like that of the acanthocephalan with respect to contraction time and relaxation time is the intestinal smooth muscle of its host.

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Plethorchis acanthus gen. et sp. n. (Trematoda: Sanguinicolidae) in Mullet, *Mugil cephalus* L., from Queensland, Australia[1]

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ABSTRACT: Testes numbering over 100 and posterior to ceca; vitellaria mainly intercecal anteriorly and mostly asymmetrical posterior to ceca; and spined eggs are distinctive characters of *Plethorchis* which is erected with *P. acanthus* as type.

During sabbatical leave (1970–71) spent in the Parasitology Department, University of Queensland, Brisbane, Australia, mullet, *Mugil cephalus* L., from the Brisbane River were found to harbor blood flukes described here as a new genus and species. Twenty-six of 34 fish from 8 to 32 cm long were infected.

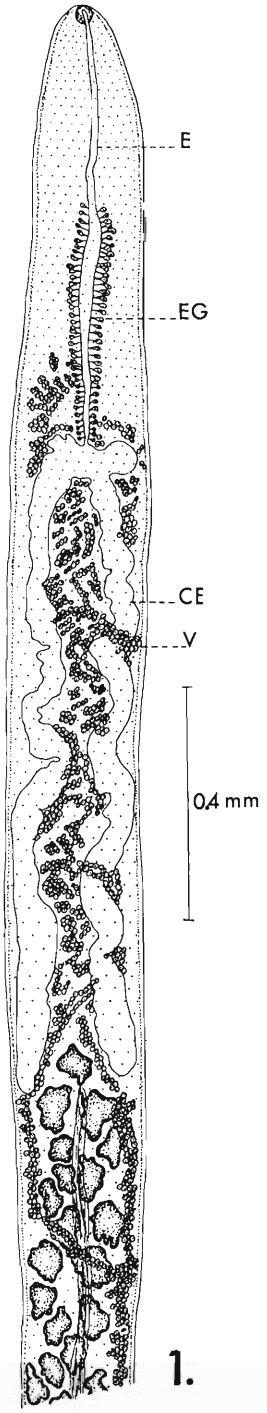
Living worms and specimens fixed without pressure in hot 5% formalin were studied. The latter were stained with Mayer's acid hemalum, cleared in methyl benzoate, and mounted in Canada balsam.

All measurements are expressed in microns unless otherwise indicated. Averages are in parentheses.

[1] Supported in part by NSF G-6962.

Plethorchis gen. n.

DIAGNOSIS: Sanguinicolidae. Body slender and elongate; tegument weakly spined. Suckers lacking. Mouth terminal or subterminal. Esophagus slender and elongate, partly glandular. Anterior cecal rami very poorly developed, posterior rami elongate, equal to subequal in length, terminating anterior to midbody. Vitellaria follicular, asymmetrical with more follicles on right side of body. Ovary on right side near posterior end of body. Uterus posttesticular. Eggs fusiform, shell delicate. Testes numerous, from cecal terminations to uterus. Cirrus pouch



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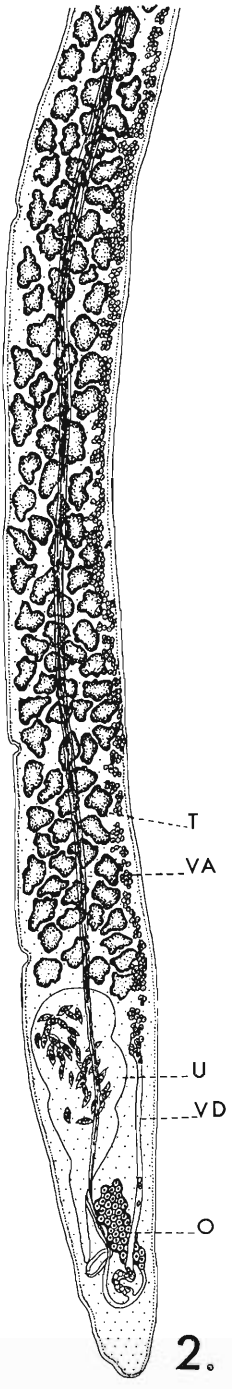

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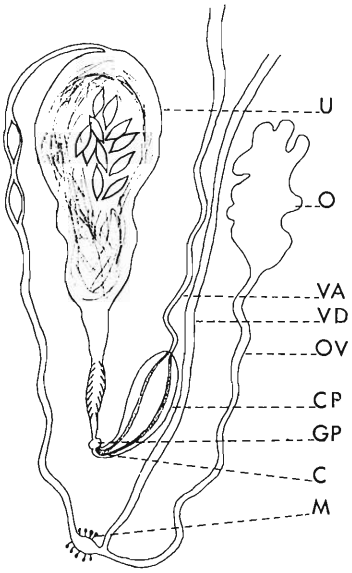

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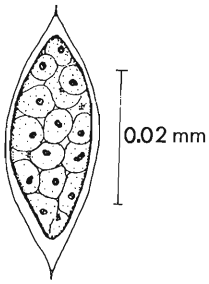

4.

small, with internal seminal vesicle and very short cirrus. Common genital pore near posterior end of body on ventral side to the left of midline. Type species:

Plethorchis acanthus sp. n. (Figs. 1–4)

SPECIFIC DIAGNOSIS: With characters of the genus. Measurements based on 20 whole mounts. Body 3.24–5.4 (4.53) mm; width 189–324 (270). Tegument laterally armed with clusters of three or four minute spines. Esophagus length 324–756 (556), posterior half glandular and occasionally expanded near ceca. Anterior cecal rami extremely short, posterior rami 810–1,755 (1,096) long. A few vitelline follicles anterior to ceca but mainly intercecal to anterior testes; follicles from left cross to right side a short distance posterior to cecal terminations, then extend posteriorly on the right side to near the ovary; vitelline duct joins oviduct near posterior end of body. Ovary lobed, 135–324 (181) long and 81–135 (97) wide. Oviduct begins at posterior end of ovary then passes posteriad to be joined by the vitelline duct. Mehlis' gland weakly developed. Uterus extends anteriad as a narrow tube then expands to form a chamber containing eggs and sperm. The exit of the chamber narrows, contains anterior projections from its lining resembling spines, and empties ventrally into common genital pore. Eggs fusiform with short, sharp spine at each end, 31–57 (46) long and 12–17 (15) wide. Uterine eggs show cleavage but no fully formed miracidia. Lobed testes number well over 100 and extend from posterior cecal terminations to expanded portion of uterus, 55–103 (75) long and 31–57 (48) wide; median vas deferens extends most of length of testes then posteriad to cirrus pouch near posterior half of ovary. Cirrus pouch with internal seminal vesicle and very short cirrus terminates at genital pore. Sperm about 376 long. Excretory bladder v-shaped.

HOST: *Mugil cephalus* L., sea mullet.

HABITAT: Coelom, blood vessels of mesenteries, intestine, and liver.

LOCALITY: Brisbane River, Queensland, Australia.

HOLOTYPE: deposited as No. 7110, Hancock Parasitology Collection, University of Southern California.

Discussion

Plethorchis shares with *Orchispirium* Madhavi and Rao, 1970, asymmetrical vitellaria, a similar location of the ovary, and a common genital pore but differs in having numerous testes instead of one, vitellaria that do not extend to near the anterior end of the body, ceca that end anterior to the testes, a more restricted uterus, and spined eggs. Madhavi and Rao (1970) placed some importance on an esophageal bulb near the cecal junction. In some of my specimens this region is somewhat swollen and contains host blood cells. Perhaps the distension of this region varies with the amount of food ingested. Probably lysis of blood cells occurs here for the ceca do not contain them but are filled with yellow fluid in life.

Some mullet were very heavily infected in the coelom and blood vessels of mesenteries, intestine, and liver. In these areas were clusters of eggs surrounded by orange brown pigment, connective tissue, and what appeared to be calcium. Some eggs contained dead embryos but most had ciliated, nonocellate miracidia that were seen pushing at the ends of the egg shells. It is likely that such action aids in working eggs through tissues.

Worms placed in physiological saline extended both ends of the body with rapid, peristaltic contractions.

The snail, *Posticobia brazieri* (Smith), is abundant in the Brisbane River and is host to at least two species of fish blood fluke larvae. Therefore, laboratory-reared *P. brazieri* were exposed to the miracidia of *Plethorchis acanthus* but none became infected.

Acknowledgments

I am greatly indebted to Professor J. F. A. Sprent, Head of the Parasitology Department,

←

Figures 1–4. 1 and 2, Holotype, dorsal view. 3, Diagram of terminal genitalia. 4, Egg. Abbreviations: C, cirrus; CE, cecum; CP, cirrus pouch; E, esophagus; EG, esophageal gland; GP, genital pore; M, Mehlis' gland; O, ovary; OV, oviduct; T, testis; U, uterus; V, vitellaria; VA, vas deferens; VD, vitelline duct.

University of Queensland, for the use of laboratory facilities and encouragement in many ways; to Dr. John Pearson, Reader in Parasitology, for furnishing laboratory-reared snails and help in many other ways; and to Jim Davie for assistance in collecting fish.

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A New Leech, *Macrobdella diplotertia* sp. n. (Hirudinea: Hirudinidae), from Missouri

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ABSTRACT: *Macrobdella diplotertia* sp. n. from Missouri is described. It differs chiefly from the other three species in the genus by having the copulatory gland pores in three rows of two each.

Seven poorly preserved and somewhat contracted specimens, representing an undescribed species of *Macrobdella*, were received together with brief penciled notes from the late J. Percy Moore. While the entire sample consists of only seven specimens (one immature), the species is so distinct that its description is warranted. The species has been known for many years, but attempts by both Moore and me to obtain additional specimens have been unsuccessful.

The genus *Macrobdella* Verrill, 1872, contains three species, all endemic to North America. The species are *M. decora* (Say, 1824), widely distributed, from Colorado east into Maine and the Maritime Provinces and from Mexico deep into Canada; *M. sestertia* Whitman, 1886, known only from the vicinity of Cambridge, Mass., and not reported since its original description; and *M. ditetra* Moore, 1936, from the southern coastal-plain states, from Texas east into Florida and north into North Carolina.

Unlike the case of *M. ditetra*, the binomen of which first appeared in the papers of other authors (in Brandt, 1936) for whom Moore had identified the species, and thus is available by indication, this has not happened with *M. diplotertia*.

DESCRIPTION (based on seven specimens, one of which was dissected): Body form similar to *Macrobdella decora* of similar size: stout, broad, depressed throughout, and generally tapering anteriorly (Fig. 1). The largest specimen in moderate contraction measures (in mm) 66 in length and 14 in width. The other six vary from 44 to 50 by 7 to 16.

The basic coloration of alcoholic specimens, which are much faded and altered, is light brownish drab dorsally, with 19 paired marginally situated metameric spots usually large and conspicuous, chiefly confined to *a2* of segments VII through XXV; also pale areas indicate the similar position of the median metamerically arranged red spots, which are faded completely. As in *M. decora*, with which *M. diplotertia* is most closely related, apparently the colors are showy in life but fade very quickly in alcohol. Ventrally the basic color is lighter and more or less blotched with irregular black spots. Sensillae may be seen occasionally but are very obscure and on many segments not visible at all. On the dorsum the head is distinctly annulated and bears the usual five pairs of eyes, which are arch-shaped in arrangement. Last pair of nephridiopores is found at the usual location, the posterior margin of *b2* in XXIV, but anteriorly cannot be traced with certainty beyond segment X.

Male gonopore, with one exception, at XI*b6*/XII*b1*. In the exception the male pore is in the middle of XII*b1*, which is enlarged, rugose, and closely connected with a similar area in the middle of XI*b6*, this entire region

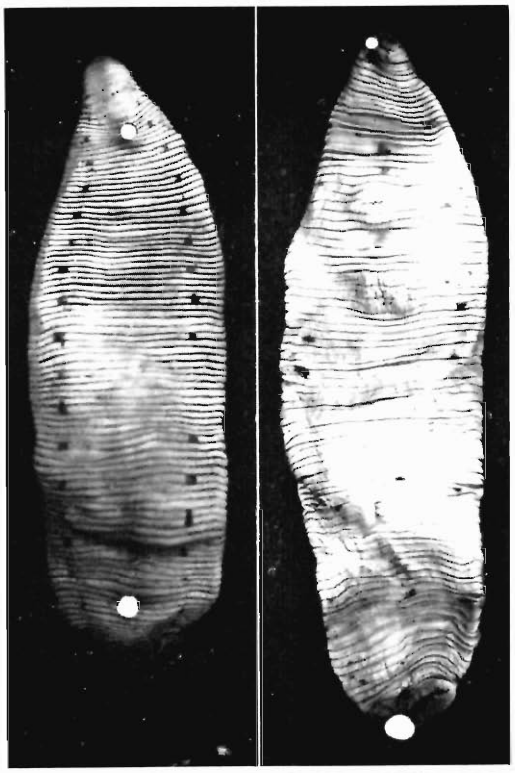


Figure 1. Photographs of *Macrobdella diplotertia* sp. n. Left, dorsal surface showing paired metameric color markings; right, ventral surface showing gonopores and copulatory gland pores. (The white spots are heads of pins.)

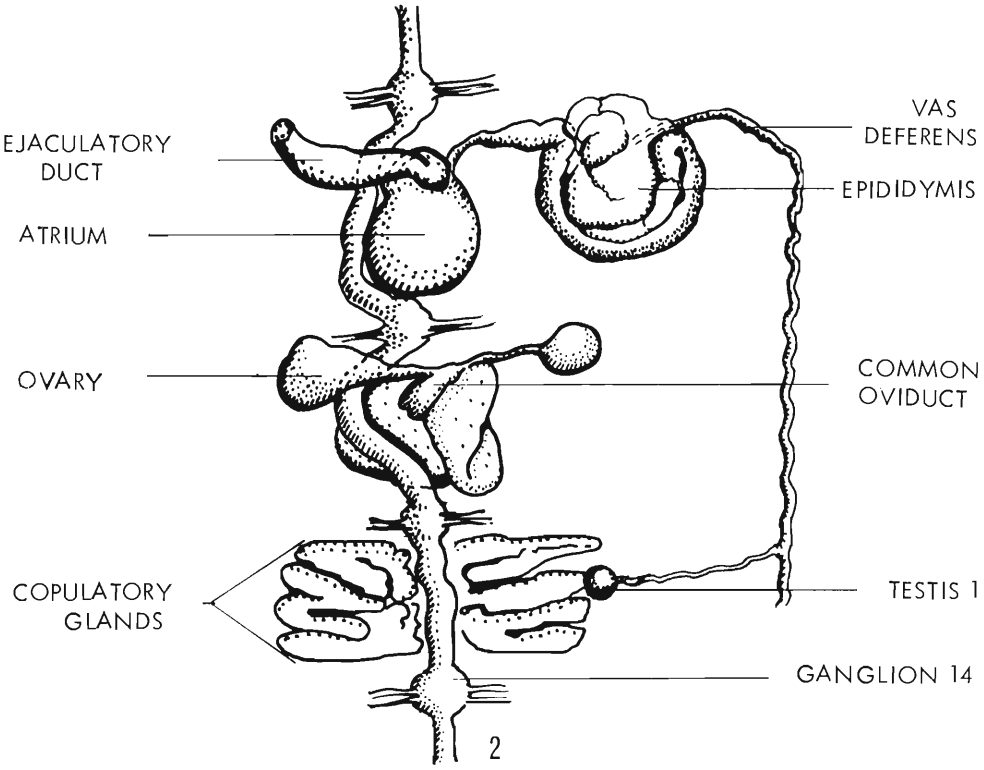
covering about five annuli being slightly elevated. A paratype specimen has a prominent forward-directed sugarloaf-shaped penis with truncately rounded summit and seven longitudinal furrows meeting at the pore.

Female gonopore at XII*b6*/XIII*b1*, elevated on a small mammilliform prominence. Copulatory gland pores are four annuli caudad of the female gonopore in three transverse rows of two each in furrows XIII*b5*/*b6*, XIII*b6*/XIV*b1*, and XIV*b1*/*b2* and each of the three pores occupies contiguous halves of adjacent annuli, which are split by shallow furrows as they approach the area. Between the pores, which lie near the lateral margins of the areas, small furrows separate small squarish areas. The additional pair of pores is added anteriorly. In all specimens the copulatory gland pores are perfectly regular, in number and position, and show none of the variations reported by Moore (1922: 10) for *M. decora*, from Ontario, and Sawyer and Pass (1972), for specimens from Georgia and South Carolina. Clitellum not apparent externally.

There is nothing peculiar about the annulation of this species to distinguish it from others of the genus. Segment I, faintly separated from II, only mesially, comprises the preocular lobe; II and III, both uniannulate, indistinctly separated from I and IV respectively, as well as from each other, have the first and second pairs of eyes; IV slightly subdivided with third pair of eyes; V large and the annuli more distinct dorsally, with the fourth pair of eyes on *a2*, ventrally forms the buccal ring; VI triannulate ($a1 < a2 < a3$) dorsally, with fifth pair of eyes on *a2*, ventrally *a1* and *a2* united to form the second postoral ring; VII triannulate both dorsally and ventrally and *a3* enlarged and with first pair of large, metameric, black spots; VIII quadrannulate ($a1 > a2 > b5 = b6$); IX through XXIII quinquannulate ($b1 = b2 = a2 = b5 = b6$); XXIV quadrannulate, $b1 + b2 + a2 + a3$, with *a3* slightly enlarged and clearly subdivided ventrally; XXV triannulate, $a1 = a2 = a3$; last pair of black spots on *a2*; XXVI biannulate ($a1 + a2$) *a3*, the first is only slightly larger than the second; XXVII biannulate, annuli about equal in size and much smaller than those of XXVI. Anus at XXVII/XXVIII.

The jaws, about twice as long as high, similar to those of other species of the genus. Teeth on an unpaired jaw 57, small and monostichodont. The three jaws of another specimen were removed and mounted, but the teeth were missing, either having become detached or destroyed by chemical action as a result of the long preservation. Pharynx short, reaching to about IX, followed by an even shorter esophagus. Each segment, X through XVII, is provided with two pairs of gastric ceca, which are large from XIII posteriorly. The last pair, which originates from the stomach in XIX, reaches posteriorly into XXIV or XXV. There is nothing unusual about the narrow intestine, which opens dorsally at XXVII/XXVIII.

No significant differences from that of the closely related *M. decora* can be detected in the reproductive systems (Fig. 2). The vasa deferentia are glandular, and follow sinuous



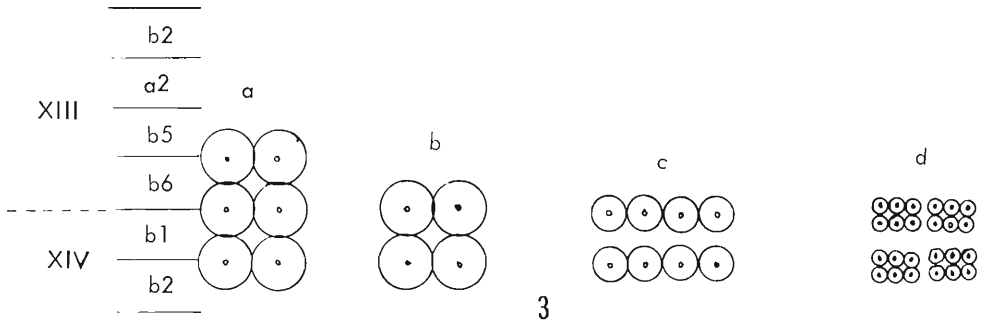


Figure 2. ***Macrobdella diplotertia*** **sp. n. Reproductive system, dorsal view. Figure 3. Diagram of copulatory gland pores of** ***Macrobdella*** **species, with segment-annuli symbols: (a)** ***M. diplotertia*****; (b)** ***M. decora*****; (c)** ***M. ditetra*****; (d)** ***M. sestertia*****.**

courses anteriorly. In segment XI they become narrow and lose their glandular coating. At the level of the 11th ganglion they turn abruptly into compact, massive, and much convoluted epididymes. From the posteromedian end of each of the latter a wide, slightly folded and coiled ejaculatory duct leads to the terminal organ, the atrium. Just before joining the muscular wall of the latter the ducts constrict, and then form a pair of slightly enlarged sacs, which proceed upward to open together into the terminal portion of the male evagination. As seen

from above the atrium appears perfectly spherical but it is clear that the pair of ejaculatory ducts bends abruptly dorsad within the muscular sheath. The male organs lie to the right of the nerve cord.

While the female organs are similar to those of *M. decora*, both unpaired oviduct and vagina are longer and more slender than in that species. The ovaries and vagina are distinct and well separated by a narrow band and the two oviducts unite in a short common slightly folded portion as in *M. decora*. The vagina is short, ventral to the nerve cord, bent on itself, and opens to the right of the nerve cord.

The copulatory glands, the external openings of which have been described above, form a conspicuous mass occupying the posterior half of segment XIII and the anterior region of the floor of XIV.

Nothing is known of *M. diplotertia*'s host preference(s) but *M. ditetra*, according to Moore (1953: 9), has a predilection for frogs and *M. decora*, for frogs, their eggs, and fish, also feeds on oligochaetes and other aquatic invertebrates (Moore, 1923: 24). Rupp and Meyer (1954) reported fish attacked by *M. decora* succumbed to their infestations.

In summarization, the more prominent characters available for distinguishing *Macrobdella diplotertia* from other species of the genus are (Fig. 3): first pair of copulatory gland pores is situated four annuli caudad to the female gonopore, pores in three transverse rows of two each (6), hence the specific appelation *diplotertia*; in *M. decora* the first pair of gland pores is five annuli posterior to the female gonopore, pores in two transverse rows of two each (4); and in *M. ditetra* the first pair of gland pores is five annuli behind the female gonopore, pores in two transverse rows of four each (8). Normally five annuli separate the gonopores in both *M. diplotertia* and *M. decora*; in *M. ditetra* and *M. sestertia* they are separated by 2 and 2.5 annuli, respectively.

REMARKS: Seven specimens available, from the Osage River, Osage County, Missouri, without further localization. Holotype (dissected) deposited U. S. National Museum Helminthological Collection, No. 51789. Paratypes (6) also in the USNMH Collection, No. 51790.

Acknowledgments

Grateful appreciation is due Albert A. Barden, Jr., whose critical reading of the manuscript contributed to its improvement, and Richard R. Eakin, who assisted with the illustrations.

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Supplement to the Life History of *Strongyloides ransomi* Schwartz and Alicata, 1930 (Nematoda: Strongyloididae) of Pigs[1]

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ABSTRACT: *S. ransomi* possesses three routes of infection: transmammary, percutaneous, and prenatal. The transmammary and percutaneous routes occur commonly in nature with prenatal occurring rarely. The larvae passed in colostrum and milk resemble filariform larvae with the exception of the genital primordium being longer, wider, and possessing more cells. Upon reaching the small intestines, the transmammary-passed larva reaches patency in 36 to 48 hr, consequently, the early egg passage by piglets. Barring further exposure, patency will persist for approximately 20 weeks. Percutaneous infection must occur for gilts and sows to store larvae in adipose tissue. Accumulation predominates in the ventroabdominal (mammary) area. Exposure prior to or as late as the final 4 to 6 weeks of pregnancy will insure transmammary passage of larvae. Without further exposure, shedding of larvae in colostrum and milk persists for at least three lactations. Hormonal changes occurring at parturition possibly influence migration and passage of larvae in colostrum and milk.

The life history of *Strongyloides ransomi* has been described in detail by Schwartz and Alicata (1930) and by Lucker (1934). Observations by Moncol and Batte (1966) revealed a transmammary route of infection.

The occurrence of intrauterine infection of pigs by *S. ransomi* has been proposed by Enigk (1952), Enigk et al. (1974), Stewart et al. (1963, 1969), Stone (1964), and Supperer and Pfeiffer (1967). The latter investigators found only one larva in the lungs of stillborn pigs or piglets killed immediately after birth, and interpreted this to mean that at birth the larvae are still in the vascular system and, therefore, scattered throughout the entire body. Yet eggs were regularly detected in the feces of baby pigs beginning the 4th day after birth. Enigk et al. (1974) observed larvae of *S. ransomi* in the tissues of only two of 47 piglets removed by Caesarean section. The sows had been infected at various stages of pregnancy, though both infected piglets occurred in sows infected in the first half of pregnancy. No patent infection followed.

Frickers (1953) observed *Strongyloides* eggs in the feces of 3- and 4-day-old piglets. Intrauterine infection was suspected yet no larvae were found in stillborn littermates. He ascertained that some other means of infection existed but did not elaborate.

Olsen and Lyons (1965) showed that larvae of *Ucinaria lucasi* were transmitted in the colostrum of the northern fur seal. The parasitic third-stage larvae were passed postpartum in the milk for only a short time. The intestinal phase of infection was shown to persist for approximately 3 months in the seal pups. The tissue phase, consisting of parasitic third-stage larvae, occurred in all age groups of seals particularly in the blubber along the belly region. In females, third-stage larvae were also present in the mammary glands and milk cisterns.

Being unable to demonstrate intrauterine infection with *S. ransomi* and having found no ova or larvae in the feces of near parturient gilts or sows, Moncol and Batte (1966) concluded that some other mechanism of infection had occurred. Larvae were observed in colostrum immediately prior to parturition and subsequently in milk. Piglets nursing these sows, or if given larvae collected from filtered milk, became infected and passed typical *Strongyloides* eggs on day 4 postpartum. Supperer and Pfeiffer (1967) and Stewart et al. (1969) subsequently confirmed the occurrence of transcolostral infection.

Other reported species of nematodes that are transmitted through milk are *Strongyloides westeri* in the horse (Lyons et al., 1969), *Strongyloides papillosus* in the sheep and cow (Lyons et al., 1970), *Toxocara cati* in the cat (Swerczek et al., 1971), and *Neoascaris vitulorum* in cattle (Warren, 1969), *Ancylostoma caninum* and *Toxocara canis* in the bitch (Stone

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and Girardeau, 1967), and *Strongyloides papillosus* in the goat (Moncol et al., 1973). Details on the biology and morphology of the transcolostral phase of S. *ransomi* as a supplement to the existing life history are presented in this paper.

Materials and Methods

Pigs with natural infections of S. *ransomi* were used as the source of ova. Larvae for experimental infections were obtained by culturing feces from infected pigs. Best results were obtained when feces were mixed with peat moss, covered with four layers of gauze, and incubated at 24 to 27 C. After incubation, only the top two layers of gauze were carefully removed to Baermann funnels for collection of filariform larvae, as this procedure proved to eliminate nearly all of the free-living forms.

Colostral larvae were obtained from naturally and experimentally infected sows. During experimental infection, pigs were always kept in concrete-floored isolation pens that were washed daily.

Following the intravenous injection of 10 IU of oxytocin, larvae were obtained from colostrum and milk that was collected by hand-stripping. The colostrum and milk were diluted with dechlorinated water and then passed through an AP-200 Millipore Filter (Millipore Filter Corporation, Bedford, Mass.). The filter was washed over a petri dish and the contents were examined under a dissecting microscope.

Larvae used for size determinations, whether from milk or tissue, were freshly collected and heat-fixed prior to being measured. Tissues to be examined for larvae were minced in a blender and then baermannized in 30 C heated funnels.

Experimental infections in most studies were accomplished either by subcutaneous injection or by direct application of larvae to the skin under moistened larvae-laden gauze pads. The exceptions to this involved experiments in which larvae were injected directly into adipose tissue.

Results

The life cycle of S. *ransomi* consists of two basic routes of infection: (1) transmammary (colostrum and milk), in which case the susceptible host (newborn piglet) receives advanced third-stage larvae that go directly to the small intestine and (2) percutaneous invasion by infective third-stage larvae (filariform) which undergo a period of tissue migration.

A. Morphogenesis

1. Morphological development of colostral (milk) larvae: Table 1 compares the measurements of filariform larvae, colostral larvae, and larvae collected from the mammary glands and adipose tissues. Larvae collected from colostrum of sows or gilts immediately prior to and during paturition measured 469 to 561 μ (519.9) long by 16 to 21 μ (19.0) wide in the region at the base of the esophagus. The length of the esophagus was 245 to 301 μ (279.0). Distance from anus to the tip of the tail was 53 to 75 μ (63.0). The genital primordium measured 12.7 to 23 μ by 4.6 to 6.9 μ (16.0 by 6.0). The trifid tail typical of the infective third-stage larva was present.

Except for being slightly larger and the genital primordium being longer, wider, and more conspicuous, colostral larvae are similar to filariform larvae. None of the larvae collected from colostrum, mammary, or adipose tissue were undergoing a molt or showed any evidence of an impending molt.

2. Development of colostral larvae within the small intestine of piglets: Table 2 compares the size of larvae collected from the small intestines of piglets at 28, 50, and 75 hr postnursing. The wide range in measurements reflects the fact that piglets continue to take in larvae with each nursing. The development of the reproductive system was quite advanced at 28 hr with ovaries extending anteriorly and posteriorly from the primordium of the vulva. Specimens were mature at 50 hr and developed ova were present in the uteri of female worms.

B. Natural infectivity

1. Duration of intestinal phase: Four pigs reared in the laboratory with only transmammary infection were shown to void eggs for at least 20 weeks. Egg production diminished to a point 12 to 15 weeks after infection where conventional flotation techniques would no longer give a positive test. After this time, eggs can only be demonstrated by centrifugation. Under normal conditions of rearing where percutaneous reinfection existed fol-

Table 1. Size comparison of *S. ransomi* filariform larvae, colostral larvae, and larvae collected from mammary glands and adipose tissues.

Measurements (μ)	Filariform larvae			Culture	Source of larvae*		
	(a)	(b)	(c)		Colostral	Subcutaneous adipose tissue	Mammary gland
Total length	504–635	500	405–420	490.6	519.9	522	526
Esophageal length	240–310	—	250–258	238.6	279.0	277	284
Esophageal width	15–19	—	13	14.7	19.0	19	19
Length of tail	60–90	—	—	62.3	63.0	67	66
Length and width of genital primordium		10–17	17	9×2.3	16×6	17×6	18×6

(a) Schwartz and Alicata, 1930.
(b) Lucker, 1934.
(c) Alicata, 1935.
* Average of 25 larvae each source.

lowing initial transmammary infection, ova production diminished rapidly and was detectable for only 8 to 10 weeks.

2. EXTENT AND DURATION OF TISSUE PHASE: The lactating sow is a vital link in the perpetuation of S. *ransomi*. For the sow to infect the next generation of pigs, it is understandable that she must be adequately exposed to infective larvae sometime prior to parturition and lactation. Ova produced by the young parasitic females during the first 3 to 4 weeks undergo homogenic development exclusively, thereby intensifying the infection in the piglet and replenishing the stores of larvae within the adipose tissues of the female host (Moncol, unpublished data). During this period, the sow develops a very transitory intestinal infection that lasts 3 to 4 weeks. Likewise, exposure to a large number (10^5) of filariform larvae immediately and within 1 week after parturition will extend the time of passage of larvae in milk. At this time only small numbers of larvae (< 1 per cc of milk) were intermittently observed. A wide variation in the degree of infection exists among litters and even among littermates.

Naturally infected sows were held for three consecutive farrowings, without additional exposure to S. *ransomi*. Larvae passed in the milk at each succeeding parturition declined at the following rate: 5 larvae per cc colostrum, 1st lactation; 1 larvae per cc, 2nd lactation; and 0.2 larvae per cc, 3rd lactation. The infection may have persisted longer, but this phase of the experiment was terminated after the third lactation.

Secretions were not taken from the mammary gland of the sow until immediately (12 to 24 hr) prior to parturition. It was not determined whether the larvae were present in the rapidly developing secretory portion of the mammary gland, or whether the larvae moved into the area during the final hours prior to parturition.

3. DISTRIBUTION OF LARVAE IN TISSUE: The location and concentration of larvae in the tissues of the naturally infected sow are compared in Table 3. Larvae were found primarily in subcutaneous adipose tissue. The adipose tissue surrounding the mammary gland contained the greatest number of larvae. Internal adipose deposits were free of larvae. Relatively high proportions of larvae were found in the adipose tissue along the rib cage and dorsum of the back. The absence of any inflammatory reaction (Fig. 1) was seen upon histological examination of biopsied adipose tissue. In essence, the tissue appeared normal, with the exception of larvae being present. The larvae appeared to locate between the fat cells.

Table 2. Measurements of colostral larvae* taken from small intestines of piglets (μ).

Areas measured	Hours postnursing 50†	75
	28	
Total length	1,064–1,694	3,486–4,494	5,264–5,894
Length of esophagus	420–658	588–756	588–924
Width of esophagus	22–29	42–70	70–84

* Based on 15 larvae each observation period.
† Eggs present in uteri.

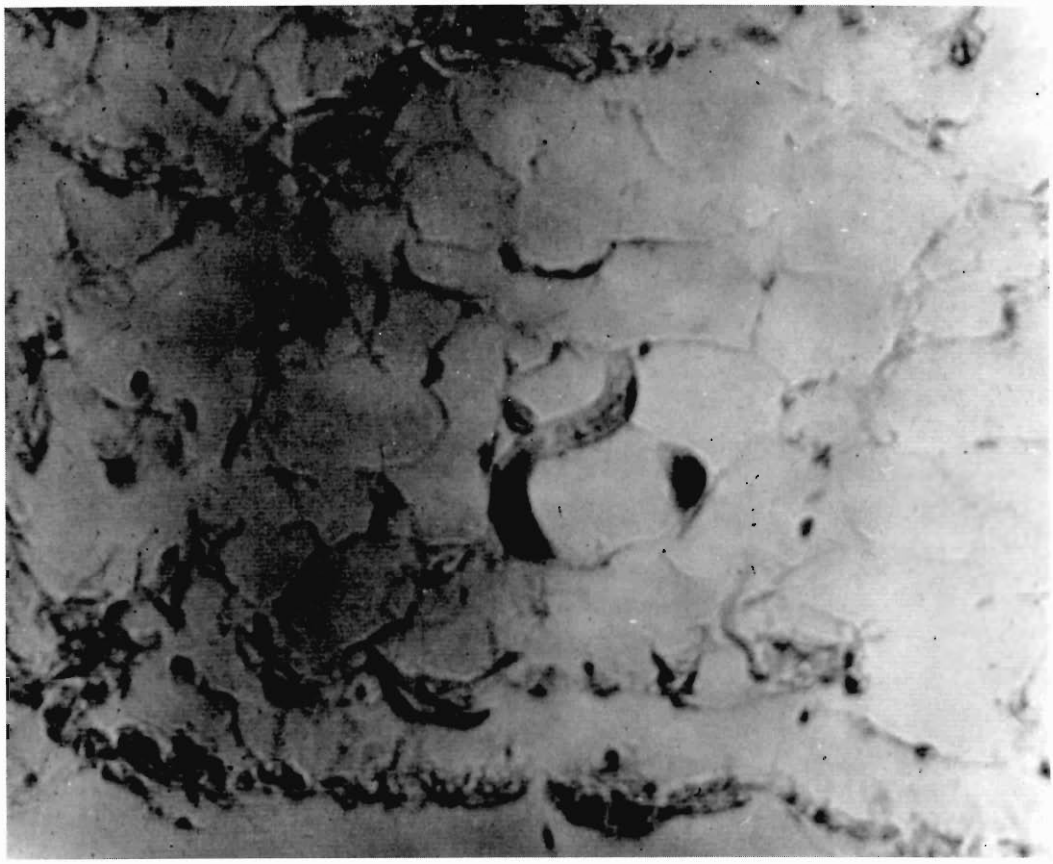

Figure 1. Photomicrograph of *S. ransomi* in adipose tissue—mammary area.

C. Experimental infections

1. RATE OF DEVELOPMENT: Table 4 compares the rate of development of larvae taken by biopsy from adipose tissue at 3 and 7 days postexposure. The only outstanding morphologic feature was the increased size (length and width) of the genital primordium. Division progressed to the eight-cell stage. The total length, total width, and length of the tail were within the normal range for those of filariform larvae. Even though the length and width of the genital primordium had increased, it had not attained the size observed in colostral larvae or larvae recovered from adipose tissue and mammary gland.

2. MIGRATION OF TISSUE LARVAE: Biopsies of mammary glands were taken 4 to 7 days prior to parturition and entire glands were surgically removed 14 days prior to parturition. Material examined histologically and by baermannization gave negative results. In each case, however, the piglets became infected. Venous blood samples taken from sows during parturition and examined by filtration were negative for *S. ransomi* larvae.

3. EXPOSURE OF THE PREGNANT FEMALE: Naturally infected gilts held in isolation for 3 to 4 months (no exposure during pregnancy) passed larvae in colostrum and milk and infected their offspring. Gilts reared with no exposure following colostral infection failed to have larvae in milk or to infect their offspring.

Five gilts reared free of colostral and percutaneous infection were exposed (4 divided

Table 3. Location of *S. ransomi* somatic larvae in tissues of naturally infected sows.

Tissues*	Larvae/100 g
Subcutaneous dorsum of back	17
Subcutaneous lateral body wall (prefemoral)	15
Subcutaneous inguinal	1
Subcutaneous lateral body wall (costal)	92
Mammary gland and fat	200
Subcutaneous abdominal wall 10 cm lateral to mammary gland	225
Omentum	Neg
Pericardial fat	Neg
Submaxillary fat	<1
Subperitoneal fat	<1
Rear leg muscle	Neg
Lung	Neg
Heart	Neg

* Five samples of 50 g each.

doses of approximately 1.3 million larvae 3 to 10 days apart) during late gestation (35 to 15 days before farrowing) had larvae in their milk and infected their offspring.

Discussion

The occurrence of transmammary infection by S. *ransomi* and a number of other nematode species has been well documented. The onset of occurrence, duration of persistence, and concentration of larvae per given volume of milk varies for each species. These variations are probably attributable to the location of stored larvae, factors responsible for inciting migration and migratory pathways.

It has been shown that larvae of S. *ransomi* are present in colostrum and later in milk of the sow. Piglets taken from the sow at birth and reared on an artificial diet were free of *Strongyloides* infection (Moncol and Batte, 1966).

Stewart et al. (1969) observed larvae in the tissues of fetuses of sows subjected to massive numbers (7 to 10 million) of filariform larvae during late gestation. However, it is not likely that the sow would naturally be exposed to such large numbers of larvae either at a given time or physiological state. Enigk et al. (1974) observed larvae in the liver, lungs, and small intestines of only two of 47 piglets taken by Caesarean section from sows heavily infected in the first half of pregnancy. The examination of fetal tissues of stillborn and live fetuses from sows reared under normal circumstances yielded no larvae.

Larvae often are present in great numbers in the colostrum prior to parturition and, subsequently, in milk. Passage of *Strongyloides* eggs

Table 4. Development of *S. ransomi* in adipose tissue.

Days after injection	Measurements* in microns (mean)			
	Total length	Width	Length of tail	Length and width of genital primordium
3	560	18.5	72.2	12.7×4.9
7	539	17.6	67.0	13.6×4.4

* Based on 10 larvae each observation period.

in the feces of piglets in 2 to 4 days postpartum substantiates the theory that early infection exists. Also, the fact that sows and gilts were not voiding eggs in feces further confirms the theory that transmammary infection is the first and most common means of infection. Furthermore, since patency does not develop until 6 to 7 days following percutaneous infection, this negates the idea that infection occurs as a result of larvae coming from a contaminated environment. Actually, this initial infection serves to contaminate the environment, whereby the piglet increases its worm burden and permits the sow to rebuild her store of larvae for subsequent lactations. If this source of infection is great enough, the time that larvae are passed in milk of the sow may be extended.

Pigs receiving the only transmammary infections continued to void eggs for 6 to 8 weeks longer than pigs maintained under constant exposure conditions. This suggests that the intestinal infection resulting from transmammary infection failed to provide an antigenic stimulus that would enable the host to develop a detectable degree of immunity or sensitization. Limited observations of S. *ransomi* females indicated that they contained few eggs. It was not possible to determine whether pigs under constant exposure eliminated significant numbers of worms in addition to the suppression of egg production. Gilts and sows without recent exposure developed an intestinal infection of short duration (3 to 5 weeks). They voided only a limited number of eggs (100 to 300 epg) when compared to young pigs (*20,000* to *100,000* epg). It would appear that a portion of the infecting larvae remained in the adipose tissues of sows and gilts. The time required for larvae to develop in tissue for transmammary passage was relatively short. This was demonstrated when pregnant gilts were exposed within the

final 4 to 6 weeks of gestation. Measurements of larvae from adipose tissue indicated that development of the genital primordium occurred quite rapidly (3 to 7 days).

Stone et al. (1967) applied *S. ransomi* larvae to the skin of 21-day-old pigs and found larvae in the lungs and other internal organs and leg muscles in 12 hr; juveniles were found in the small intestine within 36 hr. Egg voiding began on day 6 postinfection. This form of migration is commonplace in the young piglet and, to some extent, in the adult pig. On the other hand, young pigs (12 to 20 weeks old) and mature gilts (8 to 12 months old) that received frequent exposure stored larvae in adipose tissue. It was not determined whether male pigs stored larvae. Even if they did, the larvae would end up in a "dead-end" host. Olsen and Lyons (1965) observed that larvae of the seal hookworm taken from males and nonpregnant cows were not infective.

The ease of recovering filariform larvae from the adipose tissues of the subcutaneous ventral abdominal area (mammary area) from gilts and sows indicated a larval predilection for this tissue and anatomical location. Even though larvae were found in adipose tissues some distance from the ventral abdomen, the mammary area appeared to be the site of choice. Larvae are known to penetrate the dermis very rapidly; consequently, the posture of the animal (ventral or lateral recumbency during exposure) may explain this wide distribution of larvae. The reason for larval concentration in adipose tissue remains unknown. One theory that seems plausible is that adipose tissue receives limited circulation and, thereby, larvae may be free to develop and roam in an environment with few macrophages and antibodies.

Stewart et al. (1973) reported the recovery of viable *Strongyloides* larvae 20 to 30 cm from the site of injection in as little as 7 to 11 weeks. Even though *S. ransomi* as well as other members of the genus *Strongyloides* possesses this phenomenal ability to migrate, the mechanism of larvae translocation from adipose tissue to milk ducts remains undetermined.

Webster et al. (1958) and Olsen and Lyons (1965) have postulated that migration is induced by hormonal changes occurring near or at the time of parturition. The fact that larvae were not found in biopsies of mammary tissues as late as 10 days prior to parturition would add support to this theory. The idea of vertical migration of larvae from adipose tissue adjacent to alveoli and ducts of the mammary glands seems most convincing.

Smyth (1962) discussed the evolution of the host–parasite relationship and cited several examples of parasites whose life cycle is apparently regulated by host hormones, particularly reproductive hormones. He further pointed out that this phenomenon leads to the synchronization of the life cycle of the parasite with that of its host. Dunsmore (1966) observed that during the breeding season the female rabbit *Oryctolagus cuniculus* developed a greater nematode infection than the male and ovariectomized females. He stated that "the host-parasite relationship has evolved in such a way that the parasite population is reproducing at a maximal rate at the most suitable time for ensuring the success of the next generation of the parasite." In the instance of *S. ransomi*, even though it is not reproducing, its presence in milk has been synchronized to infect the piglet, the most susceptible host. The role of hormones on parasitism has been the subject of numerous reports: Salisbury and Arundel (1970), Gibbs (1967), Dunsmore (1971), and Oshima (1961).

The reproductive state of the host, especially lactation, has been shown to inhibit the onset of the self-cure reaction (Connan, 1967, 1970, 1972). Dunsmore (1965) showed that a marked *Ostertagia* spp. egg count rise occurred in all lambing ewes at or closely associated with parturition. The ewes had not themselves picked up many *Ostertagia* spp. larvae during the latter part of their pregnancy; consequently, the parturient egg count increase resulted from larvae ingested prior to or early in pregnancy. Penned ewes given repeated oral doses of *Ostertagia* spp. larvae during midpregnancy also delayed output of eggs till parturition.

A different model of host–parasite relationship probably exists for each species of parasite. The complexity of the relationship existing between the pig (sow) and *S. ransomi* is probably no greater than with others. In fact, it bears resemblance to the relationship existing between the ewe and *Ostertagia*.

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Anthelmintic Efficacy of Injectable Levamisole in Sheep[1]

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ABSTRACT: A controlled anthelmintic trial was conducted to evaluate the efficacy of injectable levamisole against gastrointestinal and lungworm helminth infections in sheep. Injectable levamisole was highly effective (88.3 to 99.8%) against abomasal and small intestinal helminths. Small numbers of cecal, large intestinal, and lungworm helminths in the experimental lambs precluded meaningful evaluation of efficacy against these parasites. The dorsolateral aspect of the neck was chosen as the most logical injection site from a labor-saving standpoint.

Early investigations of the broad-spectrum anthelmintic, levamisole, demonstrated high efficacy when the drug was administered either orally or subcutaneously (Forsyth, 1966, 1968; Thienpont et al., 1966; Turton, 1969; Walley, 1966). It was noted, however, that the hydrochloride salt was very irritating to tissues and when injected intramuscularly or subcutaneously in cattle, moderate to severe reactions occurred at the site of injection (Anonymous, 1973).

Subsequently, a relatively nonirritating injectable form of levamisole for bovine use has been developed and marketed in the United States, but studies have not been reported on this product (Anonymous, 1973). Parenteral administration offers a distinct advantage since the appropriate dose is given each animal, in contrast to the uncertainties of oral administration. Expectoration frequently occurs with oral medication, which can result in decreased efficacy and/or increased treatment cost. A parenteral drug such as levamisole would, therefore, be economically advantageous to both livestock producers and the general public. This paper reports the results of a controlled experiment designed to investigate the anthelmintic efficacy and tissue reaction of an injectable formulation of levamisole in sheep.

Materials and Methods

Experimental animals

Twelve lambs, originating from one flock, were selected in mid-September on the basis of suitably high quantitative fecal egg counts (epg) (Whitlock, 1948a), as well as diversity of parasite species. Although present, *Strongyloides papillosus* and *Trichuris* spp. eggs were not counted during epg examinations. The lambs were individually identified with numbered ear tags at the initial time of sampling.

The experimental lambs were transported to Corvallis by truck and were confined to an earth floor corral at this laboratory for 6 days prior to initiation of the experiment. The lambs were fed a mixture of grain and hay so as to maintain body weight. Water was provided ad lib.

On experimental days -6, -2, and -1, a rectal fecal sample was collected from each lamb. Quantitative epg counts for gastrointestinal nematodes and quantitative larvae per gram counts (lpg) (Walters and Andersen, 1973) for lungworm larvae (*Dictyocaulus filaria*) were performed on each sample.

Administration of anthelmintic

On experimental day 0, the mean epg was calculated for each animal and the lambs were randomized on this basis into two groups of six animals per group (Gardiner and Wehr, 1950). Lambs in Group I were used as negative controls (placebo-treated); lambs in Group II were treated parenterally with levamisole at a dose rate of 8 mg/kg (Ripercol L–levamisole phosphate, 18.2% solution; American Cyanamid Company, Princeton, N. J.). The calculated dose of placebo (saline) or levamisole was administered subcutaneously to two lambs in each group in each of three body sites, i.e., right neck, right ventrolateral thorax (area devoid of long wool posterior to olecranon), and right flank. The injection sites were examined on experimental day 2 and again on experimental day 8 when all the lambs were killed.

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At necropsy, each carcass was skinned, the injection site was examined, and lesions were recorded. Major body organs, i.e., heart, kidneys, liver, lungs, and spleen, were examined grossly for lesion suggestive of drug toxicosis.

At necropsy, the gastrointestinal tract of each lamb was collected and subdivided into abomasum, small intestine, and cecum and large intestine. Each organ was placed in a separate pan, incised longitudinally, and the contents were collected. The mucosa of each organ was scraped, then washed three times. The contents, scrapings, and washing from each organ were collected in gallon jugs and preserved in 5% formalin. Abomasums were subjected to acid–pepsin digestion for recovery of immature nematodes (Herlich, 1956). The abomasal digest material also was washed over a 150-mesh sieve and then added to the abomasal material.

After 3 days fixation, the abomasal and small intestinal contents were washed separately over a 150-mesh Tyler analytical sieve. The material remaining on the sieve was collected in a 3-liter beaker and sufficient tap water was added to make a final volume of 2 liters. A magnet was added to the beaker and the beaker was placed on a magnetic stirrer. After thorough mixing and during agitation, five 50-ml aliquots were removed and placed in separate beakers.

The five abomasal and five small intestinal aliquots were examined from treated lambs (Group II). Sufficient abomasal and small intestinal aliquots were examined from control animals (Group I), so that a minimum of 100 worms were represented. Iodine was used to assist in recovery of worms (Whitlock, 1948b).

The cecal and large intestinal contents were washed over a 20-mesh sieve with a 150-mesh sieve beneath. Adult worms were picked off the 20-mesh sieve with forceps, enumerated, collected, and preserved in vials containing 5% formalin. Three 50-ml aliquots were removed after the material remaining on the 150-mesh sieve was diluted to 2 liters and mixed. These aliquots were examined for immature nematodes which may have passed through the 20-mesh sieve.

The contents from each vial containing picked worms were poured into a ruled petri dish, mixed, and the first 50 nematodes found were removed. Each parasite was transferred to a clean glass slide, cleared in lactophenol, identified as to the stage of development, and the males were speciated. Female worms and larvae were identified to the generic level. The percentage and actual number of each nematode stage and species for each organ was calculated from the above data.

The respiratory tract, including trachea and lungs, was collected from each lamb. The trachea, bronchi, and small air passages were incised longitudinally and examined grossly for nematodes. Nematodes found were collected with forceps, counted, and preserved in 5% formalin. The lungs were then placed with the opened surface down in a 2-liter pan of warm isotonic saline. Crystalline penicillin G was added to each pan to reduce bacterial putrefaction (Baker et al., 1972). The pans were incubated overnight at room temperature. The following morning, the pulmonary tissue was rinsed and removed from the pan. The contents of the pan were washed through a 150-mesh sieve and the material remaining on the sieve was collected and examined for lungworms under magnification (20×).

Results and Discussion

Fifteen minutes after treatment, frothy salivation and coughing were noted in one levamisole-treated lamb. The reaction, however, was transient and the lamb appeared normal the following morning and for the remainder of the experiment. Untoward reactions were not noted in the remaining 11 lambs following treatment.

On experimental day 2, slight subcutaneous edema (3 × 0.25 cm) was evident at the injection site in the two lambs treated in the right axilla with levamisole. Visible reactions were not evident at this time in the lambs treated in the neck or flank areas.

One control lamb died on experimental day 4 from a *Clostridium septicum* infection. Parasitologic examinations were conducted on the carcass and the data derived from this animal were used.

At necropsy, tissue reactions were not evident at the injection sites of the six control lambs. Tissue reactions consisting of moderate subcutaneous edema and hemorrhage (3.0 × 0.25 cm) were noted in one lamb in each of the axillary- and neck-treated groups, and a small localized necrotic area (1.0 × 0.25 cm) was

Table 1. Average worms counts, ranges, and percentage efficacy of injectable levamisole against nematode parasites in lambs.

Organ and parasite stages	Treatment groups: Control average (range)	Treatment groups: Levamisole-treated (8 mg/kg) average (range)	Percentage efficacy
Abomasum			
Total, all stages combined	12,699.0 (2,688–24,430)	210.8 (33–858)	98.3
Total, adult worms	11,258.6 (2,605–23,830)	121.8 (3–375)	98.9
Total, immature worms	1,440.0 (82–6,752)	89.0 (3–483)	93.8
Small intestine			
Total, all stages combined	9,932 (2,131–24,208)	41.7 (0–120)	99.6
Total, adult worms	9,703.5 (1,953–24,208)	15.0 (0–40)	99.8
Total, immature worms	228.7 (0–4,748)	26.7 (0–96)	88.3
Cecum and large intestine			
Total, all stages combined	43 (4–102)	0.5 (0–3)	—
Total, adult worms	34 (4–102)	0.5 (0–3)	—
Total, immature worms	9 (0–53)	0	—
Lungs			
Total, all stages combined	14.8 (0–52)	1.0 (0–4)	—
Total, adult worms	8.8 (0–22)	0.5 (0–1)	—
Total, immature worms	6.0 (0–36)	0.5 (0–3)	—

evident in one lamb treated in the right flank. Evidence of skin damage was not present, and it did not appear that skin sloughing would have occurred. Gross lesions, possibly indicative of drug toxicity, were not found in the tissues of any levamisole-treated lambs.

There appeared to be little difference in tissue reaction among the three treatment sites. The skin area devoid of long wool immediately posterior to the olecranon and the medial aspect of the flank were judged as more suitable injection sites when compared to the neck since the long wool on the neck impaired accurate subcutaneous placement of the anthelmintic. The injection site on the neck was located at the cervicothoracic junction, where a fold of skin could easily be raised. This site and the technique of injecting at the base of a raised fold of skin assured, so far as was practical, subcutaneous injection. The axillary area was selected as the preferred injection site since it overlies the ribs, a relatively inexpensive cut, in the event abscission occurred after treatment and trimming was necessitated at slaughter. Also, the axillary area provided easier access than the flank. The obvious disadvantage of either ventral locations was the necessity of handling the lambs individually. Hence, it would appear that from a labor-saving standpoint, a dorsal body location will be selected by sheepmen as an injection site.

The average worm counts, ranges, and overall efficacy data are listed in Table 1. The average number of the various nematodes recovered from the experimental lambs and efficacy of injectable levamisole against individual species are listed in Table 2.

Very high efficacy (98.3%) was achieved against the combined stages of abomasal parasites, with corresponding efficacies of 93.8 and 98.9% against immature and adult stages, respectively. Hence, most stages of medium and small stomach worms were very susceptible to injectable levamisole. A possible explanation for the poor efficacy against L-4 *Trichostrongylus axei* is that a preponderance may have been

Table 2. Average number and species of nematodes and percentage efficacy of injectable levamisole against nematode parasites in lambs.

Organ and species	Control No. worms	Treated groups: Levamisole-treated (8 mg/kg) No. worms	Treated groups: Percentage efficacy (% reduction)
Abomasum			
Adults			
Ostertagia circumcincta	8,564.2	87.2	99.0
Ostertagia trifurcata	603.2	6.2	99.0
Teladorsagia davtiana	1,503.6	4.0	99.7
Trichostrongylus axei	587.6	21.2	96.4
Immature			
Ostertagia spp., L-4	1,407.6	60.8	95.7
Trichostrongylus axei, L-4	32.3	31.5	2.5
Small intestine			
Adults			
Cooperia oncophora	100.7	0	100
Nematodirus abnormalis	296.0	0	100
Nematodirus filicollis	335.3	0	100
Nematodirus helvetianus	385.5	0	100
Nematodirus spathiger	2,454.0	3.0	99.9
Strongyloides papillosus	212.6	12.0	94.4
Trichostrongylus vitrinus	5,919.3	0	100
Immature			
Nematodirus spp., L-4	30.8	22.7	—
Trichostrongylus vitrinus, L-4	197.8	4.0	98
Cecum and large intestine			
Chabertia ovina	23.0	0	—
Oesophagostomum venulosum	5.3	0	—
Trichuris ovis	5.7	0.5	—
Lungs			
Dictyocaulus filaria, adult	8.8	0.5	—
Dictyocaulus filaria, L-4	6.0	0.5	—

inhibited larvae at the time of treatment. Inhibited L-4 probably are not actively metabolizing, and, consequently, are insusceptible to anthelmintics (Baker and Walters, 1971; Reid and Armour, 1972).

Very high efficacies (94.4 to 100%) were achieved against all stages of small intestinal parasites.

Small numbers of cecal, large intestinal, and lungworm parasites present in the experimental lambs precluded meaningful evaluation of injectable levamisole against this group of parasites (Tables 1, 2). Efficacy values, therefore, were not calculated. It would appear from inspection of the data, however, that this group of parasites was susceptible to injectable levamisole.

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A Left-handed *Grubea* sp. from the Pacific Coast, Baja California, Mexico

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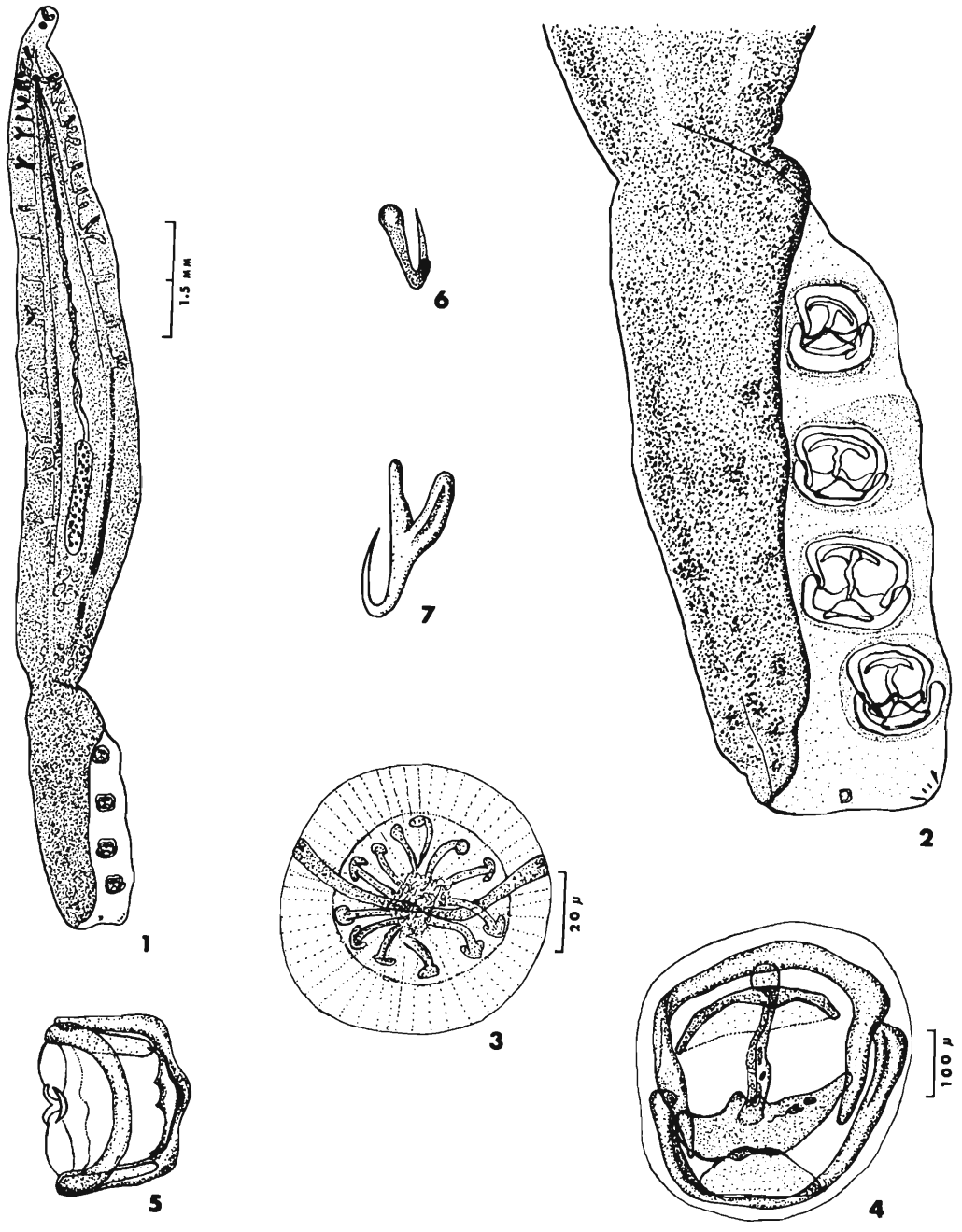
ABSTRACT: A *Grubea* sp. collected from the gills of the Pacific bonito, *Sarda chiliensis*, is described. It is the first *Grubea* sp. reported from the proximity of the Pacific Coast of the USA and from the bonito host. It differs mainly from the other species described, namely *G. cochlear* from Europe and *G. pneumatophori* from the Woods Hole region, Massachusetts, in that the opisthohaptoral clamps are on the left side, rather than on the right, and there is an extra subtriangular piece in the clamps. A new species designation is not made because only one specimen is available for study and some structures are not discernible.

Two species of *Grubea* of the family Mazocraeidae have been described. They are *Grubea cochlear* Diesing, 1858 (syn. *Octobothrium scombri* Nordm. ? of Grube, 1855; *Pleurocotylus scombri* van Beneden et Hesse, 1863), on the gills of *Scomber scombrus* of Europe, and *Grubea pneumatophori* Price, 1961 (syn. *Pleurocotyle scombri* of Linton, 1940), on the gills of *Pneumatophorus grex*, Woods Hole, Mass. The latter species description is based on a single specimen of poor condition collected in 1908 and is of doubtful specific status (Price, 1961). *G. cochlear* has previously been reported from the Mediterranean on the gills of the mackerel, *Scomber scombrus*, from Genoa, Italy, and on *S. colias* from Naples, Italy. *G. pneumatophori* of the Atlantic from the Woods Hole region, Massachusetts, was found on the gills of the "chub mackerel," *Pneumatophorus grex* (= *Scomber colias*).

Apparently this form is seen very infrequently as descriptions are based on few specimens. Manter (1956, pers. comm.) found it strange that *Grubea* is always found in such small numbers, and was inclined to believe that the true or most favorable host has not yet been found.

Sproston (1946) reports that "Though the gills of over a thousand mackerel have been examined for this trematode throughout the year, from various places off the S. W. coast of England, none has been found."

In June 1966, during the examination of marine fishes collected at Ensenada, Baja California, Mexico, a single specimen of *Grubea* sp. was found on the gills of the Pacific bonito, *Sarda chiliensis*. The specimen was observed alive in saline and it was noted that the clamps of the opisthohaptor were on the left side rather than on the right side as described for the *Grubea* sp. Upon examination of the stained and mounted specimen it became apparent that



Figures 1–7. *Grubea* sp. of the Pacific. 1. Whole mount, dorsal view (body), ventral view (opisthohaptor). 2. Opisthohaptor enlarged, showing the four sinistral clamps, ventral view, and the dextral small clamp and anchors. 3. Genital corona. 4. Haptoral clamp, one of four. 5. Small clamp. 6. Small, inner anchor. 7. Outer anchor.

Table 1. Measurements in mm and structural details of the known *Grubea* spp.

	Pacific *Grubea*	*Grubea cochlear*	*Grubea pneumatophori*
Body			
Length × width	12 × 1.5	10–15 × ?	8.6 × 1.5
Prohaptoral Suckers	0.120 × 0.075	—	0.096 × 0.056
Opisthohaptoral			
Clamps (large) width	0.40 (sinistral)	0.518 max. (dextral)	0.43 (dextral)
Clamp (small) width	0.060 (dextral)	0.070 (sinistral)	0.044 (sinistral)
Anchors			
Large, outermost, length	0.024	—	0.040
Small, innermost, length	0.013	—	0.028
Pharynx	0.084 × 0.087	—	0.09 × 0.08
Genital aperture (from ant. end)	0.73	—	0.6
Genital Corona			
No. hooks (inner)	12	13–16	13–14
Inner hooks	0.015	0.012	0.02
2 outer hooks	0.033	—	0.02 (on pad)
Testes	Probably numerous	Numerous	Few
Ovary	Elongated	Cylindrical	U-shaped
Vagina	Probably double	Double	Double
Vitelline follicles	Beginning 0.39 below genital aperture. Dense posteriorly	Absent anterior portion; diffuse, extending into caudal disc	Occupying almost entire body from genital aperture into opisthohaptor

the large clamps were indeed in the sinistral position, in ventral view, but that the body of the trematode was twisted over on itself so that it was presented in dorsal view (Figs. 1–7). The tear that is seen in the haptor above and below the four main clamps may have been present before the worm was removed, or the tear may have occurred upon removal from the gills, and the haptor was folded over before fixation.

A description of this form is given in the hope that it may help to clarify the taxonomic status. Also, the locality record in the proximity of the Pacific side of the USA and the listing of the new host may be of value and interest.

Although the specimen is significantly different from the two species described (which may be synonyms), no new species designation will be made because only one specimen is available for study, some structures are not clearly discernible, and because prior descriptions of *Grubea* are inadequate and based on few forms seen. The Pacific *Grubea* (Figs. 1–7) is compared with the two described species in Table 1, based on descriptions by Diesing (1858), Palombi (1949), Sproston (1946, after Parona and Perugia), Linton (1940), and Price (1961).

The most significant differences of the Pacific *Grubea* sp. as compared to the other species are: (1) Reversed position of the four large sinistral clamps and one small dextral clamp as compared with the two described species. (2) The large anchors are more robust and the roots appear more nearly equal in length than those of the two described species. (3) The large clamps have an extra element, a subtriangular piece. (4) There are 12 atrial or genital hooks, as compared with 13 or 16 in *G. cochlear* and 13 or 14 in *G. pneumatophori*. (5) The clamps are of equal size and shape, whereas in *G. pneumatophori* the posterior clamp is conspicuously smaller than the other three.

The subfamily Grubeinae Price, 1961, is to be emended to read, in part ". . . with four modified mazocraeid clamps in a vertical row along right margin and a single minute clamp on the left side, or with the four main clamps on the left side, and the small clamp on the right side, the single clamp comparable in position to most posterior on opposite side, . . ."

As noted by McMahon, 1964, reversal of the opisthohaptor or larval end in asymmetrical monogenids has been noted in other forms, such as in *Scomberocotyle scomberomori*.

Acknowledgments

Grateful acknowledgment is made to the late Harold W. Manter who examined the specimen described here, and to Mary Hanson Pritchard who did a critical reexamination of the form and gave valuable points of information.

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The Effect of Anthelmintic Therapy upon Early Parasitic Stages of the Dog Hookworm, *Ancylostoma caninum*

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ABSTRACT: Twenty-five beagle dogs, 5½ to 6 months of age with no history of prior exposure to hookworm, were infected percutaneously with 6,000 infective *Ancylostoma caninum* larvae per dog. Groups of five dogs each were treated with vincofos (18 mg/kg) on 1, 2, 6, or 10 days post-exposure to the infection. One group remained as the nonmedicated controls. Due to the severe hookworm infection, most of the dogs in the days 1 and 2 treatment groups and the nonmedicated controls died within 12 to 18 days of exposure. All dogs in the days 6 and 10 treatment groups survived to termination of the experiment on the 23rd day of infection. Based upon the numbers of worms collected from the nonmedicated controls versus those from the treatment groups, therapy on the 1st day of infection reduced the hookworm population by 8%, on the 2nd day, 51%, on the 6th day, 93%, and on the 10th day, 94%. It was concluded that the drug was not effective for the third-stage larvae migrating in the tissues, but was effective for those larvae (late third or early fourth stages) returning to the intestinal lumen, and highly effective for the fourth-stage larval and juvenile hookworms.

The organophosphorous anthelmintic, vincofos (2, 2-dichlorovinyl, methyl, n-octylphosphate), has shown a high degree of anthelmintic efficacy for the nematode and cestode parasites of dogs (Hass and Collins, 1974). During one of the clinical trials, a limited number of dogs were treated and held for a period of time to determine the anthelmintic effect upon immature or juvenile hookworm populations. No hookworm eggs were subsequently found in the feces which suggests that the drug could have an effect upon the immature forms of this parasite. This report presents the data obtained from a study wherein dog hookworm infections were treated at timed intervals to determine anthelmintic efficacy for the immature parasites.

Materials and Methods

Twenty-five beagle dogs, with no history of prior exposure to hookworm infections, 5½ to 6 months of age and weighing 6.08 to 7.44 kg, were used in this study. Each dog was given a single percutaneous exposure to 6,000 infective

hookworm, *Ancylostoma caninum*, larvae, (kindly supplied by Wm. M. Stone, University of Georgia, Veterinary Diagnostic and Investigational Laboratory, Tifton, Georgia 31794). These dogs were randomly divided into five groups of five dogs each with subsequent allowance for uniform distribution of the sexes. The dogs were group-housed in indoor pens with dry dog chow and water available ad lib.

The first group of dogs was treated with a 10% vincofos in mineral oil formulation at 18 mg/kg on the 1st day, a second group was treated on the 2nd day, a third group was treated on the 6th day, and the fourth group was treated on the 10th day postexposure to the hookworm infection. The fifth group of dogs remained as the nonmedicated controls.

Some of the test dogs died due to the hookworm infection during the period of study and a necropsy of these dogs was made as soon as possible after their death. Necropsy of the surviving dogs was conducted on the 23rd day of infection. At necropsy, the intestinal tracts were opened and washed onto a 60-mesh screen, and the washings were retained in a formalin solution. The worms from these washings were then identified and counted. No attempt was made to collect worms passed in the feces following treatment of the infected dogs.

As a matter of interest, 50-g samples of muscle were taken from the right thigh of each dog at necropsy and subjected to artificial gastric digestion (20 g pepsin, 50 ml HCl, 2,000 ml H_2O @ 37 C). The digests were concentrated to 10 ml and viewed microscopically for the presence of any parasitic hookworm larvae.

Results and Discussion

As evidenced by the numbers of hookworms recovered at necropsy (Table 1), this strain of hookworm larvae was highly infective and populations representing approximately 25 to 60% of the larval exposure were established in these dogs. The dogs had been preselected to fit a narrow weight and age range so as to make a uniform group of test animals. The exposure dose of 6,000 larvae (represents an approximate 400 larvae per pound of body weight) and the use of dogs with a history of no prior exposure to hookworm infection (i.e., "hookworm naive" dogs) also added to uniformity of test conditions. The results were a dramatic case of clinical canine hookworm infection.

Blood-tinged and black tarry stools were observed on infection day 8 or 9 and were followed, in some cases, by a bloody diarrhea. Manifestation of these severe aspects of clinical hookworm infection continued in the host until death occurred or they were altered by chemotherapeutic intervention. These responses show the direct pathologic capacity of fourth-stage larvae and juvenile hookworms beginning approximately 1 week before eggs from patent infections are first found in the feces. The death of some dogs at 12 to 14 days of infection further exemplifies the pathogenicity of this infection prior to patency at 14 to 16 days. As will be noted in Table 1, several thousand hookworms were recovered from the nonmedicated control dogs and on the 12th day of infection, this group had lost about 0.6 kg of body weight. In addition, all five dogs died during the period of the 13th through 18th day of infection.

When treated on the 1st or the 2nd day of infection, the hookworm populations were reduced but the loss of body weight and mortalities followed a pattern similar to the nonmedicated controls. However, more than 50% of the hookworm populations were affected by drug therapy in dogs treated on the 2nd day of infection. It is at this time that the larvae are entering the intestinal lumen following a migration from the site of injection to the heart and through the lungs. Thus, it is speculated that the drug therapy had little effect upon the third-stage larvae in the tissues but appears to be highly efficacious for those precocious larvae which have migrated into the intestinal lumen at the time of treatment on the 2nd day of infection. It is thought that these larvae would represent the late third or early fourth stages of larval development. Hookworms migrating into the intestinal lumen after the passage of the drug are thought to be the probable cause of the continued morbidity and mortality that was observed with this group.

None of the dogs treated on infection day 6 showed clinical signs of morbidity and all five dogs survived to termination of the experiment. A 93% reduction of mean hookworm populations was accorded this treatment group which

Table 1. Anthelmintic data from dogs treated with vincofos (18 mg/kg at timed intervals following exposure to infective *Ancylostoma caninum* larvae.

Dog No.	Sex	Kg body weight–day*			Necropsy		
		−1	+12	+23	Age days	No. worms	% take†
Group 1 treated 1 day postexposure to infective larvae							
261	F	6.76	5.90		14	2,126	35.4
277	F	6.24	5.70	5.45	23	2,024	33.7
278	F	7.26	6.60		13	3,168	52.8
295	M	7.36	6.75		15	1,293	21.5
303	M	7.10	6.80		12	3,802	63.4
Mean		6.94	6.35		15.4	2,483 (8.3%)‡	41.4
Group 2 treated 2 days postexposure to infective larvae							
272	F	6.88	6.10		18	657	10.9
274	F	6.18	5.50		17	1,273	21.2
282	M	7.32	6.10		15	1,238	20.6
284	M	7.42	7.00	5.75	23	1,583	24.4
304	M	6.50	6.65		14	1,832	30.5
Mean		6.86	6.67		17.4	1,317 (51.4%)‡	21.9
Group 3 treated 6 days postexposure to infective larvae							
265	F	7.28	7.60	7.35	23	375	6.3
275	F	6.60	6.65	6.80	23	182	3.0
276	F	7.44	7.80	7.75	23	129	2.1
297	M	6.52	7.00	7.10	23	156	2.6
305	M	6.58	7.55	7.35	23	5	0.1
Mean		6.88	7.32	7.27	23	169 (93.7%)‡	2.8
Group 4 treated 10 days postexposure to infective larvae							
264	F	6.76	6.40	6.80	23	116	1.9
266	F	7.28	6.25	6.35	23	66	1.1
267	F	6.98	6.60	6.85	23	53	0.9
290	M	6.40	5.80	5.65	23	384	6.4
298	M	7.20	6.00	7.25	23	182	3.0
Mean		6.92	6.21	6.58	23	160 (94.1%)‡	2.6
Group 5 nonmedicated controls							
258	F	6.12	5.86		18	1,855	30.9
263	F	6.08	5.50		13	3,317	55.3
271	F	6.64	5.80		18	1,518	25.3
292	M	6.74	5.90		14	3,233	53.9
301	M	6.24	5.70		13	3,619	60.3
Mean		6.36	5.75		15.2	2,708	45.1

* Days relative to age of hookworm infection.
† Based upon an exposure of ~6,000 infective larvae and the numbers of adult worms recovered at necropsy.
‡ Calculated mean per cent reduction.

characterized the fourth stage of hookworm development. Further, this group showed the only increase of body weight on infection day 12 as compared with all other treatment groups. These results emphasize the utility of a drug effective for the fourth larval stage of canine hookworm infections and the elimination of the pathologic responses associated with this parasitic infection.

All of the dogs in the group treated on infection day 10 also survived the entire study period and were cleared of 94% of their overall populations which represented the late fourth, early fifth, and juvenile stages of canine hookworm development. These dogs, however, did not receive their treatment until after severe signs of morbidity were manifested by the parasitic infection. At the time of treatment, all of the dogs were showing a bloody diarrhea and/or black tarry stools and on infection day 12 these dogs had lost the greatest amount (avg = 0.7 kg) of body weight making this a most unique treatment group. Regardless of the adverse conditions of parasitism, all of the dogs survived the entire study period. Also, these dogs were regaining their body weight and, in some

cases, had returned to their preinfection weights. Their recovery to a state of generally good health is remarkable relative to the morbidity and mortality observed with the nonmedicated controls or dogs treated on infection days 1 and 2. Further, it is of interest that dogs showing clinical signs of canine hookworm parasitism can apparently be successfully treated as late as 1 or 2 days prior to that time when death would be imminent.

It is thought that the digest solutions would not adversely affect the somatic larvae although none were recovered from the thigh digest solutions for all of the dogs. No attempts were made to check other host tissues. Therefore, it is assumed that the use of "hookworm naive" dogs permitted extensive larval migration via the lungs and the subsequent development of the large numbers of hookworms which were recovered from the gastrointestinal tracts of these dogs.

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Fine Structure of the Larval Stage of *Paragordius varius* (Leidy, 1851) (Gordioidea: Paragordidae). II. The Postseptum

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ABSTRACT: The structure of the postseptal portion of the larval stage of *Paragordius varius* has been examined by means of electron microscopy. The postseptal body wall consists of a cuticle, an underlying hypodermis, and six longitudinal muscles arranged peripherally. Contained within the postseptal area are two glands and two groups of undifferentiated cells. The postseptal gland consists of several vacuole-filled cells whose contents apparently collect in a cuticularized duct. This duct traverses the septum and opens at the tip of the proboscial portion of the preseptum. The other gland, the pseudointestine, consists of four cells enclosing a large cavity. These cells secrete small granules into the lumen of the gland and coalesce into larger entities, the refringent granules. The lumen of the pseudointestine opens to the exterior via a canal (exit duct) onto the posterior ventral surface of the body.

In this study the electron microscope was employed to obtain a more accurate concept of the larval anatomy of *Paragordius varius*. The anatomy of the preseptum (anterior portion of the larva) and a brief historical review are presented in Zapotosky (1974).

Materials and Methods

Larval stages of *Paragordius varius* were collected by incubating egg strings collected from two ovipositing females. Egg strings with fully developed larvae were fixed in ice-cold, phosphate-buffered 1% osmium tetroxide (pH 7.2) for 4 hr. The specimens were dehydrated in a graded series of ethanol and then placed in a graded series of epoxy resins and 100% ethanol. Tissue sections were counterstained with uranyl acetate and lead citrate, then examined with the electron microscope. For a more detailed description of specimen treatment see Zapotosky (1974).

Observations

General features

The larval body of *Paragordius varius* consists of two major areas: a preseptum (an-

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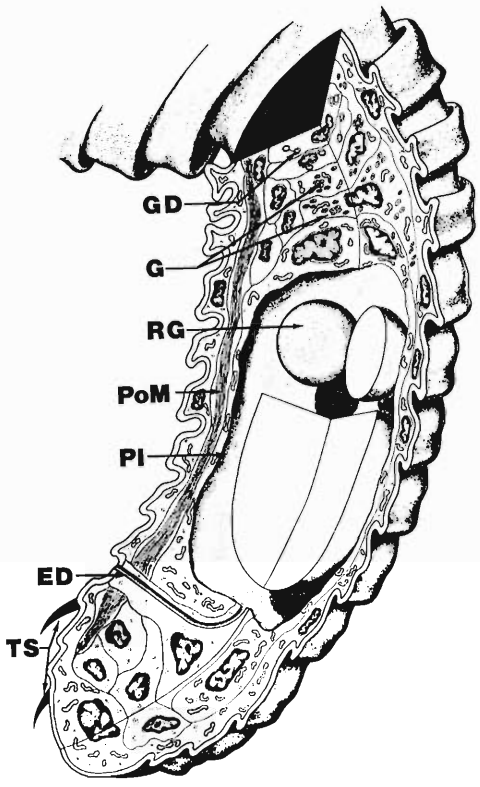

Figure 1. A three-dimensional diagram of the postseptum of the larva of *Paragordius varius*. A section has been removed to show internal structures.

terior) and a postseptum (posterior) divided externally by a constriction and internally by the septal complex. The postseptum (8–10 μ in diameter and 30–38 μ in length) is generally cylindrical, possessing a somewhat rounded and slightly enlarged posterior end (Fig. 4). Two pair of spines are located on the posterior ventral portion of the postseptum (Figs. 3–5). For an overall arrangement of internal structures see Figure 1.

Body wall

The surface of the postseptum is thrown into folds giving the body an annulate appearance. This annulation is superficial and involves only the cuticle and the hypodermis. The folds are irregular and in longitudinal sections the numbers of folds on each side of the postseptum do not correspond. The ultrastructure of the cuticle is the same as found for the postacanthal region of the preseptum (see Zapotosky, 1974). However, the hypodermis consists of a nucleate layer immediately underlying the cuticle (Fig. 8). No cell membranes were observed between hypodermal nuclei, although bounded internally and externally by these membranes. It is assumed to be of a syncytial nature.

A large pair of motile tail spines (3 μ long) are located laterally and posterior to a smaller (2 μ long) pair of ventrolateral spines (Figs. 1, 3–5). These spines are modifications of the cuticle and have a similar makeup as reported for the spines of the preseptum (see Zapotosky, 1974).

Muscles

The postseptal musculature consists of a set of longitudinal muscles (six peripheral cells), the mucles of the lateral tail spines, and fibrils found in association with the exit duct of the pseudointestine.

The postseptal parietal muscles originate on the septal complex and insert near the posterior

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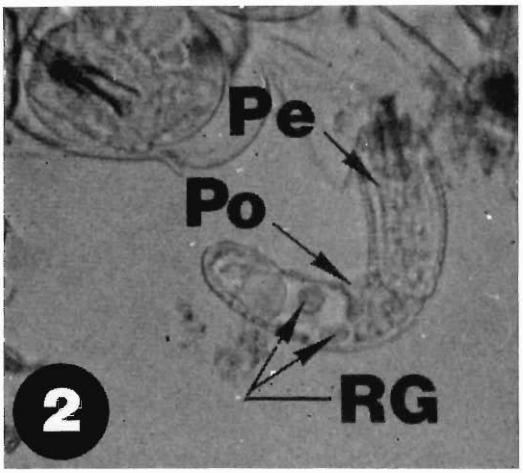
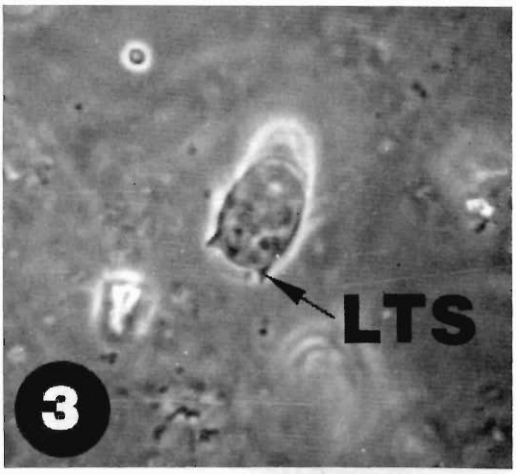
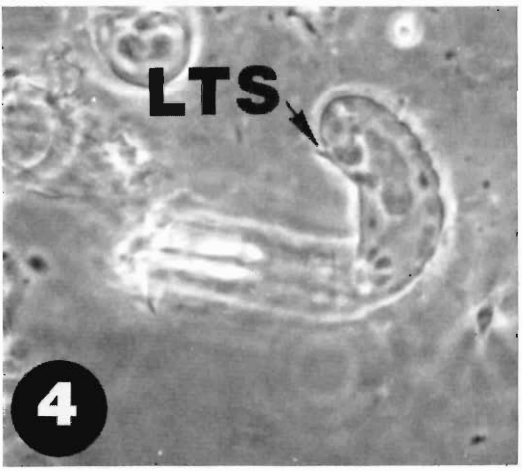
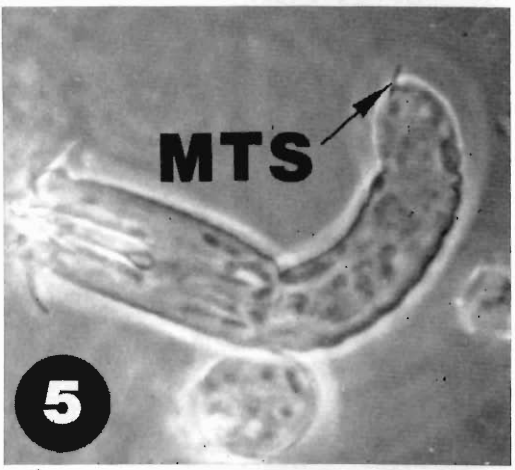
Figure 2. Light micrograph of a living larva escaping from egg membranes. 800 ×.
Figure 3. Phase contrast micrograph of a living larva, dorsal view. 800 ×.
Figure 4. Phase contrast micrograph of a living larva, lateral view. 800 ×.
Figure 5. Phase contrast micrograph of a living larva, lateral view. 800 ×.
Figure 6. Cross section through the gland duct. 67,300 ×.
Figure 7. Cross section through exit duct of the pseudointestine. 31,890 ×.

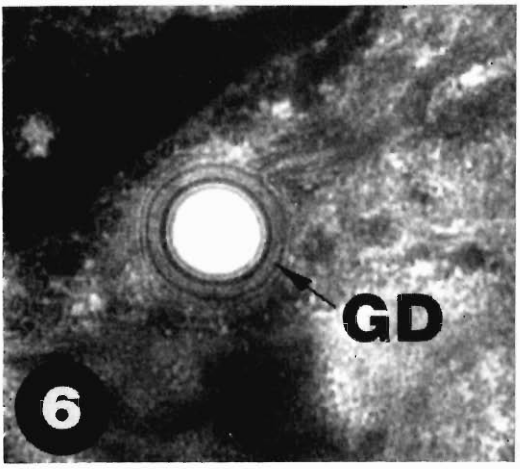
Abbreviations: C, cuticle; ED, exit duct; EM, egg membrane; G, postseptal gland; GD, gland duct; GDC, gland duct cell; H, hypodermis; LTS, lateral tail spine; MTS, median tail spine; Par, partition containing exit duct; Pe, preseptum; PI, pseudointestine; PIC, pseudointestinal cell; Po, postseptum; PoM, postseptal muscle; Se, septum; TS, tail spine.

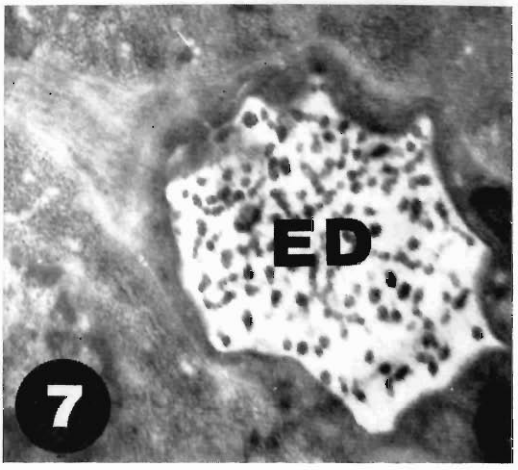


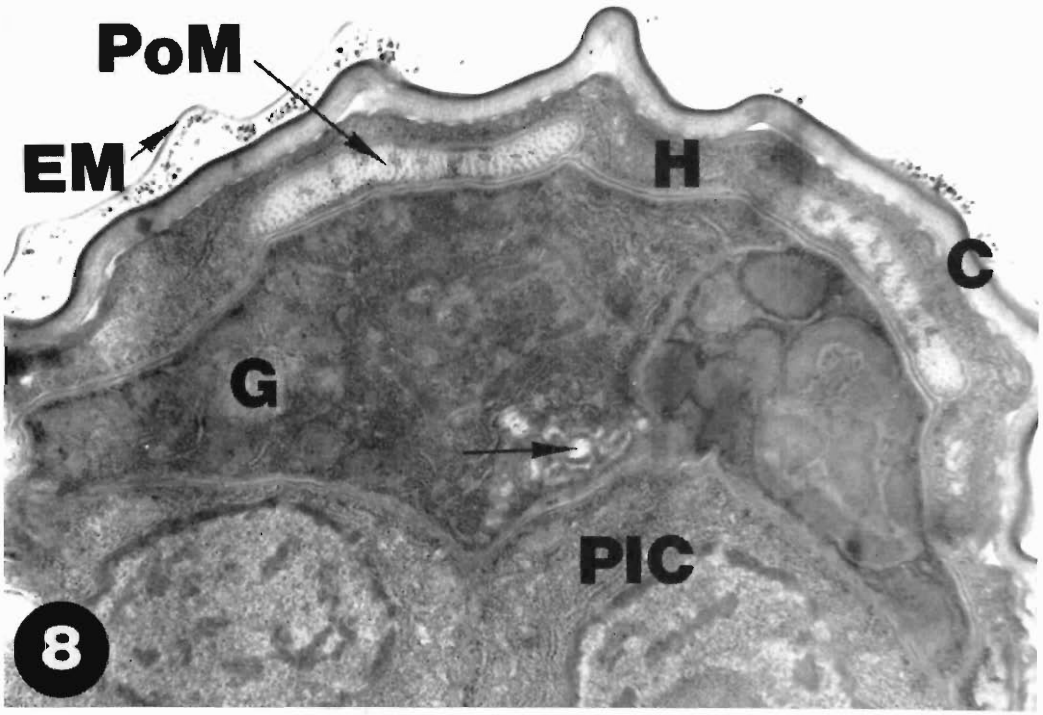


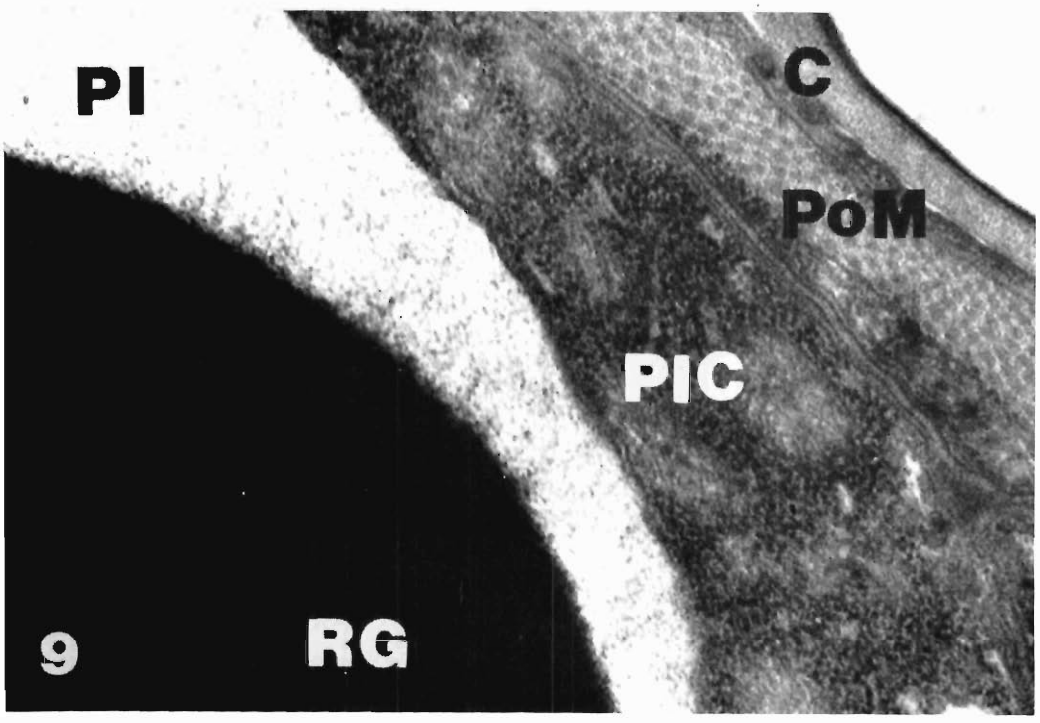












extremity of the postseptum. The muscle tracts are oblong in cross section (0.3 by 2–2.5 μ) and contain fibrils 200–300 Å in diameter (Figs. 8, 9). The nuclei are located in a slightly swollen portion of the cell adjacent to the septum.

Postseptal gland and gland duct

The postseptal gland consists of several cells located between the gland duct cells and the anterior end of the pseudointestine. The bulk of the gland occupies a median position in the postseptum but also partially envelops the lateral margins of both the gland duct cells and the pseudointestine (Figs. 8, 10). The gland cells contain a moderate amount of endoplasmic reticula and numerous membrane-bound vesicles. The vesicles are of two types, or perhaps phases, a large irregular type vesicle with finely granular contents, the other with a relatively clear lumen and large granules (Fig. 10).

The gland duct and its enveloping cells originate on the anterior portion of the postseptal gland, traverse the septum, and open at the tip of the proboscis. There are at least three cells in association with the duct: one located at the posterior end of the preseptum and two tandem cells immediately posterior to the septum (Fig. 10). Light microscopic examinations indicate a coiled structure in these two areas and a balled, stringlike structure enveloping and somewhat posterior to the postseptal gland. In cross sections the duct appears cuticular, consisting of three circular lamellae. The lumen of the duct is of relatively constant diameter throughout its length (0.18–0.21 μ) (Fig. 6). The duct is enclosed by a unit membrane, thrown roughly into a u-shaped loop. The curved portion of the "u" surrounds the canal while the free ends are apparently continuous with the cell membrane of the enveloping cell. Although a direct connection between the gland cells and the lumen of the cuticular duct was not observed, all the gland cells examined did possess a reticular-like structure (Figs. 8, 10).

Pseudointestine

The pseudointestinal sac is a sausage-shaped structure composed of four cells. The cells enclose a large central cavity in which are contained two anterior granules and a large posterior mass. The lumen of this organ opens to the exterior via a ventromedial duct (Fig. 1).

The pseudointestine is thin-walled in its middle portion, while both the anterior and posterior ends are enlarged and contain nuclei. The two anterior cells are adjacent, forming a complete cap over the anterior end of the pseudointestine (Fig. 8). These cells are located over, and secrete, the anterior two refringent granules (Fig. 9). The posterior two cells of the pseudointestine are larger than the anterior cells and are found adjacent only in their ventral and posterior portion. Between the posterior cells there is a mesentery-like partition which contains the exit duct of the pseudointestine (Fig. 11). Both anterior and posterior cells have large open nuclei with relatively large amounts of ribosomes and rough endoplasmic reticula.

The lumen of the pseudointestine is round to oval in cross section (4.5–6 μ in diameter) and sausage-shaped in longitudinal sections (12–13 μ long). The refringent granules enclosed within the lumen are intensely osmiophilic and are composed of even smaller entities of about 33–45 Å in diameter. The refringent granules are apparently formed by coalescence of the small entities secreted by the cells (Fig. 9). The anterior two granules usually appear spherical or spherical with truncated adjacent sides (Fig. 2), while the shape of the large posterior mass (usually not readily seen in light microscopic studies) follows that of the enclosing sac.

The lumen of the sac opens onto the median ventral surface of the body by means of an L-shaped duct approximately 1–1.5 μ in diam-

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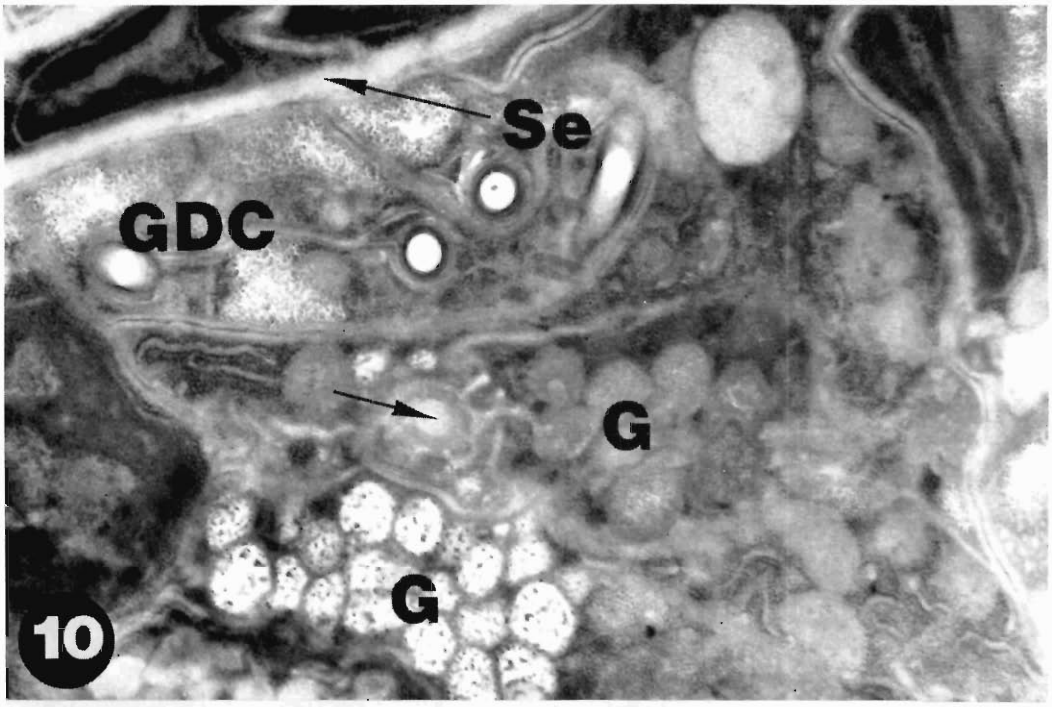
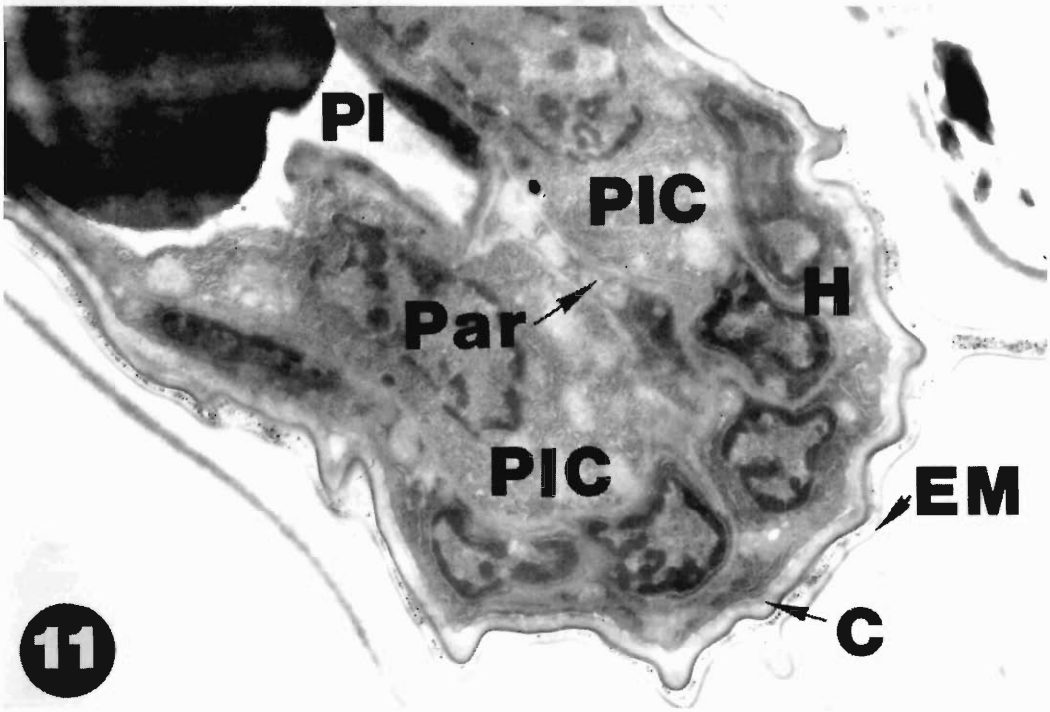
Figure 8. Cross section through the anterior cells of the pseudointestine; arrow indicates reticularlike structure. 23,300 ×.

Figure 9. Cross section through a portion of the pseudointestine and the postseptal body wall. 50,800 ×.





eter. There is a short portion of the canal located medially and parallel to the longitudinal axis of the body (Fig. 11). This then turns perpendicular to the longitudinal axis about 6 μ from the end of the body and continues to the ventral surface (Fig. 1). The short leg of the duct apparently possesses fibrils (210–220 Å in diameter) attached to one of its sides (Fig. 7). The duct is lined by a cuticle which is structurally different from the external body wall cuticula or the lining of the gland duct. Located within this duct are granules or filaments in cross section. The duct and its fibrils are enclosed by a complex of cells (at least two or three) which forms the partition separating the two large posterior cells of the sac.

Undifferentiated cells

There are two groups of "undifferentiated" cells (i.e., showing no specialized organelles—such as fibrils, vesicles, etc.) found in the postseptum. One set of cells are located in the space between the posterior aspect of the pseudointestinal complex and the hypodermis. The other set of cells fill the space between the body wall and the postseptal gland and its duct.

Discussion

Montgomery (1904) and later Inoue (1958) correctly observed the presence of muscles beneath the postseptal hypodermis of *Paragordius varius* and *Chardodes japonicus*, respectively. They could not, however, determine the muscular arrangement or structure. Montgomery's observations were supported by both Dorier (1930) and Muhldorf (1914) when they induced the presence of a postseptal musculature from flexions and irregular torsions in the postseptum of *Gordius aquaticus*.

The postseptal gland of this study corresponds, although denoted differently, to a structure similar to that reported by earlier authors. Inoue (1958) and Montgomery (1904) referred to the structure as, simply, gland, while Dorier (1930, 1932, 1935) dubbed it "appareil glandulaire." Following the lead of Vejdovsky, Muhldorf (1914) called it the "braune druse" or brown gland. In 1884, Vejdovsky (Muhldorf, 1914) described a brown organ in the anterior end of the parasitic stage of a gordioid, supposedly developing from this larval gland. In light microscopic examinations, the larval postseptal gland of *P. varius* was colorless. Indeed, the parasitic stage of *P. varius* did not possess any brown structure, except the remains of the preseptum.

Both Montgomery (1904) and Dorier (1930) described the larval postseptal gland of *P. varius* and *G. aquaticus* as a cellular mass with eight large nuclei. Additionally, Dorier (1930) noted longitudinal lobes and transverse grooves in the gland of *G. aquaticus*. In a similar study Muhldorf (1914) correlated changes in the gland with its state of development. The final form of the gland was greatly elongate with its flattened or pointed end extending deep into the body cavity. The light and electron microscopic examinations of this study showed many nuclei in the area between the septum and the anterior end of the pseudointestine. However, the electron photomicrographs indicated the gland to consist of two or three uninucleate cells possessing a vacuole-packed cytoplasm.

In *P. varius*, Montgomery (1904) described a duct that arose near the base of the postseptal gland, extending through the gland on its course through the septum. Inoue (1958) noted a similar arrangement for *C. japonensis*. However, Dorier (1930, 1932, 1935), in his studies on *Parachordodes gemmatus*, *Parachordodes alpestris*, *Parachordodes violaceus*, and *G. aquaticus*, reported a proximal ball-like terminus that also ran across the septum to the tip of the proboscis. Zapotosky (1974) noted that when the proboscis of the preseptum was retracted the duct appeared coiled at the pro-

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Figure 10. Longitudinal section through the anterior portion of the postseptum, arrow indicates reticularlike structure. 30,000 ×.

Figure 11. Longitudinal section through the posterior portion of the postseptum; star indicates the proximal portion of the exit duct. 12,500 ×.

boscial base. Additionally, this study indicates both a ball-like terminus and some coiling of the duct posterior to the septum. Also it is shown here that three or four nucleated cells envelope the gland duct throughout its course. The reticular structure shown within the cells may represent a collection system (ball-like terminus) for the glandular secretions.

The function of the gland could not be determined by this study. Still, by virtue of the gland's cytology (large amounts of ribosomes and membrane-bound granular vesicles), it does not appear to be an excretory structure as hypothesized by Muhldorf (1914). Its relationship to the proboscis seems to indicate a function associated with the perforating apparatus of the preseptum (see Zapotosky, 1974).

Dorier (1930) correctly described the "intestinal sac" or pseudointestine as an elongated tube which occupies a large part of the postseptum. As he observed, the walls of this tube are thin except at the nucleated anterior and posterior end. The posterior end of the "intestine" of *G. aquaticus* was reported by Muhldorf (1914) to connect to the posterior ventral wall by a solid cuticular style or closed blastopore. Montgomery (1904) recorded the same condition in *P. varius* noting additionally the presence of two to four nuclei apposed to the surface of the stalk. However, both Dorier (1935) and Inoue (1958) observed that the larvae of *P. gemmatus* and *C. japonensis* possessed a small open canal to the ventral surface. As shown here, *P. varius* has a dorsoventral partition between the two posterior cells. This nucleated partition contains a definite canal which opens on the ventral surface. In addition, fibrils were found in association with this exit duct, suggesting a possible sphinctor or opening device for the canal.

Two or more large granules develop within the pseudointestinal sac of most gordioid larvae. Muhldorf (1914) described such globules in *G. aquaticus* and speculated that they were the remains of mesenchyme cells absorbed by the "intestine." Similar granules were noted in *C. japonensis* by Inoue (1958), in *P. gemmatus* by Dorier (1935), in both *P. alpestris* and *P. violaceus*, also by Dorier (1932), *G. robustus* by May (1919), and *P. varius* by Montgomery (1904). Montgomery (1904) believed these to be granules of metabolic waste. He suggested the "intestine" served as a waste reservoir until the next stage was reached. The cells of the pseudointestine contain large amounts of ribosomes and rough endoplasmic reticulae. Thus, these cells are apparently active in the production and secretion of proteinaceous materials into the lumen of the pseudointestine. Dorier (1935) noted that the larvae of *P. gemmatus* while encysting emits from the orifice of the pseudointestinal sac a viscous substance. While the secretion of the cyst was accomplished, he saw the two inclusions (granules of the pseudointestinal sac) diminish progressively. Inoue (1958) notes a similar condition in *C. japonensis*. These observations and the cytology indicates that the pseudointestine is in reality a gland used in larval encystment.

Dorier (1930) and Muhldorf (1914) referred to the mesenchymatous or rest mesenchyme cells which appear as large numbers of nuclei between the posterior extremity of the intestine and its posterior end. Similar cells are noted in the larvae of *P. gemmatus*, *C. japonensis*, and *P. varius* (Dorier, 1935; Inoue, 1958; Montgomery, 1904). In this study of *P. varius* many of the nuclei in the tail region can be accounted for, i.e., there are numerous hypodermal nuclei, two cells in association with the ventral tail spines, muscle cells to the lateral tail spines, and various nuclei in association with the exit canal of the pseudointestine. Still, there are several cells which do not have a readily apparent role, or association with another structure. Further developmental studies are necessary to determine the role of these cells.

No excretory organs or nervous system were found; however, a nerve primordium consisting of a double ventral row of large ectodermal cells has been reported for the larvae of *G. aquaticus* by Dorier (1930) and Muhldorf (1914), *G. robustus* by May (1919), and *P. varius* by Montgomery (1904). These cells were not seen here; still a complex of several cells with no structural peculiarities were observed on the ventro-anterior side of the pseudointestine. These cells did not appear to be in a double row and structurally resembled the "rest mesenchyme" cell noted earlier.

Muhldorf (1914) also reported a body cavity which develops from the blastocoel of *G. aquaticus*. In light and phase microscopic examination, a cavity was observed in both the preseptum (when the perforating apparatus was extruded) and in the postseptum (in permanent mounts only) of *P. varius*. Electron photomicrographs of larvae with the proboscis invaginated showed no cavity in either the pre- or postseptum.

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Metazoan Parasites of *Fundulus heteroclitus* (Linnaeus, 1766) from Insular Newfoundland[1]

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ABSTRACT: Five hundred and fifty-seven *Fundulus heteroclitus* (Linnaeus, 1766), from four locations on the west coast of insular Newfoundland, were examined for metazoan parasites, using conventional parasitological techniques. Six genera of parasites were recovered (two Monogenea, two Digenea, one Cestoda, one Acanthocephala). It was found that the parasite burden (*Gyrodactylus prolongis, G. stephanus, Urocleidus angularis, Homalometron pallidum,* and *Neoechinorhynchus rutili*) of *F. heteroclitus* was not homogeneous for sample area, sex of host (Monogenea), and length of host. Significant differences in the preferred site of attachment of the three species of Monogenea were noted. Seasonal variations in incidence and intensity were noted for some parasite species. Seasonal cycles were related to seasonal variations in selected environmental factors, and possible changes in host diet and physiology.

The mummichog, *Fundulus heteroclitus* (Linnaeus, 1766), a cyprinodont fish, is widespread in western Atlantic coastal and brackish waters (Leim and Scott, 1966; Scott and Crossman, 1973). It is a popular laboratory animal and is the host for a number of metazoan parasites (Dillon, 1966; Hoffman, 1967). To date only two papers (Gowanloch, 1927; Fantham and Porter, 1948) concerned with the parasites of members of the genus *Fundulus* from the Atlantic provinces of Canada have been published. *F. heteroclitus* is found at several localities in southwestern Newfoundland (Scott and Crossman, 1964).

In May 1973 a study was initiated to determine the occurrence, distribution, and seasonal dynamics, in relation to selected environmental parameters, of the metazoan parasites of *F. heteroclitus* in Newfoundland.

[1] This paper consists largely of material submitted by the senior author in partial fulfillment of the requirements for the degree of M.Sc., Memorial University.

Table 1. Numbers of *Fundulus heteroclitus* per length class from the four areas sampled during the present study.

Length class (cm)	Sample area				Total
	Clark's Brook	Frenchman's Cove	Mummichog Park	Seal Cove Brook	
3–3.9	17	6	14	1	38
4–4.9	63	47	23	2	135
5–5.9	34	63	17	14	128
6–6.9	23	25	5	26	79
7–7.9	32	38	4	12	86
8–8.9	13	14	3	21	51
9–9.9	7	0	6	8	21
10–10.9	5	1	3	5	14
11–11.9	0	0	4	1	5
Totals	194	194	79	90	557

Materials and Methods

Sampling was carried out at approximately monthly intervals in four areas, namely Clark's Brook (49°46′ N, 58°08′ W), Frenchman's Cove (49°03′ N, 58°11′ W), Mummichog Park (46°46′ N, 59°16′ W), and Seal Cove Brook (47°48′ N, 58°28′ W). The sample sites, details of which are given in Dickinson (1974), were chosen on the basis of three criteria: namely, as being representative of the known range of *F. heteroclitus* in Newfoundland; to exhibit as wide a range of environmental variation as possible; and to be easily accessible by road for rapid sampling in the short time periods available.

Fish were caught in the period May–December 1973, adverse environmental conditions preventing sampling in the other months. A 10-meter seine (4 mm, on the diagonal, mesh) was used and fish were transported back to the laboratory alive, using the method of Abbott and Schwartz (1968). Examination of the fish, using standard parasitological techniques, commenced on the day following arrival back at the laboratory. Any parasites recovered were fixed, stained, and mounted utilizing techniques presented in Fernando et al. (1972).

Results and Discussion

Five hundred and fifty-seven *F. heteroclitus* of various lengths (Table 1) were examined during the study. Fish from Seal Cove and Mummichog Park had significantly greater mean total lengths ($P < 0.001$) than fish from the other areas sampled.

Fluctuations in temperature and salinity (as indicated by specific conductivity) were noticeable between areas and months, while little variation was noticed in pH values (Table 2).

Three species of Monogenea were recovered during the present study: *Gyrodactylus prolongis* Hargis, 1955; *G. stephanus* Mueller, 1937; and *Urocleidus angularis* Mueller, 1934.

Gyrodactylus prolongis occurred most frequently on fish in the 5–5.9-cm class (26% of worms recovered from this length class) and less frequently on fish in the 3–3.9-cm (3%) and 11–11.9-cm (6%) classes. The preferred site of infestation was the fins, with the caudal and anal fins being most often infested and the dorsal fin less so. Anthony (1969) demonstrated that the distribution of *Gyrodactylus elegans* Nordmann, 1832, on *Cyprinus auratus* L. was influenced by water temperature and that this helminth showed a lack of preference for the anal fin which is different than in the present case where this fin was preferred. *G. prolongis* showed a marked seasonal cycle with maximum numbers of fish (84%) being infested in May and minimum numbers (5%) in early September. A rise to 30% infestation in late September was noted, the incidence of infestation then remaining steady at this level until December.

Cushing (1942) has suggested that the rate of antibody production in fish is greater in warm water (28 C) than in cold (14 C) and thus at high environmental temperatures the high rates of antibody production will lead to a decline in the host's parasite load, as was seen in the above case.

Table 2. Selected environmental parameters, according to month and sample area.

Month	Sample area											
	Clark's Brook			Frenchman's Cove			Mummichog Park			Seal Cove Brook		
	(a)*	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
May	—	—	—	14	6.9	159	14	7.3	12,927	14	7.0	959
June	19	7.3	149	20	6.8	449	18	7.2	21,068	16	—	—
July	22	7.6	22,036	26	7.3	9,165	—	—	—	20	6.7	201
September (1)†	23	7.5	1,591	21	7.4	8,072	—	—	—	18	7.0	2,637
September (2)	11	8.5	786	18	7.2	12,746	19	8.4	25,179	14	7.3	18,553
October	7	7.4	36,126	8	7.7	11,529	—	—	—	8	7.4	36,126
November	3	7.8	8,670	2	7.6	642	4	7.0	160	3	7.3	11,910
December	0	8.5	9,128	1	7.5	674	—	—	—	2	7.1	1,176

* (a) = surface water temperature, °C. (b) = pH. (c) = Specific conductivity, micromhos/cm³ @ 20 C.
† 2 samples taken in September (one early, one late) due to senior author being unable to get August sample due to Canadian National strike.

Little information on the effect of salinity changes on seasonal cycles of Monogenea is available. Gowanloch (1927) showed that a *Gyrodactylus* sp. from *F. heteroclitus* was unaffected by the lowering of the salinity down to freshwater. Isakov (1970) showed that *G. arcuatus* exhibited paranecrosis during changing of the salinity from freshwater to marine which may partially account for the decline in incidence of *G. prolongis* during the summer months when environmental salinity was increasing. Dartnall (1972), however, stated that *Gyrodactylus* sp. exhibited a wide range of tolerance to salinity changes. However, since he did not identify the parasites he examined to the species level, it is possible that more than one species of *Gyrodactylus* may have been involved.

Gyrodactylus stephanus has not previously been recorded from *F. heteroclitus* in Canada. Fewer fish in the 3–3.9-cm (3.3% worms recovered from this length class) and 10–10.9-cm (5.2%) classes were infested than in the 5–5.9-cm (32%) class. The majority of worms were recovered from the gills. The incidence of infestation was low in May (10%) and rose to a peak in November (65%). In December a slight drop in the numbers of fish infested (58%) was noted, this perhaps being the start of a winter decline to a spring low.

The recovery of *Urocleidus angularis* from *F. heteroclitus* represents a new host record. The preferred site of infestation was the gills, with the 4–4.9-cm (29% worms recovered from this length class) length class being the most frequently infested. The number of fish infested rose from 6% in May to a high of 74% in early September. It then declined rapidly to 8 and 10% infested in November and December, respectively. This fluctuation may be explained in a manner similar to that proposed by Paling (1965) for *Discocotyle sagittata* Leuckart on *Salmo trutta* L. At low winter temperatures any eggs that are present probably remain dormant. As the temperatures rise in spring, hatching commences and the oncomiracidia invade the host population. The rate of egg production at temperatures above 15 C is high and the incubation period is short, resulting in a midsummer peak. The rate of egg production, incubation, and larval development declines in the fall with decreased temperatures.

The three species of Monogenea were not found in the same proportions in the four areas sampled. *G. stephanus* formed 71.5% of the monogenean population on fish from Clark's Brook, the rest of the population being *G. prolongis* (28.5%). At Frenchman's Cove the proportions were *G. prolongis*, 24.0% of the population; *G. stephanus*, 60.5%; *U. angularis* 15.5%; while at Mummichog Park they were 56.4, 7.8, and 35.8%, respectively. The only area where *U. angularis* was the dominant form (78.4%) was Seal Cove with *G. prolongis* and *G. stephanus* forming the rest of the population (16.2, 5.4%, respectively).

A trend was noted for larger fish to have a greater parasite burden than smaller fish (3–3.9-cm length class, mean number parasites per infested fish 4.8; 11–11.9-cm length class,

16.6). No differences were noted between the numbers of male and female fish infested when individual species of parasites were considered. However, when data on the total monogenean fauna were analyzed, it was found that male fish from Clark's Brook were more frequently infested than females ($P < 0.005$); this may be due to females having a greater hormonal-controlled physiological resistance to infestation than males as shown for other parasites (Lees and Bass, 1960; Dobson, 1961; Paling, 1965). However, females from Mummichog Park and Frenchman's Cove showed a significantly greater incidence of infestation than males in May and June, respectively; since the females were gravid at the time, it may have been due to the reasons proposed by Thomas (1964).

The distribution of the Monogenea between the gills and fins showed a significant ($P < 0.005$) variation, 80.9% (1,122) occurring on the former, and 19.1% (265) on the latter. No significant preferences were noted for the various gill arches on the left or right side of the body. Arch I on each side was a less preferred site ($P < 0.005$) than the other arches, between which no significant differences were noted. In single parasite gill infestations, no significant preferences were noted for any gill arch (gill arch I, 20.4% of Monogenea recovered; II, 20.6%; III, 26.8%; IV, 32.3%). The preferences of *Gyrodactylus* spp. for a particular gill arch have been studied by MacKenzie (1970) and by Tedla and Fernando (1970) for *Urocleidus adspectus* Mueller, 1936, while Llewellyn (1956) suggested that the pattern of distribution of monogenean larvae on the gills may be due to the pattern of flow of the gill ventilation current.

No preferences existed between the incidence of occurrence of Monogenea on the dorsal, anal, and caudal fins (15.7, 17.5, 13.8%, respectively). The pectoral fins were significantly less infested (0.3%; $P < 0.005$). No Monogenea were found on the pelvic fins probably due to their small surface area.

Analysis of the total mean intensity of infestation of each fin showed that the caudal fin was subject to a greater mean parasite load ($P < 0.005$) than the other fins, and that pectoral fins were the least infested of the infested fins ($P < 0.005$). Monogenea were significantly more prevalent ($P < 0.005$) on the fins of the fish from Clark's Brook than from any other sample area, whereas they were the least prevalent ($P < 0.005$) on the fish from Seal Cove. Analysis of the sample area data showed fish from Mummichog Park and Seal Cove to have a significantly greater mean fin parasite load (13.7:3.7, respectively) than fish from the other sample areas, (Clark's Brook 2.1: Frenchman's Cove 2.5; $P < 0.005$).

The distribution of Monogenea on the fins of the host has been studied by Anthony (1969) who noted that the intensity of infestation was inversely proportional to the fin area, which is in direct contrast to this study where the distribution was proportional to fin area.

The only adult digenean recovered, from all localities except Clark's Brook, was *Homalometron pallidum* Stafford, 1904 (22% infection: mean 2, range 1–8, per infected fish). The helminths were slightly smaller (length and width) than those of Linton (1901, 1940) and Miller (1941). A gradual increase in the incidence of infection was noted with increasing host length (3–3.9-cm length class, 5.2% infected; 11–11.9-cm length class, 40.0%). A seasonal variation in the number of fish infected with this parasite was also noted—37.9% infected in May, gradually dropping to 5.2% in early September and rising to 23% by late September, a drop to 3.3% occurred in October, increasing to 46.6% in December. The existence of a seasonal cycle, with spring and fall peaks, for this worm may be due to the detrimental effect of high summer temperatures, as shown for other trematodes, on the eggs (Rowcliffe and Ollerenshaw, 1960), miracidia (Varma, 1961), or adult trematodes (Vernberg and Hunter, 1961). Similarly the higher summer water salinities may retard the hatching of the eggs (Standen, 1951).

The gill filaments of all the fish from Frenchman's Cove (194) and Mummichog Park (79) were found to be infected with a metacercaria. Stunkard and Uzmann (1955) and Lillis and Nigrelli (1965) noted the presence of metacercariae on the gills of this host and worked on the life cycles of the parasites involved.

Thirty-five percent of the fish were infected with a *Proteocephalus* sp. (mean 2, range 1–16, per infected fish). Only immature specimens were found, the measurements

of which were as follows [mean (range)]: body length 1.47 mm (0.36–6.18 mm); body width 0.16 mm (0.08–0.36 mm); maximum scolex width 0.19 mm (0.11–0.27 mm); maximum sucker diameter 0.08 mm (0.05–0.11 mm); maximum apical organ diameter 0.02 mm (0.02–0.04 mm).

Neoechinorhynchus rutili (Mueller, 1780) previously found in Newfoundland in threespine sticklebacks (Hanek and Threlfall, 1970), was recovered from *F. heteroclitus* for the first time. Five fish (1% infected) from Clark's Brook were infected with one to 32 parasites (mean 9.6). The only months in which infected fish were found were November (4 fish) and December (1), Walkey (1967) suggesting that such cyclic life cycles may be related to decreases in environmental temperatures.

As may be seen from the foregoing a wide variety of factors may affect the incidence and intensity of infection with various parasite species. Before any conclusions may be drawn about the causes of variations in these parameters a great deal more ecological and laboratory work must be performed.

Acknowledgments

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Studies on *Acanthocephalus jacksoni* Bullock, 1962 (Acanthocephala: Echinorhynchidae). III. The Altered Behavior of *Lirceus lineatus* (Say) Infected with Cystacanths of *Acanthocephalus jacksoni*

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ABSTRACT: The behavior of noninfected isopods and those infected with cystacanths of *Acanthocephalus jacksoni* was observed in laboratory experiments. The amount of time spent by the isopods under leaves, on leaves, on floating material, wandering, and/or fighting was recorded and analyzed. Nonparasitized (pigmented) isopods spent significantly more time under leaves than did parasitized (nonpigmented) isopods. Parasitized isopods spent significantly more time "wandering" than did nonparasitized isopods. Parasitized males spent significantly more time on leaves than infected females. It is suggested that these altered behavioral responses and increased conspicuousness of infected *Lirceus lineatus* help the transmission of the cystacanth to the definitive host.

The study of the behavior of intermediate hosts infected with cystacanths of acanthocephalans is a relatively new area in the field of parasitology. Holmes and Bethel (1972) and Bethel and Holmes (1973) have investigated the behavior of *Gammarus lacustris* infected with *Polymorphus paradoxus*. Holmes and Bethel (1972) have reviewed the literature concerning the modification of intermediate host behavior by parasites.

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The present study was undertaken to determine what modifications, if any, occurred in the behavior of *Lirceus lineatus* infected with cystacanths of *Acanthocephalus jacksoni*.

Materials and Methods

Isopods were collected at Jackson Cutoff, Wood County, Ohio, an area described by Muzzall and Rabalais (1975a).

Parasitized and nonparasitized isopods were easily differentiated by pigmentation as demonstrated by Muzzall and Rabalais (1975a, b). Parasitized isopods are nonpigmented and nonparasitized isopods are pigmented. Cystacanths of *Acanthocephalus jacksoni* were easily detectable through the carapace of living *Lirceus lineatus*.

Each experiment involved the observation of the behavior of six or four isopods (three infected and three noninfected or two infected and two noninfected). Groups of six isopods were observed in eight 1-hr periods. These 48 isopods were observed for 48 isopod hours. Groups of four isopods were obesrved in six 1-hr periods. These isopods were observed for 24 isopod hours. A total of 72 isopods were observed for 4,305 min out of a possible 4,320 (72 isopod hours of observations × 60 min). All isopods in a given experiment were of the same sex. Size of the isopod and its effect on behavior was not investigated. Isopods were never used in more than one experiment. Two observers were present at each experiment; one watched infected isopods, and the other, noninfected ones. The observers reversed their positions at the beginning of each subsequent observation period.

Isopods were placed in clear pans with mud from Jackson Cutoff on the bottom. Five large leaves were placed in the center of the pan and floating vegetation was placed on the sides of the pan. The isopods were given 5 min to acclimatize before their actions were recorded by the Time-Interval Method. The Time-Interval Method is a method in which a mark is placed in a category every 30 sec, recording the organisms' actions. Isopods could be found under leaves, on leaves, on floating vegetation, wandering, and/or fighting. Fighting is a term that describes prolonged physical contact between two isopods.

Sokal and Rohlf (1969) was used as a reference for statistical procedures.

Results and Discussion

Holmes and Bethel (1972) stated that larval parasites alter the behavior of their intermediate host by reducing their stamina, increasing their conspicuousness, and disorienting and altering their responses.

The results of laboratory experiments and statistical comparisons made by the Mann–Whitney U-Test, shown in Table 1, suggest that cystacanths of *Acanthocephalus jacksoni* alter the behavioral responses of *Lirceus lineatus*. Nonparasitized (pigmented) isopods spent significantly more time under leaves than did parasitized (nonpigmented) isopods. Parasitized isopods spent significantly more time "wandering" than did nonparasitized isopods. All other comparisons made were nonsignificant.

"Wandering" is a term that describes a very active isopod, one that never really comes to rest. It seemed as if these "wandering" infected isopods were attempting to get out of the pan. This "wandering" activity of infected isopods may be similar to the "skimming" activity of *Gammarus lacustris* infected with *Polymorphus paradoxus*. Holmes and Bethel (1972) state that "skimming" is so pronounced that it almost appears to be an effort to get out of the water and causes an obvious surface disturbance.

Some infected isopods were observed to rear up on their back legs and wave their anterior ends vigorously when on the bottom and on floating material. Noninfected isopods were not observed to do this.

Infected isopods crawled over objects, such as leaves and twigs, instead of going under them. Noninfected isopods generally crawled under objects.

Infected and noninfected isopods were seen clinging to floating material at Jackson Cutoff on several occasions. There was no statistical difference in the time spent on floating material between infected and noninfected isopods in laboratory experiments.

Infected isopods positioned themselves on floating material with their ventral side up. The operculum would open and the gills would

Table 1. Time in total minutes spent in various actions by infected and uninfected *Lirceus lineatus*.*

	Infected	Uninfected	Infected		Uninfected	
			♂♂	♀♀	♂♂	♀♀
Number isopods observed	36	36	(17)	(19)	(19)	(17)
Action:						
Under leaves	37	1,011†	23	14	487	524
On leaves	224	314	169	55†	216	98
On floating material	620	439	237	383	235	204
Wandering	1,262	377†	583	679	186	191
Fighting	14	7	10	4	6	1
Total minutes	2,157	2,148	1,022	1,135	1,130	1,018

* Half minutes were rounded off to whole minutes.
† Significantly different from expected 50:50 distribution at $P < .05$ by Mann–Whitney U-Test.

move very rapidly. This was also noted in noninfected isopods, but to a lesser extent.

Infected male isopods spent significantly more time on leaves than infected females (Table 1). This, however, may have been because male isopods were looking for a mate. Studies concerned with the behavior of asellids were not found in the literature; therefore, no comparisons of normal and abnormal behavior could be made.

It is suggested that cystacanths of *Acanthocephalus jacksoni* increase the vulnerability of *Lirceus lineatus* to fish predation by increasing their conspicuousness and altering the responses of *L. lineatus*.

Muzzall and Rabalais (1975b) found that 204 (97.6%) out of 209 nonpigmented isopods examined were infected with cystacanths. Pigmented isopods were not found to be infected with cystacanths. This increased conspicuousness and altered behavioral responses of infected *Lirceus lineatus* seems to help the transmission of the cystacanth to the definitive hosts. Muzzall and Rabalais (1975a) found adult *Acanthocephalus jacksoni* in 15 species of fish and found it to be the dominant helminth at Jackson Cutoff. It is believed that this increased conspicuousness and altered behavior of infected *L. lineatus* may account for *A. jacksoni* being the dominant helminth at Jackson Cutoff.

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Helminths of Wild Turkeys in Florida[1]

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ABSTRACT: Examination of 220 wild turkeys (*Meleagris gallopavo*) from eight localities in Florida revealed 34 species of helminths, including 10 trematodes, six cestodes, 17 nematodes, and one acanthocephalan. Seventeen of these represent new host records and five are undescribed species. Data are presented on the prevalence and intensity of infection by each species along with comments on taxonomy, pathology, and relationships of some species with other hosts. The helminth fauna of turkeys from the Fisheating Creek study area in southern Florida was compared with data from the Mississippi Delta (Prestwood, 1968). Twelve of the 30 helminth species found in Fisheating Creek birds were also reported from Mississippi turkeys. Cestodes were the most similar group while trematode and nematode faunas showed greater differences. Turkey populations in the Mississippi area were considerably higher than those of Fisheating Creek and helminth burdens in turkeys there were correspondingly higher and exhibited loads almost seven times larger than the Florida population.

Florida's wild turkey populations consist of the Florida turkey, *Meleagris gallopavo osceola* Scott (peninsular Florida), which intergrades with the eastern turkey, *M. g. silvestris* Vieillot, in the western panhandle (Aldrich and Duvall, 1955). A marked decline in Florida's turkey populations was observed by management biologists between 1964 and 1968 and the statewide harvest of turkeys by hunters in 1968 was the lowest on record during recent years. This prompted a cooperative study between the University of Florida and the Florida Game and Fresh Water Fish Commission in July 1969 to study the prevalence, distribution, and impact of parasites and diseases of wild turkeys in Florida. The present study on helminths was undertaken as part of that program. A report on the blood protozoans has been published (Forrester et al., 1974).

Materials and Methods

From September 1969 through March 1972, 220 wild turkeys were collected from eight localities in the state (Fig. 1). These birds consisted of 126 poults (hatch date through 31 December of the same year), 52 juveniles (1 January through hatching season or approximately 1 year), and 42 adults (over 1 year old). Most of these birds (181) were obtained during 1970 and 1971. Very young poults were collected by hand. Older turkeys were livetrapped, using orally administered drugs (Williams et al., 1973) or cannon nets (Austin, 1965), while some were collected with shotguns. Intestinal tracts from nine hunter-killed birds were collected from the Glades County study area in November 1970.

Techniques for recovering, killing, fixing, preserving, and staining helminths were similar to those described by Kinsella and Forrester (1972).

Parasite communities were analyzed using the index of similarity from Holmes and Podesta (1968). With this index a value of 100% indicates that the two groups being considered are identical. If no helminths are shared, the index is zero. Between these extremes relatively high values denote similarities in parasite groups and lower values indicate greater differences in species composition. Helminth dominance was studied by using parasite profiles similar to those of Uhazy and Holmes (1971). Such profiles reflect dominance by showing the percentage each species contributes to the total helminth population.

Results and Discussion

Thirty-four species of helminths (10 trematodes, six cestodes, 17 nematodes, and one acanthocephalan) were found. Seventeen of these species are reported from wild turkeys for the first time. Five species are apparently undescribed.

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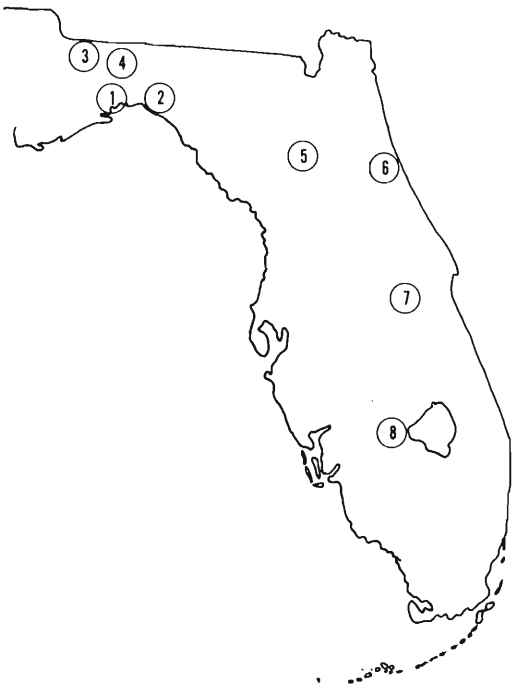

Figure 1. Collection areas for wild turkeys in Florida. Numbers of birds collected from each area are as follows: (1) Wakulla Co. (n = 3); (2) Taylor Co. (n = 2); (3) Gadsden Co. (n = 1); (4) Leon Co. (n = 1); (5) Alachua Co. (n = 19); (6) Flager Co. (n = 4); (7) Osceola Co. (n = 18); (8) Fisheating Creek study area, Glades Co. (n = 172).

The prevalence and intensity of infection of each helminth species for the composite state sample are given in Table 1.

Trematoda

Ten species of trematodes representing nine families were encountered, occurring in 72 (33%) of the birds examined. Most fluke burdens were small and the majority were single species infections. The mean trematode burden was 4.8 worms per bird while the mean number of species encountered in an infection was 1.1.

Echinoparyphium recurvatum was the most commonly encountered trematode both in prevalence and number of individuals. Annereaux (1940) reported debilitation and death in domestic turkey poults resulting from heavy infections of *E. recurvatum*. The largest infection of *E. recurvatum* found in the present study (109 worms) was in a juvenile gobbler from Glades County. No clinical signs were associated with this infection.

Echinoparyphium recurvatum has a cosmopolitan distribution, wide host specificity, and is listed by McDonald (1969) as a characteristic helminth of waterfowl. Kinsella and Forrester (1972) found *E. recurvatum* in 40 of 74 Florida ducks (*Anas platyrhynchos fulvigula*) from Glades County in which the mean infection encountered was 32 worms (range 1 to 575). From their work it appears that the Florida duck, which is the most common waterfowl species on the study area, is the main host of *Echinoparyphium*. Turkeys are probably auxiliary hosts to the parasite.

The second most prevalent trematode encountered, *Zygocotyle lunata*, is another characteristic helminth of waterfowl (McDonald, 1969). Infections ranging from one to 21 worms (mean 3) were found in 34 of 74 Florida ducks from Glades County (Kinsella and Forrester, 1972).

Thirteen young turkeys (12 poults and one juvenile) from Glades County were infected with *Stomylotrema vicarium*. Bush (1973) found 29 of 31 white ibises (*Eudocimus albus*) from Glades County infected with *S. vicarium*. The same parasite was also found in three of 15 Florida sandhill cranes (*Grus canadensis pratensis*) from southern Florida (Forrester et al., 1975). Most worms from turkeys were gravid. The turkey may be an accidental host of *S. vicarium*, but one which is not appreciably inhibitory to its growth.

Strigea elegans meleagris was described by Harwood (1931) as *S. falconis meleagris* from a domestic turkey from Houston, Texas. On the basis of specimens collected in this study, however, it has been redescribed by Dubois and Hon (1973) as *S. e. meleagris*.

One specimen of *Prosthogonimus ovatus* was found in each of five turkeys and one specimen of *Echinostomum revolutum* was found in each of three turkeys from Glades County. Both species are characteristic helminths of waterfowl (McDonald, 1969) and have been reported as common in Florida ducks in Glades County (Kinsella and Forrester, 1972).

Four other flukes (*Brachylaima virginianum*, *Ascocotyle* sp., *Tanaisia* sp., *and Zonorchis* sp.)

Table 1. Helminths recovered from 220 wild turkeys collected in Florida.

Helminth	Per cent prevalence	Number of worms per infection	
		Mean	Range
Trematoda			
Echinoparyphium recurvatum (4, 5)*	11	8	1–109
Zygocotyle lunata (6)	10	3	1–20
Stomylotrema vicarium (8)†	6	3	1–9
Strigea elegans meleagris (4)†	4	5	1–14
Prosthogonimus ovatus (7, 8)	2	1	–
Echinostoma revolutum (5)†	1	1	–
Ascocotyle sp. (4)†	<1	2	–
Brachylaima virginianum (5)	<1	3	–
Tanaisia sp. (13)†	<1	3	–
Zonorchis? sp. (12)†	<1	1	–
Cestoda			
Metroliasthes lucida (4, 5)	55	32	1–453
Raillietina georgiensis (4, 5)	23	60	1–340
Raillietina ransomi (4)	9	15	1–73
Raillietina cesticillus (4)	4	11	1–39
Davainea meleagridis (4)	2	14	1–28
Hymenolepis carioca (4, 5)	1	1	–
Nematoda			
Strongyloides sp. (4, 5, 6)	48	38	1–783
Trichostrongylus tenuis (6)	33	20	1–201
Dispharynx nasuta (2)	28	4	1–18
Cyrnea eurycerca (3)†	25	3	1–16
Ascaridia dissimilis (4, 5)	23	3	1–17
Capillaria sp. 1 (4, 5)†	20	2	1–10
Cyrnea sp. (3)	8	4	1–12
Singhfilaria hayesi (14)	7	2	1–9
Heterakis gallinarum (6)	5	2	1–10
Aproctella stoddardi (11)†	4	2	1–5
Synhimantus sp. (3)†	3	1	1–2
Aulonocephalus pennula (6)†	<1	4	–
Capillaria sp. 2 (5)†	<1	2	–
Chandlerella sp. (10)†	<1	3	–
Cheilospirura spinosa (3)†	<1	1	–
Splendidofilaria sp. (9)†	<1	1	–
Splendidofilariinae (12)†	<1	1	–
Acanthocephala			
Mediorhynchus papillosum (5)†	<1	1	–

* Numbers in parentheses indicate location in hosts: (1) upper esophagus and crop, (2) proventriculus, (3) gizzard lining, (4) duodenum, (5) lower small intestine, (6) ceca, (7) bursa of Fabricius, (8) cloaca, (9) heart, (10) lungs, (11) body cavity (12) liver, (13) kidneys, (14) connective tissue.
† Not previously reported from wild turkeys.

were found in one host each and are probably accidental parasites of turkeys. Specimens of the latter three species were recovered in poor condition which prevented identification beyond genus.

Cestoda

Six species of cestodes were recovered with 69% of the birds harboring infections of one or more species. Mixed infections of two or more species occurred in 31% of the infected birds.

The most common cestode was *Metroliasthes lucida*. This tapeworm is a very common parasite of domestic turkeys in the United States (Wehr, 1972).

The next most frequently encountered cestode was *Raillietina georgiensis*. A closely related species, *Raillietina williamsi*, was found in 39% of 390 wild turkeys collected in 10 southeastern states by Maxfield et al. (1963). One of 67 turkeys collected from Florida during that study was infected with *R. williamsi*. Prestwood (1968) encountered *Metroliasthes lucida* and *R. williamsi* in 50% of 216 wild turkeys from Arkansas and Mississippi. From these findings and those of Maxfield et al. (1963), Prestwood et al. (1973) concluded that *M. lucida* and *R. williamsi* are the two most common cestodes of southeastern wild turkeys. This appears not to be the case in Florida. Maxfield et al. (1963) found that *R. georgiensis* was more prevalent in Florida turkeys than *R. williamsi* (22.4% vs. 1.5%). No *R. wil-*

liamsi infections were found during the present study.

Nematoda

These were the most commonly encountered helminths, occurring in 88% of the birds. Mixed infections of two or more species were found in 69% of the hosts infected with nematodes. Seventeen species were recovered including 10 new host records for wild turkeys.

The most prevalent nematode was *Strongyloides* sp. Culturing of the free-living generation from feces of a young bird revealed male specimens which conformed closely to measurements given by Cram (1929) for S. *avium*. Parasitic females recovered ranged in length from 1.9 to 5.7 mm. This variability may be due to the presence of more than one species of *Strongyloides* in the sample. The original description by Cram (1929) gave a parasitic female length of 2.2 mm. Cram (1936) listed a range of 2.61 to 4.39 mm for worms identified as S. *avium* in chickens from Puerto Rico. Positive species identification and determination of the number of *Strongyloides* species recovered during the present study were not accomplished. This would require culture of free-living stages from each infected bird.

Maxfield et al. (1963) reported S. *avium* in wild turkeys from Glades County, Florida. Prestwood (1968) found 60% of 216 wild turkeys from the delta region of Arkansas and Mississippi infected with *Strongyloides* sp.

Trichostrongylus tenuis was most common in the birds from Osceola County. This nematode may be of pathological significance since it has been reported to have a serious effect on populations of red grouse (*Lagopus scoticus*) in Great Britain (Committee of Inquiry, 1911). No lesions were found associated with this infection in the present study, however. Maxfield et al. (1963) found 15 of 67 wild turkeys from Florida infected with *T. tenuis*, but a low incidence of the nematode was encountered in Arkansas and Mississippi turkeys (Prestwood, 1968).

Dispharynx nasuta was found in birds from each of the study areas. Maxfield et al. (1963) recovered *D. nasuta* from a pen-raised wild turkey in South Carolina. The first report of *D. nasuta* parasitizing free-ranging wild turkeys was made by Prestwood (1968) for birds from the delta region of Mississippi. The nematode occurred infrequently in that study and only poults were parasitized.

Although *D. nasuta* infections have been associated with pathological changes and deaths in ruffed grouse, *Bonasa umbellus* (Goble and Kutz, 1945), blue grouse, *Dendragapus obscurus fuliginosus* (Bendell, 1955), and feral pigeons, *Columba livia* (Hwang et al., 1961), nothing is known of their pathogenicity in wild turkeys. During the present study, *Dispharynx* was the first helminth to appear chronologically, with poults being infected as early as 3 days after hatching. Lesions at the attachment sites of the nematodes resembled those described by Hwang et al. (1961). Involvement of 25% or more of the glandular surface of the proventriculus was encountered in 1- to 2-week-old poults harboring as few as three *Dispharynx*.

Fisheating Creek turkeys experience about 50% mortality during the first 4 weeks after hatching (L. E. Williams, Jr., unpublished data). Factors responsible for this mortality are unknown. Dead or dying birds are practically impossible to find because of the habitat and the activities of predators and scavengers. One sick poult 8 to 12 days old was recovered. This bird was weak, unable to fly, and easily captured by hand. At necropsy, 18 *Dispharynx* were recovered from the proventriculus; it is possible that the infection either caused or contributed to the poult's debilitated condition.

Numbers of *D. nasuta* per bird were small compared to the findings of Bendell (1955) in blue grouse (severe infections of 100 to 300 worms) and those of Goble and Kutz (1945) in ruffed grouse (infections ranged from 10 to 246). Even so, the number of poults infected with *D. nasuta* was high, with the prevalence reaching 100% in 2-week-old birds collected in 1971. Higher worm burdens and environmental stress may have weakened and eliminated some birds from the population. Further investigation is needed to determine the numbers of *D. nasuta* needed to produce morbidity and mortality in young poults. *Dispharynx* appears to be maintained on the Fisheating Creek area by a number of avian species besides turkeys. Bush and Forrester (unpublished data) found nine of 28 bobwhite

quail (*Colinus virginianus*) from Fisheating Creek infected with *Dispharynx nasuta*. A small survey of two other common avian species on the area (present study) revealed that four of six common crows (*Corvus brachyrhynchos*) and four of five blue jays (*Cyanocitta cristata*) harbored *D. nasuta* infections. The possibility of additional species of passerines serving as reservoirs of infection remains to be investigated.

Two species of *Cyrnea* were encountered. One of these, *Cyrnea eurycerca*, has been reported from Europe, Asia, and Africa, where it occurs normally in Galliformes, being found only rarely in Coraciiformes and Anseriformes (McDonald, 1969; Skrjabin and Sobolev, 1963). Domestic turkeys from Bulgaria have been found to harbor *C. eurycerca* (Vasilev, 1964). To our knowledge, this is the first report of *C. eurycerca* from the Western Hemisphere.

An undescribed species of *Cyrnea* was harbored by 18 turkeys. Maxfield et al. (1963) found an unidentified species of *Cyrnea* in two turkeys from Florida. Specimens from the U. S. National Museum (cataloged as *Seurocyrnea* species, No. 56894) which were submitted by Maxfield have been examined and are identical to specimens of *Cyrnea* sp. collected during the present study.

Turkeys from the Glades County and the northwestern study areas harbored *Ascaridia dissimilis*. Maxfield et al. (1963) recovered *A. dissimilis* from 66% of 390 wild turkeys collected in 10 southeastern states. *Ascaridia galli* was found in 1.5% of those birds. Prestwood (1968) found *A. dissimilis* in 96% of 216 turkeys from three Arkansas and Mississippi study sites. *Ascaridia dissimilis* and *A. galli* demonstrate a marked host specificity for turkeys and chickens, respectively (Kates and Colglazier, 1970). This high degree of specificity is obviously maintained in southeastern wild turkey populations.

Two undescribed species of intestinal capillarids were recovered. *Capillaria* sp. 1 appears closest to *C. nyrocinarum* reported from diving ducks and snipe in Europe, Asia, and North America (McDonald, 1969). Spicule lengths averaged 1.29 mm and females contained barrel-shaped vulvar appendages.

One male and one female of the second *Capillaria* sp. were recovered. Characteristics of the species include the absence of caudal alae, a spiny spicule sheath, a spicule length of 672 μ for the male, and a simple vulva in the female. This undescribed species appears to be a duck parasite, being found also in 47 of 78 Florida ducks by Kinsella and Forrester (1972). The turkey infection was apparently accidental.

Cecal worms, *Heterakis gallinarum*, were found in low prevalence and numbers in birds from each of the study areas. Maxfield et al. (1963) reported a 62% incidence of *H. gallinarum* in 390 wild turkeys from 10 southeastern states. Of 67 wild turkeys collected in Florida for that study, 26 (39%) harbored *Heterakis*. A 97% incidence of *Heterakis gallinarum* and *Heterakis* sp. was reported for Arkansas and Mississippi turkeys by Prestwood (1968).

Heterakis is an important parasite of wild turkeys, since *Histomonas meleagridis*, the etiologic agent of blackhead, is transmitted through the nematode's eggs. Two cases of blackhead, one in a juvenile turkey from Glades County and another in a pen-raised "wild" bird from northwest Florida, were diagnosed during the present study. Blackhead does not appear to be a serious disease problem in wild turkeys in Florida at present, judging from the low incidence of *Heterakis* and few reported cases of blackhead in the populations sampled.

The actual prevalence of the subcutaneous tissue filarid, *Singhfilaria hayesi*, may have been higher; due to difficulty of locating worms in the tissues of the neck and crop region, some infections may have been overlooked. This nematode was described from wild turkeys in Alabama and Mississippi by Anderson and Prestwood (1969).

Aproctella stoddardi was originally described from the bobwhite quail in Georgia by Cram (1931), and has since been found in other gallinaceous and passerine birds (Anderson, 1957, 1961). Bush and Forrester (unpublished data) have found seven of 62 quail from Alachua and Glades counties infected with *A. stoddardi*. The turkey appears to be an accidental host of this nematode.

Six turkeys from Glades County were each infected with one or two specimens of an un-

described species of *Synhimantus*. Forrester et al. (1974, 1975) have found the same nematode in one of 34 greater sandhill cranes (*Grus canadensis tabida*) and two of 15 Florida sandhill cranes. Bush (1973) recovered what is apparently the same species from the white ibis in Florida. Male nematodes from turkeys are 3.9 to 4.9 mm long with spicules 600 to 620 μ and 135 to 160 μ. Females range in length from 5.7 to 10.4 mm; eggs are 29.7 to 31.9 by 20.9 μ.

Four specimens of *Aulonocephalus pennula* were found in one poult. The worms were mature indicating that the turkey, even if an accidental host, is not appreciably inhibitory to the nematode. This species has been reported from various species of quail in the southwestern United States (Lewin and Holmes, 1971). Those authors found *A. pennula* in California quail, *Lophortyx californica*, which had been introduced onto the Puuwaawaa Ranch, Hawaii. Webster (1947) found the nematode occurring in 265 of 300 bobwhite quail examined from Texas. Bush and Forrester (unpublished data) found no infections of *A. pennula* in 62 quail from Florida. The possibility exists, however, that Florida quail do harbor the nematode. This seems to be a plausible explanation for the presence of *A. pennula* in Florida since it is a common parasite of quail in other areas and has been reported only from gallinaceous birds.

Acanthocephala

One immature male specimen of *Mediorhynchus papillosum* was found in the small intestine of a Glades County poult. Prestwood (1968) found *Mediorhynchus* sp. in 20 of 216 turkeys from Mississippi and Arkansas.

Mediorhynchus papillosum was originally described from the eastern wood pewee, *Myiochanes virens*, and has since been reported from several other passerine species, the sora rail (*Porzana carolina*), and the greater prairie chicken (*Tympanuchus cupido americana*) in the United States (Yamaguti, 1963).

Helminth fauna comparisons

One comprehensive study dealing with helminths in a specific wild turkey population is available for comparison with the present

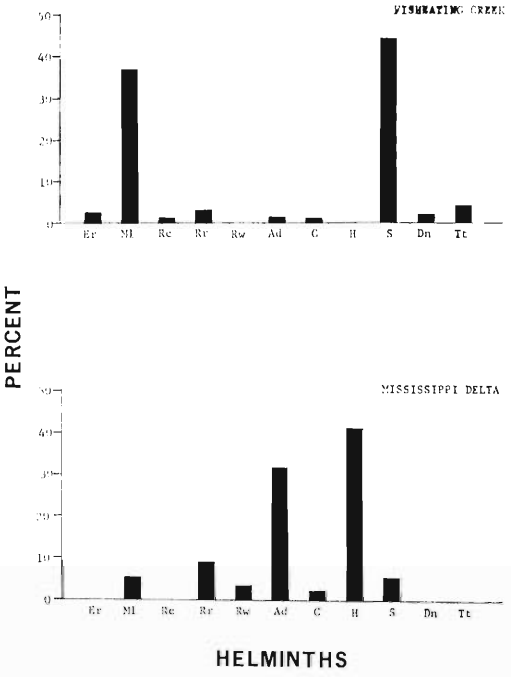

Figure 2. Comparison of helminth profiles for Fisheating Creek and Mississippi Delta turkeys. Er = *Echinoparyphium recurvatum*; Ml = *Metroliasthes lucida*; Rc = *Raillietina cesticillus*; Rr = *Raillietina ransomi*; Rw = *Raillietina williamsi*; Ad = *Ascaridia dissimilis*; C^1 = *Capillaria* sp. 1 (present study); C_2 = *Capillaria* spp. (Prestwood's study); H = *Heterakis*; S = *Strongyloides* sp.; Dn = *Dispharynx nasuta*; Tt = *Trichostrongylus tenuis*.

study. Prestwood (1968) conducted a survey of helminth parasites over a 3-year period, examining both poults and adults. Her work on turkeys from the Mississippi River Delta provides quantitative data on helminth parasites in that particular population. Since our Fisheating Creek study area (Glades County) is the only area from which adequate samples of various age birds were obtained, all comparisons with Prestwood's study are concerned with that population.

Sixteen of the 30 helminth species found in Fisheating Creek birds are listed by Prestwood et al. (1973) as previously reported from wild turkeys. Of these 16 species, 12 were shared with Delta birds. An index of similarity for all helminths was 40%. Table 2 presents a

Table 2. Comparison of helminth faunas for Fisheating Creek and Mississippi Delta turkeys.

	Helminth groups												
	Trematoda			Cestoda			Nematoda			Acanthocephala			All helminths
	F*	G	S	F	G	S	F	G	S	F	G	S	S
No. taxons													
Fisheating Creek	7	8	8	3	3	5	9	14	16	1	1	1	30
Mississippi Delta	5	5	5	2†	5	7	9	9	12	1	1	1	25
Shared	4	3	2	2	3	4	7	7	6‡	1	1	0	12
Index of similarity (%)		26			53			38			0		40

* Designations are: F = families; G = genera; S = species.
† Prestwood (1968) found scolices of an unknown cestode, order Cyclophyllidea, in one turkey. No further classification was made.
‡ This number of species shared assuming Prestwood's *Strongyloides* sp. and *Cyrnea* sp. are identical to the species recovered during present study.

comparison of taxonomic groups between the two areas and corresponding indexes of similarity. Cestodes appear to be the most similar group, while the trematode and nematode faunas showed more differences in species composition.

Holmes and Podesta (1968) found a similar trend for helminths of wolves from different regions of North America; cestodes demonstrated less geographic variation than did trematodes and nematodes. All trematode species of the Fisheating Creek population apparently are supported by other host species on the area, and occur in turkeys either secondarily, or, as in most cases, accidentally. Evidently this is true also for several nematode species occurring infrequently in the population. Judging from the small prevalences of trematodes in Mississippi Delta turkeys (Prestwood, 1968) the same phenomenon exists there. Differences in ecological settings such as intermediate and other definitive hosts on the areas and habitat probably account for many of the differences in helminth distributions.

Figure 2 presents a comparison of profiles of helminth species in the two populations; only helminths with relative abundances of 1% or greater in each population were graphed. In this manner the dominant species in each population were selected and compared using the index of similarity; an index of 56% was obtained. This value is higher than the indexes for any of the taxonomic groups, but still shows substantial variation in helminth faunas between Delta and Fisheating Creek turkeys. Dominance in terms of the most prevalent and most abundant worms appears to be held by different species in the two populations. From Figure 2 it can be seen that *Strongyloides* sp. and *Metroliasthes lucida* were the two most abundant helminths in the Fisheating Creek population. This is in contrast to the Delta population in which *Heterakis* spp. and *Ascaridia dissimilis* were most abundant.

Odum (1959) points out that predators, parasites, and pathogens often act through density-dependent pathways. As the population density of host or prey increases, the incidence of infection or attack increases since greater numbers can be located. Turkey populations on the Mississippi Delta areas were considered very high with an estimated density of one turkey per 10 acres (Prestwood, 1968). In 1970, Williams (unpublished data) estimated the prenesting season turkey population on the Fisheating Creek area to be no greater than one bird per 35 acres. This difference in host densities was reflected in differences in worm loads, with Delta turkeys having helminth burdens almost seven times larger than those of Fisheating Creek birds (Table 3). Heavy nematode burdens in Mississippi turkeys were largely responsible for the difference, although parasitism by cestodes and acanthocephalans was also greater in Delta birds. From the parasite profiles, *Strongyloides* sp. and *Metroliasthes* appear to be much more important in the Fisheating Creek pop-

Table 3. Comparison of helminth burdens for Fisheating Creek and Mississippi Delta turkeys.

	Fisheating Creek (present study)	Mississippi Delta (Prestwood, 1968)
Total no. of worms	8,960	78,564
No. hosts examined	172	216
Per cent of total No. comprised by:		
Trematodes	3.8	0.2
Cestodes	41.3	19.0
Nematodes	54.9	80.8
Acanthocephala	0.01	0.04
Prevalence % – avg. burden:		
Trematodes	36.6– 5.5	8.8– 7.0
Cestodes	61.6–34.9	88.0– 78.7
Nematodes	87.8–32.6	100.0–293.7
Acanthocephala	0.6– 1.0	9.3– 1.9
All helminths	91.9–52.1	100.0–364.0

ulation than in Delta birds. Actually, prevalence and numbers of *Strongyloides* and *M. lucida* in the two populations were almost equal, a fact overshadowed by the high prevalences and heavy infections of *Heterakis* and *Ascaridia* in Mississippi birds.

Helminth species and burdens on the two areas present different host population implications. Prestwood (1968) states that because turkeys on the Delta area were heavily parasitized, several management recommendations were made. An increased hunter harvest and conversion from a "gobblers only" to an "either sex" fall season were advocated. Reduced turkey densities would tend to lessen the likelihood of disease transmission and winter food shortages. In view of present parasitologic findings, these recommendations are not applicable to the Fisheating Creek population.

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Report on the Brayton H. Ransom Memorial Trust Fund

Balance on Hand, 1 January, 1974	$3495.75
Receipts: Interest received in 1974	226.84
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Disbursements: Grant to Helminthological Society of Washington	10.00
On Hand, 31 December, 1974	$3712.59

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Critical Tests of Levamisole Alone or in Mixtures with Piperazine or Trichlorfon Against Internal Parasites of Horses[1]

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ABSTRACT: Critical tests were conducted in 11 horses with levamisole at the dose level of 8 mg/kg alone or as a mixture with piperazine at the dose level of 88 mg base equivalent/kg or with trichlorfon at the dose level of 40 mg/kg. No toxicosis was seen in any of the treated horses.

Levamisole hydrochloride via stomach tube in one horse removed 100% of *Strongylus vulgaris*, 47% of *Strongylus edentatus*, and 24% of small strongyles. Removals of immature *Oxyuris equi*, *Gasterophilus intestinalis*, and *Gasterophilus nasalis* were nil. *Parascaris equorum* and mature *O. equi* were not present.

A mixture of levamisole hydrochloride and piperazine dihydrochloride administered via stomach tube to two horses removed 33% of *S. vulgaris*, 100% of mature *P. equorum*, and 70% of mature *O. equi*. There was no activity on *G. intestinalis* and *G. nasalis*; *S. edentatus* and immature *P. equorum* were not present, and removals of small strongyles and immature *O. equi* were not determined.

A mixture of levamisole hydrochloride and piperazine phosphate in alfalfa pellets was fed to three horses. Time for consumption varied from 30 min to 32 hr. In the aggregate, per cent removals were 97% of *S. vulgaris*, 90% of *S. edentatus*, 98% of small strongyles, 100% of mature and immature *P. equorum*, 17% of mature and 76% of immature *O. equi*, and 99% of *Probstmayria vivipara*. Removal of second- and third-instar *G. intestinalis* was unexpectedly high in one horse, but of low order against this species in the other horses and *G. nasalis* in all three horses.

A mixture of levamisole hydrochloride and trichlorfon via stomach tube in five horses removed in the aggregate 99% of *S. vulgaris*, 64% of *S. edentatus*, 83% of *Strongylus equinus*, 83% of small strongyles, 100% of mature *P. equorum*, 0% of mature *O. equi*, 99% of immature *O. equi*, 100% of *P. vivipara*, 99% of second-instar *G. intestinalis*, 89% of third-instar *G. intestinalis*, and 100% of second- and third-instar *G. nasalis*.

There was no evidence of activity against migrating fourth- and fifth-stage larvae of *S. vulgaris* in the cranial mesenteric artery, *Habronema muscae* in the stomachs, *Anoplocephala perfoliata* or *Anoplocephala magna* in the gastrointestinal tracts of any of the treated horses.

Anthelmintic activity of *dl*-tetramisole (Lyons and Drudge, 1970), levamisole (Clarkson and Beg, 1971), and a mixture of levamisole and piperazine (Drudge et al., 1974) has been reported in horses. In general, the *dextro-levo* and *levo* forms of tetramisole are highly efficacious against *P. equorum* and *S. vulgaris*, but much less effective for removing *S. edentatus*, small strongyles, mature *O. equi*, and immature *O. equi*. For the mixture of levamisole and piperazine, aggregate removals were 97% of *S. vulgaris*, 63% of *S. edentatus*, 97% of small strongyles, 100% of mature and immature *P. equorum*, 33% of mature *O. equi*, 76% of immature *O. equi*, and 97% of *P. vivipara*. Some activity on *H. muscae* was in evidence.

Published data on antiparasitic efficacy of trichlorfon indicate excellent activity against *Gasterophilus* spp. (Drummond et al., 1959; Trace et al., 1962) and *P. equorum*, mature *O. equi*, large strongyles (*S. vulgaris* and *S. edentatus*), and small strongyles (Trace et al., 1962). In a summary of unpublished critical test data (Drudge, 1965), essentially the same activity of trichlorfon was reported as by Trace et al., 1962, at the dose level of 80 mg/kg of the bolus formulation except that he found removal of *S. edentatus* was lower than that of the other major parasites. At the dose level of 40 mg/kg (Drudge, 1972) technical trichlorfon had excellent activity on bots, ascarids, and mature pinworms, but activity was ineffective on *S. vulgaris*, *S. edentatus*, and small strongyles.

Purposes of the present critical tests were

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(1) to provide additional data on antiparasitic efficacies for levamisole alone via stomach tube or a mixture of levamisole and piperazine in stomach tube and feed additive formulations, and (2) to investigate the compatibility and spectrum of activity against the gastrointestinal parasites of a mixture of levamisole at the dose level of 8 mg/kg and trichlorfon at the dose level of 40 mg/kg administered by stomach tube.

Materials and Methods

The critical tests were done in 1971, 1972, and 1973 in the total of 11 horses which ranged from 4 to 12 months of age and were of mixed light-horse type breeding. All of the parasitic infections were naturally acquired.

Individual doses of each drug (supplied by American Cyanamid Company, Princeton, NJ) were measured by weight or volume before administration. The horses were not fasted before treatment; hay and water were available ad lib.

Levamisole hydrochloride, which was formulated as a 4.49% active ingredient drench powder, was dissolved in 0.5 liter water for the treatment of one horse (No. 1582) at the dose level of 8 mg/kg, via stomach tube. This was followed by 0.5 liter water rinse. A solution of levamisole hydrochloride (1.2% w/v active ingredient) at the dose level of 8 mg/kg, and a solution of piperazine dihydrochloride (13.31% w/v active ingredient as base) at the dose level of 88 mg base equivalent/kg, was administered to two horses (Nos. 1718 and 1726) via stomach tube. This was followed by 0.5 liter water rinse.

Alfalfa pellets (each 454 g containing 3.6 g levamisole hydrochloride as the resinate and 40.0 g piperazine base as the phosphate) were fed to deliver levamisole hydrochloride at the dose level of 8 mg/kg and piperazine phosphate at the dose level of 88 mg base equivalent/kg to three horses (Nos. 72-1435, 1705, and 1713).

Levamisole hydrochloride (90% active ingredient) soluble drench powder, at the dose level of 8 mg/kg, and trichlorfon powder (90% active ingredient) at the dose level of 40 mg/kg were dissolved in 100 ml water and administered via stomach tube, followed by 0.5 liter water rinse, to five horses. Levamisole was given first, followed immediately by the trichlorfon to two horses (Nos. 1745 and 1747) and the levamisole and the trichlorfon were mixed together immediately before administration to three horses (Nos. 1787, 1800, and 1804).

Two types of critical tests were carried out in the 11 horses (Table 1). Complete critical tests in which evaluation included the "small parasites" (small strongyles, immature *O. equi* and *P. vivipara*) and the "large parasites" (*G. intestinalis*, *G. nasalis*, *P. equorum*, mature *O. equi*, *S. vulgaris*, *S. edentatus*, and *S. equinus*) were done in nine horses. For two horses (Nos. 1718 and 1726), treated with a mixture of levamisole and piperazine via stomach tube, critical tests involved evaluation only against the large parasites. Procedures for recovering and enumerating the parasites in these critical tests were reported previously (Drudge et al., 1963).

Small strongyles recovered from the feces and the large intestinal contents at necropsy of three horses (Nos. 1745, 1747, and 1800) that were treated with the mixture of levamisole and trichlorfon via stomach tube were identified. Species of small strongyles that were recovered were compared with the checklist of Becklund (1964) (Table 4).

Procedures for pre- and posttreatment worm egg (EPG) and differential larval (LPG) counts on feces of the test horses were the same as reported previously (Drudge, 1963). One count was made on the day of treatment and one count was done when the horses were killed at 5 to 7 days posttreatment.

Results and Discussion

Ascarid and strongyle EPG's and LPG's for the major nematodes in the 11 critical test horses are summarized (Table 1). Additionally, data on *Strongyloides* indicated eggs and larvae were present in feces of three horses (Nos. 1726, 72-1435, and 1747) before, but not after, treatment. No evidence of toxicosis was seen in any of the treated horses.

Parasite recovery data on horses treated via stomach tube with levamisole alone or in the mixtures with piperazine are recorded (Table 2).

In the single test with levamisole alone, the excellent removal of *S. vulgaris* and limited activity on *S. edentatus*, small strongyles, immature *O. equi* and *Gasterophilus* spp. is in

Table 1. Egg and larval counts on feces of 11 critical test horses treated with single doses of levamisole hydrochloride (8 mg/kg) alone or mixtures containing piperazine (88 mg base equivalent/kg) or trichlorfon (40 mg/kg).

Horse No.	Type of critical test	Eggs per gram of feces				Larvae per gram of feces							
		Ascarid		Strongyle		Small strongyle		*S. vulgaris*		*S. edentatus*		*T. axei*	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Levamisole via stomach tube													
1582	C*	0	0	810	1,320	40	310	0	0	20	20	0	0
Mixture of levamisole and piperazine via stomach tube													
1718	L†	160	0	1,230	20	450	1	0	0	0	0	0	0
1726	L	20	0	580	0	165	0	0	0	0	0	0	0
Mixture of levamisole and piperazine alfalfa pellets via feed													
72–1435	C	1,020	0	1,270	0	ND	ND	ND	ND	ND	ND	ND	ND
1705	C	0	0	170	0	ND	ND	ND	ND	ND	ND	ND	ND
1713	C	0	0	1,540	0	ND	ND	ND	ND	ND	ND	ND	ND
Mixture of levamisole and trichlorfon via stomach tube													
1745	C	0	0	430	10	135	7	0	0	0	0	0	0
1747	C	0	0	430	60	45	50	0	0	0	0	0	0
1787	C	60	30	210	10	350	2	30	0	0	0	10	0
1800	C	0	0	1,090	40	195	26	0	0	25	7	0	0
1804	C	0	0	1,200	120	80	33	70	0	85	0	10	0

* = C—Complete critical test where evaluation was on both small and large parasites.
† L—Large parasite critical where evaluation was only on large parasites.
ND = No data.

Table 2. Critical test data on six horses treated with single doses of levamisole hydrochloride (8 mg/kg) alone or in a mixture containing piperazine (88 mg base equivalent/kg).

Horse No.	*G. intestinalis* 2nd instar	*G. intestinalis* 3rd instar	*G. nasalis* 2nd instar	*G. nasalis* 3rd instar	*P. equorum* Mature	*P. equorum* Immature	*O. equi* Mature	*O. equi* Immature	*S. vulgaris*	*S. edentatus*	Small strongyles	*P. vivipara*
					Total No. removed/total No. Present							
Levamisole via stomach tube												
1582 Totals	0/0	2/343	0/0	0/66	0/0	0/0	0/0	0/500	15/15	38/81	67T/278T	0/0
% Efficacy	—	< 1	—	0	—	—	—	0	100	47	24	—
Mixture of levamisole and piperazine via stomach tube												
1718	5/64	0/231	0/0	0/21	9/9	1/1	1/2	ND	0/0	0/0	ND	ND
1726	4/125	0/53	4/48	1/6	11/11	0/0	84/120	ND	5/15	0/0	ND	ND
Totals	9/189	0/284	4/48	1/27	20/20	1/1	85/122	—	5/15	0/0	—	—
% Efficacy*	5	0	8	4	100	100	70	—	33	—	—	—
Mixture of levamisole and piperazine alfalfa pellets via feed												
72–1435	8/41	1/61	0/0	0/0	89/89	41/51	0/0	0/0	0/0	0/0	25T/27T	0/0
1705	17/39	98/173	0/0	6/168	4/4	4/4	0/0	2,044/2,744	40/40	5/9	3.5T/3.9T	0/0
1713	1/67	0/0	0/43	0/39	0/0	0/0	1/6	1,805/2,305	109/113	47/49	1,379T/1,399T	34.5M/35.0M
Totals	26/147	99/234	0/43	6/209	93/93	45/45	1/6	3,849/5,049	149/153	52/58	1,407.5T/1,429.9T	34.5M/35.0M
% Efficacy*	18	42	0	3	100	100	17	76	97	90	98	99

M = Millions.
T = Thousands.
ND = No data.
* Aggregate.

agreement with that reported previously for *dl*-tetramisole and levamisole by Lyons and Drudge (1970), and Clarkson and Beg (1971), respectively. Partial removal of mature *H. muscae* was evidenced by finding 22 specimens in the feces and 36 specimens at necropsy. Immature *H. muscae* was not present.

For the two horses treated via stomach tube with a mixture of levamisole and piperazine, removal was 100% for mature *P. equorum* in both horses, 50% and 70% for mature *O. equi* in the two horses, respectively, and 33% for *S. vulgaris* in the one infected horse. A few *Gasterophilus* were recovered from the feces. These two horses were too young to harbor mature large strongyles in the large intestine, except for a few *S. vulgaris*. The low per cent removal of *S. vulgaris* is probably ascribable to the emergence of the young adults into the gastrointestinal tract between treatment and necropsy. The excellent removal of ascarids and lower activity on mature *O. equi* compares to the report (Drudge, 1974) for a mixture of levamisole and piperazine administered via stomach tube.

Time required for consumption of the alfalfa pellets containing the levamisole and piperazine varied for the three treated horses. Horse No. 72-1435 ate about 33% of the mixture in 45 min, about 66% in 6 hr, and 100% in 10 hr. Horse No. 1705 consumed the mixture within about 30 min. Horse No. 1713 consumed only about 33% of the mixture in 24 hr, and the remainder within 32 hr. Removal of *P. equorum* was 100% in the two horses harboring both mature and immature stages. Mature *O. equi* were present in only one horse (No. 1713) and removal was 17%. This low per cent removal of mature *O. equi* may have been due to the prolonged time (32 hr) for consumption of the pellets. However, the removal of *P. vivipara* was 99% in this, the only horse infected with this species. Efficacy against *S. vulgaris* was 100 and 96%, and against *S. edentatus* 56 and 96%, respectively, for the two infected horses. These values for these large strongyles are comparable to those reported (Drudge et al., 1974) for levamisole and piperazine via stomach tube except the 96% removal of *S. edentatus* from one horse was much higher than they reported. The 44 and 57% removals of second- and third-instar *G. intestinalis*, respectively, coupled with 4% removal of third-

Table 3. Critical test data on five horses treated with single doses of a mixture of levamisole (8 mg/kg) and trichlorfon (40 mg/kg) administered by stomach tube.

Horse No.	*G. intestinalis*		*G. nasalis*		*P. equorum*	*O. equi*		*S. vulgaris*	*S. edentatus*	*S. equinus*	Small strongyles	*P. vivipara*
	2nd instar	3rd instar	2nd instar	3rd instar	Mature	Mature	Immature					
	Total No. removed/total No. present											
1745	179/180	205/210	3/3	80/80	0/0	0/0	545/545	259/259	51/88	3/3	89T/93T	0/0
1747	4/4	203/239	0/0	0/1	0/0	0/0	209/209	67/70	0/0	0/0	1.5%/9.2T	0/0
1787	0/0	82/139	0/0	0/0	0/0	0/0	0/0	25/25	6/6	0/0	70T/82T	0/0
1800	0/0	163/163	0/0	59/59	0/0	0/1	73/123	40/40	22/39	2/3	14T/40T	0/0
1804	0/0	211/217	0/0	23/23	2/2	0/0	4,945/4,945	181/181	116/172	0/0	39T/42T	229T/229T
Totals	183/184	864/968	3/3	162/163	2/2	0/0	5,772/5,822	572/575	195/305	5/6	213.5T/257.2T	229T/229T
% Efficacy*	99	89	100	100	100	0	99	99	64	83	83	100

T = Thousands.
* Aggregate.

instar *G. nasalis* for horse No. 1705 cannot be explained. The test was run in August which could account for the presence of third instars but not the second instars in the feces as spontaneous discharges. Mature *H. muscae* was not found in any of the horses. Immature *H. muscae* were found (20 to 500) in all three horses at necropsy but none of these forms was recovered from the feces of the three horses.

Critical test data on the five horses treated with a mixture of levamisole hydrochloride and trichlorfon via stomach tube are summarized (Table 3).

Removal of second-instar *G. intestinalis* was 99 and 100%, respectively, from the two infected horses. Third-instar *G. intestinalis* were present in all five horses and removal varied from 59 to 100%. All of the second-instar *G. nasalis* were removed from the single horse that was infected. There was 100% removal of third-instar *G. nasalis* from three infected horses but the one specimen present in one other horse was not removed. The removal of the two species of bots was similar to that reported by Drudge (1972) for trichlorfon at the dose level of 40 mg/kg except for the low (59%) efficacy against third-instar *G. intestinalis* from one horse.

Both of the *P. equorum* present in one horse were removed. The single mature specimen of *O. equi* in one horse was not removed. Removal of immature *O. equi* was 100% from three horses and 60% from one other horse. Efficacious removal of immature *O. equi*, except for one horse, indicates that activity was either from trichlorfon for which published data are not available at the 40 mg/kg dose level or a synergistic effect, because levamisole alone is ineffective on this stage of *O. equi* (Lyons and Drudge, 1970; Clarkson and Beg, 1971). Removal of *P. vivipara* was 100% in the one infected horse.

Activity on *S. vulgaris* varied from 96 to 100% in these five horses, and was probably largely attributable to the levamisole component since trichlorfon is ineffective on large strongyles (*S. vulgaris* and *S. edentatus*) according to Drudge (1972) at the dose level of 40 mg/kg. Removal of *S. edentatus* from the four infected horses varied from 56 to 100%; this level of removal activity is similar to that reported for levamisole except that the highest efficacy (100%) in the one horse is unusual. *S. equinus* was present in low numbers in two horses and removal was 67 and 100%, respectively.

Efficacy against small strongyles in these five horses varied widely, ranging from 16 to 96%. The efficacious removal in the three horses suggests a synergistic or additive effect, because *dl*-tetramisole or levamisole alone (Lyons and Drudge, 1970; Clarkson and Beg, 1971) and trichlorfon at 40 mg/kg alone (Drudge, 1972) are relatively ineffective against these parasites. No reason for the low level of efficacy on small strongyles in two horses can be given. To determine if the difference in susceptibility to the mixture was attributable to the species of small strongyles present, identification was completed on the species present in one horse where removal was high (No. 1745) and those from the two horses where removal was low (Nos. 1747 and 1800) (Table 4). No definite pattern emerged from these observations that would indicate certain species of small strongyles were relatively more "resistant" or "susceptible" than others to the mixture of levamisole and trichlorfon. Possibly a subtle relationship between the two drugs, based on some delicate factor such as acidity or alkalinity of the gastrointestinal tract, plays a determinate role in the activity against small strongyles.

Twenty-seven mature *H. muscae* were found at necropsy but none was recovered from the feces of the single infected horse, while immature *H. muscae* were not found in the feces or the stomachs of any of the horses treated with the levamisole and trichlorfon mixture.

Aneurysms of the cranial mesenteric artery and its main branches from all of the 11 test horses were examined for migrating fourth- and fifth-stage larvae of *S. vulgaris*. Neither of these stages was found in the one horse treated with levamisole alone or in one of the five horses treated with the mixture of levamisole and trichlorfon. All of the other nine treated horses had both of these stages of *S. vulgaris* in their aneurysms. The numbers of fourth- and fifth-stage larvae were: 43 and 27 and 4 and 22, respectively, for the two horses treated with a mixture of levamisole and piperazine via stomach tube. The number of fourth- and fifth-stage larvae varied from 31 to 255 and 12 to 69, respectively, for the three horses treated with a mixture of levamisole and piper-

Table 4. Species of small strongyles in feces and at necropsy of three horses treated with a mixture of levamisole and trichlorfon.

Checklist of species*	Occuring in horses in central Kentucky	Occuring in feces			Occurring at necropsy		
		Horse No. 1745	Horse No. 1747	Horse No. 1800	Horse No. 1745	Horse No. 1747	Horse No. 1800
Craterostomum acuticaudatum	–	–	–	–	–	–	–
Craterostomum mucronatum	x	x	–	–	–	–	x
Cyathostomum coronatum	x	x	–	–	x	–	x
Cyathostomum labiatum	x	x	–	x	x	–	x
Cyathostomum labratum	x	x	–	–	x	–	x
Cyathostomum ornatum	–	–	–	–	–	–	–
Cyathostomum tetracanthum	–	–	–	–	–	–	–
Cylicobrachytus brevicapsulatus	–	–	–	–	–	–	–
Cylicocercus alveatus	–	–	–	–	–	–	–
Cylicocercus catinatus	x	x	–	x	x	–	x
Cylicocercus goldi	x	x	–	–	–	–	–
Cylicocercus pateratus	x	–	–	x	–	–	–
Cylicocyclus auriculatus	–	–	–	–	–	–	–
Cylicocyclus elongatus	x	x	–	–	–	–	–
Cylicocyclus insigne	x	x	–	x	x	x	x
Cylicocyclus leptostomus	x	–	–	–	–	–	–
Cylicocyclus nassatus	x	x	–	x	x	x	–
Cylicocyclus radiatus	x	x	–	–	–	–	–
Cylicodontophorus bicoronatus	x	x	–	–	–	–	–
Cylicodontophorus euproctus	x	–	–	–	–	–	–
Cylicodontophorus mettami	x	–	–	–	–	–	–
Cylicodontophorus ultrajectinus	x	–	–	–	–	–	–
Cylicostephanus calicatus	x	x	–	x	–	–	x
Cylicostephanus hybridus	–	–	–	–	–	–	–
Cylicostephanus longibursatus	x	x	x	x	x	x	x
Cylicostephanus minutus	x	x	–	x	–	–	x
Cylicostephanus poculatus	x	x	–	x	–	–	–
Cylicotetrapedon asymetricus	x	x	–	–	–	–	–
Gyalocephalus captitatus	x	–	–	–	–	–	–
Oesophagodontus robustus	x	–	–	–	–	–	–
Poteriostomum imparidentatum	x	x	–	–	–	–	–
Poteriostomum ratzii	x	–	–	–	–	–	–
Triodontophorus brevicauda	x	x	x	–	–	–	–
Triodontophorus minor	x	–	–	–	–	–	–
Triodontophorus serratus	x	x	–	–	–	–	–
Triodontophorus tenuicollis	x	x	x	x	–	x	–
Immature fourth stage	x	x	x	x	x	x	x

* According to Becklund (1964).

azine in the alfalfa pellets and 18 to 41 and 11 to 30, respectively, for four of the horses treated with a mixture of levamisole and trichlorfon via stomach tube.

Tapeworms were present in only three of the 11 treated horses. *A. perfoliata* was present in two horses; one specimen was present but not removed from the single horse treated with levamisole alone, and one specimen was recovered from the feces of one of the five horses treated with the mixture of levamisole and trichlorfon. One specimen of *A. magna* was present but not removed from one of the three horses fed the alfalfa pellet formulation of levamisole and piperazine.

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Efficacy of Oxibendazole as an Anthelmintic in Cattle

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ABSTRACT: In a critical test of the activity of oxibendazole at dose rates of 5 and 10 mg/kg in cattle, efficacy against adult *Haemonchus contortus, Ostertagia ostertagi, Trichostrongylus axei, Trichostrongylus colubriformis, Cooperia oncophora,* and *Oesophagostomum radiatum* ranged from 85 to 100%. Efficacy was slightly poorer against *H. contortus* and *C. oncophora* at the lower dosage. Activity at 10 mg/kg against 3- and 7-day-old worms of these species was assessed in a controlled test. Efficacy was above 90% against *H. contortus, T. colubriformis,* and *C. oncophora,* 76 and 87% against *T. axei,* and 34% against *O. ostertagi*; there was no activity against *O. radiatum.*

A new anthelmintic, oxibendazole (methyl 5 n-propoxy-2-benzimidazole carbonate), has been reported (Theodorides et al., 1973; Theodorides and Chang, 1974) to be highly effective against the adult stages of the nematodes, *Haemonchus contortus, Ostertagia ostertagi, Trichostrongylus axei, Trichostrongylus colubriformis, Cooperia* spp., and *Oesophagostomum radiatum* in cattle. They (loc. cit.) also reported that the drug was highly effective against immature and adult stages of species from the same genera that parasitize sheep. Their results were based on controlled tests in which limited numbers of animals were used. This report presents the results of critical tests with oxibendazole against adult nematodes in cattle and a controlled test against immature stages.

Procedure

Ten 4-month-old calves were used for the critical tests. Each was inoculated with a single dose of the following mixture of nematode larvae: 17,000 *H. contortus,* 180,000 *O. ostertagi,* 120,000 *T. axei,* 90,000 *C. oncophora,* 100,000 *T. colubriformis,* and 10,000 *O. radiatum.* After 28 days, oxibendazole (supplied by Smith, Kline and French, Inc., Philadelphia, Pennsylvania) was given per os to five calves at a dose rate of 5 mg/kg body weight and to five calves at 10 mg/kg. Feces were collected every 24 hr for 4 days and examined by the technique of Swanson et al., (1940). After 4 days, residual worm counts were determined at necropsy by techniques of Porter (1942) and Herlich (1956).

Twelve 3-month-old calves were used in controlled tests against 3- and 7-day-old worms. Each calf was inoculated with a single dose of the following infective larvae: 15,000 *H. contortus,* 40,000 *O. ostertagi,* 40,000 *T. axei,* 30,000 *T. colubriformis,* 50,000 *C. oncophora,* and 5,000 *O. radiatum.* Three days after inoculation, four calves were treated with oxibendazole at 10 mg/kg; 7 days after inoculation four other calves were similarly treated; and four calves were left untreated to serve as controls.

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Table 1. Anthelmintic activity of oxibendazole against adult gastrointestinal nematodes in 10 calves treated at a dosage of 5 or 10 mg/kg.

Dosage and animal No.	Worms eliminated after treatment					
	H.c.	O.o.	T.a.	T.c.	C.o.	O.r.
5 mg/kg:						
714	270	18,900	42,000	2,400	31,080	2,072
715	190	30,800	42,000	2,800	27,160	196
730	530	26,180	33,600	4,200	16,520	1,568
731	460	39,100	51,200	3,300	14,180	1,210
732	420	17,500	29,800	3,090	28,420	1,415
Avg.	374	26,496	39,720	3,158	23,472	1,292
	Worms recovered at necropsy*					
714	27	13	0	0	1,960	0
715	0	133	0	0	4,620	0
730	80	93	0	0	6,160	0
731	45	122	0	0	1,820	0
732	50	60	0	0	3,200	0
Avg.	40 (89)	84 (99)	0 (100)	0 (100)	3,552 (85)	0 (100)
	Worms eliminated after treatment					
10 mg/kg:						
668	480	37,925	47,930	1,800	14,485	1,680
693	260	49,105	75,325	4,200	15,520	4,600
694	340	34,645	50,600	6,000	13,795	230
695	240	39,905	41,745	3,800	21,850	4,280
696	360	30,900	52,600	3,900	14,120	1,760
Avg.	336	38,496	53,640	3,940	15,954	2,510
	Worms recovered at necropsy*					
668	0	1,620	840	0	30	0
693	0	270	60	0	60	0
694	0	360	120	0	90	0
695	0	210	0	0	0	0
696	0	460	0	0	20	0
Avg.	0 (100)	584 (98)	204 (99)	0 (100)	40 (99)	0 (100)

H.c. = *H. contortus*; O.o. = *O. ostertagi*; T.a. = *T. axei*; T.c. = *T. colubriformis*; C.o. = *C. oncophora*; O.r. = *O. radiatum*.

* Figures in parentheses represent percentage of efficacy.

Necropsy was performed on all calves 28 days after inoculation to determine residual worm counts.

Results and Discussion

Worm count data for calves in the critical tests are shown in Table 1. Nearly all worms (90% or more) were expelled within 48 hr. Most of the worms recovered during the next 48 hr were *C. oncophora* and lesser numbers of *O. ostertagi*.

The drug was 85 to 100% effective against adults of all species at both dose rates; it was slightly less active against *H. contortus* and *O. ostertagi* at the lower dosage. Because intact specimens of *H. contortus* were infrequently recovered, the estimate of numbers expelled was based on posterior ends of worms only. The fact that this species was so severely affected by hostal digestion suggests that estimates of the numbers expelled were conservative and that the efficacy of oxibendazole at 5 mg/kg was probably greater than 89%. Calf 732, which was passing eggs of *Bunostomum phlebotomum* at the time of inoculation, expelled seven hookworms and had none at necropsy. These results confirm those reported by Theodorides et al. (1973) and Theodorides and Chang (1974).

Table 2. Anthelmintic activity of oxibendazole against 3- and 7-day-old gastrointestinal nematodes in eight calves treated at a dosage of 10 mg/kg.

Animal No.	Worms recovered at necropsy*					
	H.c.	O.o	T.a.	T.c.	C.o.	O.r.
			Group I—Treated 3 days postinoculation			
751	400	18,400	6,200	0	180	1,320
756	820	18,200	3,600	0	60	1,980
767	140	11,400	1,600	0	150	2,380
768	1,060	33,600	7,600	60	600	2,135
Avg.	605 (91)	20,400 (34)	4,750 (76)	15 (99)	248 (99)	1,954 (0)
			Group II—Treated 7 days postinoculation*			
757	520	27,200	2,600	0	720	2,960
758	280	29,600	3,800	0	2,460	1,170
769	240	6,200	600	0	1,290	1,980
770	480	18,600	2,800	0	1,060	2,425
Avg.	380 (94)	20,400 (34)	2,450 (87)	0 (100)	1,383 (96)	1,748 (0)
			Group III—Untreated controls			
759	6,480	38,200	15,400	1,500	26,400	1,710
771	9,140	27,400	21,800	4,500	33,900	1,800
773	3,560	27,600	21,200	3,900	39,900	2,100
774	6,740	29,800	19,200	4,500	24,900	1,380
Avg.	6,480	30,750	19,400	3,600	31,275	1,748

H.c. = *H. contortus*; O.o. = *O. ostertagi*; T.a. = *T. axei*; T.c. = *T. colubriformis*; C.o. = *C. oncophora*; O.r. = *O. radiatum*.
* Figures in parentheses represent percentage of efficacy.

Results of the controlled test with immature worms are shown in Table 2. Efficacy against 3- and 7-day-old *H. contortus*, *T. colubriformis*, and *C. oncophora* ranged from 91 to 99%, comparing favorably with the anthelmintic action against adults. However, activity against *O. ostertagi* and *T. axei* was poor, and the drug was totally ineffective against *O. radiatum*. The results with these three species are in sharp contrast to the reported (Theodorides et al., 1973) activity of oxibendazole against immature stages of the same or closely related parasites in sheep. However, precise details of the developmental stages and species of nematode were not given for that study, and subsequent work by that group has yielded results (pers. comm.) similar to mine.

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Effects of Refrigeration, Cooking, and Freezing on *Sarcocystis* in Beef from Retail Food Stores

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ABSTRACT: *Sarcocystis* organisms in ground bovine hearts survived storage at refrigeration temperatures for 3 days. Dogs fed this meat became infected and passed sporocysts in their feces. Ground beef from a small local grocery store was fed raw to dogs, and ground beef from a supermarket was fed as raw, rare, medium, and well-done hamburger patties. All dogs fed raw and rare meat became infected and passed sporocysts. Ground beef from a supermarket was fed raw to dogs on the day of purchase and meat from the same batch was fed to other dogs after it had been frozen for 7 days. Only those dogs fed unfrozen meat became infected and passed sporocysts.

Workers recently have found that *Sarcocystis*-infected meat obtained fresh from abattoirs and ingested by dogs, cats, humans, or coyotes results in the development of coccidian stages in the intestine and elimination of sporocysts and/or oocysts in the feces (Rommel et al., 1972; Heydorn and Rommel, 1972; Mahrt, 1973; Fayer and Leek, 1973; Rommel and Heydorn, 1972; Fayer, 1975). Although 22,194 carcasses of cattle and sheep were condemned at slaughter in the United States in 1971–73 because of sarcosporidiosis and eosinophilic myositis (a loss of over $6.7 million),[1] most carcasses harboring cysts of *Sarcocystis* show no gross signs of infection and therefore pass inspection. Indeed, in three surveys in the United States, 75 to 98% of the cattle examined at slaughter were found to be infected with *Sarcocystis* (Levine, 1973). The experiments described herein were undertaken to determine whether *Sarcocystis*-infected beef obtained at slaughter would remain infectious after refrigeration, whether beef obtained from retail stores contained infectious *Sarcocystis* organisms, and whether such organisms remained infectious after cooking and after freezing.

Materials and Methods

In three of four experiments, tissue fluid expressed from meat samples was found to contain bodies that could not, in all samples, be positively identified as *Sarcocystis* zoites although they were similar in size and shape. The beef was fed to 28 5- to 17-month-old coccidia-free beagles. These dogs had never previously eaten raw meat and, except for the time of infection, were fed dry pelleted dog food. Dogs were housed in individual cages and fecal samples collected daily from each animal were examined microscopically for the presence of oocysts and sporocysts.

The first experiment was conducted to determine whether *Sarcocystis* organisms would survive storage at refrigeration temperatures for several days. Eight dogs were divided into four groups of two. Several bovine hearts were obtained from a local abattoir and ground in a commercial meat grinder, and part of this ground meat was fed within 4 hr of slaughter to Group 1. The rest of the ground heart was refrigerated, and part was fed to Group 2 at 24 hr, to Group 3 at 48 hr, and Group 4 at 72 hr.

The second experiment was conducted to determine whether *Sarcocystis* organisms were still viable and infective in meat sold by a retail store. Six dogs were divided into two groups of three. Three pounds of lean ground beef were purchased daily for 5 days from a small local grocery store, and 1 pound was fed to each of the dogs in Group 1. Dogs in Group 2 received the normal dry food ration.

The third experiment was conducted to determine the effect of cooking on viability and infectivity of the organisms. Eight dogs were divided into four groups of two. Eight pounds of lean ground beef were purchased daily for

[1] Calculated from whole carcass condemnations due to sarcosporidiosis and eosinophilic myositis (a possible result of sarcosporidiosis) in Federal Meat and Poultry Inspection Statistical Summary for 1971, ibid. 1972, ibid. 1973, and estimating value of cattle at $500 and sheep at $50.

5 days from a large supermarket in the Maryland suburbs of Washington, D. C. This meat was formed into 16 uniform ½-pound patties with the aid of a hamburger press. Patties were divided into four groups of four. Meat for Group 1 was not cooked; meat for the other groups was oven cooked at 400 F (204.4 C), until it was either rare, medium, or well done. Criteria for determining rare, medium, and well-done meats included color and temperature of the meat at the center of the patty. Rare patties were red, medium were pink, and well done were brown. The temperature in the rare patty was 100–128 F (37.8–53.3 C), that in the medium was 140 F (60 C), and that in the well done was 160–166 F (71.1–74.4 C). Temperature was recorded on a Honeywell Brown Electronik potentiometer[2] with a heat-shielded thermistor cable inserted into the center of two patties in each group. Patties were removed from the oven when they reached the desired temperature. Each day, two raw patties were fed to each dog in Group 1, two rare patties were fed to each dog in Group 2, two medium patties were fed to each dog in Group 3, and two well-done patties were fed to each dog in Group 4.

The fourth experiment was conducted to determine the effect of freezing on the viability and infectivity of the organisms. Six dogs were divided into three groups of two. Four pounds of lean ground beef were purchased daily for 5 days from a large supermarket. Each day, 1 lb. of ground beef was fed to each dog in Group 1 and the remaining 2 lb. placed in the freezer section of a refrigerator. Each 2-lb. package of ground beef was thawed 7 days after freezing and 1 lb. was fed to each dog in Group 2 for 5 successive days. Dogs in Group 3 were fed only dry pelleted feed.

Results

Effect of storage at refrigeration temperature (Experiment 1): Dogs were fed ground meat within 4 hr of slaughter and after refrigeration for 24, 48, and 72 hr. Beginning 15–18 days after the infective meat was fed, all eight dogs passed sporulated *Sarcocystis* sporocysts.

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may be suitable.

Infectivity of ground beef from grocery store (Experiment 2): All three dogs fed ground beef began to pass sporulated sporocysts in their feces 19–21 days after the initial feeding of ground beef. None of the three dogs fed the normal dry food ration passed such stages.

Effect of cooking on infectivity of organisms (Experiment 3): All dogs fed raw or rare patties began to pass sporocysts 16–17 days after the initial feeding. None of the dogs fed medium or well-done patties passed such stages within 25 days after the initial feeding.

Effect of freezing on infectivity of organisms (Experiment 4): Both dogs fed raw unfrozen ground beef began to pass sporulated sporocysts in their feces 15–16 days after the initial feeding. Neither the two dogs fed ground beef from the same batches, but frozen for 1 week, nor the two dogs fed dry pelleted food passed such stages within 22 days after the initial feeding of thawed ground beef.

Discussion

Because of the high percentage of cattle infected with *Sarcocystis* and the present findings that indicate that viable, infectious organisms are present in fresh beef sold in retail stores (Exps. 2–4), the potential for transmission of *Sarcocystis* to humans and their pets by fresh beef clearly exists.

Five of six German investigators who ate *Sarcocystis*-infected raw beef or raw pork seasoned with onions and spices passed sporocysts in their feces beginning 9–17 days later (Rommel and Heydorn, 1972). One suffered a severe influenzalike infection with fever and mild diarrhea. The others were asymptomatic. Ingestion of meat containing greater numbers of organisms, ingestion of larger quantities of infected meat, or ingestion of meat containing other species or strains of *Sarcocystis* could significantly alter the degree of pathogenicity.

Toxoplasma gondii, a protozoan parasite pathogenic for many animals, including man, is closely related to *Sarcocystis* and has also been found in cattle, swine, and sheep in the United States and other countries (Levine, 1973). Transmission to man by means of undercooked meat was strongly suggested

when patients in a hospital in France where such meat was given for therapeutic purposes became infected (Desmonts et al., 1965) and in the U. S. where five medical students who had eaten hamburgers at the same time and place concurrently suffered acute lymphadenitic toxoplasmosis (Kean et al., 1969). The finding that cats and certain other Felidae produce oocysts after ingesting *Toxoplasma*-infected mice and that these oocysts infect all birds and mammals tested and, circumstantially, humans (Miller et al., 1972) suggests that the feeding of raw or undercooked meat to pets may result in human infection as a result of contact with organisms from pet feces. Although we do not know yet whether *Sarcocystis* sporocysts from dogs or cats are infectious to humans, conceivably, transmission from pets could occur similarly.

Both freezing and cooking appear to reduce the infectivity of *Toxoplasma* and *Sarcocystis*. Dubey (1974) found that *Toxoplasma*-infected mice that had been stored at –9 and –20 C for as little as 3 hr were not infectious for cats. Jacobs et al. (1960) found that the viability of *Toxoplasma* organisms in mouse brain decreased after 1 hr at 113 F (45 C) and was lost after 30 min at 122 F (50 C) and 10–15 min at 132.8 F (56 C). Similarly, the effect of cooking decreases infectivity of *Sarcocystis*-infected meat. Gestrich (1974) fed cats bovine diaphragm stored at 35.6 F (2 C) for 14 days and heated to different temperatures. All cats fed meat heated to 113 F (45 C) for 5–6 min passed sporocysts, as did one of four cats fed meat heated to 131–140 F (55–60 C) for 6 min. No cats fed meat heated to 149–158 F (65–70 C) for 10 min passed sporocysts. Gestrich also found that of two groups of cats, one fed fresh meat and the other fed meat that had been stored at –20 C for 3 days, the latter group passed no sporocysts. These results are closely paralleled and substantiated by the results obtained in the present study, and together they indicate that *Sarcocystis* organisms remain infective for long periods of time and that proper cooking or freezing may be effective controls.

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Range Extension Records for *Cooperia curticei, Ostertagia ostertagi, Setaria yehi,* and *Trichuris ovis* in White-tailed Deer from Kentucky

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ABSTRACT: In order of prevalence, the following helminths (all nematodes) were recovered from 31 white-tailed deer (*Odocoileus virginianus*) from Kentucky: *Ostertagia ostertagi, Oesophagostomum venulosum, Gongylonema pulchrum, Dictyocaulus viviparus, Ostertagia mossi, Trichuris ovis, Pneumostrongylus tenuis, Capillaria bovis, Setaria yehi,* and *Cooperia curticei. Cooperia curticei, O. ostertagi, S. yehi,* and *T. ovis* are new state and range extension records.

There are very few reported accounts of helminths from the white-tailed deer in Kentucky. As a result, it was felt that a more complete study of the helminths should be done on the white-tailed deer in Kentucky and is the basis for this report.

Materials and Methods

By a special permit from the Director of the Kentucky Department of Fish and Wildlife Resources, 14 deer were taken from the Lexington Blue Grass Army Depot (Madison County) and 17 deer from Land Between the Lakes recreation area in western Kentucky. The deer obtained were aged and weighed. They were all young bucks (1½–2½ years of age) and taken during the fall of 1973 and 1974.

The viscera and heads were placed in plastic bags, perforated, and flooded with 10% formalin (for preservation of contents until examined). The bags were placed inside garbage cans and returned to the laboratory for examination.

The heads were examined for meningeal worms by the technique used by Prestwood and Smith (1969). Internal organs were separated and examined externally for the presence of helminths. The liver and lungs were cut into small sections and placed under a 3× dissecting microscope. The ducts were severed longitudinally and the passageways examined. In addition, the lungs were washed into a 500-ml beaker of water which was then poured through a No. 100 mesh screen in order to expose fourth- and fifth-stage larval lungworms (Prestwood et al., 1971).

The esophagus was split longitudinally and examined according to Samuel and Beaudoin (1966). The majority of the contents of the rumen, reticulum, omasum, and abomasum were flushed through a No. 20 sieve and examined for stomach worms. Afterwards, the Baermann technique was used on the remainder of the stomach contents. The contents of the cecum, large and small intestines, were washed through a No. 20 sieve and examined. Feces were removed and examined according to Levine et al. (1960). The kidneys and pancreas were sectioned and examined under a 3× dissecting microscope.

Nematodes found were stored in 7% formalin. They were later stained with Mayer's paracarmine, cleared with methanol and xylol, and mounted in permount (White, 1973). Adult nematodes were identified by using the descriptions of Becklund and Walker (1968, 1969), Levine (1968), and Olsen and Fenstermacher (1943). Confirmation of *S. yehi* and *T. ovis* was done by Dr. A. K. Prestwood.

Results and Discussion

The only helminth parasites infecting white-tailed deer in this study were nematodes. No trematodes, cestodes, or acanthocephala were found. Those nematodes recovered are given in Table 1. Levels of parasitism for each nematode were low even though the prevalence of three (*O. ostertagi, O. venulosum,* and *G. pulchrum*) was high. This may be due to

[1] This work was supported in part by an EKU faculty research grant No. 42-80.
[2] To whom reprint requests should be sent.

Table 1. Summary of nematodes recovered from white-tailed deer in Kentucky.

Parasite	% infected*	Mean intensity of infection	Location in deer	New state record
Ostertagia ostertagi	75	10	Abomasum	X
Oesophagostomum venulosum	53	11	Cecum	
Gongylonema pulchrum	47	4	Esophagus	
Dictyocaulus viviparus	28	13	Bronchi	
Ostertagia mossi	21	17	Abomasum	
Trichuris ovis	15	3	Cecum	X
Pneumostrongylus tenuis	11	6	Meningeal surface of brain	
Capillaria bovis	9	24	Small intestine	
Setaria yehi	9	13	Peritoneal cavity	X
Cooperia curticei	4	4	Small intestine	X

* Sample size was 31 deer.

either the isolated nature of the habitats, the dry elevated nature of the habitats, the efficient management of the deer herds, or the absence of human inhabitants with their accompanying domestic or feral animals. Since worm burdens and egg counts were low, there was no apparent effect of parasitism on the deer examined. No anomalies related to parasitism were observed.

The finding of *C. curticei*, *O. ostertagi*, *T. ovis*, and *S. yehi* constitute new state and range extension records for the white-tailed deer. With the aforementioned exceptions, all other nematodes recovered have previously been reported in white-tailed deer from Kentucky (Prestwood and Smith, 1969; Prestwood et al., 1970, 1971).

No appreciable differences existed in the presence or prevalence of nematodes from the two geographic populations sampled. Other studies in the United States, such as the one by Beaudoin et al. (1970) in Pennsylvania, have shown differences in parasite prevalence from two geographically separated populations of white-tailed deer.

In summary, this report gives additional geographic distribution records of nematodes from the white-tailed deer. However, more regional distribution studies need to be done in Kentucky before any definite conclusions (re: specific parasite distribution and prevalence) can be made for the state as a whole, as has been done for several southern states (Prestwood and Smith, 1969; Prestwood et al., 1971, 1973).

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A Description of the Male and Redescription of the Female of *Philometra cylindracea* Ward and Magath, 1916 (Nematoda: Philometridae)[1]

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ABSTRACT: The male and mature female are described for the first time and the gravid female description is emended to include three lips and some additional measurements.

Philometra cylindracea is a nematode which occurs in the body cavity of a wide range of fishes. It has been reported from 17 species of fishes in North America from 12 genera and eight families. In Lake Erie *P. cylindracea* occurs in the yellow perch, *Perca flavescens*. The known range of geographical distribution in North America extends from northwest Ontario (Dechtiar, 1972) southward to Ohio (Hare, 1943) and from New York (Van Cleave and Mueller, 1932) westward to Wisconsin (Fischthal, 1953).

The original description by Ward and Magath was based exclusively on gravid female specimens. Mature females, with unatrophied vulva and vagina, and males have never been described. As a result of this study material is now available for a more complete description.

Nematodes recovered from *Perca flavescens* were killed in AFA and fixed in AFA for 24 hr. They were preserved in 10% glycerin alcohol, cleared, and studied in glycerin. Several en face views were studied in glycerin jelly and agar. Drawings were made with the aid of a light microscope fitted with a drawing tube. All measurements are in microns unless otherwise indicated. The range is followed by the average in parentheses.

[1] Supported by The Ohio Division of Wildlife under Federal Aid in Fish Restoration Act, Project No. F-48-R-3.

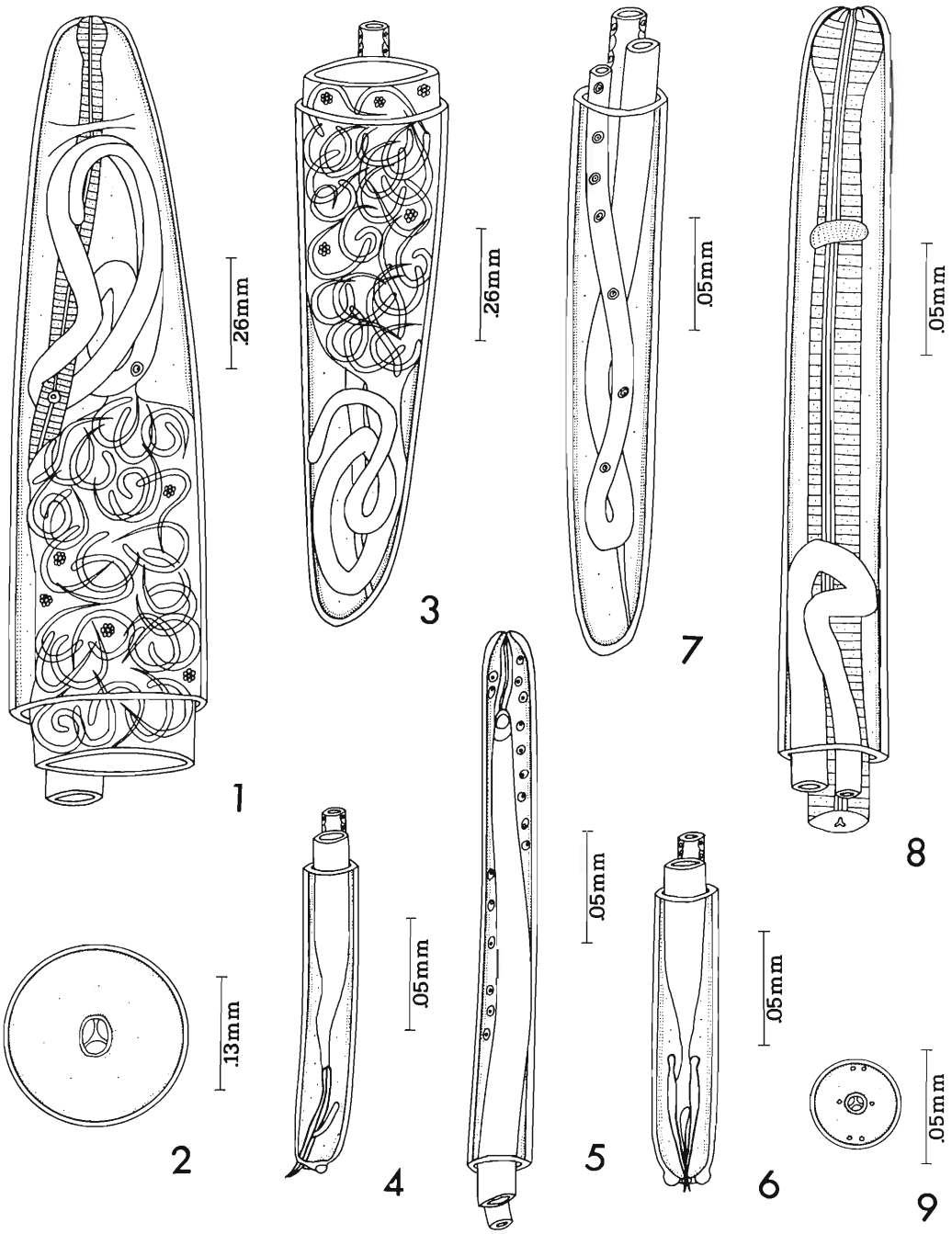
Philometra cylindracea Ward and Magath, 1916 (Figs. 1–9)

Description

Philometridae Baylis and Daubney, 1926. Slender nematodes. Living worms red in color or transulcent. Oral opening with three liplike structures.

Male (seven specimens): Length 1.87–2.4 mm (2.20 mm), width 22–25 (24). Cuticle smooth. Esophagus 98.0–135 (110) long. Nerve ring 88–104 (100) from anterior end. One testis directed anteriorly, not reflexed. Two spicules 54–73 (61) equal in length. Gubernaculum present, arcuate, sharply pointed, one-third as long as spicules. Two protuberances on the caudal end of the body. Terminal anus.

Mature female (10 specimens): Length 1.98–3.83 mm (2.62 mm), width 32–101 (61). Cuticle smooth. Six circumoral papillae. Oral opening with three small liplike structures. Esophagus 529–889 (654) long. Nerve ring 101–120 (109) from anterior end. Vulva 625–1,160 (863) from posterior end, lips slightly salient. Vagina directed posteriorly. Uterus didelphic: one uterine branch extending anteriorly, one posteriorly. Two ovaries—one anterior, one posterior, both reflexed.



Figures 1–9. 1. Lateral view of anterior end of gravid female. 2. En face view of gravid female. 3. Lateral view of posterior end of gravid female. 4. Lateral view of posterior end of male. 5. Lateral view of anterior end of male. 6. Ventral view of posterior end of male. 7. Lateral view of posterior end of mature female. 8. Lateral view of anterior end of mature female. 9. En face view of mature female.

GRAVID FEMALE: Length 92.6–154 mm (122.2 mm). Width 356–594 (448). Cuticle smooth. Oral opening with three small liplike structures. Esophagus 0.976–1.53 (1.27) long. Nerve ring 136–221 (163) from anterior end. Vulva and vagina atrophied, uterus occupying most of body volume. Ovaries coiled and reflexed at anterior and posterior ends of the body. Posterior end rounded. Ovoviviparous; larvae (avg) 638 long and 14 wide.

HOST: *Perca flavescens*.

LOCALITY: Lake Erie between Rattlesnake Island and South Bass Island, Put-in-Bay Twp., Ottawa Co., Ohio.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 73669.

Discussion

The present description serves to supplement the original one by Ward and Magath which was based solely on gravid females. The characters useful for comparison are length, width, esophagus length, and cephalic structures of the gravid female. The larger measurements in the present description are attributed to further development of the embryos and the corresponding increase in body size. Ward and Magath describe the anterior end of the gravid female as having no lips or papillae. In the present description three small liplike structures at the oral opening are described for the gravid female as well as the mature female.

In the literature there are two other descriptive references to *Philometra cylindracea*, one by Van Cleave and Mueller (1932) and one by Rasheed (1963). Van Cleave and Mueller describe a greater length for gravid females than that of the original description. This is also attributed to further development of the embryos and the corresponding increase in the body volume of their specimens. Rasheed, in her revision of the genus *Philometra*, includes a key to the genera of the family Philometridae in which primary importance is given to the structure and arrangement of cephalic papillae of gravid females. In the key she describes the genus *Philometra* as having "more than four cephalic papillae, obscure, small or large." She includes *Philometra cylindracea* in the genus *Philometra* despite the fact that the original description of gravid females by Ward and Magath describes *P. cylindracea* as having no papillae. The present authors accept the generic description given by Yamaguti (1962): "head and tail papillae present or absent."

Moravec (1963) stated that "mouth papillae are little appreciable in nematodes of the genus *Philometra* and there are numerous interstages in their arrangement and structure."

The present data bear this out as cephalic papillae are present in mature specimens but inapparent in the gravid ones.

Acknowledgments

We would like to express appreciation to Michael Gray and Jean Sprinkle-Fastzkie for assistance in collecting fish. We would like to acknowledge Dr. C. E. Herdendorf and the Center for Lake Erie Area Research for use of facilities.

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Cleidodiscus ektyphus sp. n. from the Roanoke Bass, *Ambloplites cavifrons* Cope, and other Ancyrocephalinae (Trematoda: Monogenea) from Some North Carolina Centrarchid Fishes

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ABSTRACT: *Cleidodiscus ektyphus* is described from the Roanoke bass, *Ambloplites cavifrons* Cope, collected in eastern North Carolina. *Cleidodiscus ektyphus* can be distinguished from its nearest relative, *C. stentor* Mueller, 1936, by differences in the morphology of the haptoral armament and by the characteristic bulblike distal expansion of the cirrus of *C. ektyphus*. Forty-four additional monogeneans collected from centrarchids in North Carolina are reported and a host–parasite list provided.

Between March 1970 and July 1971 375 fishes representing eight genera and 12 species of the family Centrarchidae were collected in Craven, Catawba, Harnett, Hyde, Nash, Tyrrell, Wake, and Warren counties in middle and eastern North Carolina. From these hosts, 45 species involving eight genera of ancyrocephaline monogeneans were recovered. This included one new genus and six new species which have been described previously (Mayes, 1973; Mayes and Miller, 1973). This paper describes a seventh, a species of *Cleidodiscus*, the first monogenean to be reported from *Ambloplites cavifrons*.

Materials and Methods

Hosts were collected by chemicals, seine, gill nets, and fish traps. The monogeneans were recovered following the procedures described by Rogers (1966) and preserved in 5% formalin. Some were mounted unstained in glycerin gel; others were stained in Mayer's HCl carmine or Van Cleave's hematoxylin and mounted in commercial resin. Measurements were made with an ocular micrometer as suggested by Mizelle and Klucka (1953) and expressed in micra. Average measurements are followed by the ranges in parentheses. Illustrations were prepared with the use of a camera lucida.

Cleidodiscus ektyphus sp. n. (Figs. 1–7)

HOST AND LOCALITY: *Ambloplites cavifrons* Cope, the Roanoke bass, Fishing Creek, Aventon, Nash County, North Carolina.

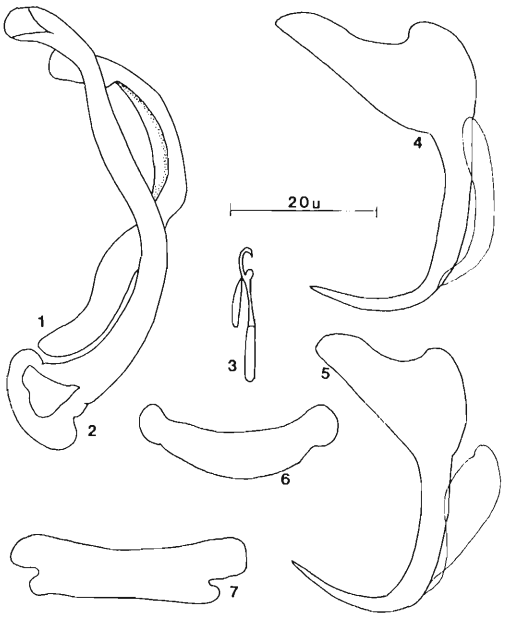
LOCATION ON HOST: Gills.

SPECIMENS STUDIED: 25 (15 measured).

TYPE SPECIMENS: USNM Helm. Coll. holotype No. 73935, paratype No. 73936. Additional paratypes, Univ. Nebraska State Mus. Helm. Coll. No. 20232 and in the collection of the authors.

Description

Length 443 (378–550), width 82 (48–166). Two pairs of eyespots, anterior pair smaller and closer together than posterior pair. Cephalic glands present. Pharynx round, diameter 25 (21–30). Gut bifurcate, ceca confluent posteriorly. Haptor rectangular, length 59 (30–72), width 70 (62–86). Two similar but subequal pairs of anchors present. Ventral anchors normally larger. Anchors composed of superficial root, well-developed deep root, and arcuate shaft leading to elongate point; anchor filament present. Ventral anchor length 41 (37–44), width 25 (20–27); dorsal anchor length 36 (30–41), width 21 (17–25). Two dissimilar, nonarticulate bars support anchors. Ventral bar rectangular with small, conspicu-



Figures 1–7. ***Cleidodiscus ektyphus.*** **1. Accessory piece. 2. Cirrus. 3. Hook. 4. Ventral anchor. 5. Dorsal anchor. 6. Dorsal bar. 7. Ventral bar.**

ous projections on posterolateral edges, larger rounded projections on anterolateral border, length 39 (26–35). Dorsal bar less robust, slightly curved, lateral edges rounded, length 29 (25–33). Seven pairs of hooks present, normal in arrangement, each composed of cylindrical base, narrow shaft, sickle-shaped point, and a prominent hook filament. Pairs 1, 2, 3, 4, 6, and 7 subequal in length 19 (17–22), pair 5 length 15 (14–16). Copulatory complex composed of cirrus and basally articulated accessory piece. Cirrus tubular, cuticularized, with expanded base and bulblike distal tip, length 56 (41–67). Accessory piece curved, with infolded lateral margins which may or may not support the cirrus distally, length 38 (27–44). Testis located in intercecal space near confluency of ceca. Vas deferens not observed. Seminal vesicle posterior to cirrus. Large prostate present, median to copulatory complex. Ovary elongate, anterior to testis. Vagina sinistral, walls cuticularized, pyriform. Vitellaria well developed, extending laterally from pharynx to posterior confluency of ceca.

Remarks

Cleidodiscus ektyphus most closely resembles *C. stentor* Mueller, 1936. *Cleidodiscus ektyphus* can be distinguished from *C. stentor* by the haptoral armament and the copulatory complex. The anchors of *C. ektyphus* have conspicuous deep roots while the anchors of *C. stentor* lack or have very short deep roots. The bars of *C. stentor* have distinct, posteriorly directed central protuberances while the bars of *C. ektyphus* lack such structures. The cirrus of *C. ektyhpus* is stout with a bulblike expansion of the distal end while the cirrus of *C. stentor* is a thin tube which is much more flexible than the cirrus of *C. ektyphus.*

The specific name is derived from Greek (ecktyphos = puffed up) referring to the bulblike distal tip of the cirrus.

Host–Monogenean List

The following is a list of the centrarchid fishes examined and the monogeneans recovered. The numbers of hosts examined are listed in parentheses and new host records are indicated by an asterisk.

Acantharchus pomotis (Baird), mud sunfish (1)
  Cleidodiscoides sulcata Mayes and Miller, 1973
  Urocleidus pomotis Mayes and Miller, 1973
Ambloplites cavifrons Cope, Roanoke bass (22)
  **Cleidodiscus ektyphus nobis*
Centrarchus macropterus (Lacépède), Flier (15)
  Urocleidus wadei Seamster, 1948
  Urocleidus flieri Putz and Hoffman, 1966
Elassoma zonatum Jordan, banded pigmy sunfish (50)
  Urocleidus udicola Allison and Rogers, 1970
Enneacanthus gloriosus (Holbrook), bluespotted sunfish (40)
  Urocleidus anchora Mayes, 1973
  Urocleidus carolinensis Mayes, 1973
  Urocleidus adsimulatus Mayes, 1973

Lepomis auritus (Linnaeus), redbreast sunfish (34)
**Acolpenteron ureterocetes* Fischthal and Allison, 1940
Actinocleidus bennetti Allison and Rogers, 1970
Actinocleidus georgiensis Price, 1966
Lyrodiscus lanceolatus Mayes, 1973
Urocleidus acer (Mueller, 1936)
Urocleidus attenuatus Mizelle, 1941
Urocleidus dispar (Mueller, 1936)
Urocleidus tuberculatus Allison and Rogers, 1970
Lepomis cyanellus Rafinesque, green sunfish (4)
Actinocleidus longus Mizelle, 1938
Actinocleidus gracilis Mueller, 1937
Urocleidus cyanellus (Mizelle, 1938)
Lepomis gibbosus (Linnaeus), pumpkinseed (28)
Actinocleidus oculatus (Mueller, 1934)
Actinocleidus recurvatus Mizelle and Donahue, 1944
Actinocleidus sigmoideus Mizelle and Donahue, 1944
**Anchoradiscus anchoradiscus* Mizelle, 1941
**Clavunculus bifurcatus* (Mizelle, 1941)
Urocleidus acer (Mueller, 1936)
Urocleidus attenuatus Mizelle, 1941
Urocleidus biramosus (Mueller, 1937)
Urocleidus dispar (Mueller, 1936)
Urocleidus ferox Mueller, 1934
Urocleidus procax Mizelle and Donahue, 1944
Lepomis gulosus (Cuvier), warmouth (14)
Actinocleidus flagellatus Mizelle and Seamster, 1939
Clavunculus okeechobeensis (Mizelle and Seamster, 1939)
Urocleidus chaenobryttus Mizelle and Seamster, 1939
Urocleidus doloresae Hargis, 1952
Urocleidus grandis Mizelle and Seamster, 1939
Lepomis macrochirus Rafinesque, bluegill (90)
Actinocleidus fergusoni Mizelle, 1938
Actinocleidus oculatus (Mueller, 1934)
Cleidodiscus nematocirrus Mueller, 1937
Cleidodiscus robustus Mueller, 1934
Urocleidus acer (Mueller, 1936)
**Urocleidus acuminatus* (Mizelle, 1936)
Urocleidus biramosus (Mueller, 1937)
Urocleidus dispar (Mueller, 1936)
Urocleidus ferox Mueller, 1934
**Urocleidus variabilis* Mizelle and Cronin, 1943
Micropterus salmoides (Lacépède), largemouth bass (62)
Actinocleidus fergusoni Mizelle, 1938
Actinocleidus fusiformis (Mueller, 1934)
Clavunculus bursatus (Mueller, 1936)
Clavunculus unguis (Mizelle and Cronin, 1943)
Urocleidus furcatus (Mueller, 1937)
Urocleidus principalis (Mizelle, 1936)
Pomoxis nigromaculatus (Le Sueur), black crappie (15)
Cleidodiscus vancleavei Mizelle, 1936
Lyrodiscus longibasis Rogers, 1967

All 45 of the monogeneans recovered in this study are restricted to centrarchid hosts, and only seven species were found on more than one host. *Lepomis gibbosus*, *L. macrochirus*, and *L. auritus* shared two species. *Lepomis gibbosus* and *L. macrochirus* shared five species. These species of *Lepomis* have naturally occurring hybrids (Childers, 1967). Our data support the general concept of host specificity of monogenetic trematodes for one species or for closely related species.

Acknowledgments

We thank Dr. John D. Mizelle for his suggestions regarding the validity of *Cleidodiscus ektyphus*; Mrs. Mary Hanson Pritchard and Dr. Brent B. Nickol for reviewing the manuscript; Mr. John Smith, Fishery Biologist, North Carolina State Game Commission, for his assistance in collection of the specimens of *Ambloplites cavifrons;* and Messrs. Charles Johnson, Kenneth Burris, and Larry Grimes for their assistance in other field collections.

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Some Digenetic Trematodes of Mammals from Taiwan[1]

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ABSTRACT: Twenty-three digenetic trematodes of mammals are reported from Taiwan. Five new species are described: Notocotylidae, *Ogmocotyle ratti*; Lecithodendriidae, *Odeningotrema ratti*; Dicrocoeliidae, *Brachylecithum taiwanense*, *Lyperosomum taipeiense*; Troglotrematidae, *Stephanolecithus taiwanensis*. Previously known species reported are: Paramphistomidae, *Homalogaster paloniae*; Gastrothylacidae, *Fischoederius elongatus*; Notocotylidae, *Ogmocotyle ailuri*, *O. capricorni*; Plagiorchiidae, *Plagiorchis muris*; Mesocoeliidae, *Mesocoelium brevicaecum*; Lecithodendriidae, *Prosthodendrium cordiforme*, *P. glandulosum*; Echinostomatidae, *Echinostoma aegyptiacum*, *E. cinetorchis*, *E. macrorchis*, *E. revolutum*; Dicrocoeliidae, *Eurytrema coelomaticum*, *E. pancreaticum*, *Platynosomoides muris*; Ophisthorchiidae, *Clonorchis sinensis*; Heterophyidae, *Haplorchis pumilio*; Diplostomatidae, *Pharyngostomum cordatum*.

The trematodes of this paper are part of a collection made by the junior author while a member of the United States Naval Medical Research Unit No. 2, Taipei, Taiwan, Republic of China. Parasites were washed in saline, killed in hot water, and transferred immediately to FAA fixative; after 4–8 hr they were stored in 70% alcohol plus 2% glycerin; staining was with carmine or hematoxylin. Host names recorded herein are those listed by Kuntz and Dien (1970). Host names preceded by an asterisk (*) represent new host records. Specimens of each trematode species have been deposited in the United States National Museum Helminthological Collection as noted. All measurements are in microns.

Ogmocotyle ratti sp. n. (Fig. 1)

HOST: *Rattus culturatus* Thomas, Formosan white-bellied rat (Rodentia: Muridae).

HABITAT: Small intestine.

LOCALITY: Ali-shan, Chia-I Prefecture.

DATE: 14 February 1962.

SPECIMENS DEPOSITED: No. 73705 (holotype); No. 73706 (paratypes).

Description

Notocotylidae. Body elongate, oval to pyriform, with lateral margins turned over ventrally and frequently overlapping, appearing canoelike, ventral groove extending to ovarian level, part of body bearing cirrus sac and uterus bulging dorsally, extremities rounded, 525–750 long by 320–370 wide. Oral sucker ventral, usually wider than long, 70–87 by 74–93, lying 5–10 from anterior extremity. Esophagus emerging from oral sucker dorsum, 70–102 long; cecal bifurcation lying 70–116 anterior to cirrus sac; ceca narrow, terminating median to testes.

Testes two, symmetrical, lateral near posterior extremity, deeply lobed, 121–172 by 85–97. Cirrus sac entirely transversely oriented (without any part of it extending posteriorly), preuterine, large, 210–250 by 83–97; bipartite, proximal part bulbous and containing

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seminal and prostatic vesicles and part of muscular ejaculatory duct, distal part narrower, directed ventrally, containing remainder of ejaculatory duct and cirrus. Seminal vesicle round to elongate oval, 30–69 by 25–53. Prostatic vesicle elongate, 87–116 by 35–53. Prostate cells surrounding anterior part of seminal vesicle, prostatic vesicle, and posterior part of ejaculatory duct. Cirrus muscular, thick, bearing papillae but number of rows not discernible as cirrus not everted. Genital pore sinistral, opening within ventral body groove. Ovary 5–8-lobed, median, at posterior end of body, 60–90 by 138–170. Vitellaria U-shaped; follicles large, arms lateral, commencing short distance pretesticular, passing dorsal to testes, uniting medianly anterior to ovary. Uterus coiling from one side of body to other between ovary and cirrus sac. Metraterm thick-walled, muscular, lying posterolateral to distal part of cirrus sac. Eggs numerous, operculate, 20 measuring 19–22 (20.9) by 10–12 (11), with single filament at each end, anopercular filament thicker than opercular one, length and thickness increasing as eggs pass through uterus.

Discussion

Our collection contains 103 adult worms from one rat; eight were measured. Our new species differs from all others in the genus in being considerably smaller, and in the cirrus sac being entirely transversely oriented. While in *O. ailuri* (Price, 1954) Price, 1960, much of the cirrus sac is transversely oriented, the proximal part containing the seminal vesicle is posteriorly or posteromedianly directed. Additionally, *O. ailuri* differs in having a proportionately much larger cirrus sac and seminal and prostatic vesicles.

Odeningotrema ratti sp. n. (Fig. 2)

HOST: *Rattus rattus* (L.), house rat (Rodentia: Muridae).

HABITAT: Small intestine.

LOCALITY: Hung T'ou Ts'un, Lan Yü or Orchid Island.

DATE: 11 March 1959.

SPECIMENS DEPOSITED: No. 73707 (holotype); No. 73708 (paratypes).

Description

Lecithodendriidae. Body elongate oval to spindle-shaped, slightly narrower anteriorly, extremities rounded, entirely spined, 252–287 long by 106–158 wide at acetabular level. Forebody 104–120 long; hindbody 108–123 long; forebody–hindbody length ratio 1 : 0.96–1.05. Oral sucker ventroterminal, somewhat flattened posteriorly, 48–58 by 46–59. Acetabulum round, 44–56 by 42–58, smaller than oral sucker. Sucker length ratio 1 : 0.90–0.97, width ratio 1 : 0.88–0.98. Prepharynx very short; pharynx diameter 22–26; esophagus 13–20 long; cecal bifurcation close to acetabulum; ceca inverted V-shaped, extending to acetabular level. Excretory vesicle Y-shaped, arms extending to testes; pore terminal.

Testes two, smooth, postacetabular, symmetrical, longitudinally elongate, right testis 65–82 by 48–54, left testis 77–80 by 40–52. Seminal vesicle bipartite, lying along anterior margin of acetabulum, commencing sinistrally. Genital pore at anterodextral margin of acetabulum. Ovary smooth, round, 47–60 by 46–58, antero- to posterodorsal to acetabulum, median to sinistromedian. Seminal receptacle postovarian, 22–26 by 22–31. Vitellaria extending from pharyngeal level to near posterior extremity, in lateral fields at acetabular and gonadal levels, confluent in part preacetabular (occasionally overlapping acetabulum) and posttesticular, follicles relatively large. Uterus ascending sinistrally from posterior part of ovary to bifurcal level, looping back on itself to near posterior extremity, next ascending medianly to acetabulum, then dextrally to bifurcal level, looping back on itself to anterior margin of right testis or more posteriorly, finally looping once again on itself to ascend to genital pore. Eggs relatively few, numbering 18, 20, 21, 28, and 37 in five worms, or operculate, most with anopercular thickening or knob, yellow near ovary, yellow–brown farther along uterus, brown distally; 12 eggs measuring 28–31 (30) by 18–20 (19.4).

Discussion

Our collection contains seven adult worms (three measured); all but the holotype were somewhat macerated. Three species, all from Malaya, are known in the genus: *O. bivesicu-*

lare Rohde, 1962, from a lorisid primate and molossid bat; *O. hypergenitale* Rohde, 1962, from an erinaceid insectivore; *O. apidion* Dunn, 1964, from a tupaiid primate. Our new species differs from the others in being considerably smaller, and in having the acetabulum smaller than the oral sucker. *O. bivesiculare* differs further from ours in having the ovary dextral, the vitellaria interrupted at the testicular level, and longer eggs (36–47). *O. hypergenitale* differs further in having the ovary dextral, and longer eggs (34–39). *O. apidion* differs further in having a pyriform-shaped body, the ovary dextral, and the vitellaria interrupted at the testicular level, and the terminal male genitalia being curved or hooked and lying dorsal to the acetabulum.

Brachylecithum taiwanense sp. n. (Figs. 3, 4)

HOST: *Hipposideros armiger terasensis* Kishida, large leaf-nosed bat (Chiroptera: Hipposideridae).

HABITAT: Small intestine.

LOCALITY: Ping-tung, Ping-tung Prefecture.

DATE: 9 July 1959.

SPECIMEN DEPOSITED: No. 73709 (holotype).

Description

Dicrocoeliidae. Body elongate, threadlike, widest at acetabular level, extremities rounded, 3,615 long by 275 wide. Forebody 610 long; hindbody 2,780 long; forebody–hindbody length ratio 1 : 4.6. Oral sucker ventroterminal, 198 by 188; preoral space 5 long. Acetabulum slightly wider than body at its level, 225 by 275. Sucker length ratio 1 : 1.14, width ratio 1 : 1.45. Prepharynx absent; pharynx 61 by 73, overlapping oral sucker dorsally; esophagus 248 long; cecal bifurcation 130 preacetabular; ceca narrow, posterior extent obscured by eggs. Excretory vesicle tubular, anterior extent obscured by eggs; pore terminal.

Testes two, smooth, tandem, 26 apart, with single dorsoventral uterine coil between them; anterior testis 145 by 167, contiguous with acetabulum; posterior testis 145 by 157. Cirrus sac elongate oval, thick-walled, muscular, filling intercecal space, 165 by 90, commencing 10 preacetabular, terminating at cecal bifurcation. Seminal vesicle coiling, filling most of cirrus sac, 138 (longitudinal extent) by 41. Prostatic vesicle 53 by 58, surrounded by prostate cells. Cirrus muscular, protruded through genital pore at cecal bifurcation. Ovary smooth, median, in tandem with testes, 110 by 198, lying 85 posterior to posterior testis and with three uterine loops between them. Seminal receptacle small, postovarian. Mehlis' gland postovarian. Vitelline follicles large, right field 380 long, follicles numbering 9, commencing 20 postovarian, left field 480 long, follicles 8, commencing 60 postovarian; postvitelline space 1,720 long, distance 61.9% of hindbody length. Uterine coils extensive, filling most of hindbody, ascending dorsal to gonads and acetabulum. Metraterm shorter than cirrus sac, muscular, ventral to cirrus sac. Eggs numerous, operculate, 10 measuring 33–36 (34.8) by 16–22 (18.6).

Discussion

Our collection contains only the holotype specimen. This is the first report of the genus from bats. Only two species are known from mammals: *B. aetechini* Dollfus, 1951, from an erinaceid insectivore from Morocco; *B. rodentini* Agapova, 1955, from a microtid rodent from Kazakh SSR. Both species differ from ours in being much longer, and having the testes much larger than the ovary. *B. aetechini* differs further in having a pyriform cirrus sac, the ovary contiguous with or very close to the posterior testis, and larger eggs (41–53 by 26–35), and in the uterine pathway relative to the gonads. *B. rodentini* differs further in having a relatively wider body, a forebody–hindbody length ratio of about 1 : 8.6 (in worm illustrated), the acetabulum narrower than the body at its level, and a relatively narrower cirrus sac.

Lyperosomum taipeiense sp. n. (Figs. 5–7)

HOST: *Melogale moschata subaurantiaca* Swinhoe, Formosan ferret badger (Carnivora: Mustelidae).

HABITAT: Small intestine.

LOCALITY: Wu-lai, Taipei Prefecture.

DATE: 13 December 1958.

SPECIMENS DEPOSITED: No. 73712 (holotype and paratypes).

Description

Dicrocoeliidae. Body elongate, narrow, extremities rounded, 3,080–3,250 long by 605–635 wide at Mehlis' gland level. Forebody 670–765 long; hindbody 2,075–2,360 long; forebody–hindbody length ratio 1 : 2.7–3.5. Oral sucker ventroterminal, nearly round to transversely elongate, sometimes longer posteroventrally than dorsally, with posterior concavity, 127–155 by 150–160; preoral space absent to 5 long. Acetabulum round to longitudinally elongate, on body protuberance in lateral view, surrounded by prominent body fold in ventrodorsal view, 220–237 by 210–220. Sucker length ratio 1 : 1.53–1.62, width ratio 1 : 1.38–1.41. Prepharynx absent; pharynx large, 119–133 by 114–119, anterior part within posterior concavity of oral sucker; esophagus 194–215 long; cecal bifurcation 145–240 preacetabular, devoid of cell lining; ceca narrow, cell-lined starting short distance postbifurcal; postcecal space 285–322 long, distances 12.4–15.5% of hindbody length. Excretory vesicle tubular, commencing 315–370 postovarian between vitelline fields; pore terminal.

Testis two, smooth, intercecal, diagonal, separated by uterus; anterior testis dextral or sinistral, 150–189 by 100–136, overlapping acetabulum 35 to lying 55 postacetabular; posterior testis 153–182 by 125–152, overlapping acetabulum 8 to lying 120 postacetabular. Cirrus sac 165–240 by 61–62, commencing 119–208 preacetabular at cecal bifurcation or short distance postbifurcal. Seminal vesicle mainly tubular, winding, 110–174 (longitudinal extent) by 30–41. Prostatic vesicle tubular, 53–72 by 15–24. Cirrus protruded from genital pore; latter submedian on right in three worms, left in one, lying 31–85 postpharyngeal. Ovary smooth, 116–148 by 111–153, lying 102–238 posterior to posterior testis, in tandem with to slightly more median than latter, separated by uterus. Seminal receptacle 63–97 by 66–97, posteromedian to posterolateral to ovary. Mehlis' gland large, posteromedian to ovary. Vitelline follicles mainly in extracecal lateral fields, commencing at testicular level, subequal anteriorly and posteriorly; right field 885–1,140 long, extending 565–645 postovarian; left field 880–1,145 long, extending 485–605 postovarian; field lengths representing 42.4–49.6% of hindbody length; postvitelline space 1,010–1,215 long, distances 48.3–51.5% of hindbody length. Uterus coiling between vitelline fields, reaching body margins postvitelline, extending to posterior extremity, few coils dorsal to acetabulum. Metraterm thick-walled, muscular, slightly longer than cirrus sac, dextro- or sinistromedian to latter. Eggs numerous, yellow–brown, operculate, 20 measuring 36–47 (40.3) by 20–24 (21.9).

Discussion

Our collection contains three entire adult worms plus fragments of three others from one host. Our new species is closest to *L. armenicum* Shcherbakova, 1942, from a glirid rodent from Armenian SSR. The latter species differs from ours in having a wider body, a greater forebody–hindbody length ratio (1 : 4.3) and sucker ratio (1 : 2), a pharynx wider than long, a longer postcecal space (28.5% of hindbody length), larger testes, and a wider cirrus sac, and in lacking a body protuberance bearing the acetabulum.

Stephanolecithus taiwanensis sp. n. (Figs. 8, 9)

HOST: *Rattus rattus* (L.), house rat (Rodentia: Muridae).

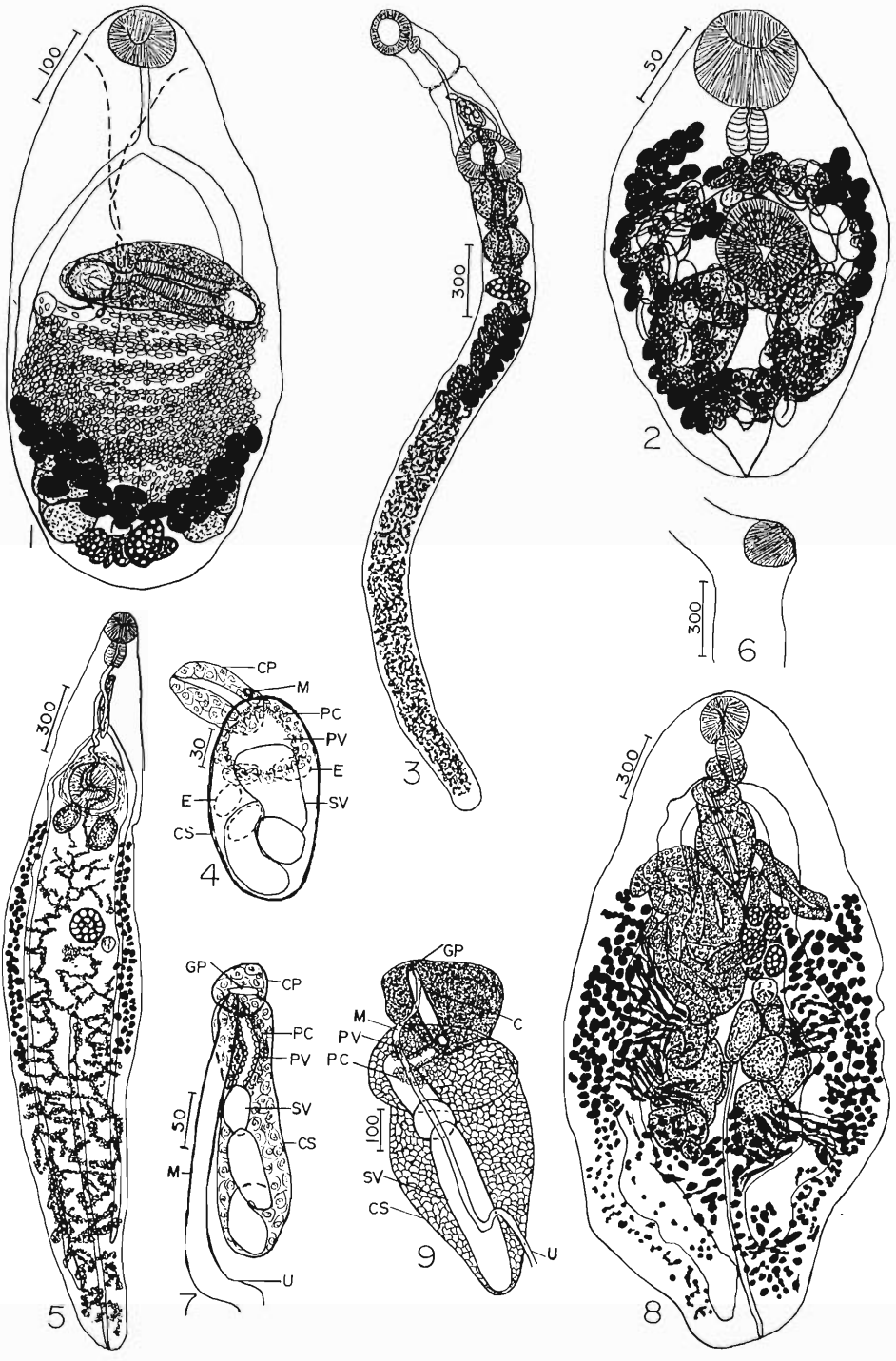
HABITAT: Liver.

LOCALITY: Hung T'ou Ts'un, Lan Yü or Orchid Island.

DATE: 14 March 1959.

→

Figures 1–9. ***Ogmocotyle ratti*** **sp. n. 1. Whole mount, holotype, dorsal view.** ***Odeningotrema ratti*** **sp. n. 2. Whole mount, holotype, ventral view.** ***Brachylecithum taiwanense*** **sp. n. 3. Whole mount, holotype, dorsal view. 4. Terminal genitalia, holotype.** ***Lyperosomum taipeiense*** **sp. n. 5. Whole mount, holotype, dorsal view. 6. Acetabular region of body showing protuberance bearing acetabulum, paratype, dextrolateral view. 7. Terminal genitalia, holotype.** ***Stephanolecithus taiwanensis*** **sp. n. 8. Whole mount, holotype, dorsal view. 9. Terminal genitalia, holotype. C, cirrus; CP, protruded cirrus; CS, cirrus sac; E, egg; GP, genital pore; M, metraterm; PC, prostate cells; PV, prostatic vesicle; SV, seminal vesicle; U, uterus.**



SPECIMENS DEPOSITED: No. 73713 (holotype and fragment of paratype).

Description

Troglotrematidae. Body phylliform, 2,940 long by 1,495 wide at testicular level; anterior extremity rounded, posterior flat to concave; spinose, spines longer posteriorly, 27 long at body margin just posttesticular. Forebody 545 long; hindbody 2,115 long; forebody–hindbody length ratio 1 : 3.9. Oral sucker ventroterminal, 230 by 240; preoral space 15 long. Acetabulum 280 by 295. Sucker length ratio 1 : 1.22, width ratio 1 : 1.23. Prepharynx very short; pharynx 195 by 145, overlapping oral sucker dorsally; esophagus short; cecal bifurcation 140 preacetabular; ceca wide, sinuous, conspicuously cell-lined, terminating subequally; postcecal space 100 long. Excretory vesicle Y-shaped, tubular, intertesticular, without muscular sphincter at terminal pore, bifurcating near anterior part of testes; arms short, extending to level of posterior margin of ovary.

Testes two, symmetrical to subsymmetrical, intercecal, deeply lobed; right testis 770 by 330, lying 55 postovarian and 555 postacetabular; left testis 745 by 415; posttesticular space 795 long. Cirrus sac large, appearing tripartite, middle part widest; posterior part dorsomedian to acetabulum, commencing 80 posterior to latter, filled with vesicular cells; turning sharply ventrally between acetabulum and arch of cecal bifurcation as middle part, also filled with vesicular cells except for prostate cells around prostatic vesicle; anterior part ventral, extending anteriorly ventral to pharynx, filled with smaller and more darkly staining cells. Seminal vesicle tubular, extending from proximal part of cirrus into middle part, 470 (longitudinal extent) by 77. Prostatic vesicle tubular, transversely oriented, 63 by 26. Cirrus muscular, commencing in middle part of cirrus sac and extending into anterior part. Genital pore ventral to sinistral part of pharynx at about its midlength. Ovary deeply lobed, in tandem with right testis, 330 by 260, lying 170 postacetabular. Seminal receptacle large, between ovary and right testis, overlapping both dorsally, 148 by 136. Laurer's canal not discernible. Mehlis' gland median to ovary. Vitellaria follicular to dendritic, in lateral fields but invading intercecal space slightly from short distance postacetabular to posterior part of testes, in extra- and intercecal fields from latter level to posterior extremity. Uterus coiling between anteroventral part of left testis and acetabular level, extending extracecal anteriorly. Metraterm short, muscular, dorsal to cirrus sac. Eggs numerous, yellow–brown, with anopercular thickening, 10 measuring 36–45 (40.5) by 20–24 (21.4).

Discussion

Our collection from one host contains one complete adult worm (holotype) and another with the body anterior to the testes missing. Three species are known in this genus: S. *parvus* Nakagawa, 1919, from experimentally infected mouse, cat, and dog from Japan; S. *beaveri* (Lee, 1965) Yamaguti, 1971, from a rat from Malaya; S. *microacetabulum* (Lee, 1965) Yamaguti, 1971, from a rat from North Borneo. All differ from our species in the nature of the cirrus sac. S. *parvus* differs further from ours in having a much rounder body, a greater sucker ratio, smooth gonads, and vitellaria extending preacetabular. S. *beaveri* differs further in having a more oval body with a posteromedian notch, a muscular sphincter at the excretory pore, the cirrus sac widest posteriorly, the genital pore at the oral sucker level, in the cirrus sac commencing dorsal to the acetabulum and the vitellaria preacetabular, and in the vitelline fields not being confluent posttesticular. S. *microacetabulum* differs further in having the acetabulum smaller than the oral sucker, the cecal bifurcation at the acetabular level, the testes smaller than the ovary, the cirrus sac widest posteriorly, and the genital pore at the oral sucker level.

Previously Described Species

1. *Homalogaster paloniae* Poirier, 1883 (Paramphistomidae) from the small intestine of domestic cattle collected 25 February 1958 at Taipei, Taipei Prefecture. Specimens deposited: No. 73714.

2. *Fischoederius elongatus* (Poirier, 1883) Stiles and Goldberger, 1910 (Gastrothylacidae) from the stomach of domestic cattle collected 4 September 1959 at the Taipei

slaughterhouse. Specimens deposited: No. 73715.

3. *Ogmocotyle ailuri* (Price, 1954) Price, 1960 (Notocotylidae) from the small intestine of the Formosan macaque, *Macaca cyclopsis* Swinhoe (Primates: Cercopithecidae), collected 14 December 1957, 11 May, 29 November 1960, and 10 January 1962 at Taipei; Pu-li, Nan-tou Prefecture; Chia-I, Chia-I Prefecture; and South Taiwan. Specimens deposited: No. 73716. Yoshimura et al. (1969) reported, with illustrations, this trematode from the same host species from Taiwan, noting that it differed from Price's (1960) description, based on the holotype only from the lesser panda, *Ailurus fulgens* (Cuvier) (Carnivora: Procyonidae), in the cirrus showing six rows of papillae rather than being unarmed, and the opercular end of the egg usually with only one filament, rarely two, rather than two as described. Examination by us of Price's holotype worm (USNM Helm. Coll. No. 27777) reveals evidence of at least five rows of papillae, possibly six, on the everted cirrus, and the eggs usually with one filament, rarely two, on the opercular end. Examination of 170 worms from three hosts in our collection show without exception that the cirrus sac is transversely oriented with the part containing the seminal vesicle posteriorly or posteromedianly directed as originally described for this species and as redescribed by Yoshimura et al.; additionally, the everted cirrus may show six rows of papillae. Yoshimura et al. (1969) noted that their report of *Ogmocotyle* species is the first from the Formosan macaque. Actually, Coil (1966) first reported it as *O. indica* (Bhalerao, 1842) Ruiz, 1946, from this host species and *Capricornis swinhoei* from Taiwan from specimens which are part of the same collection as ours. It is possible that *O. indica* reported, without description or illustration, by Bezubik and Furmaga (1959) from *Macaca rhesus* Audeb. (*M. mulatta* Zimmermann, according to Yoshimura et al.) from China actually is *O. ailuri*.

4. *Ogmocotyle capricorni* Machida, 1970, from the small intestine and stomach of the Formosan serow, *Capricornis swinhoei* Gray (Artiodactyla: Bovidae), collected 11 May, 26 October 1960, and 27 January 1961 at Wulai, Taipei Prefecture; Pu-li, Nan-tou Prefecture; I-lan, I-lan Prefecture; and Tai-chung Prefecture. Specimens deposited: No. 73717. Machida (1970) did not indicate whether the cirrus had papillae. Our worms show six rows of papillae similar to that described by Yoshimura et al. (1969) for *O. ailuri*. Also, the eggs, usually with one filament on the opercular end, rarely have two. Coil's (1966) *O. indica*, a part of the same collection as our worms from the same host species and locality, is actually *O. capricorni*; Machida did not refer to this paper.

5. *Plagiorchis* (*Multiglandularis*) *muris* (Tanabe, 1922) Shults and Skvortsov, 1931 (Plagiorchiidae), from the small intestine of *Rattus rattus* collected 10, 11, 14, 15, 18, 21, 27 March 1959 and 1 September 1960 at Tai-tung Prefecture and at Hung T'ou Ts'un, Lan Yü or Orchid Island. Specimens deposited: No. 73718.

6. *Mesocoelium brevicaecum* Ochi in Goto and Ozaki, 1929 (Mesocoeliidae), from the small intestine of the small Formosan civet, **Viverricula indica pallida* Gray (Carnivora: Viverridae), collected 11 December 1959 at Lo-tung, I-lan Prefecture. Specimen deposited: No. 73719. This is the first record of this genus from mammals; it is usually found in amphibians and reptiles, but has been reported twice from marine fishes. *B. brevicaecum* has been reported in amphibians from Japan and Korea. It probably is an accidental infection in the civet.

7. *Prosthodendrium* (*Prosthodendrium*) *cordiforme* (Braun, 1900) Macy, 1936 (Lecithodendriidae), from the small intestine of the long-winged bat, *Minopterus schreibersii* Kuhl (Chiroptera: Vespertilionidae), and **Hipposideros armiger terasensis* collected 9 July 1959 and 2, 3 March 1960 at Hsin-sheh, Tai-chung Prefecture and Ping-tung, Ping-tung Prefecture. Specimens deposited: No. 73721 (*M. schreibersii*); No. 73710 (*H. armiger*).

8. *Prosthodendrium* (*Paralecithodendrium*) *glandulosum* (Looss, 1896) Bhalerao, 1936, from the small intestine of **Hipposideros armiger terasensis* collected 9 July 1959 at Ping-tung, Ping-tung Prefecture. Specimens deposited: No. 73711.

9. *Echinostoma aegyptiacum* Khalil and Abaza, 1924 (Echinostomatidae) from the small intestine and stomach of the Formosan brown country rat, **Rattus losea* Swinhoe; the Norway rat, **R. norvegicus*; and *R. rattus* col-

lected in 1958, 1960, and 1961 from Taipei, Chang-hua, and Ping-tung Prefectures. Specimens deposited: No. 73722 (*R. losea*); No. 73723 (*R. norvegicus*); No. 73724 (*R. rattus*).

10. *Echinostoma cinetorchis* Ando and Ozaki, 1923, from the small and large intestines of **Rattus losea*, *R. norvegicus*, and **R. rattus* collected in 1958, 1960, and 1961 at Chang-hua, Tai-chung, Taipei, Kao-hsiung, and Ping-tung Prefectures. Specimens deposited: No. 73725 (*R. losea*); No. 73726 (*R. norvegicus*); No. 73727 (*R. rattus*).

11. *Echinostoma macrorochis* Ando and Ozaki, 1923, from the small intestine of *Rattus norvegicus* and *R. rattus* collected in 1958 and 1960 at Chang-hua and Taipei Prefectures. Specimens deposited: No. 73728 (*R. norvegicus*); No. 73729 (*R. rattus*).

12. *Echinostoma revolutum* (Froelich, 1802) Looss, 1899, from the small intestine of **Viverricula indica pallida* collected 11 December 1958 and 11 December 1959 at Wulai, Taipei Prefecture, and Lo-tung, I-lan Prefecture. Specimens deposited: Nos. 73730, 73720. Our collection contains 10 worms from one host and 13 from another with 37 collar spines. It was most difficult allocating them to species as they closely resemble *E. lindoense* Sandground and Bonne, 1940. Most of our worms have weakly lobed testes, but in some they are smooth. In *E. lindoense* the testes are always lobed, even in young specimens not yet producing eggs. Most importantly, life cycle studies are necessary to determine whether our specimens are indeed *E. revolutum*. There are other species with 37 collar spines very similar to ours in adult morphology which have been separated from one another by differences in larval characters.

13. *Eurytrema coelomaticum* (Giart and Billet, 1892) Looss, 1907 (Dicrocoeliidae), from the pancreas of the domestic goat collected 24 May 1957 from Taipei, Taipei Prefecture. Specimens deposited: No. 73731.

14. *Eurytrema pancreaticum* (Janson, 1889) Looss, 1907, from the body cavity of **Capricornis swinhoei* collected 24 February and 25 April 1959 at Pu-li and Chuo-sheh, Nan-tou Prefecture. Specimens deposited: No. 73732.

15. *Platynosomoides muris* (Shcherbakova, 1942) Yamaguti, 1971 (Dicrocoeliidae), from the liver of the Formosan striped field mouse, **Apodemus agrarius insulaemus* Tokuda (Rodentia: Muridae), **Rattus losea*, and **R. rattus* collected 3, 4 May and 11 August 1960 at Ali-lou and Ali-lao, Taipei Prefecture. Specimens deposited: No. 73733 (*A. agrarius*); No. 73734 (*R. losea*); No. 73735 (*R. rattus*). One worm was obtained from each host species. Those from the first two listed hosts are more like those compared by Uzhakhov (1963) from *Apodemus sylvaticus* L. from Armenian and Dagestan SSR, but differ in having a larger pharynx (165–170 by 147–165 rather than 90–110 by 90–120), and the testes and ovary approximately the same size (testes 155–220 by 175–190; ovary 195–230 by 180–200) rather than the testes being larger than the ovary (testes 300–420 by 180–520; ovary 150–210 by 150–300). The worm from *R. rattus* is a large one (about 6,700 by 1,625), more like that illustrated by Lee (1965) from feral rats from Malaya.

16. *Clonorchis sinensis* (Cobbold, 1875) Looss, 1907 (Opisthorchiidae), from the liver, gall bladder, and bile duct of the domestic cat collected from 1959–62 at Taipei and Yang Ming Shan, Taipei Prefecture, and Sin-shwei, Chang-hua Prefecture. Specimens deposited: No. 73736.

17. *Haplorchis pumilio* (Looss, 1896) Looss, 1899 (Heterophyidae) from the small intestine of the domestic cat collected 8 January 1959 and 20 January 1960 at Hsin-chu and Hsin-lo, Hsin-chu Prefecture. Specimens deposited: No. 73737.

18. *Pharyngostomum cordatum* (Diesing, 1850) Ciurea, 1922 (Diplostomatidae) from the small intestine of the domestic cat collected from 1958–60 at Taipei, Tai-chung, Nan-tou, and I-lan Prefectures. Specimens deposited: No. 73738.

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The Metazoan Parasites of Green-winged Teal (*Anas crecca* L.) and Blue-winged Teal (*Anas discors* L.) from Eastern Canada[1]

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ABSTRACT: One hundred and forty-eight ducks (87 *Anas crecca* Linnaeus, 61 *Anas discors* Linnaeus) collected from three localities in eastern Canada were examined for parasites. Ninety-five per cent of the *A. crecca* were infected, 23 parasite species being represented. Four are new host records and six are new records for *A. crecca* in North America. One hundred per cent of the *A. discors* were infected, 21 parasite species being represented, including eight new host records.

The number and percentage of each sex and age group of both host species infected, and mean and range of parasite numbers per infected bird is given. Parasite species are discussed with regard to incidence and intensity of infection, location of parasites within host, host records, authorities used in specific determination, and minor variations, if any, from previous descriptions. Infections in the two hosts are compared and differences, if any, are discussed.

Due to the economic and aesthetic value of waterfowl, many aspects of their biology have been studied, including their helminth fauna. Anatid helminths have been investigated by workers in many parts of the world and the published literature is extensive. Lapage (1961) and McDonald (1969a, b) have provided host–parasite and parasite–host lists, respectively, and both reviews include a bibliography.

The green-winged teal (*Anas crecca* Linnaeus) and blue-winged teal (*Anas discors* Linnaeus), closely related and sympatric over

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a large part of their ranges, are common waterfowl species in North America. Little work, particularly quantitative, has been done on the parasites of these duck species. One of the more recent publications on teal is that of Buscher (1966), who worked on the intestinal helminths of a population of *A. discors* on its breeding grounds.

Relatively few works dealing with the parasites of ducks from eastern Canada have been published, the most recent being those of McLaughlin (1970) and McLaughlin and Burt (1973) who worked on black duck (*Anas rubripes* Brewster) in New Brunswick and Bishop and Threlfall (1974) who examined common eider ducks [*Somateria mollissima* (Linnaeus)] from Newfoundland.

A study was therefore undertaken to determine the helminth fauna of *A. crecca* and *A. discors*, from areas in eastern Canada and in particular two areas that are being managed by the Canadian Wildlife Service and the Provinces of Nova Scotia and New Brunswick, and to note any similarities and/or differences in the parasite fauna of the two host species which occupy basically similar habitats.

Materials and Methods

The viscera of 148 ducks (87 *A. crecca*, 61 *A. discors*) were collected during August–October 1972 and 1973). The majority (133) of the specimens (75 *A. crecca*, 58 *A. discors*) were obtained as banding casualties or from hunters at biological check stations on the Tintamarre National Wildlife Area (45°57′ N, 64°15′ W) and Missaquash Marsh (45°57′ N, 64°10′ W) in the Nova Scotia–New Brunswick border region. Fifteen specimens (12 *A. crecca*, 3 *A. discors*) were collected on the Magdalen Islands (47°22′ N, 61°55′ W).

Both species of ducks were aged and sexed by cloacal examination (Kortright, 1942) and/or characters of the plumage (Carney, 1964). Three age classes were recognized: (1) local —a locally hatched bird, unable to fly; (2) immature—a young bird capable of flight but taken during the same calendar year as hatching; and (3) adult—a bird taken during its second calendar year of life or later.

All material collected was frozen and examined when time permitted, using conventional parasitological techniques (Andrews, 1974). The small intestine was divided into four regions (namely the duodenum S1, S2, and posterior region S3) to note the linear distribution of any parasites found therein. When whole birds were available, the nasal sinuses and external body surfaces were also checked for parasites.

In all cases an attempt was made to obtain an accurate count of the parasite population. When the parasite burden was less than 100 for any particular helminth species, specimens were counted individually. In instances where the parasite numbers exceeded 100, the population size was estimated. A grid of 1-cm^2 blocks was placed beneath a Petri dish of known area which contained parasites. An average number, obtained from counts of five random blocks, was used to calculate the total parasite numbers.

Infections are recorded as incidence (i.e., per cent of ducks infected) and intensity (i.e., average number of helminths per infected duck). Data were analyzed using standard statistical tests (Chi-square, t test).

All measurements are given in microns unless otherwise specified.

Sample Sites

The Tintamarre National Wildlife Area, located 5 miles to the north of Sackville, New Brunswick, is one of several areas acquired by the Canadian Wildlife Service in eastern Canada for the preservation of waterfowl habitat. The area has been subjected to intense waterfowl management and at present contains 7 man-made impoundments, ranging from 1 to 7 years in age. Also included in the area are four natural lakes as well as numerous potholes, both natural and man-made. Since the creation of the impoundments waterfowl have tended to concentrate on these areas resulting in decreased utilization of the natural lakes (Whitman, 1974). Whitman (1974) studied the macroinvertebrate fauna of the areas and found considerably higher densities of invertebrate populations in artificial impoundments as compared to the natural lakes on the area. Particularly prominent was the high density of gastropod populations. Five genera of snails have been collected and at least two (*Physa* and *Lymnaea*) are known to carry infective stages of echinostomes (shown experimentally

Table 1. Details of trematode infections (excluding *Echinostoma revolutum* and *Echinoparyphium recurvatum*) in *A. crecca*.

	Apatemon gracilis	*Cotylurus platycephalus**	*Cotylurus cornutus***	*Notocotylus ctenoatus*	*Microphallus primus**	*Paramonostomum alveatum***	*Hypoderaeum conoideum*	*Prosthogonimus cuneatus***	*Zygocotyle lunata***	*Psilostomum* sp.*	*Trichobilharzia queraqueduae**	*Psilochasmus oxyurus***
Adult male												
No. (%) birds infected	2(18)	–	–	4(36)	2(18)	1(9)	–	–	1(9)	–	–	–
Range of parasite Nos.	4–15	–	–	1–4	5–40	32	–	–	1	–	–	–
Mean No. parasites/infected bird	10	–	–	3	23	32	–	–	1	–	–	–
Total No. parasites	19	–	–	12	45	32	–	–	1	–	–	–
Adult female												
No. (%) birds infected	5(56)	–	–	2(22)	–	–	–	1(11)	1(11)	1(11)	–	1(11)
Range of parasite Nos.	2–320	–	–	3–5	–	–	–	1	1	2	–	1
Mean No. parasites/infected bird	87	–	–	4	–	–	–	1	1	2	–	1
Total No. parasites	433	–	–	8	–	–	–	1	1	2	–	1
Immature male												
No. (%) birds infected	10(38)	–	–	8(31)	1(4)	1(4)	1(4)	–	–	–	–	2(8)
Range of parasite Nos.	2–198	–	–	1–36	51	882	1	–	–	–	–	1–3
Mean No. parasites/infected bird	53	–	–	11	51	882	1	–	–	–	–	2
Total No. parasites	531	–	–	88	51	882	1	–	–	–	–	4
Immature female												
No. (%) birds infected	17(43)	1(3)	1(3)	13(33)	2(5)	4(10)	1(3)	1(3)	–	2(5)	1(3)	3(8)
Range of parasite Nos.	1–77	1	2	1–29	16–23	3–1,675	1	1	–	1–9	6	1–2
Mean No. parasites/infected bird	13	1	2	7	20	409	1	1	–	5	6	1
Total No. parasites	220	1	2	85	39	2,199	1	1	–	10	6	4
Total												
Total No. (%) birds infected	35(40)†	1(1)	1(1)	27(31)	5(6)	6(7)	2(2)	2(2)	2(2)	3(3)	1(1)	6(7)
Range of parasite Nos.	1–320	1	2	1–36	5–51	3–1,675	1	1	1	1–9	6	1–3
Mean No. parasites/infected bird	35	1	2	7	27	519	1	1	1	4	6	1
Total No. parasites recovered	1231	1	2	193	135	3,113	2	2	2	12	6	8

† Includes one bird of unknown age and sex. * New host record. ** New record for *A. crecca* in N. America.

during the present study, utilizing Pekin ducks).

The Missaquash Marsh, located 5 miles to the northeast of Sackville, New Brunswick, was acquired by the province of Nova Scotia to provide nesting and staging areas for waterfowl. The history of the area is not unlike that of the Tintamarre National Wildlife Area. Originally the area consisted of a single large impoundment but recently three smaller compartments have been added to the marsh complex.

Whitman (1974) reported on the invertebrates of the oldest impoundment (8 years) and found a decrease in the densities of invertebrate populations but an increase in species diversity. However, the populations of gastropod genera were relatively high.

Results and Discussion

Twenty-four species of parasites (excluding Cestoda) were recovered from the two host species during the present study. Among those collected were 14 species of Trematoda (13 genera), 6 of Nematoda (6 genera), 1 of Acanthocephala, and 3 of Mallophaga (3 genera). Eighty-three (95%) of the *A. crecca* were infected and 20 helminth species were collected from this host (range 1–7; mean 2 per infected bird). Sixty-one (100%) of the *A. discors* were infected with representatives of 18 helminth species (range 1–8; mean 2 per infected bird).

Trematoda

Fourteen trematode species (Table 1) were collected from the *A. crecca* (range 1–6; mean 1 per infected bird) and 11 (Table 2) were collected from *A. discors* (range 1–5; mean 2 per infected bird).

Specimens of *Apatemon gracilis* (Rudolphi, 1819) were recovered from 35 (Table 1) *A. crecca* and 36 (Table 2) *A. discors*. The incidence of infection was similar in the two hosts

Table 2. Details of trematode infections (excluding *Echinostoma revolutum* and *Echinoparyphium recurvatum*) in *A. discors*.

	Apatemon gracilis	*Cotylurus platycephalus**	*Notocotylus attenuatus*	*Microphallus primus**	*Pavanonostomum alveatum**	*Hypoderaeum conoideum*	*Prosthogonimus cuneatus**	*Trichobilharzia querquedulae*	*Psilochasmus oxyuru**
Adult male									
No. (%) of birds infected	–	–	2(67)	1(33)	–	1(33)	–	1(33)	–
Range of parasite Nos.	–	–	5–46	14	–	3	–	3	–
Mean No. parasites/infected bird	–	–	26	14	–	3	–	3	–
Total No. parasites	–	–	51	14	–	3	–	3	–
Adult female									
No. (%) of birds infected	3(50)	–	1(17)	–	–	1(17)	–	3(50)	–
Range of parasite Nos.	1–120	–	49	–	–	8	–	1–4	–
Mean No. parasites/infected bird	54	–	49	–	–	8	–	3	–
Total No. parasites	161	–	49	–	–	8	–	10	–
Immature male									
No. (%) of birds infected	13(65)	–	10(50)	–	1(5)	3(15)	2(10)	6(30)	1(5)
Range of parasite Nos.	1–201	–	1–50	–	23	2–29	1	1–5	23
Mean No. parasites/infected bird	51	–	16	–	23	11	1	3	23
Total No. parasites	664	–	164	–	23	34	2	17	23
Immature female									
No. (%) of birds infected	18(72)	–	14(56)	1(4)	–	3(12)	1(4)	13(52)	–
Range of parasite Nos.	1–256	–	1–114	15	–	1–27	1	1–7	–
Mean No. parasites/infected bird	34	–	20	15	–	14	1	3	–
Total No. parasites	617	–	277	15	–	41	1	41	–
Local male									
No. (%) of birds infected	–	1(33)	1(33)	–	–	1(33)	–	–	–
Range of parasite Nos.	–	2	1	–	–	32	–	–	–
Mean No. parasites/infected bird	–	2	1	–	–	32	–	–	–
Total No. parasites	–	2	1	–	–	32	–	–	–
Total									
Total No. (%) birds infected	36(59)†	1(2)	30(49)†	2(3)	1(2)	9(15)	3(5)	23(38)	1(2)
Range of parasite Nos.	1–256	2	1–114	14–15	23	1–29	1	1–7	23
Mean No. parasites/infected bird	40	2	18	15	23	13	1	3	23
Total No. parasites recovered	1442	2	549	29	23	116	3	71	23

† Includes two birds of unknown age and sex. * New host record.

($P > 0.05$) and did not differ with age and sex. The intensity of infection was also similar in the two host species ($P > 0.05$).

The majority of specimens were collected from the duodenum, S1, and S2 but also occurred in decreasing numbers in S3, the large intestine, and the ceca. McDonald (1969b) cites the duodenum and anterior portion of the small intestine as the preferred habitat of this parasite. Occasionally, these helminths were recovered from the coelom and air sacs, probably having moved to these locations through gunshot perforations of the digestive tract.

A common and widely distributed parasite (*vide* McDonald, 1969b), *A. gracilis*, was first reported in eastern Canada from the American goldeneye [*Bucephala clangula* (Linnaeus)] by Cannon (1939), who did not present quantitative data but merely stated that "a considerable infestation of these strigeids occurred in the small intestine. . . ." Beverly-Burton (1972) noted an incidence of 14.3% and range of intensity of 0–72 for this trematode collected from *A. crecca* in England. Both values are considerably lower than those obtained during the present study (Tables 1, 2).

Measurements and morphological characters compare favorably with those of Dubois (1968) and Beverly-Burton (1961).

Both the incidence and intensity of infection with *Cotylurus platycephalus* (Hughes, 1928) were quite low. Only two birds, an immature female *A. crecca* and a local male *A. discors*, were infected, harboring one and two specimens, respectively. All three trematodes were collected from the bursa of Fabricius, a lym-

phoepithelial organ occurring in young ducks (8 months or younger).

This parasite has not previously been recorded from either host, being normally found in lariform birds. Interestingly, the single infected *A. discors* was a local thus suggesting an available intermediate host within proximity of the collection site.

Van Haitsma (1930) successfully infected a freshwater snail (*Lymnaea emarginata* Sowerby) with miracidia of this species, while Olivier and Cort (1942) located metacercariae encysted on yellow perch (*Perca flavescens* Mitchill). Whitman (1974) reported on the population of *Lymnaea* sp. and also (Whitman, pers. comm.) confirmed the presence of yellow perch at the collection site. Since the herring gull (*Larus argentatus* Pontopiddan) and great black-backed gull (*Larus marinus* Linnaeus) are frequent visitors to the collection site it is quite conceivable that *C. platycephalus* was introduced via these hosts.

Measurements and morphological characters of specimens obtained during the present study agree with those presented by Dubois (1968).

Only two specimens of *Cotylurus cornutus* (Rudolphi, 1808) were recovered, both from S1 of the small intestine of an immature female *A. crecca* (Table 1). Although McDonald (1969b) listed this strigeid as a common and characteristic helminth of waterfowl, an extensive literature search revealed no evidence of this parasite having previously been recorded from *A. crecca* in North America.

Notocotylus attenuatus (Rudolphi, 1809) was collected from 27 (31%) of the *A. crecca* and 30 (49%) of the *A. discors*. Although the incidence of infection was independent ($P > 0.05$) of age and sex within a single host species, a significant difference ($P < 0.05$) did exist for incidence and intensity of infection between the two hosts, *A. discors* showing the higher value in both cases.

Specimens occurred almost exclusively in the intestinal ceca. Infrequently, small numbers were recovered from the large intestine but this can probably be attributed to postmortem migration.

Buscher (1966) reported an incidence of 23% and a mean intensity of 13 per infected bird for this parasite in a population of *A. discors* on their breeding grounds in Manitoba. Values for the same parameters are higher in the present study, particularly in the case of *A. discors*.

Considerable confusion has been expressed over the identity of *Notocotylus attenuatus* and *Notocotylus imbricatus* (Looss, 1893), the two having often been regarded as synonyms (Beverly-Burton, 1961). Szidat (1935) separated *N. attenuatus* from *N. imbricatus* on differences in the pattern of the ventral adhesive glands. Beverly-Burton (1961) recognized the two forms and suggested that several descriptions of *N. attenuatus* may have been based on a mixture of these two species. On the basis of Szidat's criterion only one species, *N. attenuatus*, is recognized in the present work in spite of the fact that some measurements fall outside the range of *attenuatus* as presented by Beverly-Burton and are similar to those of *N. imbricatus*. However, most of these differences occur as larger measurements and are confined to specimens from *A. crecca* suggesting a host reaction or increased parasite size associated with a smaller parasite burden. Measurements of specimens from both hosts are compared to those of Beverly-Burton (1961) and are presented in Table 3. The numbers of ventral adhesive glands ranged from 13–16 in lateral rows and 11–14 in median rows.

Microphallus primas (Jagerskiold, 1908) was collected from five (6%) of the *A. crecca* and two (3%) of the *A. discors*.

M. primas normally occurs in charadriiform birds (*vide* McDonald, 1969b) and has not previously been recorded from any members of the Anatini. It has, however, been recorded from the common eider, greater scaup [*Aythya marila* (Linnaeus)], and three species of Mergini (*vide* McDonald, 1969b) all of which are exposed, by the nature of their marine habits, to metacercariae which occur encysted in marine decapod and amphipod crustaceans. The salt marshes in the vicinity of the collecting sites probably serve as the focus of infection for this trematode.

Measurements and morphological characters of the present specimens agree with those given by Deblock and Pearson (1969).

Paramonostomum alveatum (Mehlis, 1846) was found in six *A. crecca* and two *A. discors* (Tables 1, 2), in the duodenum and three sections of the small intestine, being most abundant in S2. *P. alveatum* has been reported

Table 3. Measurements of *Notocotylus attenuatus* recovered from *A. crecca* and *A. discors* during the present study compared with those of Beverly-Burton (1961).

	A. crecca		*A. discors*		Beverly-Burton (1961)
	Mean	Range	Mean	Range	Range
Body length	3,630	2,040–4,810	2,580	1,850–3,080	2,010–2,960
Body width	799	591–1,050	534	479–724	480–830
Esophagus length	155	125–200	103	60–150	Up to 170
Oral sucker length	141	100–160	104	78–125	100–170
Oral sucker width	151	88–170	121	90–157	100–220
Cirrus sac length	1,140	765–1,430	846	765–1,001	750–940
Cirrus sac width	122	63–143	83	45–100	60–90
Left testis length	530	469–663	338	255–377	280–420
Left testis width	230	160–275	170	132–204	110–330
Right testis length	520	510–632	338	242–395	270–470
Right testis width	233	153–285	187	132–220	140–330
Ovary length	255	200–316	207	242–395	110–340
Ovary width	235	190–265	168	125–197	110–320
Mehlis' gland length	131	83–200	115	102–122	40–260
Mehlis' gland width	157	125–220	149	112–204	110–240
Egg length	21	20–22	20	18–22	19–20
Egg width	11	10–12	10	10–12	11–13

from many species of waterfowl including four *Anas* spp., four *Aythya* spp., five Anserini, two Somateriini, and three Mergini (*vide* McDonald, 1969b). However, it has not previously been recorded from *A. crecca* or *A. discors* in North America.

Stunkard (1967) and Kulachkova (1954) investigated the life cycle of this parasite and found that cercariae develop in marine snails and encyst, after emergence, on the surface of marine molluscs and crustaceans. In view of this it is not surprising that anatid species which occupy the littoral zone of the seacoast are the normal hosts of this parasite. Interestingly, six of the eight ducks infected with this trematode in the present study were collected on the Magdalen Islands.

Measurements of specimens from the present study compare favorably with those given by Stunkard (1967).

Hypoderaeum conoideum (Bloch, 1782) was collected from both host species. The intensity of infection was similar ($P > 0.05$) in both hosts but the incidence of infection was significantly higher ($P < 0.05$) in *A. discors* (15%) than in *A. crecca* (1%).

H. conoideum, listed by McDonald (1969b) as a very common and characteristic helminth of waterfowl, has been reported throughout the Holarctic region from at least five orders of birds. Beverly-Burton (1972) reported this parasite from *A. crecca* collected in England, and Buscher (1966) recovered it from *A. discors* in Manitoba. This parasite was first recorded in eastern Canada by Cannon (1939) from the black duck.

The majority of specimens were recovered from S2 (81%) of the small intestine with smaller numbers being found in S3 (6%), S1 (3%), and the large intestine (1%). The remaining 9% were found free in the coelom and/or on the surface of visceral organs, probably having migrated through gunshot perforations in the intestinal wall.

The measurements of mature specimens agree with those given by Beverly-Burton (1961).

Prosthogonimus cuneatus (Rudolphi, 1809) was recovered in small numbers from the two host species (Tables 1, 2). Specimens from immature birds were located in the bursa of Fabricius while the single specimen from an adult female *A. crecca* was found in the cloaca.

This species has not previously been recorded from *A. discors*, nor has it been found in *A. crecca* in North America.

Measurements of the present specimens are similar to those provided by Beverly-Burton (1961), although in some instances the measurements of the testes were larger. The differences can probably be attributed to the method of preparation (Ulmer, 1952) since *P. cuneatus* is quite large and can be easily distorted.

Single specimens of *Zygocotyle lunata* (Diesing, 1836) were recovered from the ceca of each of an adult male and an adult female *A. crecca* (Table 1). Cannon (1938) first re-

Table 4. Measurements of *Trichobilharzia querquedulae* obtained during the present study compared with *Pseudobilharziella querquedulae* of McLeod (1937) and *Trichobilharzia physellae* of McMullen and Beaver (1942).

	Trichobilharzia querquedulae Present study		*Pseudobilharziella querquedulae* McLeod (1937)	*Trichobilharzia physellae* McMullen and Beaver (1942)
	Immature	Adult	Adult	Adult
Body length	4.49–4.70 mm	5.1 mm	3.7 mm	3.18–5.71 mm
Body width	95–125	112–152	150	63–97
Oral sucker	60–80 × 42–60	53–70 × 45–53	56 × 64	31–40 × 38–47
Ventral sucker	47 × 78	53	73 (immature)	31–49 × 49–51
Distance between oral and ventral sucker	306–530 from anterior end	224–275 from anterior end	274 from anterior end	226–370
Distance between ventral sucker and gynecophoral fold	1,020 from anterior end to ventral sucker	780 from anterior end to ventral sucker	678 from anterior end to ventral sucker	230–536

ported this parasite in eastern Canada from the black duck and the domestic goose (*Anser anser* Linnaeus) collected on Montreal Island.

Representatives of the genus *Psilostomum* were recovered from three *A. crecca*. Five *Psilostomum* spp. have been reported from waterfowl, namely *P. anserum* Oshmarin, 1963; *P. borealis* Ryzhikov, 1963; *P. brevicolle* (Creplin, 1829); *P. cygnei* Southwell and Koishner, 1937; and *P. marilae* Price, 1942. Only one of these, namely *P. marilae*, recorded by Price (1942) from the lesser scaup, has ever been reported from North America. *P. marilae* was later synonymized with *Gyrosoma marilae* by Byrd et al. (1961) who recovered this species from the raccoon [*Procyon lotor* (Linnaeus)]. No *Psilostomum* sp. has previously been reported from a dabbling duck.

The method of preservation of the host (freezing) did not permit accurate counts of schistosome populations since specimens entangled in coagulated blood were easily overlooked and usually of little value. As a result, the hosts were not examined systematically for this group but whenever specimens were encountered they were collected. One per cent of the *A. crecca* (Table 1) and 38% of the *A. discors* were infected with *Trichobilharzia querquedulae* McLeod, 1937 (males only).

T. querquedulae was first described by McLeod (1937) as *Pseudobilharziella querquedulae*. The validity of the genus *Pseudobilharziella*, erected by Ejsmont (1929) largely on the presence of a gynecophoral fold, has been queried by McMullen and Beaver (1942) who state: "Ejsmont (1929) established the genus *Pseudobilharziella* largely on the presence of a gynecophoral canal but a study of the genera in question indicates that this structure is present in both. Consequently *Pseudobilharziella* Ejsmont, 1929 becomes a synonym of *Trichobilharzia* Skrjabin and Zakharow, 1920."

More recently these authors (McMullen and Beaver, 1945) stated that *P. querquedulae* was a synonym of *T. physellae* Talbot, 1936. Wu (1953) disputed this contention and suggested that *P. querquedulae* be retained as a separate species, *Trichobilharzia querquedulae* (McLeod, 1937). In his opinion: "There seems little doubt, however, that they are congeneric and McLeod's species is accordingly placed in the genus *Trichobilharzia* as *T. querquedulae* (McLeod, 1937)."

Yamaguti (1958) followed the work of McMullen and Beaver (1945) but later (Yamaguti, 1971) recognized *Pseudobilharziella* as a valid genus on the grounds of differences out by Ejsmont (1929).

The measurements of *T. querquedulae* obtained during the present study are compared with those of *T. querquedulae* given by McLeod (1937) and those of *T. physellae* given by McMullen and Beaver (1942) (Table 4). In view of differences between *T. querquedulae* and *T. physellae*, the contention of Wu (1953) is accepted and *T. querquedulae* is regarded as a valid species.

Small numbers of *Psilochasmus oxyurus* (Creplin, 1825) were recovered from the large intestine of both host species (Tables 1,

2). Six (7%) of the *A. crecca* and one (2%) of the *A. discors* harbored this parasite. *P. oxyurus* has not previously been reported from *A. discors*. It has been recovered from *A. crecca* in Europe but this is the first record of its occurrence in this host from North America.

Measurements and morphological characters of specimens obtained in the present study agree with those given by Stunkard and Dunihue (1931) and Beverly-Burton (1961).

The similarity of both mature and immature *Echinoparyphium recurvatum* (von Linstow, 1873) to immature *Echinostoma revolutum* (Frolich, 1802), compounded by the presence of large numbers of these parasites, eliminated any possibility of accurate assessment of numbers of each species. Fully mature *E. revolutum*, readily distinguished by size, were recovered from 70% of each host species. A detailed examination of a random sample of 10 parasites from each infected bird provided an indication of the incidence of infection with *E. recurvatum* (13% of the *A. crecca* and 31% of the *A. discors*). Since the majority of the parasites in this group were not assigned to any particular genus, both species are considered collectively as "echinostomes."

The incidence and intensity of infection were significantly higher ($P < 0.05$) in *A. discors* [82% infected with 1 to 2,144 (mean 386) worms] than *A. crecca* [36% infected with 1 to 69 (mean 9) worms]. Immature birds of both host species were the most heavily infected. The duodenum harbored the largest number of parasites, with smaller numbers occurring in S1, S2, S3, large intestine, and ceca.

The measurements of specimens recovered during the present study agree with those of Beverly-Burton (1961).

Nematoda

Four nematode species (Table 5) were collected from *A. crecca* (range 1–3, mean 1 per infected bird) and five (Table 5) from *A. discors* (range 1–4, mean 2 per infected bird). Thirty-one per cent of the *A. crecca* and 84% of the *A. discors* harbored nematodes.

Amidostomum acutum (Lundahl, 1848) was recovered from 13% of the *A. crecca* and 62% of the *A. discors*. Although the intensity of infection did not differ significantly ($P > 0.05$) between the two host species, *A. discors* had the higher incidence of infection ($P < 0.05$). All specimens were collected from beneath the keratinous lining of the gizzard and occurred most abundantly at its junctions with the proventriculus and duodenum.

Buscher (1966) reported infection rates of 63 and 20% for *Amidostomum* sp. in immature and adult *A. discors* on their breeding grounds in Manitoba. In the present study 66% of the immature and 44% of the adult *A. discors* were infected.

The measurements of mature specimens from *A. crecca* and *A. discors* agreed with those given by Czaplinski (1962).

Epomidiostomum uncinatum (Lundahl, 1848) was recovered from beneath the gizzard lining of one immature male *A. crecca* (1% infected: one specimen) and 22 *A. discors* (36% infected: Table 5). Beverly-Burton (1972) reported this nematode from *A. crecca* in England, 14% of her sample being infected with from one to seven parasites, while Buscher (1966) reported on the degree of infection with this parasite in a population of *A. discors* on their breeding grounds. He noted that 8 and 61% of the adults and immatures were infected, respectively, while the mean intensity of infection per infected bird was three in both age classes. While *E. uncinatum* is a characteristic helminth of waterfowl (*vide* McDonald, 1969b), this is the first record of its occurrence in *A. crecca* in North America.

The measurements of mature specimens of both sexes agreed with those given by Czaplinski (1962).

Capillaria anatis (Shrank, 1790) was recovered from two (2%) of the *A. crecca* and 20 (33%) of the *A. discors* (Table 5). The intensity of infection was similar in both host species ($P > 0.05$) but the incidence of infection was significantly different ($P < 0.05$), *A. discors* being the more frequently infected. Specimens were usually found in the ceca but occasionally individuals were collected from S3 of the small intestine.

The measurements of mature specimens of each sex from both hosts agree with those given by Czaplinski (1962), Mettrick (1959), and Wakelin (1965).

Specimens of *Capillaria contorta* (Creplin, 1839) were found in the esophageal mucosa of

Table 5. Details of infection of *A. crecca* and *A. discors* with Nematoda and Acanthocephala.

	A. crecca						*A. discors*						
	Amidostomum acutum	*Epomidiostomum uncinatum*	*Capillaria anatis*	*Capillaria contorta*	*Tetrameres ryjikovi**	*Corynosoma constrictum*	*Amidostomum acutum*	*Epomidiostomum uncinatum*	*Capillaria anatis***	*Capillaria contorta*	*Tetrameres ryjikovi**	*Streptocara crassicauda***	*Corynosoma constrictum*
Adult male													
No. (%) birds infected	1(9)	–	–	–	–	1(9)	1(33)	2(67)	–	–	1(33)	–	1(33)
Range of parasite Nos.	1	–	–	–	–	1	3	1–4	–	–	1	–	1
Mean No. parasites/infected bird	1	–	–	–	–	1	3	3	–	–	1	–	1
Total No. parasites	1	–	–	–	–	1	3	5	–	–	1	–	1
Adult female													
No. (%) birds infected	2(22)	–	–	–	1(11)	3(33)	3(50)	–	2(33)	–	1(17)	–	4(67)
Range of parasite Nos.	1	–	–	–	1	3–4	4–12	–	1–7	–	2	–	1–6
Mean No. parasites/infected bird	1	–	–	–	1	3	8	–	4	–	2	–	4
Total No. parasites	2	–	–	–	1	10	24	–	8	–	2	–	14
Immature male													
No. (%) birds infected	3(12)	1(4)	1(4)	3(12)	6(23)	8(31)	15(75)	9(45)	8(40)	–	6(30)	–	18(85)
Range of parasite Nos.	1–5	1	1	1–4	1–6	1–8	1–15	1–8	1–8	–	2–4	–	1–22
Mean No. parasites/infected bird	2	1	1	2	4	5	4	3	2	–	3	–	6
Total No. parasites	7	1	1	6	24	37	64	30	18	–	17	–	102
Immature female													
No. (%) birds infected	5(13)	–	1(3)	2(8)	8(21)	11(28)	15(60)	10(40)	9(36)	1(4)	1(4)	1(4)	15(60)
Range of parasite Nos.	1–8	–	2	1–2	1–2	1–6	1–16	1–4	1–9	1	1	1	1–22
Mean No. parasites/infected bird	3	–	2	2	1	2	6	2	4	1	1	1	5
Total No. parasites	14	–	2	3	11	25	88	15	36	1	1	1	68
Local male													
No. (%) birds infected	–	–	–	–	–	–	2(67)	–	–	–	–	–	2(66)
Range of parasite Nos.	–	–	–	–	–	–	7	–	–	–	–	–	2
Mean No. parasites/infected bird	–	–	–	–	–	–	7	–	–	–	–	–	2
Total No. parasites	–	–	–	–	–	–	14	–	–	–	–	–	4
Local female													
No. (%) birds infected	–	–	–	–	–	–	1(50)	–	–	–	–	–	1(50)
Range of parasite Nos.	–	–	–	–	–	–	1	–	–	–	–	–	23
Mean No. parasites/infected bird	–	–	–	–	–	–	1	–	–	–	–	–	23
Total No. parasites	–	–	–	–	–	–	1	–	–	–	–	–	23
Total													
Total No. (%) birds infected	11(13)	1(1)	2(2)	5(6)	15(17)	23(26)	38(62)†	22(36)†	20(33)†	1(2)	10(15)†	1(2)	43(70)‡
Range of parasite Nos.	1–8	1	1–2	1–4	1–6	1–8	1–16	1–8	1–8	1	1–4	1	1–23
Mean No. parasites/infected bird	2	1	2	2	2	3	5	2	4	1	3	1	5
Total No. parasites recovered	24	1	3	9	36	73	194	51	75	1	25	1	214

* New record for *A. crecca* in North America. ** New host record. † Includes one bird of unknown age and sex. ‡ Includes two birds of unknown age and sex.

five (6%) of the *A. crecca* and one (2%) of the *A. discors*.

Measurements of specimens obtained in the present study agree with those given by Czaplinski (1962) and Mettrick (1959).

Tetrameres ryjikovi Khuan Shen-i, 1961, was found in 15 *A. crecca* and 10 *A. discors* (Table 5). The incidence and intensity of infection were similar in both host species ($P > 0.05$). All specimens were recovered from the mucous glands of the proventriculus.

T. ryjikovi has not previously been reported from North America nor has it ever been recovered from *A. discors*. The measurements of both males and females agree with those presented by Khuan Shen-i (1961) who first recovered and described this nematode from anatids in the USSR.

A single, mature female *Streptocara crassicauda* (Creplin, 1829) was found beneath the gizzard lining of an immature female *A. discors*. Measurements of the specimen agree with those given by Gibson (1968) in his review of the genus *Streptocara* Railliet et al., 1912. This species has not previously been recorded from *A. discors*.

Acanthocephala

Only one species, *Corynosoma constrictum* Van Cleave, 1918, was recovered, from both host species, during the present study (Table 5). Although the intensity of infection was similar in both host species ($P > 0.05$) the incidence of infection was significantly higher in *A. discors* ($P < 0.05$). The majority of specimens were collected from S3 of the small intestine (64% in *A. crecca*, 76% in *A. discors*) of both host species. S2 was the next most heavily infected region (33% in *A. crecca*, 22% in *A. discors*). Small numbers also occurred in S1 and the large intestine. Buscher (1966) reported this helminth in *A. discors* in Manitoba where 59% of the adult and 69% of the immature birds were infected, harboring an average of four and nine worms, respectively.

Measurements of specimens obtained during the present study fell within the range given by Van Cleave (1918, 1945).

C. constrictum has never been incriminated as the cause of mortality nor has the pathology of infection by this helminth been described. During the present study the loss of villi and the formation of a nodule at the site of attachment were noted.

Cestoda

Cestodes were recovered from 60 (69%) and 53 (95%) of the *A. crecca* and *A. discors*, respectively. Among the *A. crecca*, immature females were most frequently infected (85%), followed by immature males (68%), adult females (56%), and adult males (36%). The most frequently infected of the *A. discors* were local birds (100%), followed by immature males (90%), immature females (88%), adult females (83%), and adult males (33%). McLaughlin (1970) reported on the cestode fauna of waterfowl collected in New Brunswick and found four and nine cestode species in *A. crecca* and *A. discors*, respectively.

Mallophaga

During the present study both host species were infested with three species of Mallophaga, namely *Trinoton querquedulae* (Linnaeus), *Anatoecus dentatus* (Scopoli), and *Anaticola crassicornis* (Scopoli). All three species are typical Mallophaga of anatids (Keirans, 1967). It is of interest to note that *A. dentatus* has not previously been reported from *A. crecca* in North America.

General Discussion

During the present study a total of 20 helminth species (Trematoda, Nematoda, Acanthocephala) were recovered from the *A. crecca* of which four are new host records and six are new records for this host in North America. Beverly-Burton (1972) recovered only five species belonging to these three parasite groups from *A. crecca* in England. *A. discors* was found to be the host for 18 species of helminths, of which eight are new host records, as compared to the 11 species recovered from *A. discors* on its breeding grounds in Manitoba by Buscher (1966). The present study thus revealed a larger species composition than previously reported by other workers.

Ninety-five per cent of the *A. crecca* and 100% of the *A. discors* were infected during the present study; however, *A. discors* had the higher incidence of infection for 11 of the 17 helminth species common to both hosts.

The intensity of infection was similar in both host species except in the cases of echinostomes, *Notocotylus attenuatus*, and *Corynosoma constrictum*. The food habits of the host could possibly explain this, Kortright (1942) stating: "The animal food of the Green-winged Teal amounts to 9 per cent of its diet so that the Blue-winged consumes more than three times as much animal food [29%] as does that species."

Although the intensity of infection with echinostomes is higher than any previously reported from both host species, *A. discors* was by far the most heavily infected. In addition to their food habits the behavior of the hosts could also account for this discrepancy. *A. discors* is an early migrant and large numbers concentrate on the Tintamarre National Wildlife Area and Missaquash Marsh from mid-August to early October at which time they continue on their southward migration. On the other hand, *A. crecca* is a later migrant, the larger numbers concentrating on the areas around the opening of the duck hunting season at which time their utilization of the marshes becomes limited by hunter activity. Since *Echinostoma revolutum* and *Echinoparyphium recurvatum* occur in the infective stage on these areas (proven experimentally by infections in Pekin ducks in 1973), the high level of intermediate host populations on artificial impoundments as compared to a natural area (Whitman, 1974), compounded with a concentration of definitive hosts, could well account for the high level of parasitism with these trematodes in *A. discors*.

It is unlikely that *A. crecca* would become heavily infected with echinostomes, in the sample area, in spring, due to the fact that this host is an early spring migrant. The first arrivals reach the Nova Scotia–New Brunswick border region around the 1st week of April and tend to concentrate on the salt marshes awaiting ice-out on the impoundments. Population numbers generally peak during the 3rd and 4th week of April and drop suddenly in early May when the bulk of the migrants continue their northward movement. The artificial impoundments are not used heavily by nesting *A. crecca* and breeding birds that do remain are usually found on small streams or ponds located within or near the wildlife areas.

Unlike *A. crecca*, *A. discors* is a later spring migrant. Small numbers reach the sampling area during the first 2 weeks of April but the population peak occurs around the 1st week of May, about 10–14 days later than *A. crecca*. The population drops gradually during May but relatively large numbers remain to nest. During the nesting and brood-rearing periods the artificial impoundments are used extensively by this species.

Although the sample sizes of adults were too small to statistically compare parasite burdens with immatures, the intensity of infections were generally higher in young birds (Tables 1, 2, 5). Buscher (1965) reported a higher incidence and intensity of infection in immature birds and suggested that young ducks have probably not developed an age-immunity to parasitic infections and are more susceptible to parasitism than adults. The heavy utilization of invertebrates by ducklings could also contribute to a higher incidence and intensity of infection.

Eight of the parasite species recovered during the present study have been incriminated as the cause of mortality among waterfowl and several others have been associated with pathological conditions (*vide* McDonald, 1969b). During the present study only two species caused observable damage, namely *A. acutum* and *C. constrictum*. It seems likely that helminth infections are a normal part of anatid biology. However, before large-scale wetland improvement schemes are undertaken it is imperative that baseline data, such as is presented above, be obtained so that the desired objectives are not negated by a buildup of potentially pathogenic parasites (Bennett et al., 1974).

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In Memoriam

John B. Poole
February 12, 1914—May 1, 1975
Member since 1966

Gerald Thorne
July 6, 1890—February 16, 1975
Member since 1923
Elected to Life Membership 1961

Research Note

The Effect of *Mesocestoides corti* Larval ES Antigens on Establishment and Development of *Hymenolepis diminuta* in Rats[1]

Although antigens extracted from larval or adult cestodes have been used to stimulate immunity, with varying degrees of success, it is generally found that immunity can best be induced by living parasites or their diffusible products. Homologous protection against challenge has been elicited using activated or implanted larval stages, and their excretory and secretory (ES) products (Weinmann, 1970, *In* G. J. Jackson et al., Immunity to Parasitic Animals, Vol. 2). There are relatively few reports, however, concerning heterologous resistance in cestode infections, although where this has been investigated it has generally been apparent, especially when tissue phases of the parasites are involved. Weinmann (1964, Exp. Parasit. 15: 514–526) demonstrated that previous infection of mice with *Taenia taeniaeformis*, *T. crassiceps*, *Hymenolepis microstoma*, or *H. citelli* enhanced resistance to *H. nana* to varying degrees. Gemmell (1964, Immunology 7: 489–499), using intramuscular inoculations of living taeniid oncospheres, showed marked cross-protection between *T. hydatigena* and *T. ovis* in sheep. Mueller (1974, J. Parasit. 60: 3–14, and pers. comm.) feels that prior infection with *T. taeniaeformis* immunizes cats against *Spirometra mansonoides*, as they are extremely difficult to infect with the latter worm.

Recently, it has been shown that supposed nonimmunogenic lumen-dwelling adult cestodes are indeed immunogenic. Tan and Jones (1968, Exp. Parasit. 22: 250–255) demonstrated resistance in mice to the bile-duct cestode, *H. microstoma*, and Harris and Turton (1973, Nature 246: 521–522) have shown by sensitive techniques an antibody response to the classically nonimmunogenic *H. diminuta*.

Kowalski and Thorson (1972, J. Parasit. 58: 732–734) showed that *Mesocestoides corti* larval ES products are very immunogenic in mice, protecting them to a substantial degree from a challenge infection. The purpose of the present investigation was to assess the effects, if any, of *M. corti* larval ES antigens on the establishment and development of *H. diminuta* cysticercoids in rats.

M. corti tetrathyridia were collected from the peritoneal cavities of infected mice, extensively washed in sterile Krebs–Ringer solution (K–R) and sterile antibiotic K–R (250 μg/ml streptomycin sulfate, 250 Oxford units/ml crystalline penicillin G), and incubated for 24 hr in vitro in sterile antibiotic K–R at 37 C. The very opalescent ES supernatant was removed and frozen in sterile serum bottles at –20 C. Protein determinations were done by the method of Lowry et al. (1951, J. Biol. Chem. 193: 265–275). Rats were given successive, graded intraperitoneal injections of *M. corti* ES antigens every other day for 10 days in the first experiment (total per rat 1,260 μg), and 14 days in the second experiment (total per rat 1,650 μg). Control rats received injections of sterile K–R. Six to 7 days later all rats were challenged by stomach tube with 10 *H. diminuta* cysticercoids each, previously dissected from infected *Tribolium confusum*. Fourteen days postchallenge the rats were necropsied, and adult *H. diminuta* removed. They were scrupulously cleansed of intestinal debris, rinsed once in distilled water, blotted, and placed into previously dried (overnight, 110 C) and weighed (0.01 mg) 12- by 75-mm test tubes, one rat's worm burden per tube. These were again dried overnight and reweighed, to assess mean worm development as dry weight of worm tissue. Mean dry weight of one adult worm per group was computed, and these values were compared using Student's *t* test, a value of 0.05 or less for *P* being considered significant.

It can be seen from Table 1 that prior immunization of rats with *M. corti* larval ES

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Table 1. Mean dry weight (mg) of *Hymenolepis diminuta* of a challenge infection recovered from rats previously injected with *Mesocestoides corti* larval ES antigens, as compared to uninjected controls.

Experiment number	Group	Number of rats	*M. corti* ES antigen dose/rat (μg protein)	Challenge no. *H. diminuta*/ rat	Mean no. *H. diminuta* recovered/ rat (total no.)	Mean dry weight *H. diminuta* recovered (mg)
1	Experimental	5	1,260	10	10(50)	69.62 ± 4.01*†
	Control	5	—	10	10(50)	72.76 ± 6.27
2	Experimental	11	1,650	10	9.45(105)	42.85 ± 3.18 †
	Control	11	—	10	9.55(104)	42.03 ± 3.76

* Standard error.
† Not significantly different from control, Student's *t* test.

antigens had no effect on the establishment or subsequent development of *H. diminuta* cysticercoids. Equal numbers of worms established in both groups, and all worms developed to essentially the same size, as expressed by dry weight of worm tissue.

Challenge immunity to cestode infections is often expressed as resistance of the intestinal mucosa to hexacanth penetration, a concept called the "intestinal barrier" (Weinmann, 1970, loc. cit.). Since *H. diminuta* has no mammalian tissue phase in its development, any homologous or heterologous resistance effects would be on cysticercoid establishment, and/or adult development. Heyneman (1962, Exp. Parasit. 12: 7–18) found that *H. nana* infections in mice and rats greatly reduced the size and infectivity of a subsequent *H. diminuta* challenge infection, and felt that the tissue phase of *H. nana* was the primary immunizing factor. In the present study, ES antigens from the tissue phase of *M. corti*, which are known to be highly immunogenic and protective in the homologous situation, did not have a similar effect on *H. diminuta*. It is felt that in the present system, larger doses of antigen or its combination with adjuvants, or concurrent infection effects would be avenues for further investigation of any cross-immune reactions between these cestode species.

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Research Note

Blood Changes in Irradiated Mice Infected with *Heligmosomoides polygyrus* (= *Nematospiroides dubius*)

Whole-body radiation has been shown to depress natural resistance and active and passive immunity of the host to nematode infections (Dunsmore, 1961, Nature 192: 139–140; Stoner and Hale, 1963, N. Y. State J. Med. 63: 691–698). Larsh et al. (1962, Am. J. Trop. Med. Hyg. 11: 633–640) have postulated that the effect is an indirect one instigated by damage to the hematopoietic system and its subsequent inability to supply adequate numbers of cellular elements necessary for an effective inflammatory response. Cypess (1972, J. Parasit. 58: 563–566) reported observations which indicated that there was an association of peripheral blood changes and immune reactions to *Heligmosomoides polygyrus* by S–W mice. The observations reported herein are concerned with the combined effects of a sublethal dose of gamma radiation and infective larvae of *H. polygyrus* on the blood picture of mice.

To establish the LD 50/30 value of gamma radiation 50 C3H/Anf Cum Agouti mice

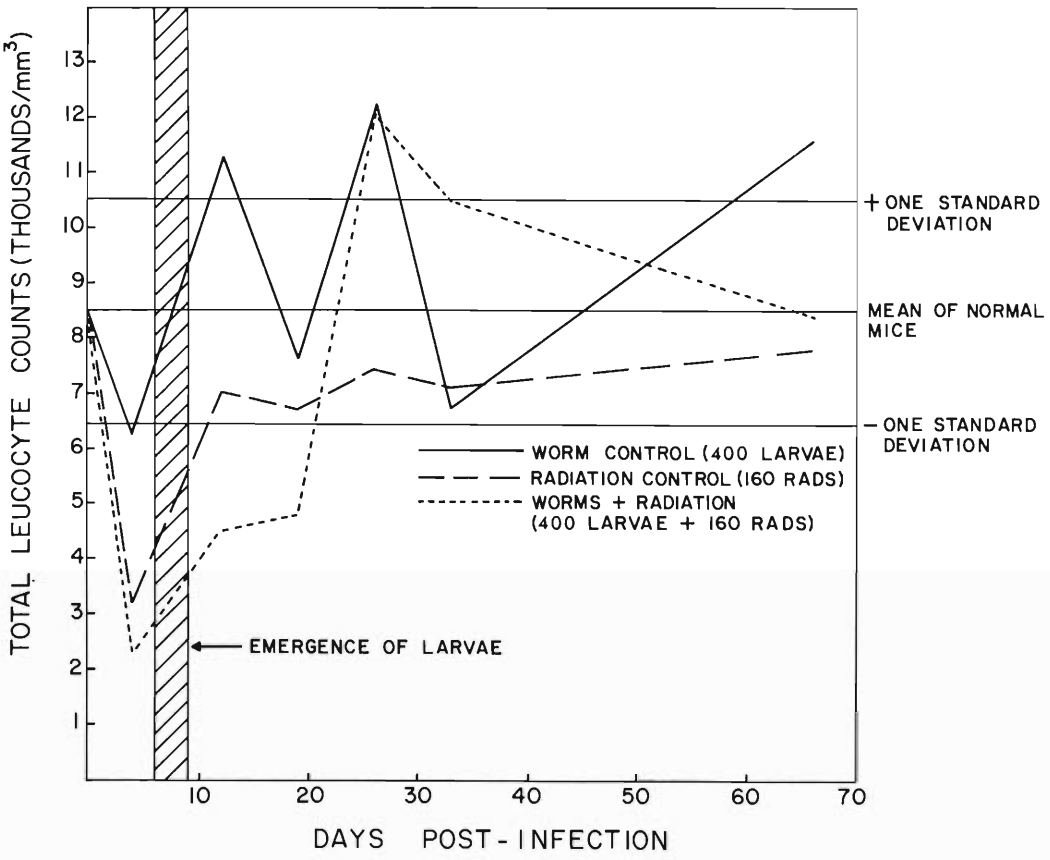

Figure 1. Total leukocyte counts of C3H mice which had been irradiated and then infected with *Heligmosomoides polygyrus*.

(25 males and 25 females) between 44 and 48 days of age were divided into five groups each containing five males and five females. One group served as controls and received no radiation. The other four groups received 200, 400, 600, and 800 rads of gamma radiation from a cobalt-60 source utilizing facilities and methods described previously (Forrester and Neilson, 1973, J. Parasit. 59: 251–255). The LD 50/30 value was calculated to be 325 rads according to the method of Reed and Muench (1938, Am. J. Hyg. 27: 493–497).

Twenty additional mice (9 females and 11 males) 42 to 49 days of age were divided into four groups of five. Group one served as normal controls and received no radiation and no infective larvae. Group two served as radiation controls and received 160 rads of gamma radiation (approximately ½ LD 50/30), but no worms. Group three was the worm control group and received 400 infective larvae of *H. polygyrus* (Strain 50 of Forrester, 1971, J. Parasit. 57: 498–503), but no radiation. Group four included mice which received 160 rads of gamma radiation and 400 infective larvae administered 5 hr after irradiation. The techniques of culturing, collecting, and administering infective larvae, obtaining adult worms at necropsy, and maintaining experimental mice followed those of Forrester (1971, J. Parasit. 57: 498–503). Every 7 days the mice were anesthetized with ether and blood was drawn from the tips of tails for packed cell volume (PCV) and for total white blood (WBC) determinations. Packed cell volumes

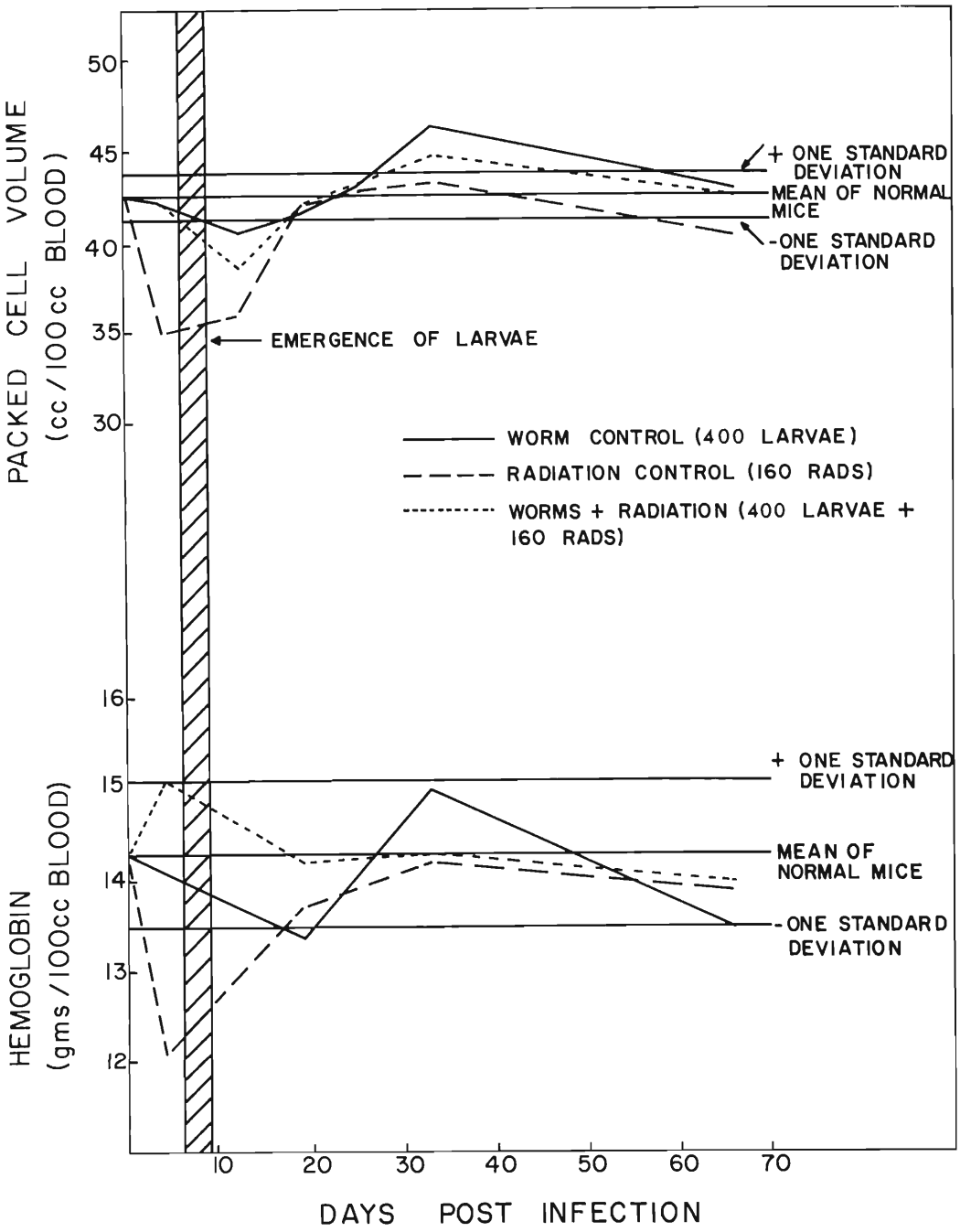

Figure 2. Packed cell volumes and hemoglobin levels of C3H mice which had been irradiated and then infected with *Heligmosomoides polygyrus*.

were determined using double oxalated microhematocrit capillaries and total white cell counts were made using a Spencer Bright Line Improved Neubauer Chamber. Every 14 days enough additional blood was drawn to determine hemoglobin concentrations (utilizing a Beckman "B" Spectrophotometer) as well as PCV and WBC values.

Observations continued for 66 days. None of the mice died due to the radiation and/or infection. The absence of death in mice receiving 400 larvae is in contrast to previously reported data (Forrester, 1971, J. Parasit. 57: 498–503) in which 60% mortality occurred within 30 days of infection for C3H mice given 400 larvae. This discrepancy is probably due to a loss of pathogenicity such as that reported recently by Hepler and Lueker (1974, J. Parasit. 60: 1057–1058). Severe leukopenia developed by day 4 in both the radiation controls and those mice with worms and radiation exposures (Fig. 1). Baker (1962, J. Parasit. 48: 438–441) recorded a sixfold increase in total white cell counts which coincided with the emergence of larvae from the intestinal wall of Webster mice infected with 400 *H. polygyrus* larvae. This phenomenon was not seen in the present experiment since blood samples were taken on days 4 and 12, but not on days 7 through 9 postinfection. Leukopenia usually occurs during the 1st week after whole-body irradiation of C3H mice (Henshaw, 1944, J. Nat. Can. Inst. 4: 485–501) and therefore was to be expected in mice receiving 160 rads of gamma radiation. What was not expected was the long duration of leukopenia which lasted through day 19 in irradiated and infected mice. The radiation controls had returned to the normal range by day 12. This prolonged leukopenia may have been a result of general debilitation of the radiated animals with their additional worm burdens.

Packed cell volumes and hemoglobin concentrations indicated that a slight anemia occurred in the radiation controls by day 4, but not in the radiated and infected mice (Fig. 2). The anemia occurred before the time of emergence of larvae from the intestinal walls (days 7 to 9) and recovery occurred by day 19. Baker (1955, Exp. Parasit. 4: 526–541) found that in Webster mice infected with 400 larvae of *H. polygyrus* the anemia produced was precipitated by the emergence of larvae from the wall of the intestine. A slight reduction in the PCV and hemoglobin levels (more than one standard deviation in both components) occurred after emergence of larvae in worm-control animals. Mice that were infected and irradiated exhibited more favorable erythrocyte patterns than those which received radiation only. It is possible, however, that the anemia observed in this experiment was partially a reflection of fluid shift or retention and not solely a hematopoietic change. Further studies beyond the preliminary one reported here are needed before a "protective effect" due to worm infections can be postulated for irradiated mice.

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Research Note

New Helminth Records from Minnesota Mammals

During July–August 1974, a general survey of helminths from assorted mammals taken in Itasca State Park, Minnesota, produced a new host record and three range extensions. All worms were recovered from intestinal contents by shaking in physiological saline. Specimens were processed by conventional methods and mounted in Canada balsam. Examples of the

four species have been deposited in the University of North Dakota Parasitology Collection, and additional specimens are in the personal collection of the second author.

The small intestine of a raccoon, *Procyon lotor* (L.), contained several dozen small flukes of two species. Microscopic examination revealed that all members of one species were immature. Although the largest specimens were only 2.5 mm long, we believe them to be *Echinostoma revolutum* (Froelich) Looss, 1899. The collar bears 37 spines of similar size, of which five occur on each corner. The small body size and poorly developed reproductive system makes it seem unlikely that *E. revolutum* is capable of maturing in raccoons. *Euparyphium beaveri* Yamaguti, 1958, is the only echinostome previously reported from raccoons (Harkema and Miller, 1964, J. Parasit. 50: 60–66).

The other species recovered from raccoon was the tiny microphallid fluke, *Maritreminoides nettae* (Gower) Rankin, 1939. With one exception, morphology and measurements are in close agreement with the original description (Gower, 1938, Mem. Michigan State Coll. Agr. Exp. Sta. 3: 1–94). In our specimens the alimentary system is not confined to the first one-fourth of the body, but rather the first two-fifths. Microphallids frequently show low host specificity, and *M. nettae* follows that pattern. It was described from ducks in Michigan, but is also known from raccoons in Georgia and North and South Carolina (Harkema and Miller, op. cit.). Its occurrence in Minnesota raccoons is an extension of its range and only the second report from *P. lotor*.

The small intestine of a short-tailed shrew (*Blarina brevicauda* Say) yielded numerous cyclophyllidean cestodes. From whole mounts and sectioned material these were identified as *Pseudodiorchis* sp. Minnesota specimens are much shorter (4 to 6 mm vs. 20 to 32 mm) than *P. reynoldsi* (Jones, 1944) as redescribed from Ohio shrews by Oswald (1957, J. Parasit. 43: 464–469). Our largest specimens possess scolex, sucker, rostellum, rostellar sac, and egg dimensions which are less than Oswald's smallest measurements, but comparable to the original description from shrews in Virginia (Jones, 1944, Tr. Am. Microscop. Soc. 63: 46–49). Despite these variations, presence of two testes per segment and a distinctive sucker-like rostellum bearing about 220 minute hooks on its rim make our specimens referable to *Pseudodiorchis* Skrjabin and Matevosian, 1948. It seems prudent to await more and better preserved specimens before attempting to decide if the Itasca material is a new species or simply a small version of *P. reynoldsi*. We are unaware of other records of this genus west of Ohio.

The final species of interest is an oxyurid nematode. Approximately 40 female specimens of *Citellina triradiata* (Hall) Manter, 1930, were recovered from the large intestine of a woodchuck (*Marmota monax* L.). This parasite has not been reported previously from Minnesota, although Read's (1957, J. Parasit. 43: 446–450) revision of the genus recognized *C. triradiata* in ground squirrels and marmots from Austria eastward through Russia, Alaska, and across much of North America. A more recent review of *Citellina* supports this extensive geographic distribution (Inglis and Ogden, 1965, J. Helm. 39: 11–17).

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Research Note

The Chromosome Number of *Philophthalmus hegeneri* Penner and Fried, 1963 (Trematoda)

The purpose of this note is to report observations on the chromosome number of *Philophthalmus hegeneri* Penner and Fried, 1963.

Batillaria minima (Gmelin) snails naturally infected with *P. hegeneri* larvae were collected in Clearwater Harbor, Florida, USA. Cercariae emitted from snails maintained in artificial seawater (30‰) encysted on the bottom and sides of finger bowls. Metacercariae were thermally excysted as described by Moseley and Nollen (1973, J. Parasit. 59: 650–654) and inoculated into the eyes of day-old domestic chicks. The testes of five worms recovered 37 days postinoculation were dissected, placed in acetoorcein for 5 to 10 min, squashed, and then examined immediately with phase optics at 1,000 ×.

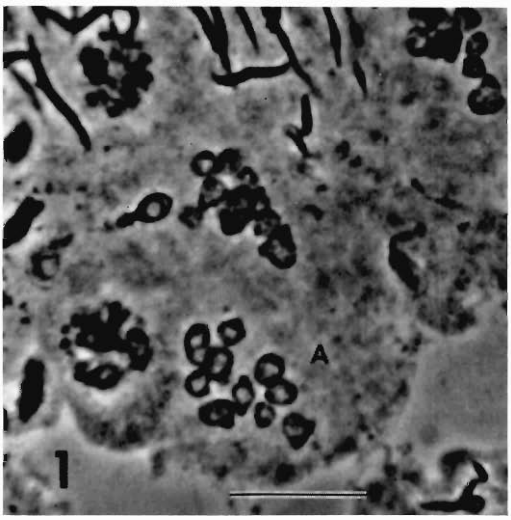

Figure 1. Photomicrograph of primary spermatocytes of *Philophthalmus hegeneri* in first meiotic metaphase. Note 10 bivalents in cell A. Scale bar equals approximately 10 μm.

Meiotic chromosomes were clearly seen in these preparations. During diakineses and metaphase I, 10 bivalent chromosomes/cell were clearly visualized in approximately 50 primary spermatocytes (Fig. 1).

A haploid chromosome number of 10 has also been reported for *Philophthalmus megalurus* (Cort, 1914) by Khalil and Cable (1968, Z. Parasitenk. 31: 211–231) and for an unidentified species of *Philophthalmus* by Reddy and Subramanyam (1971, Cur. Sci. 40: 578–579). Supported in part by a research grant from the Lafayette College Committee on Advanced Study and Research.

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Research Note

New Hosts and Localities for Turtle Helminths

Recent examination of the parasite collection of the University of Maryland's Department of Zoology has revealed three new host records and several new locality records for turtle helminths.

Specimen (#790)—*Spirorchis elegans* Stunkard, 1923, from blood of *Pseudemys elonae* = *Chrysemys concinna concinna* (Le Conte, 1830) from Duke University, Durham, North Carolina, represents a new host record for this

trematode which has only been reported from *Chrysemys picta* by Stunkard (1923, Bull. Am. Mus. Nat. Hist. 48: 165–221), Schroeder and Ulmer (1959, Trans. Iowa Acad. Sci. 66: 443–454), Goodchild and Kirk (1960, J. Parasit. 46: 219–229), and Martin (1973, Trans. Illinois Acad. Sci. 65: 61–67) and *Chrysemys scripta* by Stunkard (1923), Martin (1973), and Harwood (1932, J. Parasit. 18: 98–101). This specimen and another (#791) from a North Carolina *Chrysemys scripta* represent new locality records, as *Spirorchis elegans* had been previously reported only from turtles in Georgia (Goodchild and Kirk, 1960), Illinois (Stunkard, 1923; Martin, 1973), Iowa (Schroeder and Ulmer, 1959), New York (Stunkard, 1923), and Oklahoma (Harwood, 1932).

Amphimerus ovalis (Barker, 1911) Barker, 1911 [Stud. Zool. Lab. Univ. Nebraska (103): 513–561] has been reported only from Mississippi River softshell turtles (Trionychidae), *Trionyx muticus* and *Trionyx spiniferus*. Representing this trematode species, specimen #759 found in *Kinosternon odoratum* = *Sternotherus odoratus* (Latreille, 1801) by D. W. Rumbold is not only a new host record, but constitutes also a new family (Kinosternidae) record. Locality data are not given, but Rumbold studied North Carolina turtle helminths and this specimen may have originated there (Rumbold, 1928, Thesis, Duke Univ., 77 p.).

Trematode specimen #755, *Telorchis robustus* Goldberger, 1911, taken from the intestines of *Chrysemys picta* by Rumbold may also have come from North Carolina, as no site collection data were given. *Telorchis robustus* has been reported in the turtles *Chrysemys scripta*, *Clemmys guttata*, *Rhinoclemys areolata*, *Sternotherus odoratus*, and *Terrapene carolina* of Louisiana, Maryland, Ohio, Texas, and Mexico (Yamaguti, 1971, Vol. 1, 1074 p.).

Henotosoma haematobium Stunkard, 1922 (#792) collected from a Maryland *Chelydra serpentina* is a new locality record. Other localities previously reported are Indiana, New Jersey, New York, and North Carolina [Stunkard, 1922, Amer. Mus. Novit. (39): 1–8], Iowa (Ulmer, 1959, Trans. Am. Microscop. Soc. 78: 81–89), Louisiana and Tennessee (Byrd, 1939, J. Tennessee Acad. Sci. 14: 116–161), Ohio (Rausch, 1947, Am. Midl. Nat. 38: 434–442), and Oklahoma (Williams, 1953, Trans. Am. Microscop. Soc. 72: 175–178).

Neopolystoma orbiculare (Stunkard, 1916) Price, 1939, has been reported from turtles caught in Florida (Price, 1939; Stunkard, 1924, Trans. Am. Microscop. Soc. 43: 97–113; Loftin, 1960, Quart. J. Florida Acad. Sci. 23: 302–314), Illinois (Stunkard, 1917, Illinois Biol. Monogr. 3: 1–114; Martin, 1973), Iowa and North Carolina (Stunkard, 1917), Louisiana (Acholonu, 1969, Proc. Louisiana Acad. Sci. 32: 20–25), Michigan (Esch and Gibbons, 1967, J. Parasit. 53: 818–821), Minnesota and New York (Price, 1939), Ohio (Rausch, 1947), Oklahoma (Harwood, 1932; Price, 1939; Williams, 1953; Everhart, 1958, Proc. Oklahoma Acad. Sci. 38: 38–43), Oregon (Thatcher, 1954, J. Parasit. 40: 481–482), and Texas (Price, 1939; Everhart, 1958). Specimen #410, representing this monogenetic trematode species, collected from a *Chrysemys picta* taken in Prince Georges County, Maryland, constitutes a new locality record.

A nematode—*Oswaldocruzia leidyi* Travassos, 1917, specimen #353—previously reported from Illinois (Martin, 1973), Louisiana (Acholonu and Aryn, 1970, Proc. Louisiana Acad. Sci. 33: 25–34), Ohio (Rausch, 1947), and Oklahoma (McKnight, 1958, Diss. Univ. Oklahoma, 47 p.), was found in a *Terrapene carolina* from College Park, Maryland.

Of the eight recorded species of the nematode genus, *Spironoura*, none have been recorded from Maryland turtles. Unidentified larval *Spironoura* sp. were found in *Chrysemys picta* from Prince Georges County, Maryland (#355–356) and *Terrapene carolina* from Montgomery County, Maryland (#351–352).

In addition to specimens indicated above, there are two slides (#246) of unidentified cyclophyllidean cysticerci from visceral cysts in *Chrysemys scripta* from Lakeview, North Carolina. These slides, bearing the designation *Tetrarhynchus* (cysticercus), an obvious error, constitute the first record of a cyclophyllidean cestode from *C. scripta* (Acholonu, 1970, J. Wildlife Dis. 6: 171–172).

Turtle binomial designations were taken from Ernst and Barbour (Turtles of the United

States, 1972, Univ. Kentucky Press, 347 p.), who place *Pseudemys* within the genus *Chrysemys* and follow McDowell (1964, Proc. Zool. Soc. London 143: 239–279), who showed these turtles to be congeneric; this arrangement has also been accepted by the editors of the Catalogue of American Amphibians and Reptiles. We thank Drs. Norman R. Sinclair, Leo A. Jachowski, Jr., and Gilbert F. Otto for offering suggestions to improve the manuscript, and also Dr. Jachowski for allowing us to examine the University of Maryland helminth collection.

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Research Note

The Occurrence of *Microphallus pygmaeus* and *Cercaria lebouri* in *Littorina saxatilis, L. obtusata,* and *L. littorea* from New England

Littorina saxatilis (Olivi), *L. obtusata* (L.), and *L. littorea* (L.) occur sympatrically on the northeast coast of the United States and Canada. Examination of these littorines for larval trematodes revealed two species not previously reported from gastropods on the east coast of North America, namely, *Microphallus pygmaeus* (Levinsen, 1881) and *Cercaria lebouri* Stunkard, 1932.

Collections of the three *Littorina* species were made at two different sites at Eastport, Maine, as well as at one site at Roque Bluffs, Maine, and at Watch Hill, Rhode Island, in August 1972, November 1972, December 1972, February 1973, and May 1973.

Examination by crushing the snails revealed that *M. pygmaeus* and *C. lebouri* occurred at the three sites in Maine but not at the one in Rhode Island.

Microphallus pygmaeus adults have been reported from a variety of shorebirds and waterfowl in many parts of the world (Deblock and Tran Van Ky, 1966, cited by James, 1968, J. Nat. Hist. 2: 155–172). Recently Threlfall (1974, Proc. Helm. Soc. Wash. 41: 25–35) found this parasite in 82% of common eider ducks (*Somateria mollissima*) examined in Newfoundland. Larval stages have been reported previously from *L. scutulata* Gould on the west coast of North America (Ching, 1962, Can. J. Zool. 40: 675–676), from *L. littorea* in Germany (Werding, 1969, Marine Biol. 3: 306–333), from *L. saxatilis* in England and Wales (James, 1968, loc. cit.), and from *L. littoralis* (L.) (= *L. obtusata*) and *L. saxatilis* in Russia (Belopolskaia, 1949, cited by James, 1968, loc. cit.). *M. pygmaeus* was most prevalent in *L. saxatilis* with 29.8% of 657 specimens infected at Eastport site #1 and 53.3% of 503 specimens infected at Eastport site #2. In contrast, only 5.1% of 642 snails were infected at the Roque Bluffs site. At Eastport #1, 44.7, 35.4, 8.4, and 21.4% of the snails were infected in August, December, February, and May, respectively, while at Eastport #2, 68.0, 53.7, 27.8, and 63.1% of the snails were infected in August, November, February, and May, respectively. During the latter 4 months at Roque Bluffs, the percentages of snails infected were 8.7, 3.8, 0.0, and 6.1, respectively. Examination of 618 *L. obtusata* from Eastport #1 revealed 9.2% infected while 11.4% of 849 snails were infected at Eastport #2. During the same period, 4.8% of 796 snails were infected at the Roque Bluffs site. As with *L. saxatilis*, there was a seasonal

fluctuation in occurrence with a high in August and a low in February. *M. pygmaeus* was absent from *L. littorea* at Eastport and was found in only one of 651 snails from Roque Bluffs.

The life cycle of *M. pygmaeus* was completed by feeding the visceral hump of infected *L. obtusata* and *L. saxatilis* to day-old chickens. Mature adults were obtained at necropsy 3 days later. The herring gull, *Larus argentatus*, may be the primary definitive host of *M. pygmaeus* at the sites studied, since it was the most abundant shorebird observed during the study period. However, in recent years eider duck populations have been increasing along the Maine and Canadian Maritime coasts (E. O. Minot, 1975, pers. comm.). Flocks of ducks (not positively identified as eider ducks) were observed at Eastport #1 in fall 1974. The possibility therefore exists that the common eider duck is a host of *M. pygmaeus* in the Eastport–New Brunswick area. Further investigation is warranted since Belopolskaia (1953, cited by McDonald, 1969, U. S. Dept. Int., Spec. Sci. Rep. Wildl. No. 126) considers *M. pygmaeus* to be potentially pathogenic to young eiders.

Cercaria lebouri has been reported from *L. littorea*, *L. neritoides*, *L. littoralis*, and *L. saxatilis* in England and Wales (James, 1968, J. Nat. Hist. 2: 329–343), *L. saxatilis* and *L. obtusata* in France (Stunkard, 1932, Parasitology 24: 321–343), and *L. littorea* in Germany (Werding, 1969, loc. cit.). *C. lebouri* was found in both *L. saxatilis* and *L. obtusata* (twice for each snail species) at Eastport site #1. In May 1973 a double infection was seen in a single *L. obtusata* in which *C. lebouri* occurred with *M. pygmaeus*. *C. lebouri* was found only in *L. obtusata* from the two other Maine sites. At Eastport site #2, eight infections were found, seven in November and one in February. At Roque Bluffs only one infection was found, in the November collection.

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Research Note

Recovery of Multiplication Stages of *Trypanosoma cervi* Kingston and Morton, 1975, in Elk Spleen[1, 2]

Trypanosomes have been reported by blood culture techniques from elk, *Cervus canadensis*, in Wyoming by Kingston and Morton (1973, J. Parasit. 59: 1132–1133), in New Mexico by Davies and Clark (1974, J. Wildl. Dis. 10: 63–65), and in Michigan by Stuht (1973, Wildl. Dis. Conf. Storrs, 22–25 Aug.). The culture forms seen were considered by some authors as being similar to *Trypanosoma theileri* Laveran, 1902, a cosmopolitan bovine species. However, bloodstream trypomastigotes from concentrated elk blood were sufficiently different in body size, flagellar length, flagellar length to body length ratios, and in other attributes, including non-cross-transmissibility to susceptible bovines to warrant description as a new species, *Trypanosoma cervi* Kingston and Morton, 1975 (J. Parasit. 61:

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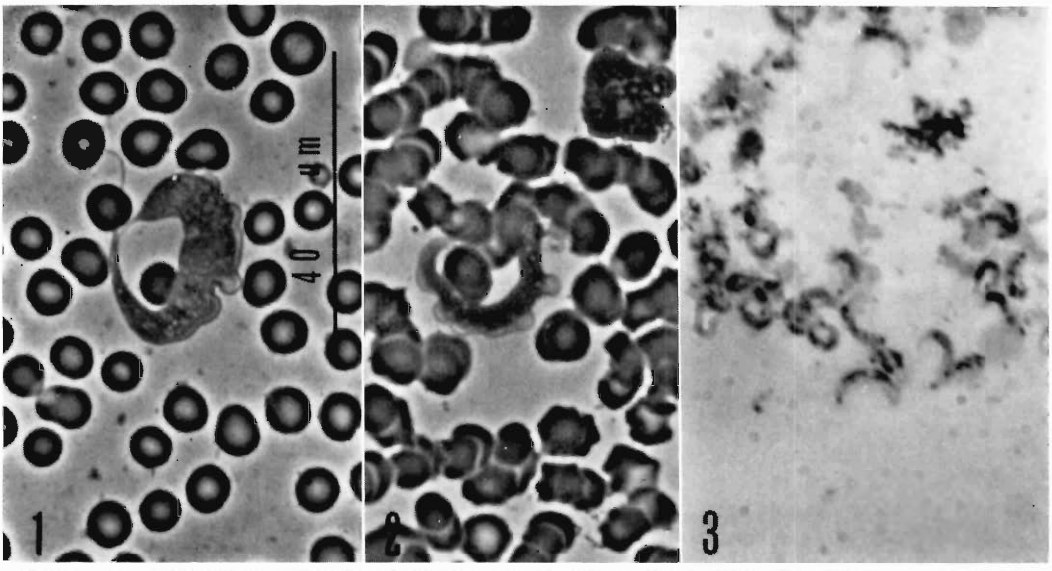

Figure 1. Bloodstream trypomastigotes of *Trypanosoma cervi* from elk. Phase contrast. The posterior refractile dot is the kinetoplast. Note elongate free flagellum.

Figure 2. Trypomastigote of *T. cervi* in blood channel in spleen of elk. Phase contrast. Note elongate free flagellum.

Figure 3. A number of small trypomastigote forms of *T. cervi* from nidus in spleen of elk. Light microscopy. Note small size of organisms; kinetoplast far posterior, nucleus far anterior. All photomicrographs to same scale.

17–23). The purpose of this note is to report the presence of what are considered division stages of this species in the spleen of the elk host. Organs of elk used in this study were made available by Dr. Tom Thorne, Research Veterinarian, Sybille Big Game Research Unit, Wheatland, Wyoming, and were taken from slaughtered elk being used in brucellosis and nutrition studies. The organs studied included liver, spleen, kidney, lung, bone marrow, and various lymph nodes (prescapular, prefemoral, popliteal, mesenteric) as well as blood films. Small portions of the organs were excised and the exposed surface was used to make imprints and smears. Organ imprints and smears and blood films were fixed in absolute methanol and stained by the method of Giemsa. Slides to be studied were coated with a thin film of immersion oil and scanned under a 16× objective. Portions of the organs and blood were cultured in veal infusion medium (VIM) and portions were preserved (10% formalin) for sectioning.

Selected organs from six known positive elk (by VIM blood culture) have been examined to date. Typical trypomastigotes have been recovered from the spleens of five of these elk and in the liver of one, usually being present in the blood channels of these organs. Imprints from other organs examined have not been positive for trypanosomes. The forms seen agree, in general, in size and dimensions, with bloodstream forms from elk recovered by the clot contraction technique of Strout (1962, J. Parasit. 48: 100). The free flagellum of these large forms has been difficult to distinguish owing to the distortion produced during the preparation of imprints. In addition, some smaller, intermediate-sized trypomastigotes have been seen in the spleen of one elk and in this spleen there has also been found a nidus of very small forms totaling more than 100 organisms. These forms were ovoid (4.5–6.2 by 2.3–4.5 μm) to elongate (6.2–10.0 by 1.5 μm), have a posteriorly to terminally located kinetoplast, and a nucleus located far

anteriorly; in a few instances a short free flagellum (measuring about 4 μm) was present on the elongate forms and rarely in some forms a single fold of the undulating membrane was seen. The smaller, ovoid forms contained a kinetoplast, a nucleus, and a vacuole which cause them to resemble amastigotes as defined by Hoare (1973, The Trypanosomes of Mammals, Blackwells, 749 p.); the more elongate forms, owing to the posterior kinetoplast, the lack of an undulating membrane, and the possession in some of a free flagellum, resemble opisthomastigotes, normally a stage seen in insects infected with *Herpetamonas*.

It is noteworthy that the larger, bloodstream trypomastigotes seen in the spleen imprints are present in greater numbers than are seen in direct blood films, or blood films made from concentrated blood (5 ml) even though smaller volumes of blood are present in the imprints. It would appear that trypansomes of this species, at least, concentrate in the spleen of the host, possibly owing to blood stasis in this organ, and there division takes place (if our interpretation of the small forms seen in the spleen is correct). Carpano (1932, Ann. de Parasit. Hum. et Comp. 4: 305–322), studying *T. theileri* from the bovine, found dividing stages similar to the above-described forms in lymphatic ganglia and in the brain but not in the spleen. Hoare (op. cit.) refers to multiplication of *T. theileri* epimastigotes in the peripheral blood. This has not been observed by us in *T. cervi* in elk blood. Further details concerning multiplication of *T. cervi* in host organs will be reported on at some later time.

We would like to express our appreciation to Ms. Barbara Bruce for alerting us to the presence of trypansomes in a bovine spleen organ culture which initiated our search for various stages in elk organs.

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Research Note

Eyeworms (Nematoda: Thelaziidae) from West Texas Quail

While surveying the helminth and acarine fauna of birds in west Texas, nematodes were recovered from the eyes of two of seven scaled quail, *Callipepla squamata*, and two of three harlequin quail, *Cyrtonyx montezumae*. None of 2 Gambel's quail, *Lophortyx gambelii*, collected in the same area, were found infected. Eyeworms were recovered from beneath the nictitating membrane of all hosts. These were identified as *Oxyspirura* (*Oxyspirura*) *petrowi* Skrjabin, 1929.

Cram (1937, *In* Papers Helm. Publ. Comm. 30 yr. Jub. K. I. Skrjabin 15th Anniver. All-Union Inst. Helm., Moscow, p. 89–98) first reported this eyeworm in North America from the ruffed grouse, prairie chicken, sharp-tailed grouse, eastern robin, and eastern meadowlark in Michigan. It was subsequently reported from the ring-necked pheasant in Nebraska by McClure (1949, J. Wildl. Mgmt. 13: 304–307) and the yellowthroat in South Carolina by Wells and Hunter (1960, J. Parasit. 46: 623). *Oxyspirura* (*Oxyspirura*) *lumsdeni* was described as a new species from the greater prairie chicken in Ontario, sage grouse in Saskatchewan, lesser prairie chicken in Oklahoma, ruffed grouse in Ontario, and sharp-tailed grouse in Ontario, South Dakota, and Montana by Addison and Anderson (1969, Can. J. Zool. 47: 1223–1227). This species was later placed in synonymy with *O.* (*O.*) *petrowi* by Pence (1972, Proc. Helm. Soc.

Table 1. *Oxyspirura* (*Oxyspirura*) *petrowi* Skrjabin, 1929, from North American birds.

Ciconiiformes	Louisiana	Pence, 1972
Butorides virescens (green heron)		
Bubulcus ibis (cattle egret)	Louisiana	Pence, 1972
Galliformes	Michigan, Ontario, North Dakota,	Cram, 1931
Pedioecetes phasianellus (sharp-tailed grouse)	Saskatchewan, Montana	Addison and Anderson, 1969
Bonasa umbellus (ruffed grouse)	Michigan	Cram, 1937
	Ontario	Addison and Anderson, 1969
Centrocercus urophasianus (sage grouse)	Saskatchewan	Addison and Anderson, 1969
Tympanuchus cupido (greater prairie chicken)	Michigan	Cram, 1937
	Ontario	Addison and Anderson, 1969
Tympanuchus pallidicinctus (lesser prairie chicken)	Oklahoma	Addison and Anderson, 1969
Phasianus colchicus (ring-necked pheasant)	Nebraska	McClure, 1941
Callipepla squamata (scaled quail)	Texas	P.S.
Cyrtonyx montezumae (harlequin quail)	Texas	P.S.
Passeriformes		
Sturnella magna (eastern meadowlark)	Michigan	Cram, 1937
	Louisiana	Pence, 1972
Agelaius phoeniceus (red-winged blackbird)	Louisiana	Pence, 1972
Cassidix mexicanus (boat-tailed grackle)	Louisiana	Pence, 1972
Quiscalus quiscula (purple grackle)	Louisiana	Pence, 1972
Tyrannus tyrannus (eastern kingbird)	Louisiana	Pence, 1972
Myiarchus crinitus (crested flycatcher)	Louisiana	Pence, 1972
Anthus spinoletta (water pipit)	Louisiana	Pence, 1972
Richmondena cardinalis (cardinal)	Louisiana	Pence, 1972
Vireo griseus (white-eyed vireo)	Louisiana	Pence, 1972
Iridoprocne bicolor (tree swallow)	Louisiana	Pence, 1972
Turdus migratorius (eastern robin)	Michigan	Cram, 1937
	Louisiana	Pence, 1972
Geothlypis trichas (yellowthroat)	South Carolina	Wells and Hunter, 1960

Wash. 39: 23–28) who also recorded it from 14 hosts in Louisiana. The latter records consisted of mostly passerines but included two new records from ciconiiform birds. Thus, this species appears to be widely distributed in a variety of species of birds over much of North America. The hosts and localities for *O.* (*O.*) *petrowi* in North America are listed in Table 1.

Eyeworms from the scaled quail and harlequin quail collected in the present study represent new host and geographic records for *O.* (*O.*) *petrowi*. The scaled quail came from Presidio County, Texas, while the harlequin quail were taken in the Davis Mountains near Fort Davis, Texas. Both series of hosts were collected June 1974. These birds are endemic to the arid southwestern United States and are predominantly species inhabiting grasslands, open scrub desert, or similar habitats. These records further substantiate the observation by Pence (loc. cit.) that *O.* (*O.*) *petrowi* is predominantly a parasite of birds occupying comparable food niches. Most of these hosts are primarily ground-feeders. Likewise, they occur in open habitats (i.e., absence of trees) such as grasslands, abandoned farms, prairies, marshlands, deserts, or similar areas. The occurrence of *O.* (*O.*) *petrowi* in a number of unrelated hosts, but all occupying a similar niche, indicates that host specificity is more dependent on the presence of a suitable intermediate host(s), which is restricted to a

particular habitat, than on the definitive host. Although its life history is unknown, this nematode probably has an arthropod intermediate host(s) which is restricted to the above particular ecological situations. Thus, it is indicated that the incidence of occurrence of these nematodes over a wide geographic area in many different host species is directly dependent on the degree of contact of these various hosts with the niches occupied by the intermediate host(s).

Nematodes collected from west Texas quail in this study conform to the redescription of *O.* (*O.*) *petrowi* by Pence (loc. cit.). Presently, there are three valid species in this genus recognized in North American birds. These include *Oxyspirura* (*Oxyspirura*) *mansoni* (Cobbold, 1879) Ransom, 1904, from domestic fowl, *Oxyspirura* (*Yorkespirura*) *pusillae* Wehr and Hwang, 1957, from a variety of arboreal piciform and passeriform hosts, and *Oxyspirura* (*Oxyspirura*) *petrowi* Skrjabin, 1929, from a variety of ground-feeding birds. A fourth species, also of the subgenus *Oxyspirura* but distinct from *O.* (*O.*) *petrowi* and *O.* (*O.*) *mansoni*, has recently been discovered in the wild turkey from West Virginia (A. K. Prestwood, pers. comm.).

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Research Note

Trypanosoma theileri in Peripheral Blood and in Bloody Vaginal Washings from a Cow

Trypanosoma theileri is a common and nominally nonpathogenic parasite of cattle. (See review by N. D. Levine, 1973, Protozoan Parasites of Domestic Animals and Man. Burgess Publishing Company, Minneapolis.) Surveys indicate incidences as high as 88% in some areas of the United States (Splitter and Soulsby, 1968, Exp. Parasit. 21: 137–148).

During a study involving an experimental treatment of *Tritrichomonas foetus* infections, one (number 6286) of three Hereford cows originally purchased from a farm near Abingdon, Virginia, developed a transitory urogenital disturbance of undetermined etiology. At the height of this infection the cow was passing bloody urine, and vaginal washings taken to confirm an experimental *T. foetus* infection were also bloody. In addition to trichomonads, a large trypanosome subsequently identified as *T. theileri* was observed in the washings. Only trichomonads were found in cultures of Diamond's medium inoculated with sediment from the washings. Washings from the other two cows contained only trichomonads.

One week later, the nonexperimental urogenital infection had subsided and the cow was asymptomatic. Vaginal washings from all three animals again were positive for trichomonads; none contained trypanosomes. Each cow was bled at this time and number 6286 was given dimetridazole intravenously as an experimental trichomonadicide. Trypanosomes were found in wet smears of blood from 6286 and 6271 but not from 6346. There was no growth or survival of trypanosomes in tissue culture medium 199 plus 10% calf serum inoculated with blood from these cows.

Seven days later, cows 6271 and 6346 were treated with dimetridazole, and all three were bled and douched. Blood and vaginal washings were processed as before. Trypanosomes were not found in any of the samples. Subsequently, only vaginal washings were examined to monitor the effectiveness of the antitrichomonal therapy.

Presumably, parasitemia was at a peak in cows 6286 and 6271 at the time trypanosomes were found in the fresh blood smears because *T. theileri* is not commonly encountered in such preparations.

Because cow 6346 was obtained at the same time and place as the others, it is possible that she was also positive but had a lower parasitemia at the time of the examinations. A more suitable medium might have allowed recovery of trypanosomes from the blood of all three cows. Cow 6346 had a normal calf approximately 4½ months after these examinations.

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Research Note

A Rapid Method of Determining Worm Burden in Intestinal Helminths[1]

Procedures for the quantitative recovery of *Nippostrongylus brasiliensis* from the intestines of laboratory rats have been described. Haley (1954, J. Parasit. 40: No. 4) employed a surface active agent which greatly facilitated the speed and accuracy of counting, although the laborious task of teasing through the material was not eliminated. Mulligan et al. (1965, Exp. Parasit. 16: 341–347) eliminated the need for teasing apart the material by cutting the small intestine into opened 3-inch segments, and allowing the worms to migrate out of the tissue. We found that while Mulligan's procedure was less time-consuming it did not yield consistently reliable worm counts due to incomplete worm migration. We present a method for obtaining quantitative worm counts that is more accurate and expeditious.

Animals to be examined are fasted overnight, killed, and the entire small intestine removed. The small intestine is slit longitudinally and placed in a petri dish containing warm saline (37 C). The petri dish and contents are held overnight at room temperature. The worms migrate into the saline and away from the intestine and are still alive 15 to 20 hr after autopsy. The effective migration during this period of time does not appear to be related to the immune status of the host, even though migration rates for shorter periods of time indicate that worms from immune hosts migrate slower (McCue and Thorson, 1965, J. Parasit. 51: 414–417). The petri dish and contents are then frozen at –20 C until it is convenient to make worm counts.

To count the worms, the contents of the petri dish are thawed and washed into a 1,000-ml graduated cylinder. Tap water is forced into the cylinder through a reduction nozzle adapter on the water outlet that greatly increases the water pressure. This results in great agitation of the preparation and separation of the intestinal layers which float to the surface, and the adult *N. brasiliensis* which settle to the bottom. The viscosity of the mucoid solution is thereby greatly reduced, thus facilitating accurate worm counts. The strips of intestine are removed with forceps and the preparation left to settle out for 30 to 45 min. (The intestinal strips may be examined under a dissecting scope at this point, if desired, although numerous examinations have revealed residual adults on rare occasions only.) The supernatant is aspirated and the cylinder refilled with tap water. This is repeated once or twice until the supernatant becomes clear. After the last aspiration, the worm sediment is decanted into a 300-ml

[1] The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Indonesian Ministry of Health or the U. S. Navy Department.
Reprint requests to Publications Office, NAMRU-2, Box 14, APO San Francisco 96263.

conical-shaped flask to facilitate settling and to prevent overflow when finally decanted into a petri dish for counting.

Since this technique utilizes freezing of the intestinal contents, the worms, when counted, are dead but almost always intact. Furthermore, since live *N. brasiliensis* adults tend to form "clumps," uncoiling after death disperses any "clumps" formed prior to freezing and individual worms are easily counted in the clear water suspension. Grids, cut into the bottom of the counting plate, facilitated both the speed and accuracy of the count.

There has been no opportunity to evaluate this technique on other intestinal helminths. With *N. brasiliensis*, this method of quantitative intestinal worm count has the advantage of being highly accurate, allows the investigator to store the intestinal contents for long periods of time when necessary, and most important, the amount of time actually spent in the counting procedure is reduced. Twenty-five to 40 experimental animals (worm burden of approximately 100) have been counted in 1 day by one person, using a mass production technique, whereas teasing the worms from the intestine required at least 2 hr per intestine.

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Editor's Note

This issue marks the completion of my five year term as editor of the Proceedings. Dr. A. James Haley has been elected by the Society to serve as editor for the term 1976–1980. Please address any correspondence relating to the January, 1976 and ensuing numbers of the Proceedings as follows: Dr. A. James Haley, Department of Zoology, University of Maryland, College Park, Maryland, 20742.

Editing the Proceedings has given me a great deal of satisfaction and pleasure. I have made many new friends and perhaps, regrettably, a few enemies. However, the work could not have been done without the help of many colleagues. I am extremely grateful to the members of the Editorial Committee for their help and advice and to other parasitologists who have contributed their time to review manuscripts. I extend a special thanks to Mr. Kenneth Blair and the staff of Allen Press, Inc. who have done an excellent job in the production of our Proceedings.

MINUTES

Four Hundred Eighty-fifth Through Four Hundred Ninety-second Meetings

485th Meeting: University of Maryland, Zoology Department, College Park, Maryland, October 11, 1974. President Sawyer reported that the Ad Hoc Committee to Select an Editor had selected A. James Haley, Zoology Department, University of Maryland for a 5-year term beginning January 1, 1976. The attending members unanimously accepted the Committee's recommendation. Slate of officers for 1975 presented: R. S. Isenstein (President); A. M. Golden (Vice President); W. R. Nickle (Corresponding Secretary-Treasurer); J. R. Lichtenfels (Recording Secretary). The Chairman of the Awards Committee, L. E. Rozeboom, announced that E. J. L. Soulsby had been selected to receive the Society's Anniversary Award. Presentation of the Award was delayed because Dr. Soulsby was traveling. Papers presented: "A catalogue for all seasons," R. H. Foote; "An informal review of the career of L. E. Rozeboom," A. O. Foster, G. F. Otto, and R. H. Foote.

486th Meeting: Animal Parasitology Institute, U. S. Department of Agriculture, Beltsville, Maryland, November 15, 1974. The slate of officers presented at the previous meeting was elected unanimously. Papers presented: "The systematic value of cuticular ridges of trichostrongylid nematodes," J. R. Lichtenfels; "The *in vitro* cultivation of *Ostertagia ostertagi* to the egg-laying adult," F. W. Douvres; "Scanning electron microscopy of *Ascaris suum* denticles from specimens of known ages," P. A. Madden and F. G. Tromba.

487th Meeting: Sponsored by Oxford Biological Laboratory, National Marine Fisheries Service; Meeting Place, Animal Parasitology Institute, Beltsville, Maryland, December 13, 1974. Papers presented: "World aquaculture disease problems," C. J. Sinderman; "Fin-rot disease in winter flounder (*Pseudopleuronectes americanus*) from the New York Bight," R. A. Murchelano; "Protozoa associated with gill fouling in crabs and lobsters from selected sites in the New York Bight," T. K. Sawyer.

488th Meeting: Laboratory of Parasitic Diseases, National Institute of Allergic and Infectious Diseases, Bethesda, Maryland, January 17, 1975. Papers presented: "Pathways of L-Serine oxidation in *Entamoeba histolytica*," T. Takeuchi, E. Weinbach, and L. S. Diamond; "Immunity and the malarial merozoite surface coat," L. Miller, M. Aikawa, T. Shiroishi, J. Dvorak, and S. Mason; "Selection of *P. berghei* resistant to clindamycin and minocycline," R. Jacobs and L. Koontz; "Genetic variation in infectivity of *S. mansoni*," C. S. Richards and P. Shade; "*Blastocystis hominis*: Pathogen or commensal?" B. P. Phillips and C. H. Zierdt.

489th Meeting: Nematology Laboratory, Plant Protection Institute, U. S. Department of Agriculture, Beltsville, Maryland; Cosponsored by Department of Botany, University of Maryland, College Park, February 21, 1975. Papers presented: "Pectinases in physiologic races of *Ditylenchus dipsaci*," D. J. Chitwood; "Potential benefits of research on nematodes of turf and pastures," J. Feldmesser, S. A. Ostazeski, and A. M. Golden; "Will insect nematodes sell?" W. R. Nickle; "Occurrence and synthesis of 18:1 fatty acids in certain nematodes," L. R. Krusberg; "Oat cyst nematode—a new threat to production of cereals in the United States," A. M. Golden.

490th Meetings: Walter Reed Army Institute of Research, Washington, D. C., March 21, 1975. Papers presented: "African trypanosomiasis"—A) "Biology," J. C. Burke; B) "Antibody variant specificity," G. H. Campbell and R. H. Perry; C) "Immunization of experimental animals," R. E. Duxbury; "Schistosomiasis"—A) "Radioisotope labeling of *S. mansoni*," W. A. Reid and S. M. Phillips; B) "Biocontrol implications of Microsporidia," A. Cali and W. A. Reid.

491rst Meeting: Naval Medical Research Institute, Bethesda, Maryland, April 18, 1975. Papers presented: "Sporozoite induced immunity to malaria," R. L. Beaudoin; "*Plasmodium berghei* and pregnancy: Some postpartum manifestations among offspring," T.

T. Palmer; "Status of oriental schistosomiasis in the Indonesian Archipelago," W. P. Carney; "Serological studies on *Nippostrongylus brasiliensis* infections in relation to the immunological response of its host," V. D. Schinski; "This wormy world—an attractive new look," (Fashion Show) W. P. Carney.

492nd Meeting: Univeristy of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania, May 10, 1975. President Isenstein announced that 5 members had been elected to Life Membership in the Society: T. von Brand, M. B. Chitwood, C. M. Herman, L. E. Rozeboom, and A. L. Taylor. The Society's Anniversary Award for 1974 was presented to E. J. L. Soulsby by A. O. Foster. Papers presented: "Acquired resistance to visceral leishmaniasis in the golden hamster," J. Farrell; "Response of different strains of mice to *Leishmania tropica*," I. Kirimi; "A role for antibody in cutaneous leishmaniasis," C. Groocock; "Importance of colostral IgA in the postnatal transfer of immunity against T. taeniaeformis in mice," S. Lloyd; "Measurement of the T cell response to *Ascaris suum* in mice," C. Johnstone; "Immunodepression by Trichinella spiralis," O. O. Barriga.

The following 37 new members were elected at the meetings indicated: *485th*: M. P. Bawden, D. T. John, S. H. Leppla, C. McCullough, M. B. Powers, D. F. Oetinger, T. P. Kistner, J. Kimpinski, S. R. Purseglove, G. R. Noel, W. P. Wergin, J. A. Meredith, J. D. Stockman II. *486th*: M. J. Simon, W. C. Grant, W. J. Pohley. *487th*: G. J. Greer, R. W. Gwadz, J. Carvajal, C. Betterton, S. J. Koepp, R. Eckerlin. *488th*: J. L. Fendrick, A. Cali, R. H. Perry, G. Kruse. *489th*: M. A. Mayes, P. R. Burn, J. E. Byram, P. M. Peterson. *490th*: B. Hörning, T. M. Seed. *491rst*: P. A. Billeter, K. A. Kinnamon. *492nd*: J. Pondick, J. M. Tarozona, H. M. Turner.

J. Ralph Lichtenfels
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