VOLUME 26 JULY, 1959 NUMBER 2

PROCEEDINGS

of

The Helminthological Society of Washington

A semi-annual journal of research devoted to Helminthology and all branches of Parasitology

Supported in part by the
Brayton H. Ransom Memorial Trust Fund

Subscription $3.00 a Volume; Foreign, $3.25

Published by
THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 26 JULY, 1959 NUMBER 2

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The Helminthological Society of Washington meets monthly from October to May for the presentation and discussion of papers. Persons interested in any branch of parasitology or related science are invited to attend the meetings and participate in the programs.

Any person interested in any phase of parasitology or related science, regardless of geographical location or nationality, may be elected to membership upon application and sponsorship by a member of the society. Application forms may be obtained from the Corresponding Secretary-Treasurer (see below for address). The annual dues for either resident or nonresident membership are four dollars. Members receive the Society's publication (Proceedings) and the privilege of publishing (papers approved by the Editorial Committee) therein without additional charge unless the papers are inordinately long or have excessive tabulation or illustrations.

PROCEEDINGS OF THE SOCIETY

The Proceedings of the Helminthological Society of Washington is a medium for the publication of notes and papers presented at the Society's meetings. However, it is not a prerequisite for publication in the Proceedings that a paper be presented before the Society, and papers by persons who are not members may be accepted provided the author will contribute toward the cost of publication. Each volume of the Proceedings consists of two numbers, issued in January and July.

Manuscripts may be sent to any member of the Editorial Committee. Manuscripts should be typewritten (double spaced) and submitted in finished form for transmission to the printer. Except in the case of preliminary papers to be published *in extenso* later, a manuscript is accepted with the understanding that it is not to be published, with essentially the same material, elsewhere. The Editorial Committee assumes no responsibility for statements appearing in authored articles. To appear in the January number, manuscripts should be received not later than November 15th; to appear in the July number, not later than May 15th.

Proof.—Whenever possible galley proof will be sent to authors for verification.

Reprints are furnished at cost in accordance with the schedule of prices printed below. Unless otherwise specified in the order, reprints are furnished without covers. The order for reprints should be submitted when proof is returned except in the case of authors not residing in the continental United States or Canada when the order for reprints should accompany the manuscript.

No. Pages	1–2	3–4	5–8	9–12	13–16	17–20
50 copies	5.88	6.25	10.00	13.50	15.19	21.44
100 copies	6.81	7.39	11.25	15.13	17.71	24.69
Add'l 100	1.88	2.25	2.50	3.25	4.81	6.50

Covers: 100 copies 6.00; Additional 100 2.38.

Proceedings of previous meetings.—Independent publication of the Proceedings began in 1934. Prior to this date the Society's proceedings were published in *Science* (1911-1913) and, later, in the *Journal of Parasitology* (1914-1933). A few sets of these early proceedings as well as the back volumes of the PROCEEDINGS are available. For information write the corresponding secretary-treasurer (see below).

Remittances should be made payable to The Helminthological Society of Washington and sent to the corresponding secretary-treasurer.

Correspondence concerning business items should be addressed to the corresponding secretary-treasurer, Edna M. Buhrer, Section of Nematology, *Plant Industry* Station, Beltsville, Md.; concerning manuscripts to the editor, Gilbert F. Otto, Parasitology Department, Research Division, Abbott Laboratories, North Chicago, Illinois.

PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 26 | JULY, 1959 | NUMBER 2

Histological Studies on the Anterior Region of *Heterodera glycines* and *Hoplolaimus tylenchiformis* (Nematoda, Tylenchida)*

HEDWIG HIRSCHMANN

In an earlier paper (Hirschmann, 1956), the presence of an apparently new structure was noted in the cephalic region of males and second stage larvae of *Heterodera glycines* Ichinohe, 1952. The structure was described as band-like, extending around the nematode body and situated between the cuticle and hypodermal layer of the body wall. Later examination revealed the same structure in larvae and males of a number of other *Heterodera* species. Since this structure appeared to be a common feature among members of the genus *Heterodera,* a detailed histological study was made of it in males and larvae of *H. glycines.* In addition, the layering of the external cuticle, cephalic structures, body and stylet musculature and hemizonid were also studied. Adults of *Hoplolaimus tylenchiformis* Daday, 1905 (syn. *Hoplolaimus coronatus* Cobb, 1923) were selected for comparative purposes.

MATERIALS AND METHODS

Heterodera glycines was propagated on soybeans, *Glycine max* (L.) Merr., in the greenhouse. Males were collected in large numbers by incubating heavily infected roots (Goodey, 1951) or by screening the soil (Christie and Perry, 1951). Second stage larvae were hatched in water from eggs in brown cysts. *Hoplolaimus tylenchiformis* was reared on cotton, *Gossypium hirsutum* L., and adult females and males were screened from the soil.

Nematode material for study was prepared by several methods.

1. The nematodes were mounted in water or 2% formalin in temporary whole mounts.
2. Whole nematodes were mounted in gylcerin for permanent slides (Thorne, 1955).
3. The layering of the cuticle was studied by treating the nematodes with 5% sodium hypochlorite. Specimens were immersed in the hypochlorite alive or dead. The dead nematodes had been subjected to 65° C. for 10 minutes.
4. The cuticular layering, cephalic framework, chords with nuclei and other anatomical features were studied in material stained with orcein. The modified technique described by Mulvey, 1955, was followed, except that the anterior region of the nematodes was severed rather than whole nematodes being squashed. This kept the body contents intact and still allowed the stain to penetrate.
5. Face views and transverse handsections were prepared according to the instructions of Buhrer, 1949. The sections were cut 10-20 microns in thickness with a surgical eye knife.

*Contribution from Plant Pathology, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director of Research as Paper No. 967 of the Journal Series.

6. Nematodes for microtome sectioning and subsequent staining were first relaxed by gentle heat. They were then transferred to a 2% agar solution and oriented with their bodies parallel in one level. After solidification, the agar was trimmed to a small rectangular block with the nematodes in the center. Several blocks were prepared and fixed in the following fixatives:

(a) A mixture of mercuric chloride and glacial acetic acid (saturated mercuric chloride solution, 100 ml; glacial acetic acid, 5-10 ml).

(b) Zenker's fluid (Potassium bichromate, 2.5 g; sodium sulphate, 1 g; water, 100 ml; mercuric chloride, 5 g. Add 5 ml glacial acetic acid before use; after fixing, wash 24 hrs. in water).

(c) Petrunkewitsch's fluid (water, 300 ml; absolute ethanol, 200 ml; glacial acetic acid, 90 ml; concentrated nitric acid, 10 ml; mercuric chloride to saturation, approx. 20 g).

After fixation (30-60 min.) the blocks were dehydrated with an ethanol series (30 min. changes) and infiltrated with paraffin. The alcohol and traces of water were removed by transferring the blocks to methyl benzoate (2 changes 30 min. each) which was replaced by benzene (30 min.), and followed by a saturated solution of paraffin in benzene at a temperature of 35-40° C. (30 min.). Finally, the blocks were placed in pure paraffin at 58° C. (1-2 hrs.) and were embedded in Fisher's tissuemat (melting point, 56-58° C.). Serial sections, 10 microns in thickness, were mounted with chromalum adhesive and stained with Mayer's haemalum (hematoxylin, 1 g; sodium iodate, 0.2 g; aluminum potassium sulphate, 50 g; chloral hydrate, 50 g; citric acid, 1 g; water, 1000 ml). Erythrosin (0.1% water solution) was used as a counterstain.

HISTOLOGICAL STUDIES

A. External cuticle

The external cuticle of males of *H. glycines* and adults of *H. tylenchiformis* consists of three distinct layers in the mid region of the body. This layering is slightly modified in the cephalic and caudal regions.

(a) *Heterodera glycines* males: In whole mounts or longitudinal sections (Fig. 1A), the outermost layer (a) shows a coarse annulation which is spaced regularly, beginning with slightly smaller annules at the constriction of the lip region, and extending posteriorly to the tail. Inwardly, layer (a) is firmly fastened to the middle layer (b), which exhibits a similar but slightly more flattened annulation. The third and innermost layer (c) appears either as a double annulation or as a row of well defined dots, depending on the level of focus, with two annules or 2 dots corresponding to one annule of the outer layers (a) or (b) (Fig. 1A 1, 2). In longitudinal optical section, the inner layer (c) appears as another annulation around the body, but the width of each annule is only half that of the outer annules. Specimens stained with orcein show particularly well the slightly wavy to zigzag course of the annules in layer (c) as dark-staining material alternating with striae of non-staining substance (Fig. 1A3).

The cross sections (Fig. 1B) show that layer (a) is as thick as layers (b) and (c) combined. At the lateral sides, layer (a) bulges out to form the lateral fields, which extend as longitudinal thickenings throughout the length of the body. In the middle of the body, they are subdivided by 4 longitudinal incisures of which the 2 inner ones are more closely spaced. Layer (a) is hyaline and does not stain with orcein or erythrosin. The juncture between layers (a) and (b) forms a dark fine line which broadens beneath the lateral fields. Layer (b) is composed of homogeneous material that stains pink with

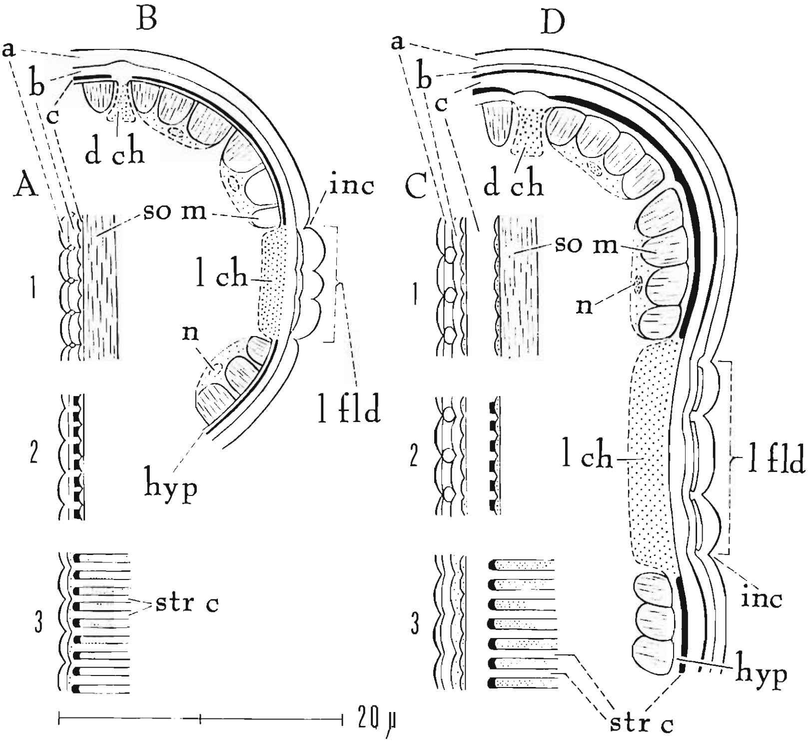


Fig. 1. Cuticular layering. A—*Heterodera glycines* male, longitudinal section of body wall; 1 and 2—different levels of focus, unstained; 3—stained with orcein. B—*Heterodera glycines* male, cross section through mid region of body. C—*Hoplolaimus tylenchiformis*, longitudinal section of body wall; 1 and 2—different levels of focus, unstained; 3—stained with orcein. D—*Hoplolaimus tylenchiformis*, cross section through mid region of body.

Abbreviations for Figures

a—outer cuticular layer
amph du—amphidial duct
amph o—amphidial pore
amph p—amphidial pouch
ant ceph—anterior cephalid
b—middle cuticular layer
bas r—basal ring
bdy cav—body cavity
c—inner cuticular layer
ce fr—cephalic framework
cut—cuticle
d ch—dorsal chord
d gl or—dorsal esophageal gland orifice
es—esophagus
es gl—esophageal gland lobe
es lu—lumen of esophagus
ex du—excretory duct
ex p—excretory pore
hem—hemizonid
hyp—hypodermis
inc—incisure
lp—lip, lip region
lp sec—lip sector
lp ppl—lip papilla
lp con—constriction of lip region
l lp gr—longitudinal lip groove
l lp str—longitudinal lip striae
l fld—lateral field
l ch—lateral chord
m f—muscle fiber
n—nucleus
nrv r—nerve ring
or ap—oral aperture
post ceph—posterior cephalid
rad b—radial blade
s—sensilla
so m—somatic musculature
su—yellowish substance
str—striation
str c—striation of inner cuticular layer
stru—8-shaped structure
sty—stylet
sty kn—stylet knobs
sty p—stylet protractor
sty sh—stylet shaft
vest—vestibule
vest ex—vestibule extension
v ch—ventral chord

erythrosin and light red with orcein. Layer (c) is a narrow layer of dense material alternating with less dense material resulting in a transverse fine annulation, i.e. striation, as described above. The annules of layer (c) show great affinity to the stains and are deep red with erythrosin or orcein. The striation of this layer is absent in the regions of the chords. In cross sections, the narrow band of dark staining material disappears where the 4 chords bulge into the body cavity (Figs. 1B; 3J, L; 6B, D). In longitudinal view, this interruption is particularly pronounced at the broad lateral chords but is also distinct at the dorsal and ventral chords (Fig. 4A). The striation of layer (c) begins at the constriction of the lip region where the muscles start and ends in the caudal region where the muscles terminate. Except for the chords, the hypodermis throughout the body is an exceedingly thin layer of tissue between cuticular layer (c) and the bases of the somatic muscles. It thus appears that the striation of layer (c) has some connection with the attachment of the muscles to the cuticle.

The cuticular layering was also studied by treating the nematodes with a 5% solution of sodium hypochlorite. The hypochlorite enters the body openings and the nematodes soon disintegrate. The thin cuticle of the cephalic region is dissolved first and this is followed by the remainder of the body cuticle. Layer (a) loosens first, then the striation of (c) is completely dissolved. Next (b) breaks loose and forms small blocks of homogeneous material which disappear rapidly. Only the outermost part of layer (a) is not dissolved. However, when specimens are heated to 65° C. for 10 min. before treating with sodium hypochlorite, this part is also destroyed.

(b) *Hoplolaimus tylenchiformis:* The cuticle of adults of *Hoplolaimus tylenchiformis* is almost 3 times as thick as that of males of *Heterodera glycines.* Basically, however, it has a similar structure (Fig. 1C, D). Layer (a) seems to be subdivided and is coarsely annulated, hyaline, and does not stain with erythrosin or orcein. It is firmly attached to the more narrow layer (b) which is flatly annulated, homogeneous in structure and stains pink with erythrosin and light red with orcein. Inwardly, layer (c) is very prominent and, in contrast to *Heterodera,* is larger in the mid region of the body than layers (a) and (b) combined. Layer (c) appears as a dense refractive material that has a slightly fibrous appearance in cross section. The boundaries of layer (c) show strong affinity for erythrosin and orcein (Fig. 1D). This is particularly true for the innermost part which exhibits a similar double annulation, i.e. striation, as layer (c) in *Heterodera* (Fig. 1C). At a certain focus in longitudinal view, there seems to be a criss-cross marking of striae in layer (c) indicating an inner and outer layer of striation. The inner striation, however, is far more prominent and always visible (Fig. 1C 1-3). Stained with orcein, the inner striation appears to be composed of heavily staining bands alternating with non-staining striae (Fig. 1C3). As in *Heterodera,* this striation is absent where the chords protrude into the body cavity (Fig. 1D). Consequently, layer (c) is more narrow in these regions. The striation of layer (c) disappears also in the caudal region where the somatic musculature ends.

Specimens of *H. tylenchiformis* exposed to sodium hypochlorite showed a similar sequence of cuticle disintegration as that described for *H. glycines* males. First, layer (a) is loosened, then layer (b) breaks off as small blocks of homogeneous material that is rapidly dissolved; almost simultaneously the inner striation of (c) disappears and the broad layer of (c) narrows down to a finely annulated line which finally is also dissolved. The outermost part of layer (a) is destroyed only when the specimens have been pretreated with heat.

Thus, it may be concluded that the cuticles of *Heterodera* and *Hoplolaimus* have the same fundamental structure except for the more developed layer (c) in *Hopolaimus*. In *Heterodera*, layer (c) is so narrow that it is actually identical with the striation of (c), whereas in *Hoplolaimus* the striation forms only the innermost part of the broad layer (c).

There are no reports in the literature regarding the cuticular layering of males of *Heterodera* and adults of *Hoplolaimus*. Some studies, however, have been made with other members of the Heteroderidae and Tylenchidae. Nagakura, 1930, describes the cuticle of males of *Heterodera radicicola* (syn. *Meloidogyne* sp.) as consisting of 3 layers but erroneously interpreted the layer of somatic musculature as the third layer of the cuticle. Elsea, 1951, reports that the cuticle of females of *Meloidogyne hapla* is composed of "at least three layers: an external cortical layer, a middle matrix layer and innermost, a somewhat thinner fiber layer" and that "the cuticle of the male is thinner than that of the female and appears to be of uniform condition." The adult female cuticle of *Meloidogyne hapla* and *M. javanica* has been studied primarily in respect to its chemical composition by Bird, 1958. In morphological respects, this author finds the cuticle of these nematodes to consist of "a thin, darkly staining surface layer covering a homogeneous substance which is divided into 3 layers by 2 darkly staining bands of material which, in some cuticles, lie close to the hypodermis and give the impression of being a single darkly stained innermost layer." Franklin, 1939, states that the cuticular layering of the cyst wall of *Heterodera* spp. comprises a surface layer which has a reticulate appearance due to shallow ridges. Underneath this layer are some pit-like markings and beneath these are 3 or 4 inner layers running around the cyst. Wieser, 1953, who made a detailed study of the construction of the cuticle of cysts of several *Heterodera* species, distinguishes an exo- and endo-cuticle. The exo-cuticle consists of a cortical layer, a fiber layer, a fibrillar layer, an inner matrix and is bounded internally by a boundary layer. The endo-cuticle appears homogeneous and more or less amorphous. This is not true for *H. rostochiensis*, in which case the endo-cuticle splits into a number of layers which resemble the fiber layers in *Ascaris lumbricoides*. The endo-cuticle is bounded by a basal lamella. In the Tylenchidae, details have been worked out for the cuticular layering of *Ditylenchus dipsaci*. Chitwood, 1938, states that the external cuticle of this nematode is a complex made up of several layers: an exterior layer (cortical layer), a fibroid matrix layer and collagenous fiber layers. He observed that the outermost part of the exterior layer is a thermolabile membrane which governs permeability. Comparing the results of our experiments with sodium hypochrolite with Chitwood's findings, it is likely that the outermost part of layer (a) in males of *Heterodera* and adults of *Hoplolaimus* constitutes also such a thermolabile membrane.

Due to the relatively small size of *Heterodera* males and adults of *Hoplolaimus*, it is difficult to compare the cuticular layering of these nematodes with that of large animal parasites, or with the layering of the highly specialized female cuticle of Heteroderidae. If such a correlation is attempted, however, it seems that layer (a) would correspond to the external and internal cortical layers, layer (b) to the matrix layer and layer (c) to the fiber layers. The striation in layer (c) would represent transverse bands of dense connective tissue. In *Hoplolaimus*, these bands form the innermost part of the prominent layer (c) which seems to be of fibrous nature throughout and appears to possess another layer of dense tissue adjacent to layer (b).

Although there is considerable variation in the construction of the cuticle among various nematode groups, the common basic pattern of cuticular structure is essentially quite similar and appears to approach a sub-division into 3 main layers.

B. Cephalic region

(a) *Heterodera glycines* males: The lip region (Figs. 2A, B; 3A-G; 4A) is distinctly set off from the rest of the body by a pronounced constriction and bears a well developed, yellowish framework. The cuticle of the cap-like lip region consists of layers (a) and (b) and when seen in lateral or median whole mounts, shows an irregular cuticular pattern with one side often exhibiting distinct annules, and the other side appearing smooth (Figs. 2A, B; 4A). This indicates the presence of longitudinal as well as transverse cuticular markings. The pattern of cuticular markings as well as the structure of the framework are best understood in "en face" mounts.

The lip region is divided into six sectors by the six radial, arched lip sclerotizations (framework) (Figs. 2A, B; 3B-E). Axial in this framework is a heavily walled hexagonal tube (vestibule) which continues into the body becoming ovoid to round in cross section (Figs. 2A, B; 3B-J). This extension resembles a lyre when viewed longitudinally and terminates with an extremely thin walled cylinder (Fig. 2A, B). The extension is made up of different material than the sclerotization of the vestibule. This is shown by its affinity to hematoxylin in stained paraffin sections. Both structures serve as stylet guides. Blade-like projections radiate toward the periphery of the lip region from each of the 6 corners of the vestibule and are attached to the corners throughout their length (Figs. 2A, B; 3B-E). The projections begin as fine short rays at the height of the second lip annule (Fig. 3B) and increase in length toward the constriction of the lip region. In longitudinal section, two opposite blades have the outline of an arch with the heaviest sclerotization on its base and periphery. The blades are connected with each other at their bases by a sclerotized ring linking the outer edge of one blade with the outer edge of another blade (Fig. 3E). The blades terminate in the approximate latitude of the last lip striation above the pronounced annulation of the constriction (Fig. 3F).

Thus, the framework divides the lip region into six sectors. The lateral lip sectors are smaller than those of the subventral and subdorsal lips (Fig. 3D, E). The oval shaped amphidial ducts are visible throughout the length of the lateral lip sectors (Fig. 3B-F). Each subdorsal and subventral lip bears medially a single obscure papilla. The nerve fibers of these papillae can be traced through the entire lip region and are no longer visible where the blades end (Fig. 3B-F).

The cuticular pattern (Figs. 3A-G; 4A) of the lip region was found to be highly irregular from specimen to specimen. In most cases, 6 distinct striations were observed (Fig. 4A). These do not include the constriction. The first lip annule is a thin, slightly convex, ovoid plate with the mouth opening in its center (Fig. 3A). At the height of the second transverse striation, the framework becomes faintly visible, and bordering this striation, the slitlike amphidial pores appear in the lateral sectors (Fig. 3B). The third striation marks the initiation of 6 longitudinal grooves, each proceeding above one of the radial blades (Fig. 3C). The fourth striation also shows 6 grooves giving the lip region a regularly lobed appearance in this vicinity (Fig. 3D). The

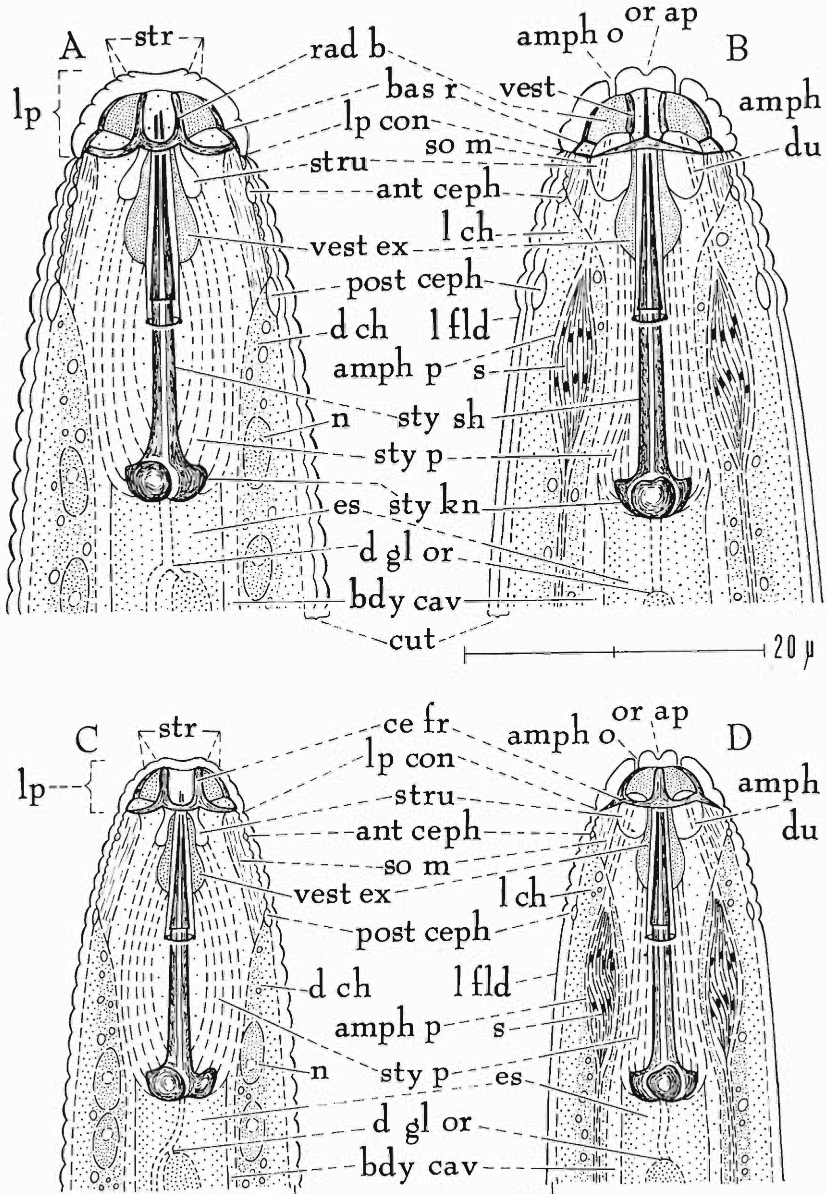


Fig. 2. Cephalic region of *Heterodera glycines*. A—male, lateral view; B—male, dorsal view. C—second stage larva, lateral view; D—second stage larva, dorsal view.

cuticular pattern becomes difficult to observe in the subsequent part of the lip region. The longitudinal grooves do not seem to keep their course over the blades, and other faint, unevenly spaced longitudinal striae arise which proceed toward the constriction (Fig. 4A). Where the basal ring begins, the 5th transverse striation surrounds the cephalic area (Fig. 3E). This striation may not complete a full circle and shows a number of unevenly spaced longitudinal grooves (Figs. 3E; 4A). These grooves vary from specimen to specimen. The 6th striation is similar, but the number of grooves is reduced in most specimens (Fig. 3F). It is followed by the constriction which is a pronounced annulation.

Starting with the constriction of the lip region and extending posteriorly, the external cuticle assumes its typical structure of 3 distinct layers described above. At the 6th to 8th annule posterior to the constriction, a highly refractive structure (the posterior cephalid) borders the inner cuticle (Figs. 2A, B; 3K). In longitudinal sections, this structure attains a more or less biconvex shape and extends around the nematode body. It may proceed horizontally following one and the same annule or it may be slightly oblique to the horizontal plane. In general, the annule exterior to this structure is much larger than the anterior annules and slightly larger than the rest of the body annules.

A similar but less conspicuous structure (the anterior cephalid) extends around the body under the second annule posterior to the constriction (Figs. 2A, B; 3I). This structure covers from ½ to the full width of the second annule. It is sometimes difficult to observe because of its small size but has been detected in every specimen critically examined.

Closer examination of the cephalids revealed that, in these regions, the striation of layer (c) as well as part of layer (b) of the cuticle are absent. The somatic muscles appear somewhat shortened in cross-section (Fig. 3K). The cephalids are well defined structures, but stain only faintly.

In search for the nature of these structures, the anterior portion of the nematodes was studied in more detail. On specimens in exact lateral position, a duct-like strand of protoplasm extending from the posterior cephalid was always observed on the dorsal and ventral sides (Fig. 2A).* On the other hand, a similar strand proceeding laterally from the anterior cephalid appeared when specimens were examined in ventral or dorsal view (Fig. 2B). Close examination of cross sections revealed that the dorsal and ventral chord begin at the level of the posterior cephalid (Fig. 3K). The 2 lateral chords start where the anterior cephalid surrounds the nematode body (Fig. 3I). Posteriorly, each of the 4 chords quickly gains size (Figs. 3I, K1, 5-7). The lateral chords have a broad base against the cuticle which narrows in the region of the stylet knobs and broadens out again posteriorly (Figs. 3I-L; 1B). The dorsal and ventral chords do not widen out to this extent. The lateral chords are considerably larger than the median chords.

Based on these observations, it is suggested that the cephalids are associated with the anterior beginning of the chords. No evidence was obtained as to the histological nature of the two cephalids.

The nuclei of the dorsal and ventral chord start at the level of the stylet knobs; those of the lateral chords start slightly further back (Fig. 2A, B). The nuclei of the lateral chords are larger than those of the median chords. The dorsal chord is soon devoid of nuclei in the remainder of the body.

---

*The terms posterior and anterior refer to the relative position of the rings to each other and not to their location on the nematode body.

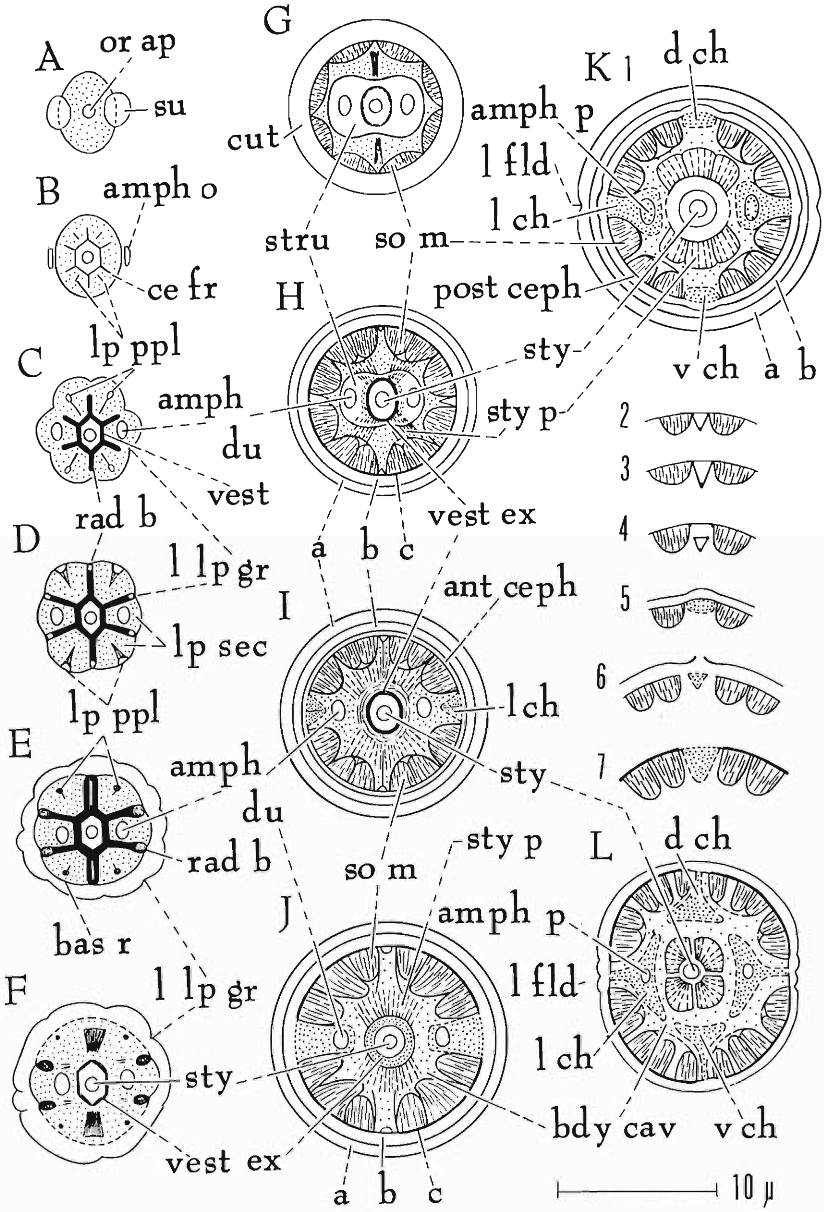


Fig. 3. Cross sections through the cephalic region of *Heterodera glycines* males. A—First lip striation. B—Second lip striation. C—Third lip striation. D—Fourth lip striation. E—Fifth lip striation. F—Sixth lip striation. G—Constriction of lip region. H—First body annulation. I—Second body annulation, level of anterior cephalid. J—Level of vestibule extension. K1—Level of posterior cephalid. K2, 3, 4—Dorsal sector 3 microns in front of posterior cephalid. K5, 6, 7—Initiation of dorsal chord (successive levels). L—Level of stylet shaft.

Two other structures that begin at the level of the posterior cephalid are the lateral fields which proceed on top of the lateral chords throughout the length of the body (Figs. 2B; 4A). Each lateral field is initiated with 2 incisures, which after 2-3 annules show a third middle incisure. The middle incisure splits in 2 at the approximate height of the median bulb. The 2 outer incisures are crenate throughout showing the same annulation as layer (a) of the cuticle. The anterior end of each lateral field is aerolated.

The chords divide the somatic musculature into 4 muscle fields, 2 of which are dorso-submedian and 2 of which are ventro-submedian (Figs. K1, L; 4B). The somatic musculature begins at the constriction with 8 muscles (Fig. 3G). At the height of the posterior cephalid, the subdorsal and subventral muscles consist of 2 fibers each; the dorsolateral and ventrolateral muscles consist of only one fiber each (Fig. 3K1). At the posterior cephalid, 4 new muscles are initiated, two of which border the dorsal and ventral chords (Fig. 4B). Four additional muscles begin lateroventrally and laterodorsally at the level of the stylet shaft (Figs. 3L, 4B). Hence, in this region there are in each field 5 fibers which belong to 4 different muscles. Each muscle field comprises 2 longitudinal rows of spindle shaped platymyarian muscles (Fig. 4B).

The protracting muscles of the stylet (Figs. 2A, B; 3H-L) are initiated at the constriction as diffuse fibers around the vestibule extension and are attached to the base of the framework. At the approximate level of the posterior cephalid, the muscles become more compact forming 3 sectors on the dorsal and 3 sectors on the ventral side (Fig. 3K1). The lateral sides are occupied by the prominent lateral chords. With the beginning of the shaft, the stylet

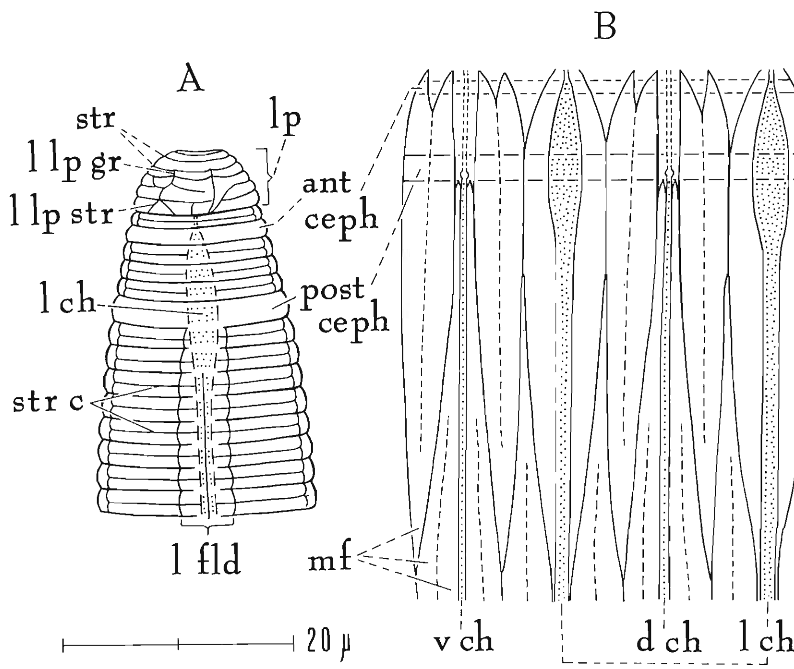


Fig. 4. Cephalic region of *Heterodera glycines* males. A—Cuticular pattern, lateral view. B—Arrangement of somatic musculature (opened out).

muscles recede from the stylet. Proceeding down the shaft, the musculature then divides into 4 submedian sectors (Fig. 3L) which finally join into one solid ring at the point at which they are attached to the stylet knobs.

In the approximate latitude of the cephalic constriction, another structure appears which surrounds the vestibule extension (Figs. 2A, B; 3G, H). In cross section, its shape resembles the figure 8, with the broad parts directed laterally. It protrudes into the body cavity about half the length of the vestibule extension. It has a clear appearance and does not absorb stain. It is penetrated by the amphidial ducts. After leaving this structure, the amphidial ducts are incorporated into the prominent lateral chords (Fig. 2B). At the approximate height of the posterior cephalid, the ducts widen out to form two spindle-shaped elongate sacs, which are the amphidial pouches that end near the stylet knobs (Figs. 2B; 3K1, L). A group of narrow sensory neurons with distinct nuclei and fine terminals form the sensilla located in the pouch. The sensilla closely resembles a taste bud, and most probably acts as a chemoreceptor. The impulses are conducted through the amphidial nerve to the amphidial ganglia. The amphidial nerves could not be traced because no special nerve stain was employed. Amphidial glands were not detected. However, it was frequently observed that the above mentioned 8-shaped structure was filled with a yellowish substance. The same substance was also present in the amphidial ducts and many times was found exuded in form of small droplets at the amphidial pores (Fig. 3A).

(b) *Heterodera glycines* larvae: The lip region of the second stage larvae of *H. glycines* closely resembles that of the male. It possesses a six-radial, strongly sclerotized framework, with one small papilla on each submedial lip and distinct amphidial ducts (Fig. 2C, D). Due to the small size of the larval head, it was not possible to determine the exact appearance of the cuticular pattern of the lip region. From longitudinal as well as "en face" view mounts, however, it seems that it is irregular and possesses longitudinal grooves in addition to transverse markings. The same is true in the male.

The location of the posterior cephalid is more constant than that in the male (Fig. 2C, D). In most specimens it occupies the 7th annule behind the constriction. This annule is not enlarged, but has the same size as the rest of the body annules. The anterior cephalid is very small and located at the second annule.

The beginning of the dorsal and ventral chords at the posterior cephalid as protoplasmic strands leading posteriorly is very distinct when viewed in exact lateral position (Fig. 2C). The cuticular layering, the lateral fields, the course of the chords, the somatic and stylet musculature, and the amphids resemble that of the male.

(c) Other *Heterodera* species: Several other *Heterodera* species were examined for th occurrence of the anterior and posterior cephalids. The cephalids were present in males and larvae of the following species:

H. schachtii, H. trifolii, H. major, H. rostochiensis, H. tabacum, H. punctata, H. cacti, H. weissi, H. carotae, H. cruciferae, and *H. fici.*

The supposition was made that the location of the cephalids, i.e. the number of annules posterior to the constriction, may be used as another quantitative character to aid in the taxonomic differentiation of *Heterodera* species.

Table 1 shows the location of the anterior and posterior cephalids in 6 different *Heterodera* species. The number of annules the 2 cephalids appear behind the constriction was the same for males and larvae except in *H. cruciferae.* The location of the 2 cephalids is rather constant for males and larvae within each species, but differences between species may not be significant.

In *H.schachtii* and *H. glycines* e.g. the number of annules is the same in males and larvae. The same holds if larvae of *H.cruciferae* are compared with males and larvae of *H. rostochiensis*. On the other hand, there are distinct differences between *H. cacti* larvae and *H. schachtii* larvae. This character may be of some taxonomic value in certain cases when used in conjunction with other characters.

(d) *Hoplolaimus tylenchiformis:* Since the anterior and posterior cephalid were common structures in *Heterodera,* they were suspected to occur also in other nematode genera. A large nematode, *Hoplolaimus tylenchiformis* was selected for comparative examinations.

In longitudinal sections and in whole mounts, the 2 cephalids were found in *Hoplolaimus* (Fig. 5A, B). In *Hoplolaimus,* the rings have a similar appearance as those of *Heterodera;* however, the anterior cephalid is the larger one. It is generally located at the 3rd to 4th annule from the constriction of the lip region and may extend as many as 2 annules in width, whereas the posterior cephalid is situated at the 9th to 10th body annule and is the same size as this annule. As in *Heterodera,* the striation of cuticular layer (c) is absent where cephalids are located (Fig. 5A, B). The structures do not occupy more layers than (c) due to the thickness of this layer in *Hoplolaimus.* The anterior cephalid causes a pronounced recession of the somatic musculature in that region (Fig. 5B).

A study of cross sections (Figs. 5B, C) showed that the origin of chords was associated with the two cephalids. As in *Heterodera,* the lateral chords begin at the level of the anterior cephalid; the dorsal and ventral chords at the level of the posterior cephalid. Each lateral chord starts as 3 strands of hypodermal tissue which unite at the 10th to 12th body annule behind the constriction.

The lateral fields begin around the 8th annule rather than at the level of the anterior cephalid as in *Heterodera.*

There have been numerous contributions which deal with the gross morphology of the cephalic region of Heteroderidae and Tylenchidae. Information concerning the finer anatomy of these groups, however, is rather limited. Some points of anatomical interest including cephalic framework, lip striation, lip papillae, amphid apertures are mentioned by Raski, 1950, in his work on the life history and morphology of *Heterodera schachtii.* In a detailed study of the morphology of the lip region of the genus *Meloidogyne,* Allen, 1952, found that in this genus the lateral lip sectors are considerably larger than those of the subventral and subdorsal lips, but the subdorsal and subventral lip sectors are the larger ones in *H. schachtii.* The histological anatomy of *Meloidogyne hapla* was described by Elsea, 1951, who emphasized the various organ systems rather than the cephalic region. The lip region

[1] The two figures are the number of annules the cephalids appear on each side of the nematode when the specimen is viewed in optical longitudinal section in lateral or median position. Different numbers within one specimen indicate the oblique position of the rings.

[2] 30 specimens were examined in each case.

[3] These species have been studied only from preserved specimens. I am indebted for material to Mr. G. Thorne, University of Wisconsin, Madison, Wisconsin; Dr. M. W. Allen, University of California, Berkeley 4, California; Dr. D. J. Raski and Dr. B. F. Lownsbery, University of California, Davis, California; Dr. M. B. Harrison, Golden Nematode Laboratory, Seaford, New York and Mr. G. Minz, Rehovot, Israel.

[*] Most of the specimens showed the cephalids in this position.

Table 1. Location of anterior and posterior cephalids in various *Heterodera* species.

Species	Anterior cephalid: Number of annules posterior to constriction[1]	Anterior cephalid: Number of specimens[2]	Posterior cephalid: Number of annules posterior to constriction[1]	Posterior cephalid: Number of specimens[2]
H. glycines males	3 – 2 2 – 2*	1 29	8 – 7 7 – 7* 7 – 6 6 – 6	2 17 7 4
larvae	3 – 2 2 – 2*	2 28	8 – 7 7 – 7* 7 – 6	1 27 2
H. schachtii[3] males	2 – 2*	30	8 – 8 8 – 7 7 – 7* 7 – 6 6 – 6	1 3 17 8 1
larvae	3 – 2 2 – 2*	4 26	8 – 7 7 – 7* 7 – 6	2 26 2
H. rostochiensis[3] males	3 – 3* 3 – 2 2 – 2	26 3 1	8 – 8 8 – 7 7 – 7* 7 – 6 6 – 6 6 – 5	1 3 19 5 1 1
larvae	3 – 3*	30	8 – 8 8 – 7 7 – 7* 7 – 6	1 6 22 1
H. cruciferae[3] males	4 – 4 4 – 3 3 – 3*	4 4 22	10 – 10 10 – 9 9 – 9* 9 – 8 8 – 8	3 5 18 3 1
larvae	3 – 3* 3 – 2	27 3	8 – 7 7 – 7* 7 – 6	3 25 2
H. trifolii larvae	3 – 3* 3 – 2	28 2	9 – 8 8 – 8* 8 – 7	2 26 2
H. cacti[3] larvae	4 – 3 3 – 3*	5 25	10 – 9 9 – 9* 9 – 8 8 – 8	1 22 6 1

of *Hoplolaimus tylenchiformis* was studied by Krueger and Linford, 1957. None of these authors mentions the existence of the anterior and posterior rings in the cephalic region.

From the present findings, it may be expected that the occurrence of both rings will not be confined to the genera *Heterodera* and *Hoplolaimus*. It is likely that further examination will reveal the presence of these structures also in other nematode genera. It seems therefore justified to name the two rings. The term "cephalid" (from the Greek κεφαλή = head) is proposed—anterior cephalid for the anterior ring and posterior cephalid for the posterior ring—for these structures in the cephalic region.

C. Hemizonid

During the study of the anterior and posterior cephalid, it was found that the hemizonid is a similar structure. The hemizonid is located in the vicinity of the excretory pore of a number of nematodes of the order Tylenchida. In

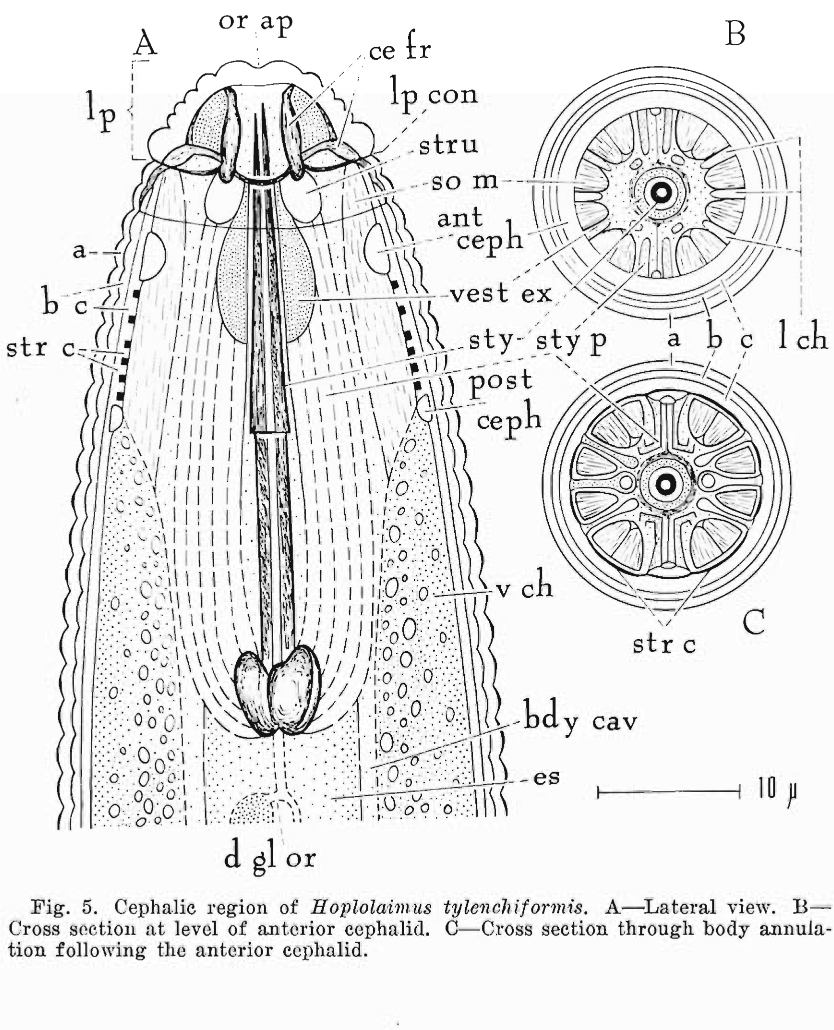


Fig. 5. Cephalic region of *Hoplolaimus tylenchiformis*. A—Lateral view. B—Cross section at level of anterior cephalid. C—Cross section through body annulation following the anterior cephalid.

longitudinal view, the hemizonids of *Heterodera* males and larvae and adults of *Hoplolaimus tylenchiformis* appear more or less biconvex and highly refractive (Figs. 6A, 7A). In contrast to the cephalids which surround the body, the hemizonid describes only half a circle on the ventral side, extending from lateral chord to lateral chord. Like the cephalids, the hemizonid is characterized by the absence of the striation of cuticular layer (c) (Figs. 6A, C; 7A, B, C).

In males of *Heterodera glycines* the hemizonid is 2 body annules in width and is located 3 to 8 annules anterior to the excretory pore (Fig. 6A). This variation in position is due, in some cases, to the hemizonid not following a

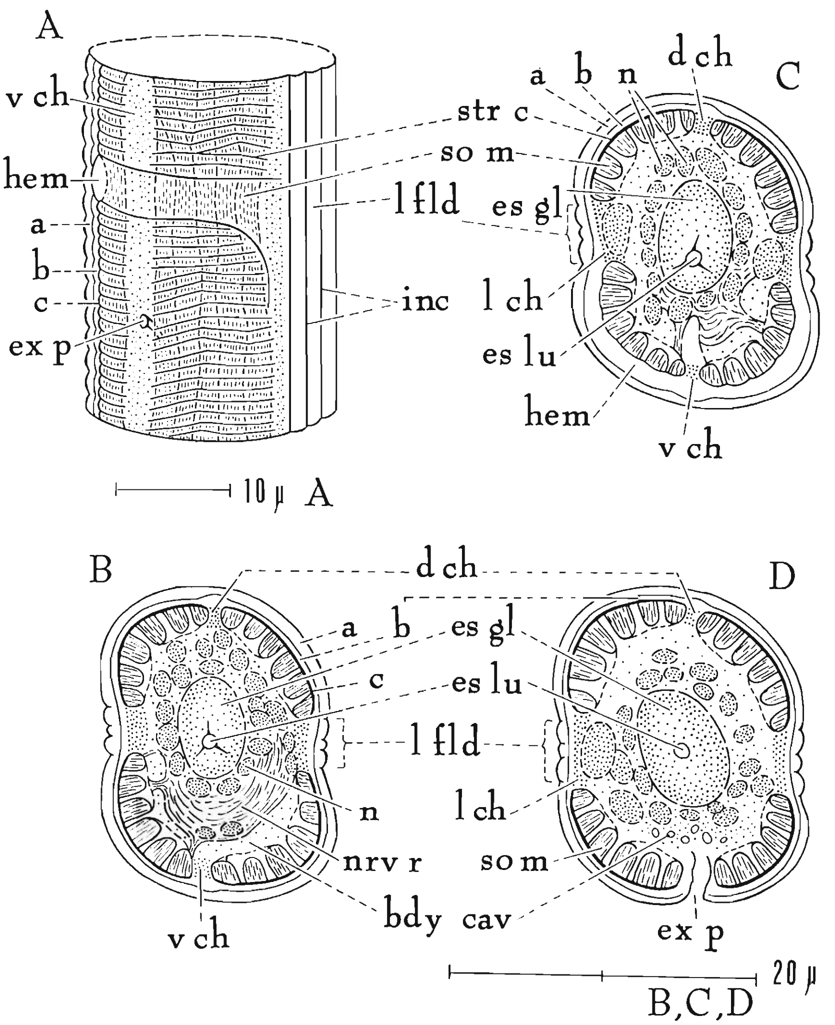


Fig. 6. Hemizonid of *Heterodera glycines* males. A—Longitudinal view. B—Cross section one annule anterior to hemizonid. C—Cross section at level of hemizonid. D—Cross section at level of excretory pore.

straight course around the body. It stops at the ventral chord and starts at a different level above or below to complete the semicircle.

In second stage larvae, the hemizonid has a rather constant position. It is situated one annule in front of the excretory pore and is the same width as this annule.

In *Hoplolaimus tylenchiformis* the hemizonid is located one body annule in front of the excretory pore and the width is equal to 2 annules.

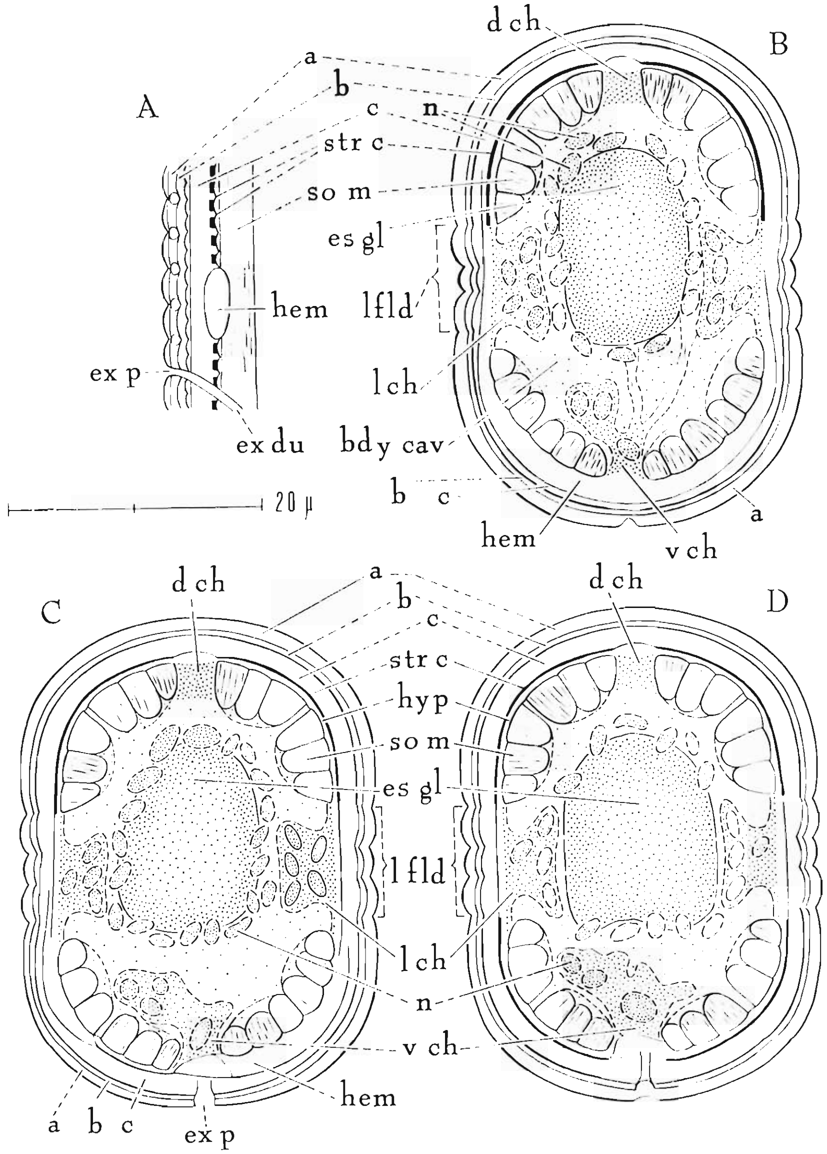


Fig. 7. Hemizonid of *Hoplolaimus tylenchiformis*. A—Longitudinal view. B, C—Cross section at level of hemizonid. D—Cross section 2 annules posterior to hemizonid.

In longitudinal sections and whole mounts of *Heterodera* males and second stage larvae, the hemizonid occupies layers (c) and (b) of the cuticle and borders layer (a) directly (Fig. 6A, C). In *Hoplolaimus*, it occupies only the striation and part of layer (c) due to the thickness of this layer (Fig. 7A, B, C).

Cross sections of both nematodes show that the muscles appear shortened where the hemizonid extends from chord to chord (Figs. 6C; 7B, C).

Since Goodey's first report on this structure, the hemizonid has been described in most Tylenchida. In most genera, its location anterior to the excretory pore is characteristic. Recently, however, Goodey reported that it was located about 20 annules posterior to the excretory pore in *Hoplolaimus proporicus*. This wide separation suggests that it is unlikely that there is any functional connection between excretory pore and hemizonid. Despite being a well defined, slightly staining structure, the biological function of the hemizonid is not yet understood. In studying the literature and from personal observations, there is a tendency for the nerve ring fibers to drift toward the ventral side into the vicinity of the hemizonid and to disappear there.

Since the hemizonid and cephalids appear to have a similar structure, one might postulate:

(a) They are associated with some sensory perception. In the case of the cephalids, stimuli are conducted alongside the chords; in the case of the hemizonid, stimuli pass through the nerve fibers to the ganglia in the nerve ring (central nervous system).

(b) The structures merely arise from the attachment of organs (chords—cephalids; nerve ring—hemizonid) to the body wall.

SUMMARY

The external cuticle of males of *Heterodera glycines* and adults of *Hoplolaimus tylenchiformis* has the same fundamental structure. In the mid region of the body it consists of three distinct layers (a, b, and c) which are likely to correspond to the cortical, matrix and fiber layers demonstrated in other nematodes. When both nematode species are treated with 5% sodium hypochlorite all layers of the cuticle except the outermost part of layer (a) are dissolved. This part also disintegrates when the specimens are pretreated with heat suggesting that it constitutes a thermolabile membrane. The innermost layer (c) shows a transverse striation, two annules of which correspond to one body annule. This striation starts at the lip constriction and ends in the caudal region. It is absent where the chords proceed the length of the body. It is also missing where cephalids and hemizonid encircle the body.

The cephalids are highly refractive structures, biconvex in longitudinal section and extend around the nematode body in the cephalic region. They are a common feature in males and second stage larvae of *Heterodera* species and have been detected also in adults of *Hoplolaimus tylenchiformis*. Their characteristic location behind the lip constriction in *Heterodera* males and second stage larvae may be of taxonomic value in certain cases when used in conjunction with other characters. Upon closer examination it was found that the hypodermal chords begin where the cephalids encircle the body: The 2 lateral chords start at the anterior cephalid, the two median chords at the posterior cephalid. No evidence was obtained as to the histological nature of the cephalids.

The hemizonid which is common in the order Tylenchida was found to be similar in structure as the cephalids. In longitudinal section it appears more

or less biconvex, highly refractive and is characterized by the absence of the striation of cuticular layer (c). The only difference from the cephalids is that it describes half a circle on the ventral side of the nematode body extending from lateral chord to lateral chord.

Although they are well defined structures, the biological functions of the cephalids and hemizonid are not yet understood. It might be postulated that they either take part in some sensory perception or arise merely from the attachment of organs to the body wall.

In addition to investigations on the cuticular layering, cephalids and hemizonid of males and second stage larvae of *Heterodera glycines* and adults of *Hoplolaimus tylenchiformis,* the lip region, somatic musculature, stylet musculature and amphids of the anterior region of males of *H. glycines* are described.

LITERATURE CITED

ALLEN, M. W. 1952. Observations on the genus *Meloidogyne* Goeldi, 1887. Proc. Helm. Soc. Wash. 19: 44-51.

ANDRÁSSY, I. 1958. *Hoplolaimus tylenchiformis* Daday, 1905 (syn. *H. coronatus* Cobb, 1923) und die Gattungen der Unterfamilie Hoplolaiminae Filipjev, 1936. Nematologica 3: 44-56.

BIRD, A. F. 1958. The adult female cuticle and egg sac of the genus *Meloidogyne* Goeldi, 1887. Nematologica 3: 205-212.

BUHRER, E. M. 1949. Technique for the beheading and "en face" examination of nematodes and similar animal types. Proc. Helm. Soc. Wash. 16: 3-6.

CAIRNS, E. J., J. N. SASSER and A. L. TAYLOR. 1955. Plant Nematode Notes, Plant Nematology Workshop. (Permanent glycerine mounts according to the method of G. Thorne).

CHITWOOD, B. G. 1938. Further studies on nemic sceletoids and their significance in the chemical control of nemic pests. Proc. Helm. Soc. Wash. 5: 68-75.

————, and M. B. CHITWOOD. 1950. An Introduction to Nematology. Section 1, Anatomy. Monumental Printing Company, Baltimore, Md. Chapter III: 28-42.

CHRISTIE, J. R. and V. G. PERRY. 1951. Removing nematodes from soil. Proc. Helm. Soc. Wash. 18: 106-108.

COBB, N. A. 1926. The species of Mermis. J. Parasitology 13: 66-72.

ELSEA, J. R. 1951. The histological anatomy of the nematode *Meloidogyne hapla* (Heteroderidae). Proc. Helm. Soc. Wash. 18: 53-63.

FRANKLIN, M. T. 1939. On the structure of the cyst wall of *Heterodera schachtii* (Schmidt). J. Helminth. 17: 127-134.

GOODEY, J. B. 1951. The "hemizonid," a hitherto unrecorded structure in members of the Tylenchoidea. J. Helminth. 25: 33-36.

GOODEY, J. B. 1957. *Hoplolaimus proporicus* n. sp. (Hoplolaiminae: Tylenchida). Nematologica 2: 108-113.

GOODEY, T. 1951. Laboratory methods for work with plant and soil nematodes. Min. Agr. and Fish. London. Tech. Bull. 2: 15.

HIRSCHMANN, H. 1956. Comparative morphological studies on the soybean cyst nematode, *Heterodera glycines* and the clover cyst nematode, *H. trifolii* (Nematoda: Heteroderidae). Proc. Helm. Soc. Wash. 23: 140-151.

KRUEGER, H. J. and M. B. LINFORD. 1957. Sex differences in the cephalic region of *Hoplolaimus coronatus* (Nematoda, Tylenchida). Proc. Helm. Soc. Wash. 24: 20-23.

MULVEY, R. H. 1955. Oogenesis in several free-living and plant-parasitic nematodes. Can. J. Zool. 33: 295-310.

NAGAKURA, K. 1930. Ueber den Bau und die Lebensgeschichte der *Heterodera radicicola* (Greeff) Müller. Jap. J. Zool. 3: 95-160.

RASKI, D. J. 1950. The life history and morphology of the sugar beet nematode, *Heterodera schachtii* Schmidt. Phytopathology 40: 135-152.

ROMEIS, B. 1948. Mikroskopische Technik. Leibniz Verlag, München. pp. 74-75, 89, 155.

THORNE, G. 1949. On the classification of the Tylenchida, new order (Nematoda, Phasmidia). Proc. Helm. Soc. Wash. 16: 37-73.

WIESER, W. 1953. On the structure of the cyst wall in four species of *Heterodera* Schmidt. Statens Växtskyddsanstalt, Meddelande 65: 3-15.

Immunization Threshold in Laboratory Rats Given a Small Initial Infection of *Nippostrongylus muris**

FRANCIS J. HURLEY

The rapid active immunity developed by laboratory rats to the nematode *Nippostrongylus muris* has been the object of many investigations. These have shown that during the second week of an initial infection the egg counts suddenly decrease; the adult worms are gradually lost from the intestine, with more rapid loss of females than of males; and a marked resistance or active immunity develops to subsequent infection manifesting itself by a lowered egg production and a retarded growth of those worms that succeed in maturing. In all these investigations large infective doses were administered. The lowest dosage thus far reported is by Graham (1934) who performed one experiment using fifty larvae. The purpose of this research was to investigate whether or not an active immunity is manifested by rats against low-grade infections and superinfections with this parasite.

MATERIALS AND METHODS

The rats used were all albino laboratory rats between seven and eight weeks of age at the time of the experiments. The infective larvae of *N. muris* were taken from moist feces-animal charcoal chamber cultures. All larvae were taken from eight to ten day old cultures, washed three times in tap water, and injected subcutaneously. The egg counts were made using a modified Stoll technique for those over 2,000 per day, and the Lane D.C.F. method for those under 2,000.

EXPERIMENTS AND RESULTS

A preliminary experiment using four rats was performed to secure comparative data on initial infections produced by four small but different sized doses of *N. muris* larvae. Rat #1 was infected with 10 larvae, rat #2 with 20 larvae, rat #3 with 40 larvae, and rat #4 with 80 larvae. The egg count curves are given in Fig. 1. On the twenty-ninth day after infection the rats were sacrificed and worm counts made. These small graded doses of *N. muris* larvae produced infections that were directly related to the number of larvae administered in terms of maximum and total numbers of eggs passed. Doses of 10 and 20 larvae resulted in egg count curves that were low and without clear indications of an acquired immunity crisis; whereas slightly higher doses of 40 and 80 larvae produced egg count curves exhibiting the classical sharp early decrease typical of acquired immunity. Rats numbers 1, 2, 3, and 4 were found to harbor on necropsy, respectively, 1, 3, 5, and 7 female worms and 4, 5, 5, and 11 males.

A second experiment using fourteen rats was performed to examine the worm burden at intervals during the course of a typical low-grade infection. All the rats were infected with 20 *N. muris* larvae and egg counts were made. Pairs of rats were sacrificed on the 7th, 9th, 12th, 16th, 20th, 24th, and 28th days of the infection. The worms were counted and a calculation was made of the number of eggs produced per adult female worm for the twenty-four hours preceding necropsy. Table 1 summarizes the data from this experiment. These data show that the egg production of the individual female worms for the twenty-four hours preceding necropsy varied with the stage of infection

*From the Department of Biology, Catholic University of America, Washington, D. C.

at which the hosts were sacrificed. The data do not indicate any expulsion of worms due to the development of acquired immunity, nor a shift in the sex ratio during the course of the infection.

A third experiment using six rats was performed to observe the effect of typical low grade initial infections upon subsequent superinfections. All but two control rats were initially infected with 20 larvae. Twenty days later two of these and one control received another dose of 20 larvae, and the course of the infection was followed for 18 more days. The remaining two infected rats and the second control were given a dose of 100 larvae 39 days after the initial infection and the course of the infection was followed for 28 more days. The egg count curves of the superinfections were characterized by a series of low peaks rather than by one definite high peak as in the initial infections. Fig. 2 is an example of this contrast as observed in rat #19. Table 2 summarizes the data from this experiment. These data indicate that the initial infections had produced an active immunity. The resistance to reinfection consisted primarily in a retardation of the maturation of the larvae with the result that the adult female worms began egg laying later than in the previously uninfected control hosts and maintained a rather steady low level

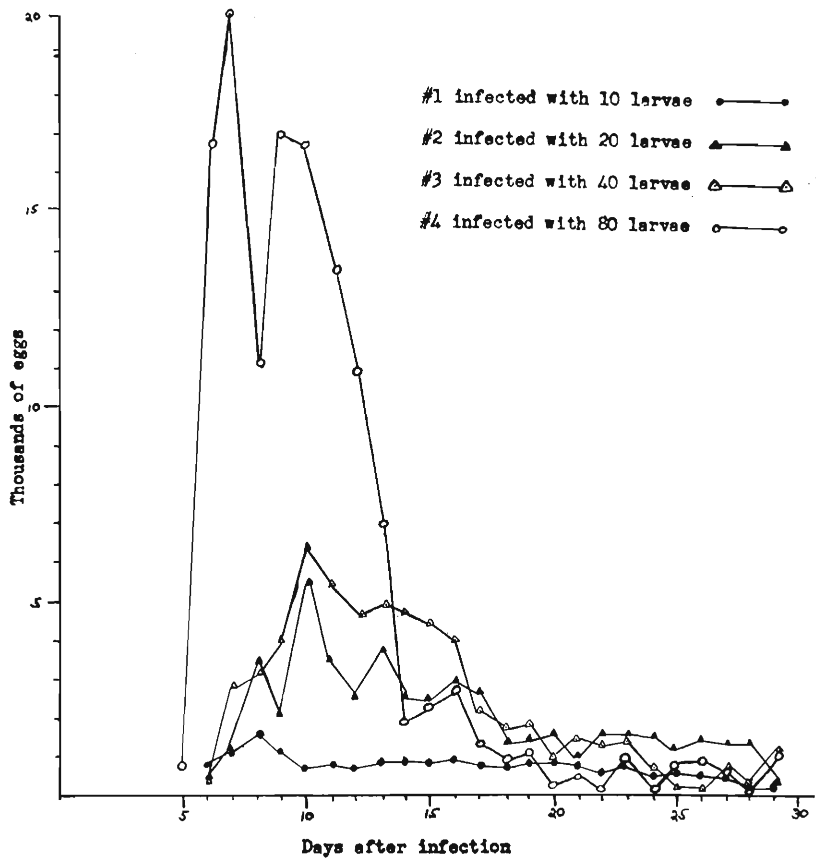


Figure 1. Comparative course of infections resulting from very small doses of *Nippostrongylus muris* larvae. Experiment 1.

Table 1. Summary of data of Experiment 2 on 14 rats each infected with 20 *N. muris* larvae and autopsied in pairs at seven different intervals after infection.

Rat No. & Sex	Duration of infection in days	Adults at autopsy Males	Females	Eggs per female on autopsy day	Average eggs per female on autopsy day
5-F	7	2	2	665	
6-M	7	1	2	215	440
7-F	9	6	5	1,200	
8-M	9	12	6	1,722	1,461
9-M	12	6	9	532	
10-F	12	1	4	1,500	1,016
11-M	16	6	4	737	
12-F	16	1	6	650	694
13-M	20	5	5	200	
14-M	20	5	8	250	225
15-F	24	4	1	800	
16-M	24	4	7	80	440
17-F	28	5	3	287	
18-F	28	1	3	283	285

of egg production. The majority of the adult worms recovered at necropsy from the test animals were pale and small in contrast to those recovered from the control animals; the only exceptions being that rat #19 harbored two normal appearing males, and rat #20 harbored two normal appearing males and two normal appearing females. These normal adults may have been from the initial infections.

A fourth experiment using five rats was performed to compare the effect of initial doses of 20 and 100 *N. muris* larvae upon a superinfection with 100 larvae, and to study the longevity of the superinfections. Two of the rats were given an initial infection with 20 larvae and two others with 100 larvae. The initial infection was followed for 38 days whereupon all 4 rats and the control were given 100 larvae and the infection followed for the next 55 days. The egg count curves of the superinfections in this experiment were similar to those of the previous experiment in that they were all characterized by a series of low peaks and a delayed maximum count, except for rat #26 which had an early maximum count. It is suspected that the rise to an early peak in rat #26 was due to the presence of adult female worms from the initial infection because the egg count at the time of superinfection was 1,000 eggs. The superinfections in the experimental rats were detectable by egg count until the termination of the experiment except in the case of rat #25 in which no eggs were detected after the 27th day of the superinfection. All of the adult worms recovered at necropsy in this experiment were pale and small in contrast to the normal appearing adults recovered from the control. Table 3 summarizes the data from this experiment. These data show that as in the previous experiment the superinfections differed quite markedly from the initial infections, indicating again that the initial infections had produced an active immunity.

DISCUSSION

In these experiments, the courses of both initial and superinfections caused by the subcutaneous injection of small numbers of *N. muris* larvae into the laboratory rat were traced and evaluated by daily fecal egg counts and worm counts at necropsy.

The prepatent period for the initial infections, no matter how small the infecting dose, was never less than five nor more than six days. This is the identical prepatent period that has always been found using higher doses of infective larvae in laboratory rats. Thus the length of the prepatent period appears to be independent of the worm burden even in minimal sized infections.

In all of the initial infections observed in these experiments the peak egg count was found to have occurred generally on the ninth day after infection. This is in keeping with the finding of Africa (1931) who gave infections with as low as 200 larvae, but it is slightly earlier than that observed by Porter (1935b) who with 300 larvae found that the peak occurred somewhere between the 12th and 14th days after infection. Chandler (1932) and Graham (1934) also found that the peak egg count occurred on about the 12th day after infection. Perhaps the reason that the present minimal doses have been found to produce earlier peaks is that the less crowded conditions in the lungs or intestine of the host allowed the parasites more opportunity for early maturity. In all of the initial infections there was a drop off in the number of eggs passed once the peak egg count had been reached. From the second experiment of this series it is evident that this falling off in the case of these rats given minimal doses of larvae is not due to a drop in the number of worms present in the rat intestine, as occurs in infections with high doses, but to a drop off in the number of eggs produced by each adult female worm. Whether this decrease in egg production is physiological or due to an immunity factor on the part of the host is not clear.

The fact that laboratory rats infected with an initial dose of *N. muris* larvae develop an acquired resistance to reinfection or superinfection has been well established by several investigators: Africa (1931); Schwartz, Alicata, and Lucker (1931); Chandler (1932); Graham (1934); Spindler (1933); and Porter (1935a,b). The third experiment of the present study revealed that this acquired resistance can also be demonstrated by using as few as 20 larvae for the initial dose and 20 or 100 larvae for the superinfective doses. The fourth experiment indicates that such a minimal initial dose of 20 larvae produces an immunity that compares equally well with the immunity produced with 100 larvae.

The active immunity produced in these experiments by small initial doses of infective *N. muris* larvae manifested itself against superinfection by: 1. An increased development period for the superinfection; 2. A lowered egg pro-

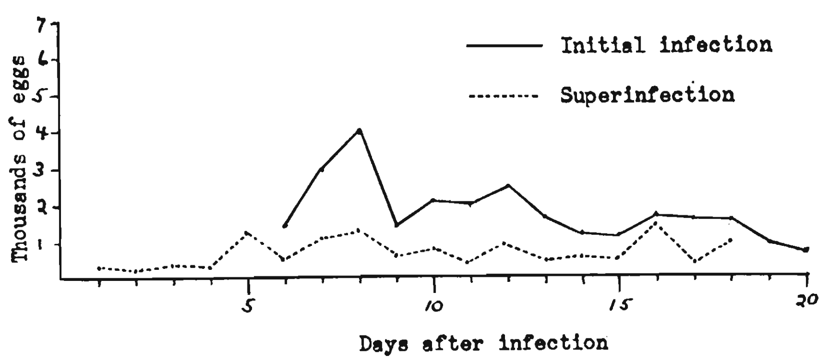


Figure 2. Effect of an initial infection with 20 *N. muris* larvae upon a superinfection of 20 larvae given on the 20th day of the initial infection. Rat # 19, Experiment 3.

Table 2. Summary of data of Experiment 3. Infections in rats initially infected with 20 *N. Muris* larvae and challenged with 20 or 100 larvae.

	Initial infection with 20 *N. muris* larvae			Superinfection with 20 *N. muris* larvae				
						Adults at autopsy (18th day after reinfec.)		
Rat No. & Sex	Day of maximum egg count	Maximum egg count	Egg count on day of superinfec. (20th day)	Maximum egg count after superinfec.	Days after superinfec. for max. egg count	Males	Females	Eggs per female on autopsy day
19-F	8th	4,000	750	1,530	16	6	3	357
20-M	10th	5,000	2,000	3,330	12	13	8	230
21-M	(Control)	-------	-------	6,000	9	8	5	378
				Superinfection with 100 *N. muris* larvae				
			(39th day)			(28th day after reinfec.)		
22-M	9th	3,000	120	2,010	28	2	4	503
23-F	9th	7,330	200	2,000	19	2	8	169
24-F	(Control)	-------	-------	24,000	9	12	6	268

Table 3. Summary of data of Experiment 4. Infections in rats initially infected with 20 or 100 *N. muris* larvae and challenged with 100 larvae.

	Initial infection with 100 *N. muris* larvae			Superinfection with 100 *N. muris* larvae				
						Adults at autopsy (55th day after superinfec.)		
Rat No. & Sex	Day of maximum egg count	Maximum egg count	Egg count on day of superinfec. (38th day)	Maximum egg count after superinfec.	Days after superinfec. for max. egg count	Males	Females	Eggs per female on autopsy day
25-F	11th	4,000	50	2,200	15	1	0	0
26-M	9th	8,330	1,000	2,850	5	1	4	105
	Initial infection with 20 *N. muris* larvae							
27-M	8th	15,000	0	2,380	24	4	1	150
28-F	8th	34,000	900	3,040	27	19	15	77
29-F	(Control)	-------	-------	27,000	8	0	0	0

duction which was maintained at a more or less constant level for a long period; and 3. A retarded growth of those worms which succeedd in maturing in the intestine.

The finding that the egg production of the worms of the small initial infections and of the superinfections was so prolonged may well explain the almost universal presence of adult *N. muris* in wild rats (*Mus norvegicus*) from dumps or other areas that provide favorable soil conditions with moist underground tunnels for the incubation of the eggs and the development of the larvae. The rapid expulsion of worms following infection with high doses of infective larvae led Africa (1931) to believe that the laboratory rat is an abnormal host for *N. muris*. It is suggested that the wild rat may be no more the normal host for this parasite than is the laboratory rat. It seems likely that the wild rat, being exposed in nature to very small initial and superinfective doses of larvae similar to those used in these present experiments, may harbor a low grade infection with the parasite even though possibly possessing some degree of immunity.

SUMMARY

Four experiments involving a total of 29 rats were performed to investigate whether or not an acquired immunity is developed in the laboratory rat by small initial subcutaneous doses of *Nippostrongylus muris* larvae. By the use of daily fecal egg counts and worm counts at autopsy, it was demonstrated that rats initially infected with as few as 20 larvae manifested evidences of acquired immunity against small superinfection doses of 20 and 100 larvae given as much as 38 days after the initial infection. These experiments indicated that there is no threshold dosage for development of acquired immunity in laboratory rats to *Nippostrongylus muris*. It was found that the adult worms resulting from these minimal initial and superinfection doses remained in the intestine and produced eggs longer than those resulting from larger doses. Similar phenomena may account for the frequent occurrence and persistence of this parasite in the wild rat, *Mus norvegicus*.

LITERATURE CITED

AFRICA, C. M. 1931. Studies on the host relations of *Nippostrongylus muris* with special reference to age resistance and acquired immunity. J. Parasitol. 18: 1-13.

CHANDLER, A. C. 1932. Experiments on resistance of rats to superinfection with the nematode, *Nippostrongylus muris*. Am. J. Hyg. 16: 750-782.

GRAHAM, G. L. 1934. Resistance studies with the nematode *Nippostrongylus muris* in laboratory rats. Am. J. Hyg. 20: 352-372.

PORTER, D. A. 1935a. Studies on the pathology of *Nippostrongylus muris* in rats and mice. J. Parasitol. 21: 226-228.

————. 1935b. A comparative study of *Nippostrongylus muris* in rats and mice. Am. J. Hyg. 22: 444-466.

SCHWARTZ, B., ALICATA, J. E., and LUCKER, J. T. 1931. Resistance of rats to superinfections with a nematode, *Nippostrongylus muris*, and an apparently similar resistance of horses to superinfection with nematodes. J. Wash. Acad. Sci. 21: 259.

SPINDLER, L. A. 1933. Relation of Vitamin A to the development of a resistance in rats to superinfections with an intestinal nematode, *Nippostrongylus muris*. J. Parasitol. 20: 72.

Experimental Infections of Cattle with the Stomach Worms, *Ostertagia ostertagi* and *Trichostrongylus axei*

Harry Herlich

Regional Animal Disease Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Auburn, Alabama

Of the three species of parasitic nematodes commonly found in the abomasum of cattle in the United States, the pathogenicity of only one, namely, the twisted stomach worm of the genus *Haemonchus*, has been extensively investigated experimentally, whereas there has been comparatively little experimental investigation into the pathogenicity of the remaining two species, namely, *Ostertagia ostertagi* and *Trichostrongylus axei*. Threlkeld (1948) found that repeated administration of infective larvae of *O. ostertagi* to calves over somewhat long intervals resulted in slight changes in some blood values, and he noted pathological lesions that varied in severity with the number of infective larvae administered as well as with the interval between their administration and necropsy of the test animal. He did not present any data pertinent to growth rate. Doran (1955) reported that an inoculum of at least 750,000 infective *T. axei* larvae was required to produce clinical symptoms of parasitism and interference with rate of growth in calves.

These results suggest that although both *O. ostertagi* and *T. axei* can be pathogenic to cattle, neither causes overt manifestation of parasitic disease unless acquired in massive numbers. Observations by Threlkeld and Bell (1952), Bailey and Herlich (1953), Andrews, Sippel, and Jones (1953), Bailey and Thorson (1954), and Dikmans and Kates (1955) have shown that enormous numbers of these nematodes may be naturally acquired by cattle. Those investigators have rather conclusively incriminated *O. ostertagi* and *T. axei* as primary or secondary causes of extensive morbidity and mortality losses in cattle.

Since *O. ostertagi* and *T. axei* so frequently occur simultaneously in cattle, the following study was conducted to observe the effects of experimentally induced mixed infections and infections with pure cultures of each species.

Procedure

Seventeen grade Jersey calves, raised free of helminth parasites other than *Strongyloides papillosus* and *Trichuris* sp., were used in four tests begun when the calves were 18 to 19 weeks old. The calves were housed in individual pens with concrete floors. All calves were fed equal rations of alfalfa hay and grain; salt and water were available *ad lib.* Each animal was weighed weekly. Feed and water were withheld for 20 hours prior to weighing, in order to preclude weight fluctuations that might have been caused by consumption of large quantities of feed and water just before weighing.

The strains of *O. ostertagi* and *T. axei* used in these tests were originally isolated from cattle and subsequently maintained at the Regional Laboratory by continuous passage through calves raised helminth-free. The number of infective larvae for inoculation was determined by dilution counts; the larvae were administered *per os* in a small quantity of water by means of a blowpipe attached to a 15-ml. rubber bulb. Single doses of larvae were administered to all infected calves.

As Doran's (1955) study showed that administration of 500,000 *T. axei* infective larvae resulted in asymptomatic infections, the following doses of larvae were used in test 1: one calf was given 500,000 *O. ostertagi* infective larvae, a second calf received a like number of *T. axei,* a third calf was given 250,000 larvae of each species, and another calf was left uninfected to serve as a control. In each of the three succeeding tests, shown in table 1, single species were established with 250,000 larvae and mixed infections with 125,000 larvae of each species.

Observations were made on the appetite, fecal consistency, and general condition of each animal three times daily. Feces were collected three times weekly and examined for helminth eggs by the DCF technic using a zinc sulfate solution as the flotation fluid. In the final two tests, blood was collected weekly for hematological determinations. These included total erythrocyte count, total and differential leukocyte counts, and packed red cell volumes; hemoglobin, serum calcium, and serum phosphorus values and total protein were determined by means of an electric colorimeter.

Except in test 1, where deaths resulted from the infections, all animals were killed six weeks after infection and the gastrointestinal tracts and associated organs were examined for evidence of pathological alterations. Nematodes were recovered by the technics described by Porter (1942) and Herlich (1956). The number of worms so recovered from each animal was estimated by dilution count; it was calculated from the average of the counts in three aliquots.

RESULTS

All of the infected animals in test 1 showed varying degrees of anorexia, and the feces passed by these calves were abnormally soft to diarrheic during the second to fourth week of infection. The calf given only *O. ostertagi* died 23 days after infection. At that time it had lost 16 pounds whereas the control had gained seven pounds. The calf infected simultaneously with the two species died 27 days after infection, at which time it had lost 21 pounds whereas the control had gained nine pounds. The *T. axei*-infected calf intermittently continued to display abnormalities in appetite and fecal consistency until it was killed 70 days after infection, when it had lost 33 pounds. The control calf gained 25 pounds in the same period. The numbers of worms recovered and percentages of inoculating dose that these represented were as follows: *O. ostertagi*-infected calf—229,840 (46); *T. axei*-infected calf—78,200 (16); *O. ostertagi*-plus-*T. axei*-infected calf—214,220 (86) and 155,780 (62), respectively.

All infected calves in tests 2, 3, and 4 (table 1) showed abnormalities in appetite and fecal consistency, generally beginning during the second week of infection, and in some cases fecal consistency ranging from slight softening to profuse diarrhea continued to be evident into the fifth week. Only the four calves infected with both species of nematodes simultaneously were unthrifty in appearance, displaying roughened hair coats, tucked-up bellies, and general emaciation. They were frequently found lying down and were extremely slow in responding to efforts to make them rise. These calves passed abnormal feces and displayed anorexia on more days than the calves with the single species infections.

At post-mortem, the abomasa of the two calves that died in test 1 showed definite evidence of gross pathology. These stomachs were extremely edematous, and the mucosa was extensively studded with nodules typical of *O. ostertagi* infections (Porter and Cauthen, 1946) and ringwormlike ulcers associated with *T. axei* (Doran, 1955). In addition, a yellowish-green fibrinonecrotic membrane covered a large portion of the fundic gland area of the abomasum of the calf infected with only *O. ostertagi.* Similar abnormali-

Table 1. Data pertaining to tests 2, 3, and 4, in which calves were infected with *Ostertagia ostertagi* or *Trichostrongylus axei* or a combination of both species.

Test No.	Calf No.	Larvae administered: Species	Larvae administered: No. (1,000)	Worms recovered post-mortem: No.	Worms recovered post-mortem: (%)*	Abnormal feces (No. days)	Anorexia (No. days)	Weight change (lbs.)	E. P. G.†: Maximum No.	E. P. G.†: No. days after infection	E. P. G.†: At necropsy (No.)
2	1437	O.o.‡	250	11,850	(5)	3	3	+20	374	30	144
	1436	T.a.§	250	53,020	(21)	16	5	+13	1,888	42	1,464
		O.o.	125	12,210	(10)						
	1428	+				27	12	− 7	2,598	36	2,451
		T.a.	125	79,320	(64)						
	1430	Control	......	......	......	0	0	+31	......	....	......
3	1452	O.o.	250	9,130	(4)	15	3	+ 7	1,362	22	228
	1447¶	T.a.	250	169,750	......	....	....	......	......	....	......
		O.o.	125	21,270	(17)						
	1449	+				21	2	− 5	690	24	274
		T.a.	125	64,380	(52)						
	1450	Control	......	......	......	0	0	+24	......	....	......
4	1509	O.o.	250	28,980	(12)	5	0	+ 6	516	37	318
	1515	T.a.	250	32,460	(13)	12	3	+ 5	612	35	579
		O.o.	125	13,740	(11)						
	1511	+				19	8	− 4	1,077	42	1,077
		T.a.	125	46,260	(37)						
		O.o.	125	16,860	(14)						
	1513	+				19	10	−11	789	37	495
		T.a.	125	51,820	(42)						
	1512	Control	......	......	......	0	0	+22	......	....	......
Averages for all three tests											
		O.o.	250	16,653	(7)	7.7	2	+11.0	......	....	......
		T.a.	250	58,815	(24)	14.0	4	+ 9.0	......	....	......
		O.o.	125	16,020	(13)						
		+				21.5	8	− 5.5	......	....	......
		T.a.	125	60,445	(48)						
		Control	......	......	......	0	0	+25.7	......	....	......

* The number of worms recovered post-mortem calculated as a percentage of the number of infective larvae administered.
† Eggs per gram of feces. ‡ *Ostertagia ostertagi.* § *Trichostrongylus axei.* ¶ Died as result of extraneous *Eimeria zurnii* infection.

ties were noted in the abomasa of the infected calves in tests 2, 3, and 4; however, the pathosis was not so extensive. In these three tests, the abomasa from calves infected concurrently with both species of nematodes were the most severely damaged, and all had a purplish discoloration.

Approximately 99 percent of the worms recovered from each calf in tests 2 to 4 (table 1) were sexually mature adults; the remainder were third- and fourth-stage larvae. The calves with the mixed infections had more worms than the calves with single species infections with the exception of calf 1447 in test 3. That animal harbored by far the greatest number of worms; however, it had accidentally acquired an infection with *Eimeria zurnii* that contributed to its death 32 days after administration of *T. axei* infective larvae. Therefore, the data for this animal are not included. When the average number of worms recovered at post-mortem is calculated as a percentage of the number of infective larvae of each species administered, the average percentage for each species was about twice as high in the calves with the mixed infections as in the calves with single species infections (table 1). In general, in both the single and mixed infections, the percentage of the *T. axei* infective larvae administered that became established and developed into adult worms was greater than that for *O. ostertagi*.

Maximum fecal egg counts ranged from 374 to 2,598 eggs per gram of feces (E.P.G.), and these were observed from 22 to 42 days after infection. Final fecal egg counts ranged from 144 to 2,451 E.P.G., with no apparent correlation between these counts and the numbers of worms recovered; *e.g.*, calves 1452 and 1449 showed almost comparable egg counts, and yet the former calf actually had only about one-ninth as many worms as the latter (table 1).

The calves infected with *T. axei* or *O. ostertagi* in tests 2 to 4 all gained weight during the six weeks of observations; however, their gains were markedly less than those of the controls (table 1). On the other hand, all four calves with the mixed infections actually lost weight. Retardation in rate of gain for all infected calves appeared with the onset of clinical symptoms of parasitism during the second week of infection. In the case of the animals with single species infections, the weights recorded two weeks after larvae were administered remained virtually unchanged during the final four weeks of the experiment, whereas the weights of the calves with the mixed infections showed this same plateau effect from the second through the fourth week of infection and then declined during the fifth and sixth weeks.

Changes occurring in the blood cell indices as well as in the levels of calcium, phosporous, and total protein in serum of the variously infected calves were not marked or consistent, did not appear to be correlated with the number of species of nematodes harbored, and were not significantly different from those observed in the uninfected controls.

DISCUSSION

The results of the foregoing experiments conclusively confirmed the pathogenicity of *O. ostertagi* and *T. axei* to cattle, and they further showed that one-half million infective larvae of the former species may be lethal to cattle, whereas a like number of the latter species was not. At sublethal levels of infection, the two species caused retardation in rate of gain of almost the same magnitude despite the fact that far more *T. axei* than *O. ostertagi* were recovered at post-mortem and in spite of the fact that the *T. axei*-infected calves pased abnormal feces and showed evidence of anorexia on more days than the *O. ostertagi*-infected calves. These results suggest that *O. ostertagi* is the more pathogenic of the two species, and indicate that some factor or factors in addition to interference with appetite may be responsible for retarded growth. Gibson (1955) reported that some factor in addition to depression in appetite operated to cause losses in weight in lambs experimentally infected with *T. axei*.

Most significantly revealed in these tests was the fact that simultaneous infections with both species were far more pathogenic to calves than pure infections with either species alone. Similar observations were made by Kates and Turner (1953 a,b) who worked with dual combinations of *Nematodirus spathiger, T. colubriformis,* and *H. contortus*. Earlier work by Fourie (1931) indicated that clinical parasitism was produced with great difficulty in lambs by administration of infective larvae of only a single species of nematode, whereas the syndrome of parasitic disease was easily reproduced by administration of a mixture of infective larvae of several species of nematodes. No explanation was offered by the aforementioned authors for the reported additive detrimental effects on the lambs. In this study, it appears that the mixed infections caused a greater pathogenic effect than the single infections because more worms became established following administration of larvae of both species than following administration of the same total number of larvae of either species alone. When the number of worms recovered was calculated as the percentage of the infective larvae administered, the average for the calves with the mixed infections was about two times greater than the average percentage for each of the respective species when given singly.

In these experiments, *T. axei* proved to be pathogenic to calves in infective doses that failed to affect calves in the study conducted by Doran (1955). That investigator and the writer used different strains of *T. axei* and different breeds of cattle, whereas the nutriments furnished were essentially the same. Concerning the strains of nematodes used, it is pertinent to note that when the writer (Herlich, in press) infected lambs with this same bovine strain of *T. axei*, the effects produced were far milder than those noted by Turner and Kates (1954) who used another bovine strain of *T. axei* in lambs. In those two studies the breed of the host animal also differed. In view of the fact that the *T. axei* strain employed by this writer was more pathogenic to calves than the strain used by Doran (1955) but less detrimental to sheep than that used by Turner and Kates (1954), one is inclined to conclude that the various breeds of cattle and sheep differ in their ability to resist the effects of different strains of this nematode. Stewart *et al.* (1937) presented evidence of a breeed difference in the resistance of sheep to *O. circumcincta*.

The absence of any consistent or significant changes in blood components in any animals in tests 3 and 4 is in agreement with the absence of any change in hemoglobin level and packed red cell volme in Doran's (1955) *T. axei*-infected calves. In contrast, however, Bailey (1955) stated that *T. axei* and *O. ostertagi* are both bloodsucking parasites and that anemia is a consistent symptom of ostertagiasis. Similarly, Threlkeld (1948) and Martin *et al.* (1957) indicated that cattle infected with *O. ostertagi* are mildly anemic. The latter authors also reported that serum proteins, notably albumin, were reduced in cattle with severe chronic diarrhea caused by *O. ostertagi*. Shumard (1957 a) found that lambs with fatal infections of *T. axei* excreted potassium, calcium, phaosphorus, and protein nitrogen in excess of the amounts consumed, and in lethal mixed infections with *H. contortus, T. colubriformis,* and *N. spathiger* (1957 b), he observed a decrease in level of serum phosphorus and total protein. The absence of similar changes in the sera of the calves in the present study suggests that such abnormalities may be associated only with fatal or chronically severe infections.

SUMMARY

In a series of experiments in which grade Jersey calves were experimentally infected with *O. ostertagi* or *T. axei* or a combination of both species, it appeared that the first-named species was the more pathogenic. The results of the tests showed that a combination of the two species was markedly more deterimental to calves than either species alone. The additive deleterious effects apparently were due to the fact that the mixed infections seemed to enhance the ability of a greater percentage of the infective larvae of both species to become established and subsequently to develop into adult worms in the host.

LITERATURE CITED

ANDREWS, J. S., SIPPEL, W. L., and JONES, D. J. 1953. Clinical parasitism in cattle in the Southeast. U. S. Livestock Sanitary Assn., 57th Ann. Proc. 228-238.

BAILEY, W. S. 1955. Veterinary parasitology problems—symposium on parasitism in southeastern United States. U.S.P.H. Reports 70: 976-982.

———— and HERLICH, H. 1953. An epizootic of parasitic gastritis in mature cattle. The Auburn Vet. 9: 105-110.

———— and THORSON, R. E. 1954. The chronology of an outbreak of parasitic gastroenterities of cattle. The Auburn Vet. 10: 97-101.

DIKMANS, G., and KATES, K. C. 1955. Trichostrongylosis in cattle. Proc. Helminth. Soc. Wash. 22: 42-46.

DORAN, D. J. 1955. The course of infection and pathogenic effect of *Trichostrongylus axei* in calves. Amer. J. Vet. Res. 16: 401-409.

FOURIE, P. J. J. 1931. The haematology and pathology of haemonchosis in sheep. 17th Report of Dir. Vet. Ser. & Anim. Ind. Union So. Afr. pp. 495-572.

GIBSON, T. E. 1955. Studies on *Trichostrongylus axei*. IV. Factors in the causation of pathogenic effects by *T. axei*. J. Comp. Path. & Ther. 65: 317-324.

HERLICH, H. 1956. A digestion method for post-mortem recovery of nematodes from ruminants. Proc. Helminth. Soc. Wash. 23: 102-103.

————. 1958. Experimental infections of sheep with three species of gastrointestinal nematodes of cattle. Trans. Amer. Micr. Soc. (In Press).

KATES, K. C., and TURNER, J. H. 1953a. A comparison of the pathogenicity and course of infection of two nematodes of sheep, *Nematodirus spathiger* and *Trichostrongylus colubriformis*, in pure and mixed infections. Proc. Helminth. Soc. Wash. 20: 117-124.

————, and ————. 1953b. An experiment on the pathogenic interaction of *Haemonchus contortus* and *Nematodirus spathiger* in lambs. J. Parasitol. 39 (4, Sec. 2): 35.

MARTIN, W. B., THOMAS, B. A. C., and URQUHART, G. M. 1957. Chronic diarrhoea in housed cattle due to atypical parasitic gastritis. Vet. Rec. 69: 736-739.

PORTER, D. A. 1942. Incidence of gastrointestinal nematodes of cattle in the Southeastern United States. Amer. J. Vet. Res. 3: 304-308.

————, and CAUTHEN, G. E. 1946. Experimental infection of calves with the small stomach worm, *Ostertagia ostertagi*. J. Parasitol. 32 (6, Sec. 2): 14-15.

SHUMARD, R. F. 1957a. The effect of the nematode *Trichostrongylus axei* (Cobbold, 1879) on the utilization and excretion of certain elements. J. Parasitol. 43 (5, Sec. 2): 12.

————. 1957b. Physiological and nutritional changes in lambs infected with the nematodes, *Haemonchus contortus*, *Trichostrongylus colubriformis*, and *Nematodirus spathiger*. Amer. J. Vet. Res. 18: 330-337.

STEWART, M. A., MILLER, R. F., and DOUGLAS, J. R. 1937. Resistance of sheep of different breeds to infestation by *Ostertagia circumcincta*. J. Agr. Res. 55: 923-930.

THRELKELD, W. L. 1948. Observations on the pathogenicity and viability of *Ostertagia ostertagi*. Vet. Med. 43: 446-452.

————, and BELL, W. B. 1952. Ostertagiasis in Virginia. The Auburn Vet. 8: 137-140.

TURNER, J. H., and KATES, K. C. 1954. The pathogenic effect on lambs of a bovine strain of the stomach hairworm, *Trichostrongylus axei*. J. Parasitol. 40 (5, Sec. 2): 13.

Symptom Expression of Fusarium Wilt Disease of the Gros Michel Banana in the Presence of *Radopholus similis* (Cobb, 1893) Thorne, 1949 and *Meloidogyne incognita acrita* Chitwood, 1949

CLIVE A. LOOS*

The association of nematodes and microorganisms in plant diseases is not a new concept. Sasser *et al* (1955) demonstrated the relationship of a root-knot nematode, *Meloidogyne incognita acrita* Chitwood, 1949, to black shank disease of tobacco. Reynolds and Hanson (1957) indicated that the incidence of postemergence damping-off disease of cotton, caused by the fungus *Rhizoctonia solani* Kuehn, was affected by the same nematode. Holdeman and Graham (1952) showed that the sting nematode, *Belonolaimus gracilis* Steiner, 1949, facilitated the development of Fusarium wilt both in susceptible and resistant cotton. Lucas *et al* (1955) found that *Meloidogyne incognita acrita* as well as mechanical wounding increased the symptom expression of Granville wilt of tobacco. Stewart and Schindler (1956) found that five root-knot species or the ectoparasitic nematode, *Helicotylenchus nannus* Steiner, 1945, increased the rate of carnation wilt in the presence of the bacterium, *Pseudomonas caryophylli* Burk. Crosse and Pitcher (1952) inoculated strawberry plants with a combination of one or other of the nematodes *Aphelenchoides ritzema-bosi* (Schwartz, 1911). Steiner and Buhrer, 1932, or *A. fragarie* Ritz. Bos, 1891) Christie, 1932, with the bacterium *Corynebacterium fascians* (Tilford) Dowson and caused typical symptoms of cauliflower disease on the plants.

Fusarium wilt disease, or the more commonly termed 'Panama disease' of bananas, caused by the fungus *Fusarium oxysporum* Schlecht. f. *cubense* (E. F. Sm.) Snyder et Hanson, is responsible for widespread destruction of Gros Michel variety bananas. A nematode survey of disease-ridden areas in the Bocas District of the Republic of Panama showed that the burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne, 1949, which occurs in lesions formed in the cortical tissues of the roots and rhizomes, was the most common plant-parasitic form present. Also a common occurrence, but less frequently encountered, were the root-knot species, *Meloidogyne incognita acrita* and *M. arenaria* (Neal, 1889) Chitwood, 1949, which usually form small galls at the tips of the smaller roots and occasionally large galls, ½ to ¾ inch in diameter, in the larger roots. Many ectoparasitic nematodes were also observed in soil and root washings, principal among them being *Helicotylenchus erythrinae* (Zimmerm. 1904) Golden, 1956, *H. multicinctus* (Cobb, 1893), Golden, 1936, *Hemicycliophora* and *Paratylenchus* spp. Bacteria-consuming forms of the genera *Cephalobus, Rhabditis, Panagrolaimus, Acrobeles* as well as the predatory *Mononchus* spp. were commonly encountered.

The two endoparasites, *R. similis* and *M. incognita acrita,* were selected for further study as possible agents in the spread of Panama disease because of their widespread distribution and the frequency with which they are found in the root system of Fusarium-wilted plants.

Rhizomes from which the Gros Michel banana is propagated may carry *R. similis* in reddish-brown to black lesions up to an inch or more deep into the soft cortical tissues. The roots may carry *R. similis,* root-knot and ectoparasitic nematodes.

*Plant Pathologist and Nematologist, Dept. of Plant Pathology, Changuinola Research Station, Chiriqui Land Company (a subsidiary of United Fruit Co.) Almirante, Rep. of Panama.
I am indebted to Dr. D. Mason of the Institute of Statistics of the North Carolina State College for his interpretation of the statistical analyses. To Mrs. C. A. Loos grateful thanks are offered for help and encouragement during the progress of the experiments and in writing the manuscript.

Loos (1957) and Stover (1957) suggested that root injury aided infection with *F. oxysporum* f. *cubense.* Stover demonstrated that injury to the root system by trenching in the presence of diseased material encouraged the early development of disease symptoms. Newhall (1958) endeavoured to prove a relationship between *F. oxysporum* f. *cubense,* the burrowing nematode and unidentified root-knot species collected from galls on tomato plants growing in the fields. He used three-foot diameter tanks to which he transferred rooted banana rhizomes and continued to replace casualties, for some considerable time, with more rooted plants. Those transfers and replacements would have caused damage to the roots, a condition which predisposes the root to infection with the fungus. Practices by which the plant roots could be mechanically injured were, therefore, eliminated in the experiment described in this paper.

MATERIALS AND METHODS

One hundred and forty six concrete tanks, 18 inches in diameter and 24 inches high, were placed on a cement floor in three rows of 50, 50 and 46 tanks respectively. A layer of horticultural asphalt was applied to the inside of each tank and a four-inch layer of washed stone rubble (½ to 1½ inches in diameter) was placed in the bottom to facilitate draining. The tanks were filled with light sandy-loam soil sifted through a one-quarter inch mesh screen and well mixed. The soil pH was 6.2. The soil in the tanks was fumigated with Dowfume MC2, at the rate of one pound of fumigant to four tanks. The soil was found to be fungus-sterile and nematode-free after fumigation.

The experiment was set up for the following treatments:

Treatment A. *R. similis* plus *F. oxysporum* f. *cubense.*

Treatment B. *R. similis* alone.

Treatment C. *M. incognita acrita* plus *F. oxysporum* f. *cubense.*

Treatment D. *M. incognita acrita* alone.

Treatment E. *F. oxysporum* f. *cubense* alone.

Treatment F. Check: No fungus or nematodes.

Two weeks after fumigation the tanks were planted with nematode-freed rhizomes, each under one pound in weight. This planting enabled a build-up of the desired nematode population in the appropriate tanks and also allowed time for a reversion of the fumigated soil to a more balanced soil floral status. *Radopholus* infected tissue was obtained from banana rhizomes from areas where Panama disease was not abundant. The rhizomes were well washed and then trimmed of roots to eliminate both root-knot and ectoparasitic nematodes, after which a thin layer of the rhizome was cut away to expose the typical *Radopholus* lesions. These lesions were cut out and chopped into small pieces. One-half pound of this inoculum, which contained about 30,000 nematodes and an undetermined number of eggs, was dug into the soil around the recently planted rhizomes in the 50 tanks of treatments A and B. *M. incognita acrita*-infected banana roots were washed, surface-sterilized for 5 minutes in 10 per cent Clorox (containing 0.525% sodium hypochlorite) and comminuted in a Waring Blendor. The comminuted material was diluted with water to facilitate handling and equal portions were added to the soil around the planted rhizomes in tanks of treatments C and D. At the same time four tomato seedlings were planted in those tanks to build up a root-knot population. Five weeks later the tomato plants were cut back to collar level, the banana plants in all the tanks were uprooted and the roots were incorporated back into the soil. The rhizomes, many of which carried severe infections of a bacterial rot, were discarded.

Table 1. Number of plants showing severe head rot, germination failure and the final total of replicates in each treatment.

Treatment	Initial no. of replicates	Cases of severe rot	Germination failures	Final total of replicates
A	25	3	1	21
B	25	4	2	19
C	25	4	1	20
D	25	4	1	20
E	25	1	1	23
F	21	1	2	18
Total	146	17	8	121

"Button seed" plants for the final plantings were grown from large rhizome sections or "bullheads" (Anon. 1957) off plants that had produced fruit. The bullheads were washed, trimmed of all roots, and then cut into discs containing about four inches of the rhizome and six inches of the pseudostem. These discs were placed 8 to 10 inches apart on a cement floor, covered with sawdust and kept damp. The "buttons" developed into vigorously growing plants after about two weeks. When these plants were about 2 feet high and had formed a rhizome about 1½ lbs. in weight they were ready for use as "seed." The plant was cut away from the disc, the rhizome trimmed of roots and discolored tissue and the pseudostem cut back to within 2 to 3 inches of the rhizome. The trimming caused a shellac-like exudation which set hard and formed a protective covering around the trimmed tissues. These nematode-freed seed were air-dried for 24 hours before they were planted in the tanks. The tanks were weeded every third day throughout the course of the experiment to avoid root disturbance of the growing banana plant.

After seven weeks, a period sufficiently long for a build-up of nematode populations and their resultant damage to the roots, the tanks in treatments A, C and E were inoculated with a culture of *F. oxysporum* f. *cubense*. The 8 week old culture was grown in sawdust-cornmeal (5%). One hundred and eighty-five grams of inoculum, each gram containing about ten million spores, was added to the soil surface and the tank was watered heavily to encourage seeping in of the spores. When the excess water drained, one-half inch of sterilized soil was added to the top of the tank. A second inoculation was made four weeks later using one pound of a 4 week old sand-cornmeal (5%) culture. In this case, too, the inoculum was spread on the soil surface and the tank watered heavily. This second inoculation brought the total spore application to about 14 million per square inch of soil.

RESULTS

Tanks in which rhizomes failed to sprout or where rhizome rot was so severe as to cause undesirable complications in the expression of results were not included in the experiment. However, 19 cases of very light rhizome rot were included. The number of replicates in each treatment at the commencement of the experiment, cases of severe rhizome rot, and the final total of replicates are summarized in Table 1.

Twenty-three plants were washed from the tanks when they were about 14 weeks old (7 weeks after inoculation with the *Fusarium* culture). All of five plants examined in the A treatment (*R. similis* plus *Fusarium*) were severely wilt diseased; two were diseased and two healthy in the C treatment (*Meloidogyne* plus *Fusarium*); four were diseased and two healthy in the E treatment (*Fusarium* alone).

TABLE 2. Results of treatments. Plants about 19 weeks old and 12 weeks after first and 8 weeks after the second, inoculation with *Fusarium* wilt fungus.

Treatments	No. of replications	No. of *Fusarium* wilted plants	No. of plants free of *Fusarium* wilt
A. *Radopholus similis* plus fungus	16	16	0
B. *Radopholus* alone	15	0	15
C. *Meloidogyne incognita acrita* plus fungus	16	16	0
D. *Meloidogyne* alone	19	0	16
E. Fungus alone	19	18	1
F. Check. No fungus or nematodes	13	0	13

Table 2 shows the results of the final examination when the plants were about 19 weeks old. Treatment A, C and E had received two inoculations with the *Fusarium* fungus. At the high level of inoculum of 14 million spores per square inch of soil all plants except one in the series to which fungus inoculum was added, were wilt diseased. None of the plants in the series B, D and F which did not receive the *Fusarium* inoculum became diseased.

TREATMENT A., *R. similis plus F. oxysporum f. cubense*: A marked feature was the rapidity of infection and the severity of disease expression. Four of the 16 plants became visually diseased only six weeks after inoculation with the fungus. Two weeks later all the plants were severely diseased. The pseudostem split longitudinally from just above the collar, the split extending 6 to 14 inches up the plant (Fig. 1-B). The lower leaves yellowed prematurely and the typical Panama disease fiber discoloration, seen in cross section of the leaf sheath, was a marked feature. The characteristic brownish-red discolorations of the vascular tissues of the rhizome were very prominent (Fig. 1-D).

Fig. 1. A. Gros Michel banana plant, 14 weeks old and 7 weeks after inoculation with *Fusarium oxysporum* f. *cubense* culture. Although the rhizome was diseased there were no wilt symptoms on the aerial portion of the plant. B. Same age as A. Inoculated with *Radopholus similis* in addition to *F. oxysporum* f. *cubense*. Note the severe splitting of the pseudo-stem which was a characteristic early symptom of wilt disease in the presence of that nematode. C. Rhizome of a check plant, 14 weeks old, split to show healthy tissue. Note profuse root formation. D. Split section of rhizome of plant shown in Fig. B. Note the wilt disease symptom shown as severe discoloration of the stelar region.

TREATMENT C., *M. incognita acrita, plus Fusarium*: The severe disease symptoms of *Fusarium* wilt, expressed as premature yellowing of the leaves and splitting of the pseudostem appeared on only a few plants. The time interval between inoculation and symptom expression was longer than in Treatment A. Five of the 16 plants developed severe visual symptoms 10 weeks after inoculation. Finally, 8 of the 16 plants became visually diseased, though at the end of the experiment all 16 carried disease symptoms in the rhizome.

TREATMENT E., *F. oxysporum f. cubense alone*: The tanks in this series carried healthy looking plants, except for three plants which developed the characteristic splitting of the pseudostem. Disease expression developed 10 weeks after inoculation. Many of the rhizomes showed only a trace of *Fusarium* infection; in many of the plants it was not possible to diagnose the wilt disease from visual appearance of the aerial growth (Fig. 1-A). Diagnosis was dependent on examination of split rhizomes. Several isolations were made from diseased material and the isolates identified as *F. oxysporum* f. *cubense*.

TREATMENTS B, D, AND CHECK F: Not one of the 47 plants not inoculated with *Fusarium* developed wilt-disease symptoms. The roots of the check plants were free of nematode infections (Fig. 1-C). There was no doubt, from the results of these examinations, that fumigation of the soil and trimming of the rhizome effectively removed fungus and nematode contaminations.

DAMAGE ATTRIBUTED TO NEMATODES

Table 3 shows the growth of plants 13 weeks old, measured from collar level to the fork at the two upper-most opened leaves. The addition of the *Fusarium* to plants already infected with a specific nematode did not affect growth, nor was there a difference between check plants and those inoculated with the fungus alone.

Table 3. Growth of Gros Michel banana plants over a 13 week period as affected by inoculations of: *Radopholus similis* plus *Fusarium oxysporum* f. *cubense* fungus (A); *R. similis* alone (B); *Meloidogyne incognita acrita* plus *F. oxysporum* f. *cubense* (C); *M. incognita acrita* alone (D); *F. oxysporum* f. *cubense* alone (E); and Checks (F).

Treatments	A	B	C	D	E	F
No. of replicates	21	19	19	21	23	18
Mean height (inches)	18.0	19.1	15.7	14.9	20.8	19.3
$S\bar{x}$	0.36	0.43	0.58	0.63	0.54	0.68
C. V. (%)	0.9	0.10	0.16	0.19	0.13	0.15

There are, however, measurable differences between the *M. incognita acrita* (C, D) and the nematode-free series (E, F). There is no doubt that the growth of these nematode-free plants was superior to that of the *Meloidogyne* infected plants (Fig. 2-A) and that such difference is real ($P<.01$). Growth differences of considerably smaller magnitude but still statistically significant were observed between *Meloidogyne* (C, D) and *Radopholus* (A, B) treatments and also in the comparison of the *Radopholus* (A, B) with the nematode-free (E, F) treatments. At the final examination of the surviving plants, after 19 weeks growth, the severely wilt-diseased plants, in treatments which had *Fusarium* inoculations, had ceased to grow. However, the significant difference between growth of *Meloidogyne* infected (treatment D) and the check plants was maintained, though visually it was not so apparent as at the observation at the 13th week. On the other hand, the *Radopholus* infected plants (treatment B) were measurably smaller and showed a significant falling off of growth in comparison with the checks. At the end of the experiment (19 weeks) the nematode infected plants were obviously smaller than the checks.

R. similis infection caused severe root destruction and mutilation (Fig. 2-B). The roots still attached to the rhizomes carried numerous lesions, many of which were up to 2 inches long and had completely girdled the root.

TABLE 4. Weights of roots and of total plants in treatment A (*Radopholus similis* plus *Fusarium oxysporum* f. *cubense*) and E (*F. oxysporum* f. *cubense* alone) at termination of experiment. Plants 19 weeks old.

	Root Weights		*Total Plant Weights*	
Treatments	A	E	A	E
No. of replicates	15	15	15	15
Range in weights (ozs.)	8-29	19-56	96-132	104-216
Mean weight (ozs.)	22.7	36.5	118.9	154.4
$S\bar{x}$	1.36	2.82	3.06	8.56
C. V. (%)	0.23	0.30	0.10	0.21

The lesions contained large numbers of *Radopholus* adults, larvae and eggs. The type of damage was typical of that described for field conditions (1957). The small roots, so abundant in the series of plants which were nematode-free, were almost completely absent or dead in the *Radopholus* infected plants. Table 4 shows the comparative root and total plant weights of treatment A (*R. similis* plus *F. oxpsporum*) and treatment E (*F. oxysporum* alone). The mean weight of the nematode-free plant roots is considerably greater than that of the *Radopholus* infected plants.

Table 5 shows the number of living roots over 3 inches long, on plants of treatment A (*Radopholus* plus *Fusarium*); B (*Meloidogyne* plus *Fusarium*) and E (*Fusarium* alone), counted when plants were 19 weeks old. The mean number of roots on plants in the *Radopholus* series is about one-third of that in the *Meloidogyne* infected plants and about one-fifth that of the nematode-free series.

Table 5. Number of living roots present on plants of treatments A (*Radopholus similis* plus *Fusarium oxysporum* f. *cubense*), C (*Meloidogyne incognita acrita* plus *F. oxysporum* f. *cubense*) and E (*F. oxysporium* f. *cubense* alone). Plants examined when 19 weeks old.

Treatment	A	C	E
No. of replicates	12	13	13
Range in number of roots	12-59	59-85	87-200
Mean of root numbers	28.8	70.9	139.5
$S\bar{x}$	4.35	2.5	9.6

The number of roots present in the *Radopholus* infected plants is significantly smaller than that of the *Meloidogyne* infected plants; in turn the root system of the *Meloidogyne* infected plants is smaller than that of the nematode-free plants. The differences are highly significant ($P<.01$).

DISCUSSION

Infection of Gros Michel plant roots with either *R. similis* or *M. incognita acrita* was not a pre-requisite to wilt disease infection when the *F. oxysporum* f. *cubense* fungus inoculum was at a high level. When the plant roots were

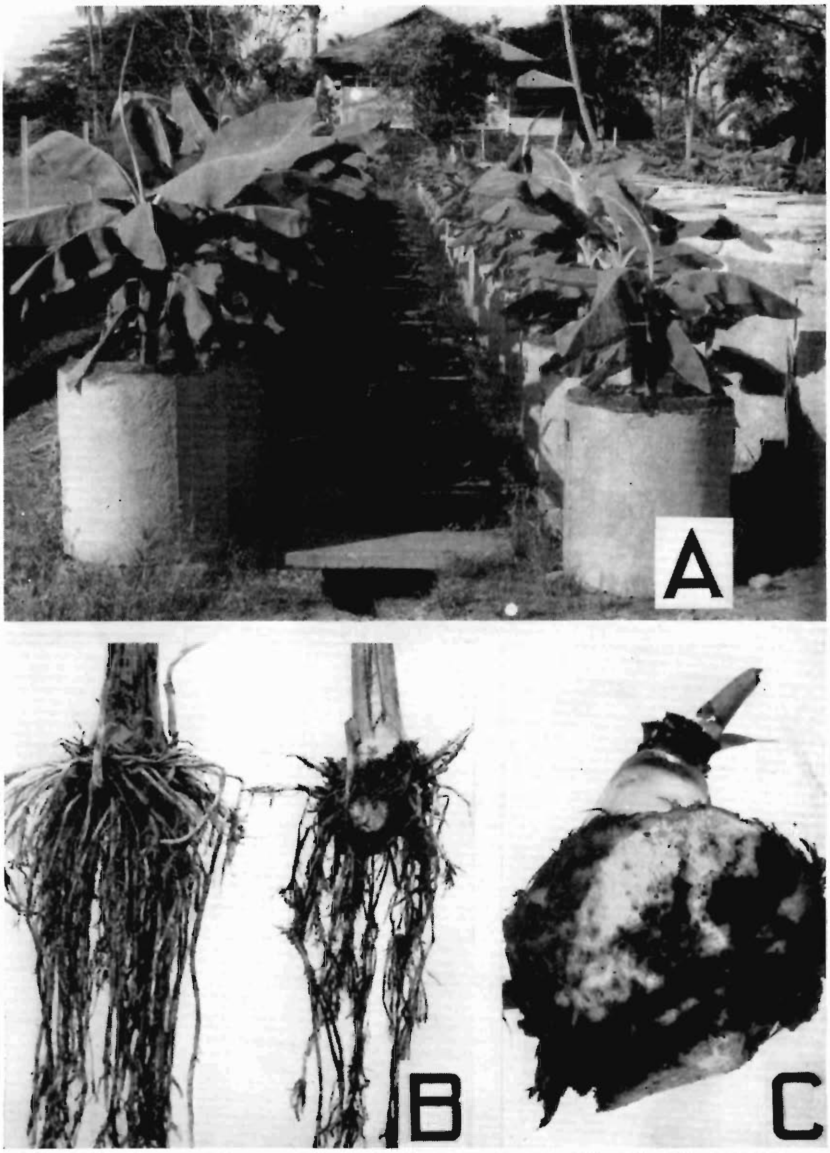


Fig. 2. A. Comparative growth of plants 11 weeks old, infected with *Meloidogyne incognita acrita* and nematode-free. Plants in the row on the right of the picture were *Meloidogyne* infected. B. Root systems of *Radopholus similis* infected (right) and Nematode-free check (left) plants. Plants 19 weeks old. C. "Button Seed" rhizome pared of outer tissue to expose *R. similis* lesions in the rhizome cortical tissues.

heavily infected with *R. similis,* the disease expression was aggravated and the period between inoculation and the appearance of marked disease symptoms was considerably shortened, though the final result of disease incidence was not affected. Unfortunately little is known of the lowest spore population in the soil capable of producing disease symptoms in banana plants. Stover (1957) considered that populations below 500 spores per gram of soil cannot be accurately determined by soil dilution plates and that the low concentrations of spores in soil makes detection on plates remote. Fourteen million spores to a square inch of soil in fairly even distribution over a confined surface is extremely heavy inoculum. Even under those conditions the difference in rapidity of onset of disease symptom expression in the presence of *R. similis* was most marked.

In these experiments, the destructive nature of *R. similis* on banana roots was manifested. The depleted root system of heavily infected plants must restrict the assimilation of plant nutrients as well as weaken the plant's anchorage. The result of this inadequate anchorage becomes abundantly evident on infested fields as plant 'uproots' or 'tip-overs.' The pull exerted by the weight of a developing fruit or the light push of a gust of wind is often sufficient to uproot the plant. On the other hand, the profuse unlesioned root system of nematode-free plants must contribute effectively to the setting of large fruit and a stand of plants which would not uproot except under extremely heavy gusts of wind.

SUMMARY

Infection of Gros Michel banana roots with either the burrowing nematode, *Radopholus similis* or the root-knot nematode *Meloidogyne incognita acrita* was not a prerequisite to wilt disease infection when spores of *Fusarium oxysporum* f. *cubense*, from culture, were added at the inoculation level of 14 million spores per square inch of soil and drenched in with water. When the plant roots were heavily infected with *R. similis*, the period between inoculation and appearance of disease symptoms was considerably shortened, though final result of disease incidence was not affected at this high inoculum level.

R. similis caused severe lesioning and destruction of banana roots. The root system was severely depleted and plant growth affected. The nematode caused large lesions which often girdled the root and extended through the cortical tissues to the stele.

LITERATURE CITED

ANON. 1957. Plant parasitic nematodes and their association with bananas. United Fruit Co. Res. Dept. Ext. News Letter 4: 8-16

ANON. 1957. Banana "Button Seed." Ibid. 4 (3): 1-8.

CROSSE, J. E. and PITCHER, R. S. 1952. Studies in the relationship of eelworms and bacteria to certain plant diseases. 1. The etiology of strawberry cauliflower disease. Ann. Appl. Biol. 39: 475-48.

HOLDEMAN, Q. L. and GRAHAM, T. W. 1952. The association of the sting nematode with some persistent cotton wilt spots in northeastern South Carolina. Phytopath: 42: 283.

LOOS, C. A. 1957. Annual Reports. Changuinola Research Station. United Fruit Company Library. Unpublished.

LUCAS, G. B., SASSER, J. N. and KELMAN, A. 1955. The relationship of root-knot nematodes to Granville wilt resistance in tobacco. Phytopath: 45: 537-540.

NEWHALL, A. G. 1958. The incidence of Panama disease of bananas in the presence of the root-knot and the burrowing nematodes (*Meloidogyne* and *Radopholus*). Plant Disease Reptr. 42: 853-856.

REYNOLDS, H. W. and HANSON, R. C. 1957. Rhizoctonia disease of cotton in presence or absence of the cotton root-knot nematode in Arizona. Phytopath. 47:256-261.

SASSER, J. N., LUCAS, G. B. and POWERS, H. R., JR. 1955. The relationship of root-knot nematodes to black-shank resistance in tobacco. Phytopath 45: 459-461.

STEWART, R. N. and SCHINDLER, A. F. 1956. The effect of some ectoparasitic and endoparasitic nematodes on the expression of bacterial wilt in carnations. Phytopath. 46: 219-222.

STOVER, R. H. 1956 . Studies on fusarium wilt of bananas. 1. The behaviour of *F. oxysporum* f. *cubense* in different soils. Canadian J. Bot. 34: 927-942.

STOVER, R. H. 1957. Progress report of studies and observations on the behaviour of *Fusarium oxysporum* f. *cubense* in bananas. United Fruit Co. Library. Unpublished.

The Histology of the Prostate Mass in the Genus *Acanthatrium* (Trematoda: Lecithodendriidae).*

THOMAS C. CHENG**

The members of the trematode genus *Acanthatrium* Faust, 1919, can be recognized by the presence of a genital atrium lined and/or bordered with spines and numerous prostate cells surrounding the atrium. The other generic characteristics were given by Cheng (1957) in the revised form. In the same paper Cheng pointed out that the main criterion for distinguishing this genus from the related genera, i.e. *Pycnoporus* Looss, 1899; *Prosthodendrium* Dollfus, 1931; *Lecithodendrium* Looss, 1899; *Paralecithodendrium* Dollfus, 1931; *Phaneropsolus* Looss, 1899; and *Parabascus* Looss, 1907, all of which are intestinal parasites of mammals, is the presence of the spinous atrium. The presence of th prostate mass also occurs in the first four related genera. This peculiar glandular mass, which is observed to lie dorsad to the genital atrium, undoubtedly serves the same function as the prostate glands which are usually found enclosed within the cirrus pouch in trematodes which possess this latter organ. The histological make-up of the prostate mass has never been reported and because of its peculiarity, warrants investigation.

Several specimens of *Acanthatrium pipistrelli* Macy, 1940 and *A. oligacanthum* Cheng, 1957, recovered from their bat hosts in Albemarle County, Virginia, were fixed in Carnoy's (6:1:1), sectioned at 10 microns in thickness, and stained with Mallory's Triple.

HISTOLOGICAL OBSERVATIONS

Observations on sections cut through the level of the genital atrium and prostate mass in both species revealed a similar arrangement of the internal structures but with some variations. The interior of the atrial wall is lined with spines. In *A. pipistrelli* this wall, measuring 0.008-0.011 mm. in thickness, is of a homogeneous, non-cellular nature and stains the same color as the atrial spines. In *A. oligacanthum* the wall is thinner and less obvious.

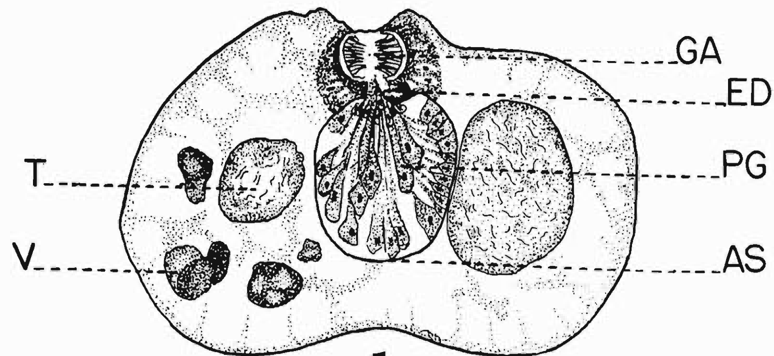
*Contribution from Mountain Lake Biological Station, University of Virginia and the Department of Histology and Embryology, Dental School, University of Maryland. The research was made possible (in part) through the National Science Foundation Award (Grant-2086), Summer, 1958.

**Present address Department of Histology & Embryology, Dental School, University of Maryland, Baltimore, Maryland.

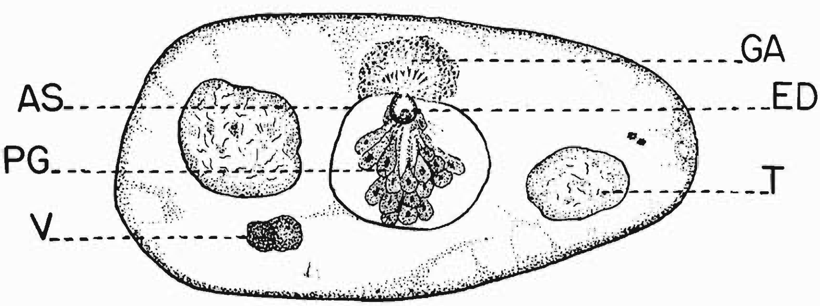
The spines, which are directed inward toward the atrial space in both species, appear as projections of the atrial wall rather than as independent structures imbedded in the wall. Surrounding the atrium is a compact layer of simple single-celled glands, 0.05-0.08 mm. in length, which empty into the atrium. These glands might be termed "auxillary prostate glands" since they presumably serve the same function as the glands of the main body of the prostate mass.

Filling the intra-glandular spaces, surrounding the atrium, are strands of connective fibers and muscle cells and which demonstrate the staining characteristics of such as described by Gatenby and Beams (1950).

Lying immediately dorsad to the atrium is the ejaculatory duct which is the distal portion of the vas deferens in *Acanthatrium* since in the members of



1



2

Figure 1. Cross section of *Acanthatrium eptesici* through the level of the genital atriu mand prostate mass. Camera lucida drawing. GA. Genital atrium; ED. Ejaculatory duct; PG. Prostate glands; AS. Prostate sac; T. Testis; V. Vitelline gland.

Figure 2. Cross section of *A. oligacanthum* through the level of the genital atrium and prostate mass. Camera lucida drawing. GA. Genital atrium; ED. Ejaculatory duct; T. Testis; AS. Prostate sac; PG. Prostate glands; V. Vitelline gland.

this genus no cirrus and/or cirrus pouch are present. This duct empties into the atrium dorsally. The diameter of the duct measures 0.022 mm. in *A. pipistrelli* and 0.016 mm. in *A. oligacanthum*.

In *A. pipistrelli*, lying immediately dorsad to the atrium, is a large saccular body, the prostate mass, which measures about 0.35 mm. in depth and about 0.25 mm. in width. The glandular mass, enclosed within an extremely thin hyaline membrane, consists of simple secretory cells which are lancet-shaped and measure about 0.05 mm. in width at the broadest point and 0.17-0.34 mm. in length. The nuclei of these secretory cells are distinct and lie in the wider extremity of the cells, which is that end distad from the point of secretion. The tubular extremities of the cells empty separately into the genital atrium through an opening on the dorsal atrial wall adjacent to the ejaculatory duct opening.

In *A. oligacanthum* the prostate mass, enveloped in a similar hyaline membrane, is approximately 1 mm. wide and 0.08-0.145 mm. deep, and the prostate cells are about 0.09 mm. wide and 0.37-0.50 mm. long. These empty individually into the ejaculatory duct before the latter enters the genital atrium. The area occupied by the secretory cells is less extensive in *A. oligacanthum* where a definite space between the cells and the membranous wall is apparent.

In both species the uterus enters the genital atrium independently through the dorsal wall of the atrium at a point posterior to the level at which the ejaculatory duct enters.

The atrial spines, which appear to be identical in composition with the noncellular atrial wall, are actually projections of and continuous with the surface of the wall. Their staining characteristics differ from that of the cuticle. The lining of the genital atrium is not continuous with the cuticle but lies within the cuticle and opens out to the body surface through the genital pore. The diameter of the atrial space was observed to be consistent in several specimens sectioned at the same level thus suggesting the non-elastic nature of the wall.

DISCUSSION

These histological observations point out several differences in the morphology of *Acanthatrium pipistrelli* and *A. oligacanthum*. In the former, the atrial wall is thick and clearly visible, the ejaculatory duct and prostate glands empty independently into the genital atrium, and the prostate glands are larger. In *A. oligacanthum* the atrial wall is not as readily visible, the prostate glands empty into the ejaculatory duct prior to the latter's entrance into the genital atrium, the prostate glands are smaller and they occupy less of the space within the membranous wall of the mass.

From the standpoint of phylogeny, the arrangement of the male reproductive system as found in the members of *Acanthatrium* is postulated to be more primitive than that found in *Phaneropsolus* and *Parabascus* where a well developed cirrus pouch, enclosing the cirrus and prostate glands, is present. The genus *Acanthatrium* is generally accepted as a member of Lecithodendriidae Odhner, 1910, and the presence of this presumably primitive prostate mass suggests that it may be considered one of the lower forms of this family.

LITERATURE CITED

CHENG, T. C. 1957. Studies on the Genus *Acanthatrium* Faust, 1919 (Trematoda: Lecithodendriidae); with the Description of two New Species. J. Parasitol. 43: 60-65.

GATENBY, J. B. AND H. W. BEAMS. 1950. *The Microtomist's Vade-Mecum* (Bolles Lee). The Blakiston Co., Phila., Penna. 753 pp.

Experimental Strongyloidiasis in Sheep and Goats. III. An Attempt to Induce Passive Immunity in Lambs; Changes in Serum Proteins after Infection; and Effect of Immune Serum on Infective Larvae*

JAMES H. TURNER

Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland

The writer (1956, 1959b) reported that, when large numbers of infective larvae of *Strongyloides papillosus,* ordinarily lethal when given as a single dose, were administered in divided doses over a period of 20 days to lambs, resistance to infection rapidly developed. Subsequently, an experiment was designed to ascertain the mechanism of the immune response in lambs to *S. papillosus* by studying the serological as well as the clinical reactions to the course of infection in lambs previously transfused with serum from a sheep refractive to infection with this nematode. The primary purpose of this paper is to present the results obtained on passive immunity, which were previously reported by the writer in abstract (1957).

A few attempts have been made to passively immunize animals to infection with *Strongyloides* spp. Sandground (1928) was unsuccessful in several attempts to immunize dogs against *S. stercoralis* by intravenous inoculation with serum obtained from an immune dog, and concluded that the mechanism of immunity did not lie in the blood. However, Lawler (1940) obtained some degree of immunity to *S. ratti* by inoculations of plasma from hyperimmune rats into susceptible rats, and concluded that the plasma probably contained protective substances.

Some investigators employed *in vitro* tests, in which infective larvae were immersed in immune serum in order to determine the presence of antibodies. Sandground (1928) concluded that sera from dogs and cats immune to *S. stercoralis* were not the lethal agents in immunized animals, as no changes were noted in larvae exposed to such sera. Lawler (1940), however, stated that *in vitro* tests indicated that the plasma from rats immune to *S. ratti* derived some of its protective value from its ability to precipitate the metabolic products of the infective larvae. Having this work in mind, the writer carried out similar *in vitro* tests with immune sera and infective larvae of *S. papillosus* during the course of this work.

In recent years electrophoresis has been employed to study host response to parasitic infection (Stauber, 1954). These studies showed that acute and chronic parasitic infections led chiefly to decreases in the albumin component of sera and some compensatory increases in one or more of the globulin fractions. The details vary with the parasite and the host species involved. It is well known that in man and animals antibodies are usually bound to, or are identical with, serum proteins of the gamma globulin fraction, whereas only a few antibodies have been identified with the beta globulin fraction (Thorbecke and Keuning, 1956). As little information is available from electrophoretic studies of serum proteins of parasitized sheep, such studies were made on the principals and controls of this experiment on passive immunization.

---

*This paper is part of a thesis submitted to the Graduate School, University of Maryland, in partial fulfillment of the requirements for the degree of doctor of philosophy.

The writer is indebted to Mr. Grant I. Wilson for assistance in many phases of this work, to Dr. Robert Rubin for aid in blood collections and serum injections, to Dr. T. B. Weber for preparation of TPN standards and assistance in the electrophoresis determinations and interpretations, and to Dr. K. C. Kates for constructive criticisms in the preparation of this paper.

EXPERIMENTAL PROCEDURES

EXPERIMENTAL ANIMALS: Nine animals were used in these studies. Four 2-month-old Shropshire lambs, which were injected with immune serum, served as infected principals; two other lambs, which were not given immune serum, served as infected controls. Two 14-month-old sheep were used as sources of immune serum. Of these sheep, one was a Columbia-Southdown-Corriedale crossbred ram (726), which had received three percutaneous dosages of larvae totaling 225,000 at various intervals over a preceding 10 months. It had been repeatedly demonstrated that this animal was refractory to reinfection with *S. papillosus*. The parasite egg count of this donor ram had remained consistently below 100 egg per gram of feces (EPG) during the preceding 2 months. At necropsy no worms were found in the lungs or in the intestine of this immune sheep. The other donor animal was a Shropshire male (728), which had been exposed to four percutaneous dosages of larvae totaling 365,000 over a 10-month period and which had shown no rise in worm egg count upon repeated exposures to infection. The number of worm eggs per gram of feces was below 2,500 at the time the animal was used as a blood donor. The ninth animal, a 3-month-old parasite-free male Shropshire lamb (738), was used as the source of normal serum.

COLLECTION AND PREPARATION OF SERUM: To obtain sufficient quantities of serum it was necessary to exsanguinate the two donor animals. Immune serum was prepared from blood collected from these sheep approximately 2 weeks after the last administration of larvae. The blood was collected in beakers and allowed to coagulate overnight in an electric refrigerator at 2°C. The serum was removed the following morning and after centrifugation was ready for use. Normal serum from the parasite-free lamb was obtained in a similar manner.

SERUM INJECTIONS AND CHALLENGE INFECTIONS: Immune serum, normal serum, or saline was injected into the external jugular vein of parasite-free lambs, which were each subsequently exposed cutaneously to 150,000 infective larvae of *S. papillosus*. As shown in Table 1, two lambs were given injections of immune serum in doses of 0.1 and 0.5 ml. per pound of body weight, respectively, at the time of infection, and two other lambs, 2.5 and 5.0 ml. per pound of body weight, respectively, 2 days before they were exposed to infection. The total amounts of serum used were 3, 13, 65, and 125 ml., respectively. One control lamb received 5.0 ml. of normal serum, and the other 5.0 ml. of physiological saline per pound of body weight, or a total of 127.5 and 108 ml., respectively.

Hemoglobin levels, hematocrit readings, and weights of the animals were determined weekly by the same methods as those previously described by the writer (1959a). The number of eggs of *S. papillosus* were estimated daily beginning 9 days after infection (the start of normal patency) until the animals were destroyed.

PAPER ELECTROPHORESIS: Qualitative and quantitative determinations of serum proteins of the infected animals were made by means of paper electrophoresis. At weekly intervals a 3.0-ml. sample of blood was obtained from the jugular vein of each animal. The blood was allowed to clot, and the serum was removed to small vials; 0.02 ml. of 3 per cent merthiolate solution was added as a preservative to each milliliter of serum. The serum was stored at 1°-5° C. until the serum protein determinations were made.

Electrophoretic determinations of the serum proteins were made under uniform conditions by standard methods. All samples were processed in a

Durrum-type, Spinco Vertical unit, using a sodium barbital buffer of pH 8.6.

TOTAL PROTEIN NITROGEN: Total protein nitrogen in the sera was determined by the biuret colorimetric method slightly modified from that of Gornall, Bardawill, and David (1949) in order to detect variations from normal, if any, and to check the validity of the results obtained by electrophoresis. Standards were prepared with pooled sera from a homogenous group of animals, and the nitrogen was determined by the micro Kjeldahl technique. Each serum sample was diluted with 19 parts of physiological saline, and 2 ml. of the resulting solution was transferred to a cuvette. Eight ml. of biuret reagent was added, the mixture resulting in a final serum dilution of 1 per cent. Determinations were made with a Coleman Jr. spectrophotometer, using a wave length of 540 mμ. A final value was obtained by subtracting the optical density of the standard from that of the unknown sample.

IN VITRO TESTS: A number of tests were conducted on the direct effect of immune serum upon infective larvae of this parasite *in vitro*. Immune sera were obtained from the two sheep refractory to repeated exposures of *S. papillosus*, and normal serum was obtained from the uninfected lamb. The sera were tested against viable, infective larvae of *S. papillosus* as follows: several drops of each serum sample were pipetted into the chamber of a depression slide and approximately 20 larvae were added. The preparations were sealed with a glass cover slip ringed with vaseline, and then placed in petri dishes, incubated at 37° C., and examined periodically over a 48-hour period. The formation of precipitates about the larvae was considered indicative of antibody activity of the serum. Normal serum and physiological saline preparations served as controls.

RESULTS

COURSE OF INFECTION: Eggs of *S. papillosus* first appeared in the feces of the lambs on the 9th or 10th day after infection (Fig. 1). Maximum numbers of eggs per gram of feces passed by control lambs 748 and 750 were 134,520 18 days after infection and 122,880 12 days after infection, respectively. The maximum numbers of eggs per gram of feces passed by the principals were somewhat lower and, except for lamb 756, were correlated with the amount of immune serum given. With the exception of the two controls and lamb 751, two peaks were noted in the number of worm eggs passed. These occurred between the 5th and 7th week after infection. Later the number of eggs decreased until they were well below 50,000 EPG at the termination of the experiment, except for lamb 749, which showed an increase in the number of eggs at this time.

WEIGHTS: Although retardation in growth rate was apparent in the two controls (748, 750) as well as in lambs 751 and 756 (Fig. 2A), only lamb 750 experienced an actual loss of weight. Four weeks after infection this lamb lost 2 pounds, and at its death, approximately 7 weeks after infection, weighed 1½ pounds less than it did at the beginning of the experiment. The total weight gained by the animals during the experiment was directly related to the amount of immune serum injected, except for lamb 756, which gained only 2 pounds. During the same period lamb 751 gained only ½ pound as compared with the 10½ and 12½ pounds gained by lambs 749 and 754, respectively. The infected control (748) that survived the infection gained only 2½ pounds.

HEMATOLOGY: The maximum decreases in the hematocrit readings were directly related to the amount of immune serum given, except for lamb 756 (Fig. 2B). The greatest decrease in hematocrit readings (49% of normal) was recorded for each of the controls. The maximum decreases in the hematocrit readings for the principals were: 751, 45 per cent; 749, 30 per cent; 754, 15 per cent; and 756, 38 per cent. Although the percentage decrease was greater for lamb 756 than for the other principals, it was less than the percentages recorded for the controls. The decrease in the quantity of hemoglobin per 100 cc. of blood was directly correlated with the decrease in the volume of packed red cells. The greatest individual decrease in hemoglobin, 5.6 grams, occurred in lamb 748. The other control (750) had a maximum hemoglobin decrease of 3.5 grams, whereas the maximum decreases for the principals were: 751, 3.7 grams; 749, 2.8 grams; 754, 1.9 grams; and 756, 4.7 grams.

SERUM PROTEIN LEVELS: The total serum protein varied within normal limits in all animals throughout the experimental period, although minor variations occurred between individuals. Since the average total serum protein was 6.0 ± 0.5 grams per 100 ml. of serum, the quantitative changes in the various serum proteins, as determined by electrophoresis, were considered to be representative of a true picture of the albumin and globulin fractions.

ALBUMIN-GLOBULIN RATIO: The A/G ratio in all animals declined rapidly during the course of the infections (Fig. 3A). It reached its lowest level by the third week after infection, rising gradually to nearly normal by the seventh week.

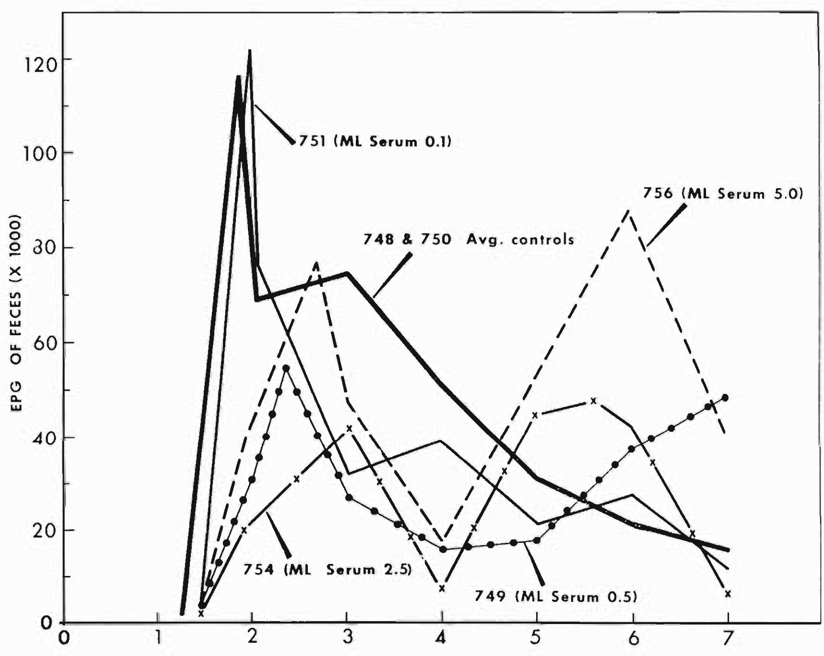


Figure 1. Number of worm eggs per gram of feces. Amount of immune serum injected per pound of body weight given opposite each lamb number (all lambs exposed to 150,000 larvae of *S. papillosus* after injections).

An increase in gamma globulin also occurred (Fig. 3B). After a sharp, initial increase the first week after infection, subsequent increases were slight. The highest level was reached the sixth week. Therefore, it may be concluded that at least one antibody was present in the gamma globulin fraction of serum of lambs infected with *S. papillosus*.

The relative quantity of the beta globulin in the infected lambs also increased (Fig. 3C). A slight increase was noted the first week following infection, and a peak was reached 2 weeks later. Thereafter, the beta globulin decreased in all infected lambs. Therefore, it also apeared that at least one antibody was present in the beta globulin fraction. There was a definite

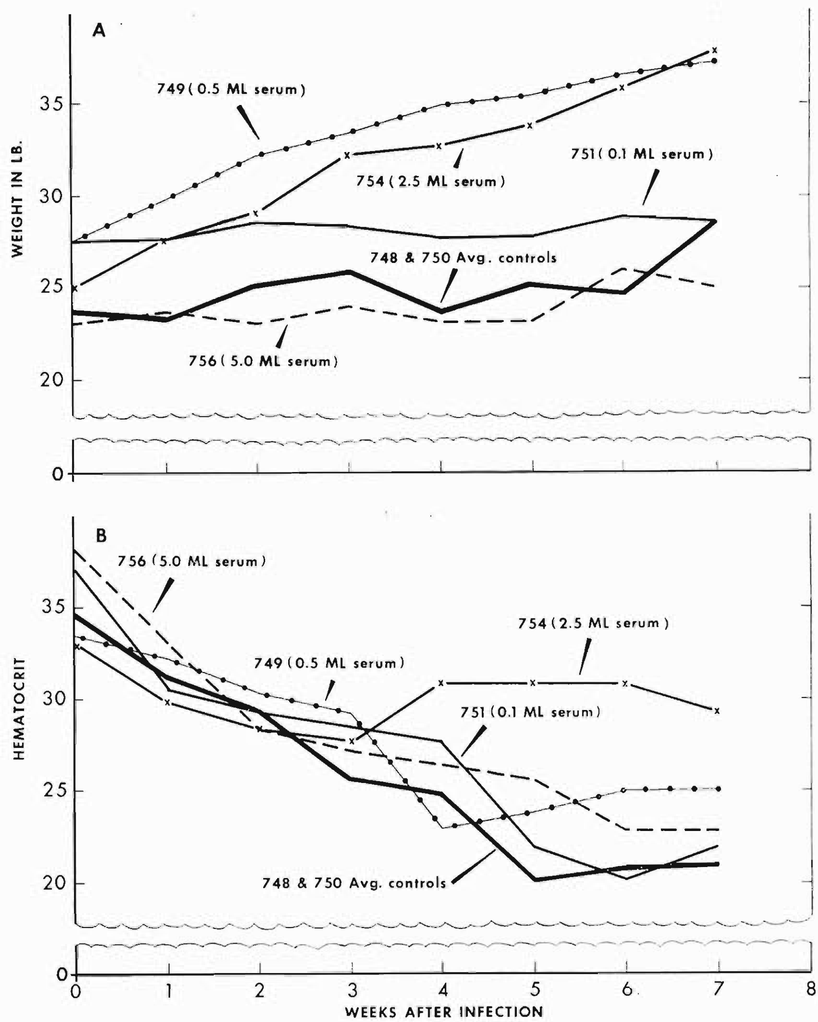


Figure 2A. Weights of infected lambs.

Figure 2B. Hematocrit readings of infected lambs.

correlation between the decrease in A/G ratio and increase in beta and gamma globulins during the second and third weeks after infection (Figs. 3A, B, and C). The A/G ratio also increased 4 to 7 weeks after infection as the beta globulin decreased. Typical electrophoretic patterns of the sera of the infected lambs at selected stages in the experiment are given in Fig. 4B and C. Fig. 4A is a typical pattern produced by serum from a worm-free sheep.

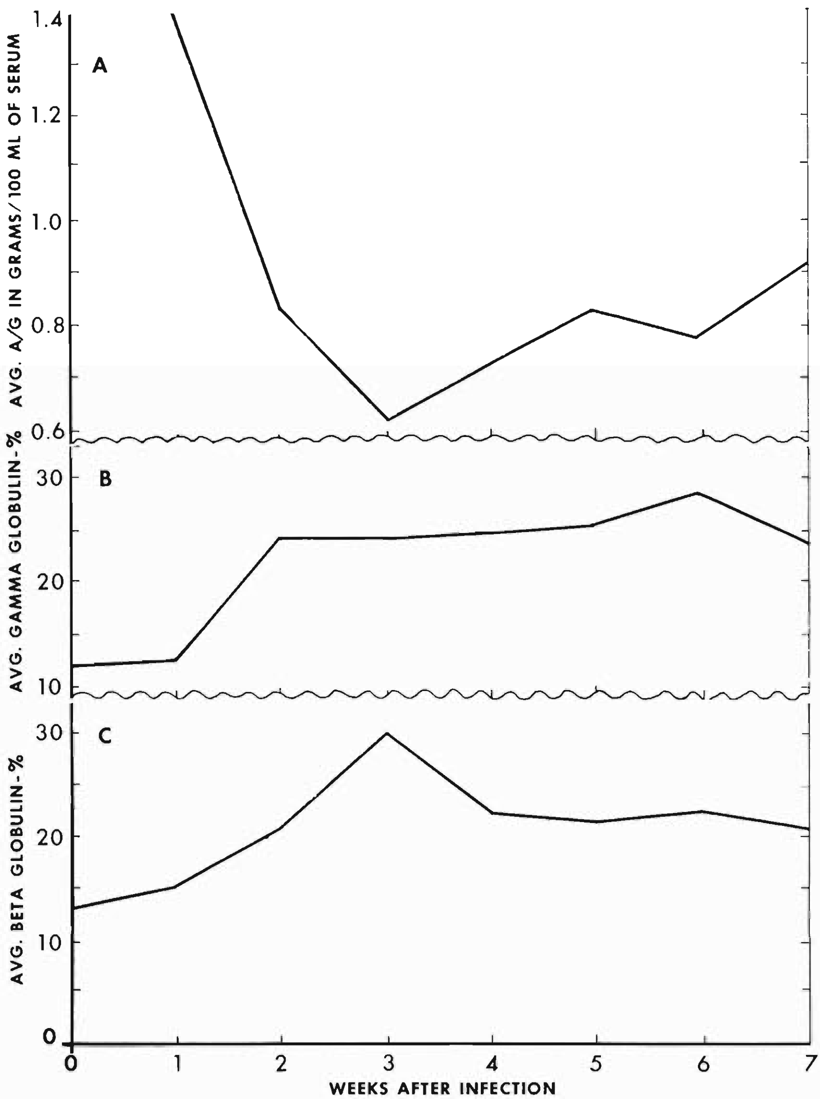


Figure 3A. Average albumin-globulin ratio of sera of infected lambs.

Figure 3B. Average per cent of gamma globulin of infected lamps.

Figure 3C. Average per cent of beta globulin of infected lambs.

Other symptoms, pathology, and parasite recovery at necropsy: Control lamb 750 died of strongyloidiasis 47 days after infection, and the other lambs were killed 7 to 10 days later in order to compare the worm burdens of all the experimental animals.

All lambs developed typical symptoms of strongyloidiasis at some time during the experimental period. Weakness and inappetence were commonly observed, and diuresis developed in the lambs about 5 weeks after infection. Stools were normal throughout the experimental period.

No gross pathology was noted in any of the animals at necropsy. Lamb 750 died during the night, and necropsy was not performed until the following morning, so any pathological lesions caused by *S. papillosus* were obscured by post-mortem changes. However, the carcass was very emaciated.

According to the data recorded in Table 1, the treated lambs were not benefited by the injection of immune serum, since more parasites were recovered from these animals than from the controls. In fact, more worms were recovered from lamb 756, which was injected with the greatest quantity of immune serum, than from the other lambs, except from lamb 751, which was given the smallest quantity of immune serum.

In vitro tests: A coarse, granular precipitate formed at the anterior end of the infective larvae as early as 4 hours after incubation in immune serum of donor sheep 726. At this time no precipitates were noted in the presence of larvae placed in immune serum of sheep 728 or in the other media. All larvae were active to some extent, but those in normal serum from lamb 738 and in saline showed greater motility than those in the immune sera. Both oral and anal precipitates formed 22 hours after incubation of larvae in the sera of immune sheep 726 and 728. All of these larvae were inactive and appeared to be dead, whereas many of the larvae in the other media were alive and motile. However, the larvae in saline showed greater motility than those in the normal serum. After 48 hours' incubation no larvae were motile, but precipitates were associated only with larvae in the immune sera.

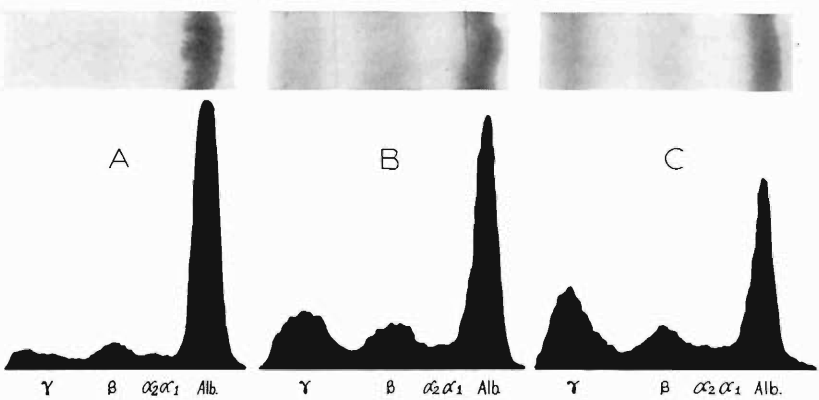


Figure 4. Typical paper electrophoretic patterns of normal serum and sera from infected lambs at two stages of the infection. Blackened portions represent the relative percentages of fractions of the serum protein deposited on the paper strips after integration. A. Lamb 754. ''Normal'', i. e., before infection. B. Lamb 754. Three to 4 weeks after infection. Marked increase in both gamma and beta globulins with concurrent decrease in A/G ratio. C. Lamb 756. Seven weeks after infection. Gamma globulin remains high, whereas beta globulin has decreased somewhat, and A/G ratio still below normal.

The A/G ratio of sera from the two immune donor sheep and the uninfected donor lamb was approximately 1.0. The gamma globulin fractions for donor sheep 726 and 728 and the uninfected control lamb 738 were 13, 30, and 17 per cent, respectively, whereas the beta globulin fractions were 28, 17, and 11 per cent, respectively. Although the sum of these two fractions was higher (47%) in sheep 728, whose serum formed a precipitate about the larvae more slowly than that from sheep 726, the latter animal had a much higher percentage of beta globulin.

DISCUSSION

The results of this limited experiment on the passive immunization of lambs against infection with *S. papillosus* by single injections of immune serum were inconclusive. The parasitological data showed that injections of immune serum produced little, if any, passive immunity against strongyloidiasis, and the serological data confirmed this. It is possible that significant passive transfer of immunity can be accomplished by serial injections of immune serum over a period of several days or weeks, and further work along this line is indicated. Jarrett *et al.* (1955) and Rubin and Weber (1955) succeeded in transferring resistance against lungworm (*Dictyocaulus viviparus*) infections in calves by the injections of immune serum, or the globulin portion thereof. These contrasting results with these two kinds of nematodes may be partially explained by the fact that adult *Dictyocaulus* live in the lungs, whereas adults of *Strongyloides* spp. live in the small intestine, and only their larval stages pass through the lungs. Furthermore, since the cattle lungworm is morphologically quite different from *Strongyloides* spp., and belongs in a different suborder, it is not surprising that these parasites differ in their immunological, host-parasite relationship.

Chandler (1947) believed that helminths which feed locally on the intestinal mucosa, such as *Strongyloides* spp., produce a localized, rapidly developing, powerful immunity, which is not passively transferable. Sandground (1928) also concluded that immunity in dogs against *S. stercoralis* was not a humoral but a local phenomenon. It appears from the results reported herein that infections of *S. papillosus* provoked a local immunity in the intestine, as some evidence of antibodies in the serum was obtained after this site was reached by the parasites. Furthermore, attempts to passively transfer the im-

Table 1. Data on lambs given injections of serum or saline prior to cutaneous exposure with larvae of *Strongyloides papillosus*.

Lamb	Age When Injected (days)	ml./lb. Body Weight of: Immune Serum	Normal Serum	Saline	No. of Larvae Administered	Days Between Exposure of Larvae and Necropsy	*S. papillosus* Recovered: Number	Per Cent of Larvae Administered
756[1]	76	5.0	—	—	150,000	52	16,160	10.7
754[1]	76	2.5	—	—	150,000	52	12,480	8.3
749[2]	65	0.5	—	—	150,000	54	11,880	7.8
751[2]	65	0.1	—	—	150,000	54	24,390	16.2
750[2]*	65	—	—	5.0	150,000	47	6,630	4.4
748[2]	65	—	5.0	—	150,000	54	9,270	6.2

[1] Given larvae 2 days after serum injections.
[2] Given larvae immediately after serum injections.
* Died; others killed.

munity by serum injections were unsuccessful under the conditions of this experiment. This does not exclude the possibility that local points of defense may occur in the skin, lungs, or other tissues, similar to those reported for *Nippostrongylus braziliensis* (*muris*) in rats by Sarles and Taliaferro (1936).

The electrophoretic studies of the serum proteins of the lambs infected with *S. papillosus* in this experiment indicated that possibly two antibodies developed in response to the presence of the parasites—one in the gamma and one in the beta globulin serum fractions. This was not an unexpected finding as Cameron (1956) stated that "parasitic organisms often possess more than one antigenic compound, and so can cause the production of several antibodies."

In the present experiment the increases in serum beta and gamma globulins of experimentally infected lambs did not occur until the parasites had reached the intestine, which indicated that an immune response to the infection was delayed until the habitat of the adult worms was attained. The appearance of these antibodies was accompanied by a decrease in the egg production of the parasites in the animals. From an experiment involving sheep infected with gastrointestinal parasites (excluding *S. papillosus*), Soulsby (1956) reported a general tendency for the antibody titer to rise after the number of worm eggs per gram had decreased. Although the "gamma antibody(s)" was present in larger quantity than the "beta antibody(s)" during the terminal weeks of the infection, it appeared that the "beta antibody(s)" was more important than the "gamma antibody(s)" in minimizing the effects of strongyloidiasis during the early patent stage of the infection. An amelioration of the infection occurred only during the peak of the "beta antibody(s)" production, *i.e.*, 2 to 3 weeks after infection. Subsequently, the clinical symptoms became more pronounced. However, toward the end of the experiment the "gamma antibody(s)" and "beta antibody(s)" possibly acted synergistically in alleviating the effects of the parasitism in some of the lambs. Whether the development of resistance in lambs to strongyloidiasis, as previously described by the writer (1959b), is due directly to a systemic antibody response or whether other mechanisms are involved remains to be determined. This study showed a definite increase in the gamma and beta serum globulins of lambs infected with *S. papillosus*, which is indirect evidence that antibodies were produced by the host's reaction to the parasites.

The precipitates formed about infective larvae which were exposed to immune sera in *in vitro* tests offered additional experimental evidence that antibodies were probably produced against infections of *S. papillosus*. The material which formed about the larvae is believed to be similar to the oral and anal precipitates which Lawler (1940) observed around larvae of *S. ratti* in the immune sera of rats. Lawler considered that these precipitates were the result of an antigen-antibody reaction and that the immune serum possessed the ability to precipitate the metabolic products of the infective larvae. According to Chandler (1953) and others, acquired immunity to *N. braziliensis* (*muris*) manifested itself in a number of ways, including stunting of growth and retardation or complete inhibition of development. Sarles (1938) demonstrated that four types of precipitates of *N. braziliensis* (*muris*) were formed when larvae were placed in serum of rats immune to infections of this worm. The precipitate designations were (1) oral, (2) cuticular, (3) excretory, and (4) intestinal. He believed that the inhibition in the development of the larvae in immune serum was due to interference in some manner with the parasite's nutrition by the precipitates. Oliver-Gonzalez

(1941) demonstrated that two antibodies formed in serum of rats infected with *Trichinella spiralis*. One antibody acted specifically on the adult worms; the other on the larvae. In immune sera, precipitates formed at the oral, anal, and vulva regions of the adult, but only at the oral region of the larvae. This investigator further demonstrated that the antiadult antibody was the active factor in the passive transfer of immunity.

Obviously, it is unwise to make generalizations concerning the precipitate-forming reaction noted in the present *in vitro* tests on larvae of *S. papillosus*. *In vitro* conditions are quite different from the normal environment of the parasite and serve only as indicators of what may occur in some phase of the host-parasite relationship. Assuming that antibodies against *S. papillosus* are present in the immune serum, it is quite possible that they are not present in sufficient quantity and/or potency to immunize animals by single injections of immune sera in moderate quantities. However, the anal and oral precipitates which appeared about these parasites in immune serum suggest the possibility that an antibody reaction against the larvae or some products thereof (the antigens) may occur in the blood of the host after infection. If this is true, the survival of the larvae in immune hosts may depend on the length of time they remain in contact with body fluids.

SUMMARY

Attempts to passively transfer immunity to strongyloidiasis to four lambs by single injections of immune serum were unsuccessful.

Concomitant quantitative determinations of total serum protein, as well as serum albumin and globulins by paper electrophoresis, indicated that at least two antibodies were probably produced in the experimental lambs in response to infections of *S. papillosus*. Beta and gamma globulins increased during the infection. The highest concentration of beta globulin appeared the third week after infection and subsequently decreased to lower levels, whereas the gamma globulin increased gradually, reaching the maximum concentration the sixth week after infection. It was postulated that the "beta antibody(s)" played a more important role in the medial stage (3 to 4 weeks) of the infection than the "gamma antibody(s)," but that both antibodies probably acted synergistically during the terminal stage of the infection. It was suggested that acquired resistance to *S. papillosus* in lambs was localized mainly in the small intestine, although antibodies developed in the blood after the parasites reached their normal location in the intestine.

Oral and anal precipitates formed about the larvae within 4 hours after contact with immune serum in *in vitro* tests. After 22 hours' exposure the larvae appeared to be dead, whereas larvae exposed to physiological saline solution and normal serum for a similar period were alive and motile.

LITERATURE CITED

CAMERON, T. W. M. 1956. Parasites and parasitism. 1st ed., Wiley and Sons, New York, 322 pp.

CHANDLER, A. C. 1947. Introduction to parasitology. 7th ed., Wiley and Sons, New York, 716 pp.

————. 1953. Immunity in parasitic diseases. J. Egyptian Med. Assoc. 36: 811-835.

GORNALL, A. G., BARDAWILL, C. J., and DAVID, M. M. 1949. Determination of serum proteins by means of the Biuret reaction. J. Biol. Chem. 177: 751-766.

JARRETT, W. F. H., JENNINGS, F. W. MCINTYRE, W. I. M., MULLIGAN, W., and URQUHART, G. M. 1955. Immunological studies on *Dictyocaulus viviparus* infection. Passive immunisation. Vet. Rec. 67: 291-296.

LAWLER, H. J. 1940. Passive transfer of immunity to the nematode, *Strongyloides ratti*. Am. J. Hyg. 31(Sect. D): 28-31.

OLIVER-GONZALEZ, J. 1941. The dual antibody basis of acquired immunity in trichinosis. J. Infect. Dis. 69: 254-270.

RUBIN, R., and WEBER, T. B. 1955. The effect of immune serum on *Dictyocaulus viviparus* in calves. Preliminary report. Proc. Helminthol. Soc. Wash. 22: 124-129.

SANDGROUND, J. H. 1928. Some studies on susceptibility, resistance, and acquired immunity to infection with *Strongyloides stercoralis* (Nematoda) in dogs and cats. Am. J. Hyg. 8: 507-538.

SARLES, M. P. 1938. The *in vitro* action of immune rat serum on the nematode, *Nippostrongylus muris*. J. Infect. Dis. 62: 337-348.

SARLES, M. P., and TALIAFERRO, W. H. 1936. The local points of defense and the passive transfer of acquired immunity to *Nippostrongylus muris* in rats. *Ibid.* 59: 207-220.

SOULSBY, E. J. L. 1956. Studies on the serological response in sheep to naturally acquired gastro-intestinal nematodes. I. Preparation of antigens and evaluation of serological techniques. J. Helminthol. 30: 129-142.

STAUBER, L. A. 1954. Application of electrophoretic techniques in the field of parasitic diseases. Exper. Parasitol. 3: 544-568.

THORBECKE, G. J., and KEUNING, F. J. 1956. Antibody and gamma globulin formation *in vitro* in hemopoietic organs. J. Infect. Dis. 98: 157-171.

TURNER, J. H. 1956. Further studies on strongyloidiasis in lambs and kids: Development of acquired resistance. J. Parasitol. 42(sect. 2): 18.

————. 1957. Further studies on ovine strongyloidiasis: An attempt to induce passive immunity. J. Parasitol. 43(sect. 2): 12.

————. 1959a. Experimental strongyloidiasis in sheep and goats. I. Single Infections. Am. J. Vet. Res., 20: 102-110.

————. 1959b. Experimental strongyloidiasis in sheep and goats. II. Multiple infections. Development of acquired resistance. J. Parasitol. 45: 76-86.

On the Misuse of the Term "Incisure"*

FIELDS E. CAVENESS and J. E. BOSHER

In descriptive terminology of the morphology of nematodes, the term "incisure" is used to designate the longitudinal cuticular clefts which divide the lateral fields. These clefts function as expansible folds in the cuticle during movement and volume increase of the nematode body due to growth or ova development. The clefts expand laterally during periods of stress and may become indistinguishable with distention of the cuticle. When the period of stress has passed, following oviposition or fat loss by starvation, for example, the body diameter contracts by the reinfolding of the clefts.

"Incisure," signifying a cut or gash, conveys the suggestion of a wound with an orifice. The root of the word is the Latin *caedere* meaning "to cut." The form and function of the lateral clefts do not lend themselves to the delimitations of "incisure."

A more appropriate word would be "involution" meaning an infolding, a return to normal after enlargement. The etymon is the Latin *involvere* meaning "to roll." The word "involution" is a more explicit term to describe the structure and function of the lateral clefts.

*Joint contribution from the Plant Pathology Department, South Dakota State College, Brookings, South Dakota, and the Plant Pathology Laboratory, Canada Department of Agriculture, Saanichton, B. C.

Journal Series No. 423 as approved for publication by the Director of the South Dakota Agricultural Experiment Station, and Contribution No. 1729 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

Studies on the Helminth Fauna of Alaska. XXXV. On the Identity of Certain Cestodes (Taeniidae) from Foxes

Robert Rausch

Arctic Health Research Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Anchorage, Alaska

Some species of the genus *Taenia* Linnaeus, 1758, are morphologically so similar in the adult stage that their separation is very difficult. Complicating this problem is the questionable validity of certain of these species which have been insufficiently characterized. There are two species, however, the adults of which possess well defined morphological differences but which have been repeatedly confused despite their having been adequately described by early investigators. These cestodes, *Taenia crassiceps* (Zeder, 1800) and *T. polyacantha* Leuckart, 1856, have for many years been recognized soleby by their larval characteristics under the misconception that the adults were indistinguishable.

It is the purpose of this paper to redefine the morphological characteristics of these two species, and to review the status of *Taenia hyperborea* von Linstow, 1905, also a parasite of foxes.

Differentiation of *T. crassiseps* and *T. palyacantha*

Although *T. crassiceps* was described by Zeder in 1800, the name frequently has been attributed to Rudolphi, who later published a synoptic description of the adult (Rudolphi, 1810; p. 172). Its larval stage—cysticerci from the thoracic cavity of *Mus arvalis* [= *Microtus arvalis* (Pallas)]—was described by Rudolphi (1819; p. 547) under the name *Cysticercus longicollis*. Leuckart 1856; p. 32) reported having established through feeding experiments the relationship of the larval and adult stages, pointing out that the close agreement in form of the rostellar hooks had convinced him previously of their identity. Leuckart (1856) also published a diagnosis of *T. crassiceps*.

T. polyacantha was adequately described by Leuckart (1856), but unfortunately, as will be seen, an error was made in the measurements of the rostellar hooks, the lengths of which were given as 53 and 34 microns for the large and small hooks, respectively. Leuckart illustrated the hooks of both *T. crassiceps* and *T. polyacantha*, demonstrating the differences in their shapes, but the large hooks of the two species were shown as being of the same length, and small hooks nearly so.

Baer (1925) studied "un flacon de Cestodes" obtained from a fox in Switzerland and identified a single species, *T. crassiceps*. Since his material did not correspond entirely with the description of *T. crassiceps* as given by Leuckart (1856), Baer undertook a redescription of the species. From his description, with the accompanying figures, it is evident that Baer's material consisted of strobilae of both *T. crassiceps* and *T. polyacantha*, but with scolices of only *T. polyacantha*. Baer's figure 1 (p. 78), "Le scolex de *T. crassiceps*," shows the typical hooks of *T. polyacantha*; his figure 2, "A" and "B," shows the details of the genital pores of both species (the differences being attributed to differences in the state of contraction of the strobilae); figure 3 (p. 79) portrays, as stated, the gravid segment of *T. crassiceps*.

Baer was aware that the number of rostellar hooks observed in his material (60 to 62) agreed with the number given by Leuckart (62) for *T. polyacantha*, but was misled by the erroneous measurements given by Leuckart for the rostellar hooks of this species. Baer (p. 78) suspected that an error had been made:

"Nous nous demandons s'il n'y a pas eu erreur, car, dans la planche II, les crochets de *T. crassiceps* et de *T. polyacantha*, dessinés à la même échelle, ont même forme et même longueur!" However, from the material that he studied, Baer concluded (p. 79), "Les deux espèces ont le même nombre de branches utérines et les dimensions des oeufs sont identiques. Nous en concluons que ces deux espèces sont identiques malgré la grande différence dans le nombre des crochets. Un example semblable nous est fourni par *T. taeniaeformis* (Batsch), où le nombre des crochets varie de 26 à 52. Nous adoptons le nom de *T. crassiceps* Rud., qui a priorité, et lui décrirons 32 à 62 crochets, dont les grands ont 186 à 207 μ de long et les petits 129 à 135 μ. L'utérus a huit à dix branches latérales, et les oeufs 25 à 28 μ de diamètre." Baer attached no significance to the differences in the shape of the hooks of the two species, as portrayed by Leuckart.

The first description of the larval *T. polyacantha* was that of Baer (1932), who made the determination on the basis of the number, form, and size of the rostellar hooks. He referred to his earlier conclusions regarding the validity of this species as follows (p. 13), "Il y a quelques années (1925a), nous proposions de réunir *T. crassiceps* Rud. à *T. polyacantha* Leuck. vu l'identité des anatomies et des hôtes, et la très grande ressemblance des crochets; cependant lorsqu'on compare les formes larvaires, on constate qu'elles sont nettement différentes." Baer and Scheidegger (1946) also studied *T. crassiceps*, remarking about the strobilar stage (p. 63), "Par la forme et la taille, ces crochets ressemblent beaucoup à ceux d'une autre espèce de Ténia du Renard,

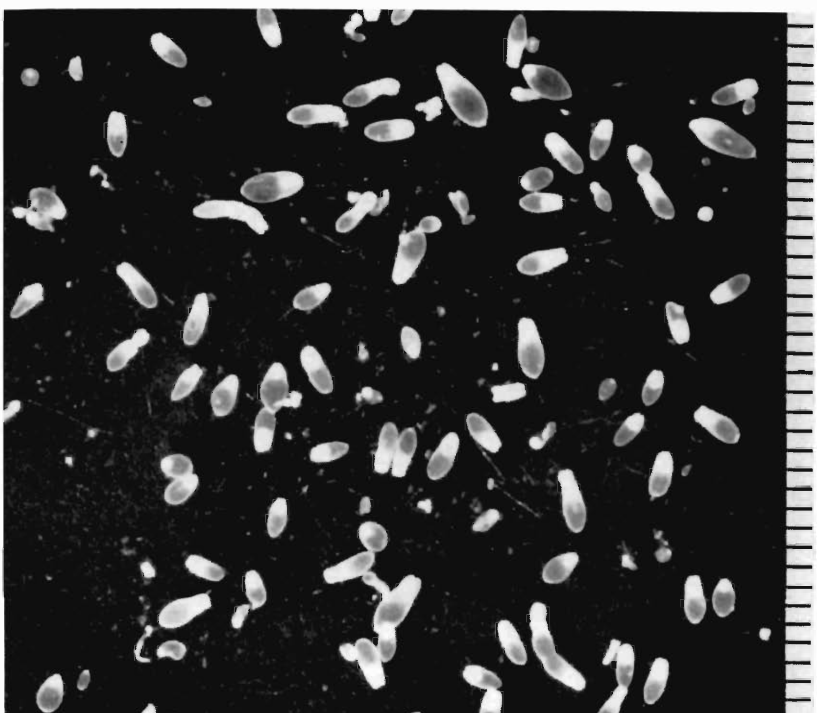


Figure 1. Larvae of *T. crassiceps* from *Lemmus*, Point Barrow, Alaska. Each space of scale has the value of 1 mm.

T. polyacantha Leuck., mais chez laquelle le nombre des crochets est presque deux fois plus élevé (60). Ces ceux espèces de Cestodes sont très voisines par leur anatomie, mais se distinguent cependant facilement par leurs formes larvaires . . ." The hooks figured by Baer and Scheidegger (p. 64, fig. 3) for *T. crassiceps* clearly include those of both species.

T. crassiceps and *T. polyacantha* have been little studied since the work of Baer and Scheidegger (1946). The former, with a description of the adult, was first recorded from North America by Rausch (1952). *T. polyacantha* had not been recorded in North America at the time, and the problem of distinguishing the two species consequently was not considered. The first North American record of *T. polyacantha*, based on larvae from *Microtus*, was that of Schiller (1953). Wardle (1952; pp. 417 and 418) listed both species under *species inquirenda*.

In the course of surveys carried on in Alaska, large numbers of cestodes of both species have been collected from foxes, and the larvae of both have been found in various species of microtine rodents. A description of *T. polyacantha*, based upon this material, is presented below. Since a description of *T. crassiceps* has been recently published (Rausch, 1952), duplication is not necessary here. The larvae of the two species are shown in figs. 1 and 2.

Taenia polyacantha Leuckart, 1856 (Figs. 3-5)

DIAGNOSIS: Strobila 140 to 235 mm long; maximum width, up to 4 mm, attained in gravid segments. Length/width ratio of segments increases with

Figure 2. Larvae of *T. polyacantha* from *Lemmus*, Point Barrow, Alaska. Each space of scale has the value of 1 mm.

age; immature segments are wider than long, mature segments are about as long as wide, and gravid segments may be two times longer than wide. Margins of strobila serrate. Scolex relatively small, measuring about 1.2 mm in diameter; well developed suckers about 450 microns in length. Rostellum armed with 44 to 50 hooks, arranged in 2 rows. Large hooks have handle and blade of nearly equal length, and measure 200 to 214 microns (av. 210 microns); small hooks, having handle only slightly developed, measure 142 to 157 microns (av. 147 microns). Genital pores irregularly alternate, situated at middle of segmental margin. Genital papillae not prominent. Subspherical cirrus sac, overlapping ventral longitudinal excretory canal, measures 140 to 215 microns long by 140 to 180 microns wide. Cirrus aspinose, measuring about 65 microns in diameter and 140 microns long when everted. Vas deferens much coiled at proximal end of cirrus sac. Testes, numbering at least 220 per segment, measure about 70 microns in diameter; they are arranged in 2 lateral fields which are confluent in anterior portion of segment. Testes not extending posterior to posterior limits of vitelline gland and not overlapping ventral longitudinal excretory canals. Vagina, opening in genital atrium posterior to cirrus sac, measures about 15 microns in diameter; it is diverted abruptly posteriad by cirrus sac, after which it takes direct course mediad and forms large seminal receptacle at mid-line of segment. Ovary consists of 2 reniform lobes, of which aporal lobe is larger. Transversely, ovary comprises about ⅓ of segment. Vitelline gland, about 8 times as wide as long, lies posterior to ovary near posterior margin of segment; its ends usually extend somewhat beyond limits of ovarian lobes. Mehlis' gland spherical, about 150 microns in diameter. Gravid uterus with 12 to 16 lateral branches on each side; these subdivide, nearly filling gravid segments within limits of ventral longitudinal excretory canals. In moderately relaxed strobilae, gravid uterus usually has well defined rectangular outline. Eggs measure 32 to 40 microns long by 30 to 35 microns wide (av. 36 by 32.5 microns).

HOSTS: *Alopex lagopus* Linnaeus (arctic fox) and *Vulpes vulpes* Linnaeus (red fox). Also occurs rarely in dogs in Alaska.

HABITAT: Small intestine of host.

DISTRIBUTION: Holarctic, but in North America known only from high-boreal regions (Alaska).

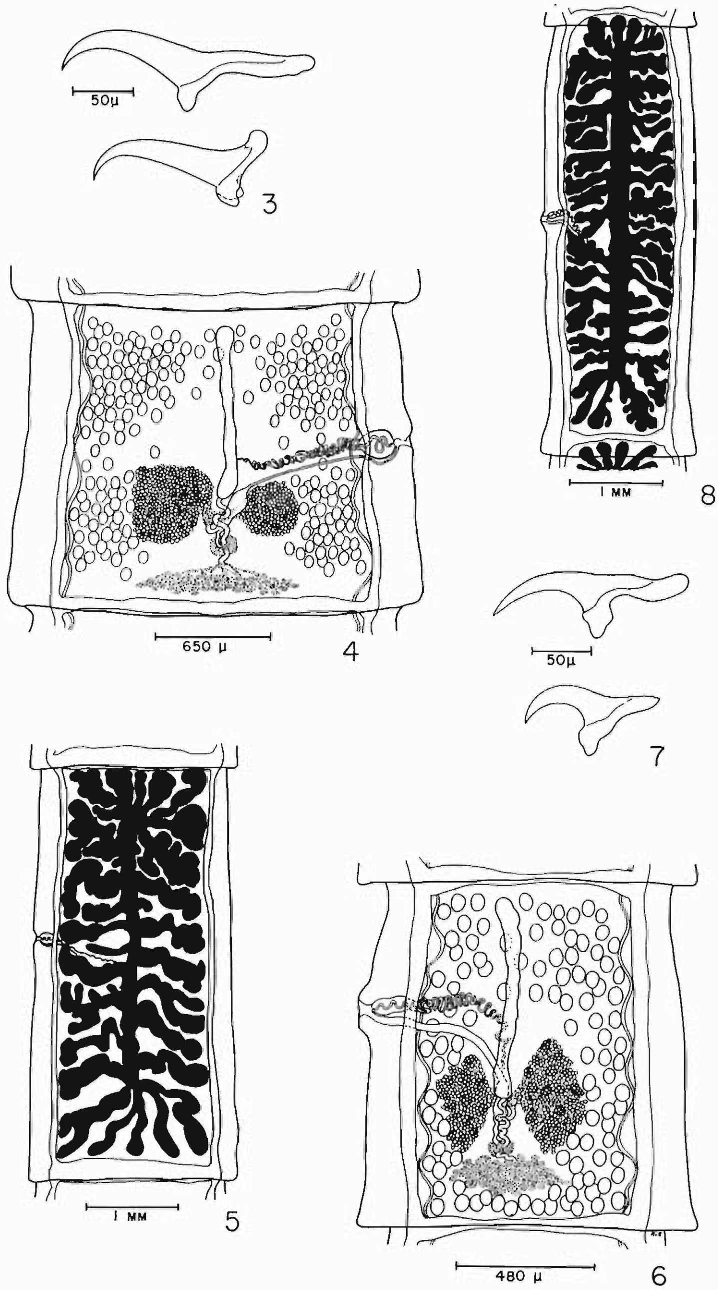
Slides containing an entire strobila of *T. polyacantha* have been deposited in the Helminthological Collection of the U. S. National Museum, No. 38398.

The specimens of *T. polyacantha* from Alaskan foxes possess a smaller number of rostellar hooks than do those from Europe (44 to 50 as compared with 60 to 62). However, I do not consider this difference sufficiently great to justify formal recognition of the Alaskan cestode at a subspecific level, particularly since the limits of normal variation in the number of rostellar hooks of *T. polyacantha* have not been determined in Eurasia, there having been no studies made on material collected east of Europe.

For comparative purposes the more important morphological details of *T. crassiceps* are shown in Figs. 6-8, and the differential characteristics of the two species are listed in Table 1.

THE IDENTITY of *Taenia hyperborea* VON LINSTOW, 1905

T. hyperborea was described from material collected from an arctic fox on the east coast of Greenland. Although von Linstow's (1905) diagnosis was adequately detailed, the status of the species has never been finally determined. The form of the rostellar hooks led Railliet and Henry (1915) to



Figures: 3. Rostellar hooks of *T. polyacantha*; 4. Mature segment of *T. polyacantha*; 5. Gravid segment of *T. polyacantha*; 6. Mature segment of *T. crassiceps*; 7. Rostellar hooks of *T. crassiceps*; 8. Gravid segment of *T. crassiceps*.

Table 1. Differential characteristics of *T. polyacantha* and *T. crassiceps*, from Alaskan material

	T. polyacantha	*T. crassiceps*
Length of strobila	140 to 235 mm	70 to 140 mm
Number of rostellar hooks	44 to 50	28 to 32
Size of rostellar hooks	Large hooks: 200 to 214 microns Small hooks: 142 to 157 microns	Large hooks: 172 to 178 microns Small hooks: 121 to 136 microns
Position of genital pore	At middle of segmental margin.	Anterior to middle of segmental margin.
Characteristics of cirrus sac	Subspherical, usually overlapping ventral longitudinal excretory canal. 140 to 215 microns long by 140 to 180 microns wide.	Elongate, usually not overlapping ventral longitudinal excretory canal. 160 to 215 microns long by 50 to 70 microns wide.
Number of uterine branches	12 to 16 on each side.	16 to 20 on each side.
Size of egg	32 to 40 microns long by 30 to 35 microns wide.	25 to 32 microns long by 22 to 27 microns wide.
Distribution of testes	Two lateral fields, confluent only in anterior half of segment.	Two lateral fields, confluent both in anterior half of segment and posterior to ovary.

conclude that *T. hyperborea* is one of the *Multiceps*-group, and most subsequent workers have accepted this opinion (cf. Meggitt, 1924, p. 89); Sprehn, 1932, p. 495). More recently, from two arctic foxes killed on the Iamal Peninsula, western Siberia, Kolmakov (1937) identified *T. hyperborea*, describing the morphological characteristics and comparing this material with von Linstow's original description.

Since both *T. crassiceps* and *T. polyacantha* have a holarctic distribution in the same species of hosts, it might be suspected that *T. hyperborea* is identical with one or the other. Now that the aforementioned species have been distinguished, it is practicable to re-examine the status of *T. hyperborea*.

It is evident from von Linstow's (1905) description and figures that *T. hyperborea* in some details resembles both *T. polyacantha* and *T. crassiceps*. However, it has several important characteristics in common with *T. crassiceps*. Comparing von Linstow's figure of the mature segment with that of *T. crassiceps*, it is seen that the testes apparently have the same distribution, being confluent across the posterior margin of the segment. However, in von Linstow's figure, the testes extend across between the ovary and vitelline gland; an error may have been made in the drawing, but it is assumed that the testes are confluent in the posterior portion of the segment. According to von Linstow, the genital pore is situated at the middle of the segmental margin, as is the case in *T. polyacantha*. This may have resulted from the contracted state of the material studied, since the relationship of the cirrus sac and vagina is the same as for *T. crassiceps*, and the shape of the cirrus sac also is typical of the latter. In von Linstow's material, the eggs measured 29 by 23 microns, corresponding in size to the eggs of *T. crassiceps*.

The rostellar hooks figured by von Linstow for *T. hyperborea* correspond in shape to those of *T. crassiceps*; they number from 30 to 32, and measure

170 and 120 microns for the large and small hooks, respectively. Kolmakov (1937) found that the hooks on his material ranged from 28 to 34 in number, and measured 172 to 188 microns and 132 to 154 microns for the large and small hooks, respectively. According to Leuckart (1856; p. 67), the specimens of *T. crassiceps* studied by him had usually 32 hooks, less commonly 34; the large hooks measured 186 microns and the small ones 135 microns. It is of interest that Leuckart (1856; p. 67) remarked that "Der Wurzelfortsatz der kleinen Haken besitzt ein sehr schmächtige Bildung, fast wie bei *T. coenurus*." This characteristic presumably led Railliet and Henry (1915) to assign *T. hyperborea* to the *Multiceps*-group. Alaskan specimens of *T. crassiceps* possess from 28 to 32 rostellar hooks, the large ones measuring 172 to 178 microns long and the small ones measuring 121 to 136 microns long.

The close agreement in morphological details between *T. crassiceps* and *T. hyperborea* seems sufficient to indicate that they are conspecific, a conclusion supported also by ecological and zoogeographic considerations. Consequently, *T. hyperborea* von Linstow, 1905, is regarded as a synonym of *T. crassiceps* (Zeder, 1800). Therefore, it appears that there are but two species of *Taenia* which commonly parasitize foxes in northern regions; these are *T. crassiceps* and *T. polyacantha*, both of which have holarctic distribution.

LITERATURE CITED

BAER, J. G. 1925. Cestodes de mammifères. Bull. Soc. Neuchâtel. Sci. Nat. 50: 77-81.

BAER, J. G. 1932. Contributions à la faune helminthologique de Suisse. (Deuxième partie.) Rev. suisse Zool. 39: 1-57.

BAER, J. G. and S. SCHEIDEGGER. 1946. Un cas intéressant de tétraplégie d'origine parasitaire. Schweiz. Zeitschr. Path. u. Bakt. 9: 61-66.

KOLMAKOV, D. V. 1937. Gel'minty pestsov Obdorskogo raiona. Rabot. Gel'mint. (Skriabin), pp. 292-295.

LEUCKART, R. 1856. Die Blasenbandwürmer und ihre Entwicklung. Zugleich ein Beitrag zur Kenntnis der Cysticercus-Leber. Giessen, 162 pp.

MEGGITT, F. J. 1924. The Cestodes of Mammals. London, 282 pp.

RAILLIET, A. and A. C. L. HENRY. 1915. Sur un cénure de la gerbille à pieds velus. Bull. Soc. Path. Exot. 8: 173-177.

RAUSCH, R. 1952. Studies on the helminth fauna of Alaska. XI. Helminth parasites of microtine rodents—taxonomic considerations. J. Parasit. 38: 415-444.

RUDOLPHI, C. A. 1810. Entozoorum sive vermium intestinalium historia naturalis Vol. 2, part 2. Amstelaedami, 386 pp.

RUDOLPHI, C. A. 1819. Entozoorum synopsis cui accedunt mantissa duplex et indices locupletissimi. Berolini, 811 pp.

SCHILLER, E. L. 1953. Studies on the helminth fauna of Alaska. XV. Some notes on the cysticercus of *Taenia polyacantha* Leuckart, 1856, from a vole (*Microtus oeconomus operarius* Nelson). J. Parasit. 39: 344-347.

SPREHN, C. 1932. Lehrbuch der Helminthologie. Eine Naturgeschichte der in deutschen Säugetieren und Vögeln schmarotzenden Würmer, unter besonderer Berücksichtigung der Helminthen des Menschen, der Haustiere und wichtigsten Nutztiere. Berlin, 998 pp.

VON LINSTOW, O. 1905. Neue Helminthen. Arch. Naturg., Berlin. 71: 267-276.

WARDLE, R. A. and J. A. MCLEOD. 1952. The Zoology of Tapeworms. Univ. of Minnesota Press, Minneapolis, 780 pp.

Immunity Against the Cattle Lungworm: Resistance Resulting from Initial Infection with Small Numbers of Larvae

Thomas B. Weber* and John T. Lucker

Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland

Michel (1954, 1955), Rubin and Lucker (1956a), and Jarrett *et al.* (1957) have shown that calves that have eliminated an initial *Dictyocaulus* infection produced by the administration of 4,000 or more normal infective *D. viviparus* larvae generally are completely immune to a patent reinfection with this lungworm. The immunity persists for several months at least and generally is strong enough to enable a calf so previously infected to survive exposure to a dose of larvae known to be lethal to previously uninfected animals of the same age.

The work here reported was undertaken to determine whether calves initially given much smaller doses of normal infective larvae would acquire enough resistance to protect them adequately against a subsequent rather heavy, but not necessarily lethal, exposure to infection. Two prior reports that contain data on immunity following initial infection with doses of fewer than 4,000 normal larvae will be discussed later in connection with our results; however, in those reports only one calf that received a dosage within the range we tested is mentioned.

Materials and Methods

Eleven young Holstein calves, reared and maintained under conditions designed to exclude extraneous infection with helminths, were used. Six of them, the test animals, were initially given small dosages of infective *D. viviparus* larvae, as shown in table 1. After they had eliminated their initial infections, they were given a challenge dose of larvae, as shown in table 2. The seventh calf (No. 49) was used exclusively as an uninfected control against which to measure gains in weight (table 1). The remaining four were used as challenge-dose controls (table 2); two of them (Nos. 7 and 9) had been previously used as uninfected controls to measure gains in weight (table 1).

The general procedures and parasitological methods used were very similar to those described by Rubin and Lucker (1956b). However, each animal's temperature was taken at least once daily, and its general clinical response to infection was observed several times each week. The total output of first-stage larvae during the course of infection was calculated from the weight of each daily fecal deposit of the calf and the number of larvae isolated per gram from a sample of the same fecal deposit.

Results

Course of "Immunizing" Infections: In table 1 are summarized the essential data pertaining to the initial infections produced in the six test calves—three, approximately 10 days old, that were differently exposed to small numbers of infective larvae and three, about 5.5 months old, that had been weaned prior to exposure to the same dosages of infective larvae. All calves acquired a patent infection. Neither the duration of the prepatent period of infection, which was relatively constant, nor that of the patent period, which was more variable, appeared to be directly correlated with the

*Resigned June 21, 1957.

total number of larvae administered or with the administration of the same number in a single dose *versus* 10 daily doses. However, in each age group, the calf that received a single dose of 500 larvae eliminated more first-stage larvae in its feces than the one that received 10 daily doses of 50 larvae.

The younger calves apparently were more susceptible to infection than the older ones. Each eliminated more first-stage larvae in its feces than the older calf correspondingly dosed with infective larvae (table 1), and evidently, therefore, acquired the greater number of mature worms. Also, the younger calves eliminated the worms more slowly than the older calves, as indicated by the longer patent period of infection in the former (table 1).

CLINICAL RESPONSE TO "IMMUNIZING" INFECTIONS: During 17 weeks post-infection, one calf (No. 48) of the younger test group gained about as much weight as the uninfected control (No. 49), whereas the other two calves of this group gained at a somewhat lower rate than the control (table 1). All three test calves of the older group gained at about the same rate as their controls. This rather long period was selected for the comparison because of the long duration of the infections in two of the younger calves and in order to minimize the influence of haphazard weight changes on the calculated rates of gain.

Of the six calves, four remained afebrile, or nearly so, during this period, one had a slight fever (max., 103° F.) for a few days, and one had a moderate fever (max., 104.0° F.) for about a month. All six calves showed some respiratory distress, which was slight to moderate in degree, except in calf 46, in which it was rather severe. The distress, which tended to be intermittent, usually began about 2.5 to 4 weeks postinfection and disappeared 3 to 4 weeks later. The peak rate of respiration was 80/min. in calf 46 and ranged from 38/min. to 62/min. among the other five calves.

INFECTIONS FROM CHALLENGE EXPOSURE: Test calf was not demonstrably resistant to patent reinfection upon challenge with 25,000 infective larvae about 3 months after the termination of its initial infection (table 2).

However, the remaining five test calves were almost completely insusceptible. Evidently only negligible numbers of mature worms developed in their lungs. The evidence for this conclusion was the fact that subsequent to the challenge exposure only small numbers of first-stage larvae were eliminated in their feces, whereas large numbers were eliminated in the feces of the controls. The calculated total outputs of such larvae in their feces (table 2) ranged from 2,900 to 55,000, whereas the least output in the feces of any control was about 2.5 million larvae.

The ratio of the number of larvae recovered from the feces of calf 47 to the number recovered from the feces of its control (No. 22) was about 1:550; for calf 48, the second of the two successfully immunized younger calves, and its control (No. 23), the corresponding ratio was about 1:280. On the average, the older controls eliminated almost 1,000 times the average number of first-stage larvae eliminated by the three older immunized calves. Also, the patent period of infection was much shorter in these five test calves than in the controls. Actually, where the duration of a patent reinfection was 6 days or more (table 2), larvae were not recovered from the feces on every day within the period.

There appeared to be no evidence that the success of immunization was dependent upon the age of the test calf when the immunizing dose of larvae was given, the use of 1,500 *versus* 500 infective larvae, or the administration of the smaller number serially *versus* in one dose.

Table 1.—Results of administration of small "immunizing" doses of infective *Dictyocaulus viviparus* larvae to six calves, including effect on increase in body weight during 17 weeks postinfection.

Calf No. and sex (m)=male (f)=female	Status at beginning of experiment		Total No. infective larvae administered	Course of infection					Weight gained (lb.)	Rate of gain (lb./day)
				Prepatent period (days)	Patent period (days)	No. of first-stage larvae eliminated during patent period				
						Per gram of feces		Total output		
	Age (days)	Weight (lb.)				Av.	Max.			
YOUNGER GROUP										
48(m)	11	100	500*	29****	87	4.0	18.7	930,000	128	1.1
47(m)	9	107	500**	29	53	19.4	90.0	2,220,000	101	0.8
46(m)	9	105	1,500***	27****	92	20.0	253.0	6,740,000	76	0.6
Average	9.7	104	.....	28.3	77.3	....	....	3,290,000	101.7	0.83
CONTROL										
49(m)	8	104	.....	....	—	....	....	.....	126	1.0
OLDER GROUP										
122(f)	160	220	500*	28****	28	1.3	4.3	137,000	150	1.3
15(f)	157	210	500**	25	35	6.0	31.8	1,279,000	143	1.2
123(f)	160	210	1,500***	25****	26	1.0	4.3	146,000	166	1.4
Average	159	213	.....	26	29.7	....	....	520,660	153.0	1.30
CONTROLS										
7(f)	171	214	.....	....	....	....	....	.....	141	1.2
9(f)	171	214	.....	....	....	....	....	.....	111	0.9
Average	171	214	.....	....	....	....	....	.....	126.0	1.05

* 50 on day 1 and 50 on each of next 9 days.
** In one dose.
*** 500 on day 1 and 1,000 nine days later.
**** Based on day first dose of larvae was given.

Table 2.—Results of administration of challenge dose of 25,000 infective *Dictyocaulus viviparus* larvae to each of six "immunized" and four previously uninfected calves, including effect on increase in body weight during 10 weeks postexposure.

Calf No. and sex (m)=male (f)=female	Status when given challenge dose: Age (days)	Status when given challenge dose: Weight (lb.)	Interval between end of "immunizing" infection and challenge exposure (days)	Course of infection or reinfection: Prepatent period (days)	Course of infection or reinfection: Patent period (days)	No. of first-stage larvae eliminated during patent period: Per gram of feces Av.	No. of first-stage larvae eliminated during patent period: Per gram of feces Max.	No. of first-stage larvae eliminated during patent period: Total output	Weight gained (lb.)	Rate of gain (lb./day)
					YOUNGER GROUP					
48(m)*	213	385	86	36	13	0.6	2.2	55,000	135	2.1
47(m)**	176	306	85	23	20	0.05	0.4	4,600	97	1.4
46(m)*	211	315	83	28	59	12.0	84.0	4,150,000	53	0.8
Average	200	335	84	29	31	----	----	1,403,600	95	1.43
					CONTROLS					
22(m)**	190***	395	----	26	31	13.3	54.5	2,535,000	75	1.1
23(m)*	228***	461	----	25	34	70.0	237.0	15,445,000	64	0.9
Average	209	378	----	25.5	32.5	----	----	8,990,000	69.5	1.0
					OLDER GROUP****					
122(f)	283	383	73	23	1	0.5	0.5	2,900	127	1.8
15(f)	287	379	73	21	15	0.25	1.5	15,200	79	1.2
123(f)	279	375	74	21	6	0.25	0.6	8,800	97	1.4
Average	283	379	73	22	7	----	----	8,967	101	1.46
					CONTROLS					
7(f)	298	395	----	22	32	34.0	140.0	4,306,000	36	0.5
9(f)	304	345	----	21	44	111.0	510.0	12,934,000	23	0.3
Average	301	370	----	21	38	-------	-------	8,620,000	29.5	0.4

* Simultaneously infected with aliquots of larvae of batch A. ** Simultaneously infected with aliquots of larvae of batch B.
*** Approximate age. **** Infected with aliquots of larvae of batch C on the following schedule: No. 123 on day 1; No. 7 on day 3; No. 122 on day 4; Nos. 15 and 9 on day 8.

Clinical Response to Challenge Exposure: In view of the result obtained in calf 46, the following account is restricted to the behavior of the remainder of the calves. Since the five resistant ones acquired only very brief, slight patent reinfections, a period of about 10 weeks postexposure was deemed adequate for comparison of their responses with the responses of the controls and detailed observations were discontinued thereafter.

All of the resistant calves outgained their controls (table 2); the average daily gain of the older three for the 10-week period was about three times that of their controls. However, only two (Nos. 48 and 122) gained as much per day as uninfected calves of the same age and breed and on the same diet usually do.

The resistant calves and the younger controls either remained afebrile or developed a slight fever lasting only a few days. The temperature of each of the older controls reached 104.2° F. One of them was feverish for about one month; the other for about one week.

Scarcely any, or only mild, respiratory distress was observed in four of the resistant calves. The fifth one coughed occasionally; its respiratory rate was 92/min. on one day and ranged from about 40 to 60/min. during much of the period. Both younger controls coughed persistently and assumed postures characteristic of lungworm disease during spells of coughing or labored breathing; their respiratory rates reached 96/min. and 112/min., being 80/min., or higher, during much of the period. One of the older controls showed a peak rate of 104/min. and respired at rates ranging from 40 to 80/min. during much of the period; the other suffered considerably less respiratory distress. However, both later developed a pneumonia which yielded to antibiotic treatment.

Other Data: Calf 46, which proved to be nonresistant, and calf 47, which proved to be resistant on initial challenge, were given subsequently a further dose of larvae. The latter calf was found to be completely immune, but calf 46 again acquired a slight patent infection.

Discussion

Reported data (Michel, 1955; Jarrett *et al.*, 1957; Rubin and Lucker, 1956b; Weber, 1958), as well as unpublished data available to us, show that there is marked variation in the magnitude, or apparent magnitude, of *D. viviparus* infections obtained in previously uninfected calves given equal numbers of larvae, even if aliquots of the same batch of larvae are given. However, all of the writers' four control calves became, as judged by total fecal outputs of first-stage larvae, much more heavily infected than five of the six test calves, and the possibility that so consistent a difference in results was due to chance seems remote. These five calves were not completely immune to a patent reinfection, whereas, according to the reports already cited, an initial infection induced by 4,000 or more normal larvae usually does result in the development of such an immunity and complete immunity also is present after recovery from naturally acquired lungworm disease (Jarrett *et al.*, 1955, 1957). However, these reinfections were insignificant from the standpoint of their potential contribution to the infectiousness of a pasture. The resistance, though definitely beneficial, did not completely prevent either respiratory distress or interference with the normal rate of gain in weight. However, even a complete immunity to a patent infection does not necessarily prevent clinical reactions (Michel, 1954; Rubin and Lucker, 1956a) following heavy reexposure to infection.

In general, our findings fit into the pattern of the results reported by others who have dealt with initial infection with small numbers of normal larvae in relation to subsequent susceptibility to a patent infection with this lungworm. Porter and Cauthen (1942) found that a calf initially infected with 200 larvae was highly susceptible to reinfection; one which they infected with 575 or 675 (?) larvae, a dosage within the range we tested, was somewhat susceptible. Jarrett *et al.* (1957) found six of 10 calves given 2,500 larvae each, a larger dosage than we tested, to be completely immune to a patent reinfection; presumably their remaining four calves became reinfected only slightly.

That calves appear to vary inherently in capacity to acquire resistance to this lungworm has been noted in previous reports (Rubin and Lucker, 1956a; Weber, 1958). This is further suggested by the lack of uniformity in results among the 10 calves tested by Jarrett *et al.* and among our own six at the time of initial challenge. Especially noteworthy, since one exposure to as few as 4,000 larvae reportedly causes most calves to become immune, is the finding that our calf 46 was still slightly susceptible after it had eliminated its second infection, which resulted from a dose of 25,000 larvae.

SUMMARY

Small doses of normal infective larvae of the cattle lungworm, *Dictyocaulus viviparus*, were tested for ability to actively immunize calves against reinfection with this parasite. Of six calves—three 10 days old and three about 5.5 months old—unexposed to this parasite previously, four were each given 500 larvae and two 1,500. Each calf was reexposed to infection about 2.5 to 3.0 months after the end of the patent period of the initial infection so induced. The reexposure was to 25,000 infective larvae. To serve as controls, four other calves, which were of comparable age and had been reared without prior exposure to this lungworm, were likewise given 25,000 larvae. Five of the six previously infected calves were found to have acquired a strong resistance, though not a complete immunity, against patent reinfection with *D. viviparus* at this level of challenge. The immunizing infections did not materially affect the growth of the calves, especially the older ones, but did cause slight-to-moderate respiratory distress. After administration of the challenge dose of larvae, the resistant calves outgained the controls; the resistance usually did not prevent respiratory distress, but did minimize it.

LITERATURE CITED

JARRETT, W. F. H., JENNINGS, F. W., McINTYRE, W. I. M., MULLIGAN, W., and URQUHART, G. M. 1955. Immunological studies on *Dictyocaulus viviparus* infection. Passive immunisation. Vet. Rec. 67: 291-296.

————. 1957. The natural history of parasitic bronchitis with notes on prophylaxis and treatment. Vet. Rec. 69: 1329-1340.

MICHEL, J. F. 1954. A contribution to the aetiology of fog fever. Vet. Rec. 66: 381-384.

————. 1955. Studies on host resistance to *Dictyocaulus* infection. I. The phenomenon of inhibited development. Jour. Comp. Path. and Therap. 65: 149-158.

PORTER, D. A., and CAUTHEN, G. E. 1942. Experiments on the life history of the cattle lungworm, *Dictyocaulus viviparus*. Amer. Jour. Vet. Res. 3: 395-400.

RUBIN, R., and LUCKER, J. T. 1956a. Acquired resistance to *Dictyocaulus viviparus*, the lungworm of cattle. Cornell Vet. 46: 88-96.

————, and ————. 1956b. The course and pathogenicity of initial infections with *Dictyocaulus viviparus*, the lungworm of cattle. Amer. Jour. Vet. Res. 17: 217-226.

WEBER, T. B. 1958. Immunity in cattle to the lungworm, *Dictyocaulus viviparus*: A test of the persistence of acquired resistance. Jour. Parasitol. 44: 244-245.

A Revision of the North American Species of the Genus *Skrjabinema* (Nematoda: Oxyuroidea)*

G. A. Schad**

During recent studies on the genus *Skrjabinema* Vereshchagin, 1926, it became apparent that published descriptions of the various species do not adequately characterize them and that the characters used for determination are unreliable. Therefore, material from all recorded North American hosts was accumulated and examined, and a partial generic revision was attempted.

The literature shows that in North America *Skrjabinema ovis* (Skrjabin, 1915) is reported from domestic sheep (*Ovis aries*), bighorn sheep (*Ovis canadensis*), and the domestic goat (*Capra hircus*). *S. oreamni* Swales, 1934, is the species currently recognized from the mountain goat (*Oreamnos americanus*) and caribou (*Rangifer* sp.), and *S. parva* Dikmans, 1942, is reported from mule deer (*Odocoileus hemionus*) in the Rocky Mountain States. Allen *et al.* (1956) recorded a pinworm as *Skrjabinema* sp. from a wild population of Barbary sheep (*Ammotragus lervia*) which was recently introduced and has become established in New Mexico.

Swales (1934) and Olsen and Tolman (1950) made taxonomic changes on the basis of nearctic materials. In a paper describing *S. oreamni* from *Oreamnos americanus* and *Rangifer* sp., Swales reduced *S. tarandi* Skrjabin and Mitskevich, 1930, to synonymy with *S. ovis*. *S. tarandi* had been previously reported as the species parasitizing reindeer in arctic Russia. Olsen and Tolman described the male of *S. parva,* emended the generic diagnosis, corrected Swales' description of the male of *S. oreamni,* and provided a key to the species of *Skrjabinema*. The present study shows this key to be unreliable.

The species discussed in the taxonomic section of this paper differ so slightly that complete morphological descriptions of each would be repetitious. Thus only in the case of *S. caprae* n.sp. will a complete morphological description be given. This description and the generic diagnosis may be consulted for morphological information not included under the other species. Measurements are given in Table 1.

The following individuals have made their collections available to the author, and to them he extends his thanks; abbreviations following their names are those used subsequently in lists of material examined:

Dr. O. A. Brunetti, Calif. Dept. Fish and Game, Sacramento, Calif. (OAB)

Dr. I. McT. Cowan, Dept. Zoology, U. British Columbia, Vancouver, B. C., Can. (IMcTC)

Dr. O. W. Olsen, Dept. Zoology, Colo. State U., Fort Collins, Colo. (OWO)

Dr. T. W. M. Cameron, Inst. Parasitol., Macdonald Col., Quebec, Can. (TWMC)

Dr. R. Rausch, Arctic Health Res. Center, Anchorage, Alaska (RR)

Dr. G. K. Sweatman, Animal Path. Div., Canada Dept. Agr., Macdonald Col., Quebec, Can. (GKS)

*From the Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture State College, New Mexico.

This work was done in cooperation with the New Mexico Agricultural Experiment Station.

**Present address, Institute of Parasitology, McGill University, Macdonald College P. O., Quebec, Canada.

THE GENUS *SKRJABINEMA* Vereshchagin, 1926

Synonym—*Oxyuris* Rudolphi, 1813 (in part)

GENERIC DIAGNOSIS (modified from *Olsen and Tolman* [1950] as emended from Monnig [1932]): Oxyuridae. Cuticle inflated around head. Body with lateral alae for greater part of length. Females with 3 anchor-shaped lips separated by digitiform interlabia; male lips triangular each with apical notch. Excretory pore postesophageal. Vulva in anterior half of body. Cuticular inflation of male tail supported by preanal and postanal pairs of ray-like propections bearing terminal papillae. Each of postanal projections with an additional basal papilla. Single spicule with gubernaculum present. Parasites of ruminants.

KEY TO THE NEARCTIC SPECIES OF *SKRJABINEMA*

1. A. Males 2
   B. Females 5

2. A. Sub-interlabial projections absent (fig. 1) 3
   B. Sub-interlabial projections present (figs. 2 & 3) 4

3. A. Adult less than 2.00 mm. in length.
   Parasite of deer .... *S. parva* (male)
   B. Adult longer than 2.00 mm. in length.
   Parasite of sheep and goats .... *S. ovis* (male)

4. A. One sub-interlabial projection (fig. 2) .... *S. caprae* (male)
   B. Two sub-interlabial projections (fig. 3) .... *S. tarandi* (male)

5. A. Posterior ending of alae abrupt and relatively close to tip of tail (figs. 4 & 5).
   Parasites of Cervidae 6
   B. Posteriorly, alae merge gradually into the tail and end relatively more to the anterior (figs. 6 & 7).
   Parasites of Bovidae 7

6. A. Tail 0.75 mm. or longer.
   Parasite of *Rangifer* sp. .... *S. tarandi* (female)
   B. Tail 0.60 mm. or shorter.
   Parasite of *Odocoileus* sp. .... *S. parva* (female)

7. A. Alae extend along tail for half its length or more. (fig. 6) .... *S. ovis* (female)
   B. Alae do not extend to mid-point of tail, but end more anteriorly (fig. 7) .... *S. caprae* (female)

THE NORTH AMERICAN SPECIES OF SKRJABINEMA

SKRJABINEMA OVIS (Skrjabin, 1915) Vereshchagin, 1926 (figs. 1 & 6)

Synonym—*Oxyuris ovis* Skrjabin, 1915; *Skrjabinema oreamni* Swales, 1934 (in part)[1]

DIAGNOSIS: *Skrjabinema.* Sub-interlabial projections absent in males. Posteriorly, alae of female merge gradually into tail. Alate tail sector about as long as, or longer than, postalar tail. Ratios of alate tail/postalar tail vary between 1:0.44 and 1:1.03. Parasite of wild and domestic sheep and goats.

[1]*S. oreamni* has been reported as *S. erami* by Brandborg (Life History and Management of the Mountain Goat in Idaho. State of Idaho Dept. of Fish and Game, Wildlife Bulletin No. 2, p. 111, 1955). This is a misprint of *S. oreamni.*

DISCUSSION: Olsen and Tolman (1950) call attention to the presence of apical lip notches (figs. 1-3; ALN) in the male *S. ovis* and maintain that this character distinguishes the species from others occurring in North America. The study of more adequate collections of the several nearctic species shows that lip notches occur in all, but may be invisible or inconspicuous depending on the angle at which the lips are viewed. However, *S. ovis* is a valid species and the males may be separated from those of *S. tarandi* and *S. caprae* n.sp. by the absence of sub-interlabial projections. *S. ovis* males are not morphologically separable from *S. parva* males, but differentiation is possible on a mensural basis. (See Table 1.)

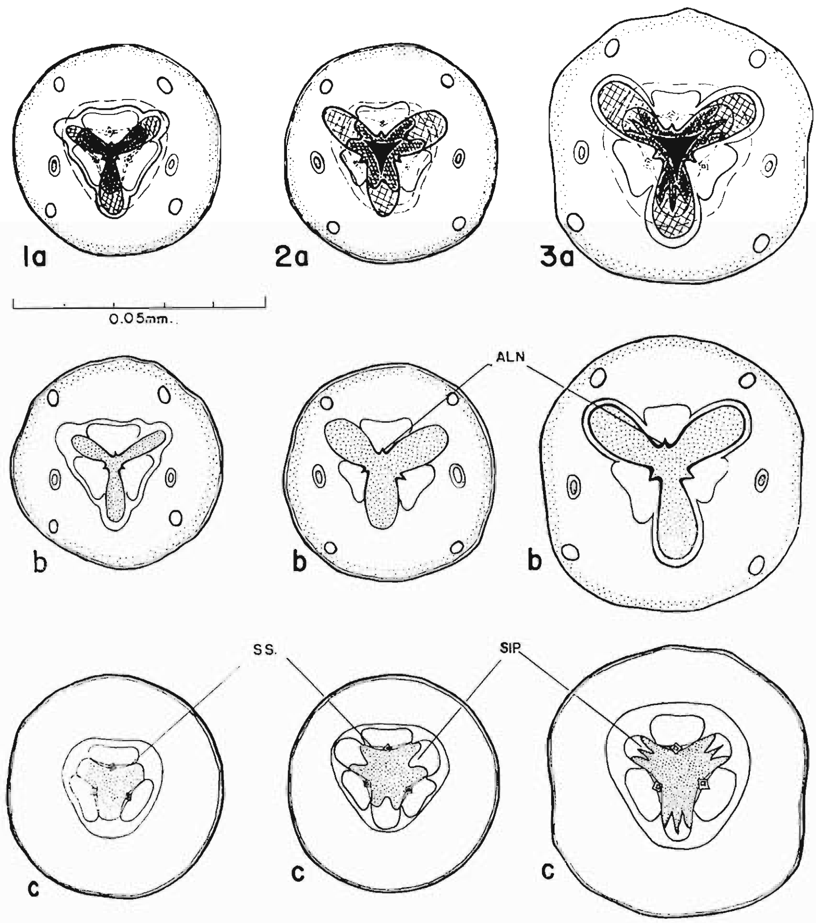


Figure 1. *Skhjabinema ovis* (male), *en face;* a. Composite of surface and intra-oral structures; b. Surface structures only; c. Optical section through oral cavity.

Figure 2. *Skrjabinema caprae* (male), *en face;* a-c. As for figure 1.

Figure 3. *Skrjabinema tarandi* (male), *en face;* a-c. As for figure 1.

Table 1. Measurements (mm.) of the North American species of *Skrjabinema*

	S. ovis	*S. caprae* n. sp.	*S. tarandi*	*S. parva*
Females				
No. of Specimens Measured	43	35	32	20
Total Length	4.99 -10.00	5.18 -8.16	7.75 -13.50	3.49 -5.48
Maximum Width	.35 - .55	.30 - .55	.45 - .75	.19 - .44
Head Diameter	.07 - .10	.06 - .11	.10 - .12	.06 - .08
Nerve Ring from Anterior	.15 - .30	.15 - .20	.16 - .30	.12 - .17
Excretory Pore from Anterior	.98 - 1.80	1.09 -1.78	1.65 - 2.63	.75 -1.26
Length of Esophagus	.56 - .80	.60 - .78	.71 - .92	.46 - .53
Width of Esophageal Bulb	.14 - .20	.11 - .20	.20 - .31	.11 - .19
Length of Esophageal Bulb	.11 - .20	.12 - .19	.20 - .29	.13 - .17
Vulva from Anterior	1.60 - 3.17	1.85 -2.88	2.63 - 4.73	1.43 -2.30
Length of Tail	.78 - 1.58	.50 -1.09	.79 - 1.39	.34 - .59
Alae End from Tip of Tail	.30 - .71	.29 - .74	.19 - .35	.08 - .16
Egg Length	.048- .063	.048- .059	.065- .080	.053- .065
Egg Width	.027- .036	.029- .037	.031- .041	.030- .035
Males				
No. of Specimens Measured	22	16	25	7
Total Length	2.34 - 3.65	2.40 -3.10	2.80 - 3.30	1.76 -1.95
Maximum Width	.11 - .18	.10 - .17	.16 - .21	.08 - .10
Head Diameter	.04 - .05	.04 - .05	.05 - .07	.03 - .04
Nerve Ring from Anterior	.14 - .16	.12 - .14	.14 - .18	.09 - .10
Excretory Pore from Anterior	.78 - .91	.68 - .79	.75 - .93	.47 - .53
Length of Esophagus	.34 - .43	.34 - .40	.35 - .41	.24 - .26
Length of Esophageal Bulb	.08 - .11	.08 - .10	.10 - .11	.07 - .08
Width of Esophageal Bulb	.08 - .11	.07 - .08	.08 - .11	.04 - .06
Length of Spicule	.06 - .09	.07 - .08	.08 - .09	.07 - .08
Length of Gubernaculum	.02	.03	.02	.01 - .02
Length of Spine	.01 - .02	.01 - .02	.01 - .02	.01

It is difficult to determine the females of the nearctic *Skrjabinema* to species. The only useful characters the author could find were (1) the nature of the posterior terminations of the lateral alae and (2) the distance of this termination from the tip of the tail relative to the over-all length of the tail (fig. 4-7). In *S. ovis* the lateral alae merge gradually into the body (fig. 6) and extend posteriorly along the tail for about one-half the length of the tail or more (*i.e.,* the anterior alate part of the tail almost equals or exceeds length of the postalar tail) (fig. 8).

Skrjabinema oreamni Swales, 1934, in part, from *Oreamnos americanus* is placed in synonymy with *S. ovis.* This action is deemed necessary upon examination of the type material (#12 in list of material examined) and additional lots of *Skrjabinema* from *O. americanus.* The differences in lip characters used by Olsen and Tolman (1950) to distinguish *S. oreamni* and *S. ovis* are not real. Both have apical lip notches. While Swales' specimens are larger than most *S. ovis,* other collections from *O. americanus* fall well within the known size range of *S. ovis* and the various measurements overlap. Thus, mensural differences previously used to distinguish *S. ovis* from *S. oreamni* also are not valid. Swales' specimens are considered as extremes at the large end of the range of variation for *S. ovis.* From figure 8 it may be seen that specimens from *Oreamnos,* though larger than many *S. ovis* from other hosts in the female tail characters plotted, are proportionally similar.

The following material was examined:

U.S.N.M. (#31434), *Capra hircus,* Beltsville, Md.
U.S.N.M. (#32083), *Capra hircus,* Beltsville, Md.

U.S.N.M. (#46870), *Capra hircus,* Beltsville, Md.

U.S.N.M. (#33976), *Capra hircus,* Beltsville, Md.

U.S.N.M. (#34045), *Capra hircus,* Boston, Mass.

U.S.N.M. (#44546), *Ovis canadensis,* Salmon, Idaho

U.S.N.M. (#47238), *Ovis canadensis,* San Andres Mts., N. M.

ADP, N.M. *Ovis canadensis,* Hachita, N. M., USDA, ARS, Animal Disease and Parasite Research Division, State College, New Mexico.

ADP, N.M. *Ovis canadensis,* Columbus, Mont.

OWO, *Ovis canadensis,* Ouray, Colo.

IMcTC, *Oreamnos americanus,* Vernon, B. C., Can.

TWMC, *Oreamnos americanus,* Berland River, Alta., Can.

U.S.N.M. (#47228), *Oreamnos americanus,* Lemhi County, Idaho.

ADP, N.M. *Ovis aries,* State College, N. M.

ADP, N.M. *Ovis aries,* State College, N. M.

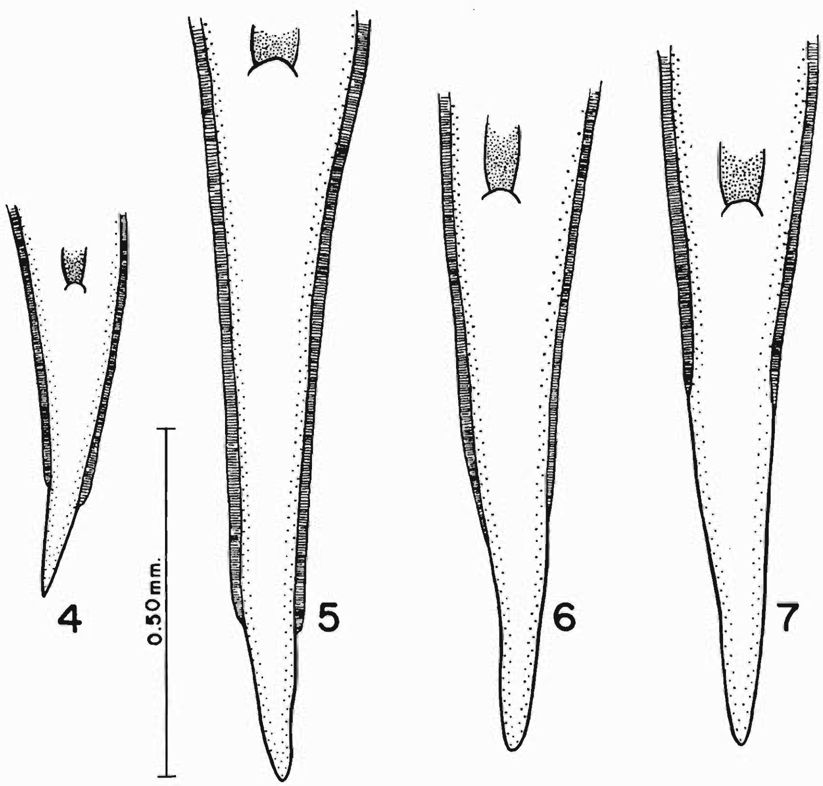


Figure 4. *Skrjabinema parva* (female), tail.

Figure 5. *Skrjabinema tarandi* (female), tail.

Figure 6. *Skrjabinema caprae* (female), tail.

Figure 7. *Skrjabinema ovis* (female), tail.

SKRJABINEMA CAPRAE n. sp. (figs. 2 & 7)

DIAGNOSIS: *Skrjabinema.* Males with one sub-interlabial projection. Posteriorly, lateral alae of female merge gradually into body. Alate tail sector shorter than postalar tail sector. Parasite of domestic goats and the Barbary sheep.

DESCRIPTION: Female longer than male, tapers at both ends, but anterior tip truncated, whereas posterior termination is a simple blunted point. Male tail complex. When extended, female straight, but male usually J-shaped, *i.e.*, tail bent ventrally.

Esophagus with single bulb similar in both sexes, as is terminal portion of excretory system. Four excretory ducts, 2 from anterior and 2 from posterior, converge on excretory sinus from which a short duct extends to ventral side, and opens through postesophageal excretory pore.

Circumoral papillae, 6 in number, similar in both sexes; amphids located close to mouth opening, remaining papillae sub-laterally and more externo-posteriorly located.

FEMALE: Anterior end with striated cuticular inflation from which striated lateral alae extend posteriorly to end along tail. Termination of alae unsymmetrical and not absolutely lateral, postalar tail longer than one-half of total length of tail. Ratios of alate tail/postalar tail vary between 1:1.13 and 1:3.19.

Mouth opening bordered by 3 anchor-shaped lips, separated from each other by elongate projections (interlabia). Free edge of each lip bears a pair of plates. Mouth wall surrounds base of lips, but free margins of lips may be elevated to project from oral opening.

Vulva, between mid-body and excretory pore; ovejector single, heavily muscularized, with wide lumen having rugose lining. Tail relatively long and evenly tapering. Eggs typically D-shaped.

MALE: Anterior with slight, striated cuticular inflation. Lateral alae present. Mouth opening surrounded by 3 apically notched, triangular lips (fig. 2). Labial pulp cavity present. Under each lip a supporting structure present (fig. 2; SS). Interlabia absent, but at a lower level in interlabial space a digitiform process (sub-interlabial projection) extends into mouth cavity (fig. 2; SIP).

Tail, usually ventrally bent, ending in spike-like point encompassed in part by cuticular inflation, supported by two pairs of projections; anterior pair laterally inserted. Posterior pair, flanking tip of tail, dorso-laterally inserted. Each projection tipped by papilla and posterior paired projections each with one additional, ventral, basally situated papilla. A pair of pads, trapezoidal in outline, one on each side of anus; each bears 3 papillae—1 medial and 1 on each of 2 distal corners.

Spicule short, simple and with slightly expanded proximal end. Gubernaculum partially surrounds tip of withdrawn spicule.

HOST: Domestic goat (*Capra hircus*).

LOCATION: Caecum and large intestine.

LOCALITY: Tortugas, New Mexico.

TYPE SPECIMENS: Holotype—male; allotype—female (U.S.N.M. #38393)
Paratypes—both sexes (U.S.N.M. #38394)
Additional material: ADP, N.M., *Ammotragus lervia*, Roy, N. Mex.

DISCUSSION: *S. caprae* closely resembles *S. ovis* but differs from the latter in the following characters: (1) In the male there is a single sub-interlabial projection which is absent in *S. ovis,* and (2) in the female, the lateral alae extend along the tail for a shorter distance. This distance is usually considerably less than one-half the length of the tail in *S. caprae,* whereas it is about one-half or more in *S. ovis.*

A difference in periodicity between *S. ovis* and *S. caprae* in *Ovis aries* and *Capra hircus* respectively has been observed. Whether this is host influenced or is an intrinsic characteristic of the pinworm species has not been determined. However, the author has found a definite nocturnal periodicity in the migration of mature female *S. caprae* to the rectum in goats, whereas periodicity was not evident in *S. ovis*-infected sheep. Schad (1957) erroneously reported this information as characteristic of *S. ovis* in sheep vs. goats. However, it is now known that actually two species were involved. Presence or absence of periodicity may prove to be a useful biological character in identifying the species of *Skrjabinema.*

SKRJABINEMA TARANDI Skrjabin & Mitskevich, 1930 (fig. 3 & 5)
Synonym: *Skrjabinema oreamni* Swales, 1934 (in part)

DIAGNOSIS: *Skrjabinema.* Two sub-interlabial projections in males. Posteriorly, alae of female end abruptly close to tip of tail. Female tail 0.75 mm. or longer. Parasite of reindeer and caribou.

DISCUSSION: This is the largest known species of the genus, and in the male is characterized by two sub-interlabial projections (fig. 3). A relatively blunt tail, bearing lateral alae ending abruptly close to the tip of the tail (fig. 5), distinguishes small *S. tarandi* females from most large *S. ovis* and *S. caprae* of like sex. *S. parva* females have a similar tail, but the species is much smaller and its males have no sub-interlabial projections.

Since Swales' report of *S. oreamni* from both caribou (*Rangifer* sp.) and the mountain goat (*Oreamnos*), *S. oreamni* has been considered as the one species parasitizing both hosts. However, the present study shows that two species actually are involved (*i.e., S. ovis* in *Oreamnos* and *S. tarandi* in *Rangifer,* both of which have *S. oreamni* in part as a synonym). The only material of Swales' original collections that is extant is a vial labeled "Columbian Mountain Goat (*Oreamnos americanus*)." A separate collection from *Rangifer* could not be found. The former is considered the type material of *S. oreamni.* However, in the author's opinion Swales' original material as a whole was probably heterogeneous; that is, the specimens from the caribou mentioned in his description were probably *S. tarandi.* Supporting this conclusion is one female specimen included with Swales' extant material. This specimen is considered as *S. tarandi.* Presumably this specimen was returned to the wrong vial (*i.e.,* the vial of specimens from *Oreamnos*).

The preceding discussion explains the author's decision to place *S. oreamni* in part in synonymy with *S. tarandi.* The latter had been incorrectly considered synonymous with *S. ovis* by Swales. Skrjabin *et al.* (1951, p. 108) have already re-instated *S. tarandi* as a valid species but without justifying their action. This re-instatement is herein justified on the basis of re-examination of part of the original material. A vial of specimens (U.S.N.M. #41996) contains a label written in Russian and is attributed to Mitskevich, one of the coauthors of the species description. The label bears his name and collection data which agree with those given in the original description.

The following material was examined:

U.S.N.M. (#41996), *Rangifer* sp. (Reindeer), Archangel, USSR
U.S.N.M. (#41268), *Rangifer* sp. (Caribou), Arctic Slope, Alaska
U.S.N.M. (#55685), *Rangifer* sp. (Caribou), Brooks Range, Alaska
TWMC, *Rangifer* sp. (Caribou), Beverly Lake, N.W.T., Can.
RR, *Rangifer* sp. (Caribou), Barrow, Alaska
RR, *Rangifer* sp. (Caribou), Brooks Range, Alaska
GKS, *Rangifer* sp. (Reindeer), Reindeer Depot, N.W.T., Alaska

All hosts are listed as *Rangifer* sp. since the various available references in mammalian taxonomy make it difficult for the non-specialist to determine the accepted trivial name for the hosts involved.

SKRJABINEMA PARVA Dikmans, 1942 (fig. 4)

DIAGNOSIS: *Skrjabinema.* Male small (less than 2.00 mm.) without sub-interlabial oral projections. Females with alae terminating abruptly near tip of tail. Female tail shorter than 0.06 mm. Parasite of mule deer, *Odocoileus hemionus.*

DISCUSSION: Although the males of *S. parva* are similar to those of *S. ovis* in not possessing sub-interlabial projections, the former is considered valid. In the collections available to date, the males are invariably smaller than males of *S. ovis.* Female *S. parva* are distinguished from female *S. ovis* by the structure of the tail, in that the alae end abruptly and the postalar tail length is very short (figs. 4 & 8). In these characters *S. parva* females re-

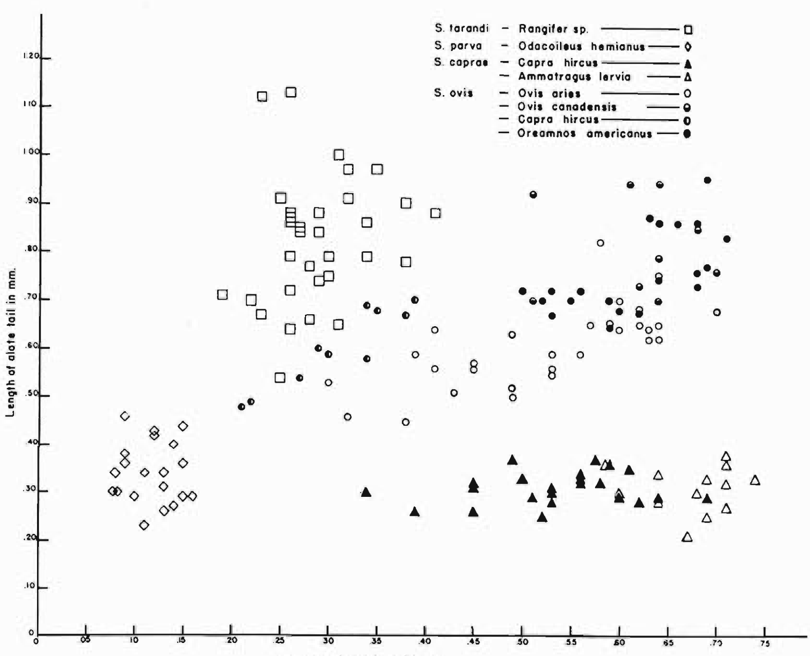


Figure 8. Relationship of length of alate tail sector to length of postalar tail sector in female *Skrjabinema* plotted by species and by host.

semble *S. tarandi,* but the latter is a much larger species and in the male *S. tarandi* lip projections are present. The following material was examined:

U.S.N.M. (#43680-1), *Odocoileus hemionus;* Boise, Idaho

U.S.N.M. (#49201), *Odocoileus hemionus;* Camp Pendleton, San Diego County, Calif.

U.S.N.M. (#46454), Odocoileus hemionus; Arkansas River Drainage, Colo.

OWO, *Odocoileus hemionus;* Arkansas River Drainage, Colo.

OAB, *Odocoileus hemionus;* Camp Pendleton, San Diego County, Calif.

DISCUSSION OF THE GENUS: Monnig (1932) divided *Skrjabinema* into two sub-genera depending on whether or not the lips protrude from the mouth opening. That the lips might be movable and capable of being drawn into, or projected from, the mouth was considered by Monnig. However, he rejected this idea on the basis of the oral morphology, believing that this left no doubt that the lips were fixed in position. Secondly, on the basis of 10 females examined and assigned by him to his 2 new species, *S. africana* Monnig, 1932, and *S. alata* Monnig, 1932, and of 18 specimens of *S. rupicaprae* Böhm and Gebauer (1930), all of which had their lips enclosed by the mouth wall, Monnig concluded that if the lips were movable some of these specimens would have had projecting lips.

In the course of the author's studies it was noted that, in any one of the species examined, the lips varied markedly in the degree of projection from the mouth. Yet, oral morphology of these species was identical with that described by Monnig. It follows then that the lips are not immovable in life or that post-mortem effects (degeneration, fixation, etc.) cause variation in this character. Monnig's second point, that no variation was found in 10 specimens of his species and 18 specimens of *S. rupicaprae,* is confusing because he includes both males and females in the same discussion and ignores the marked sexual dimorphism in oral structures that does exist. The literature indicates that this dimorphism was not recognized until a later date. However, how is the uniformity of the female part of the material cited by Monnig explainable if the lips are not fixed in a wholly intra-oral position? In the author's experience, young specimens and those freshly collected and uniformly fixed show little variation within lots in the degree of labial projection. It is believed that the lack of variation in the specimens cited by Monnig can be accounted for in this way. Thus, in the author's opinion, the subgenera *Skrjabinema* and *Chylocrypta* Monnig, 1932, are not valid.

In believing these subgeneric characters to be of value, Monnig precluded identication of his species *S. alata* and *S. africana* with *S. ovis,* since the first two fell in *Chylocrypta* while *S. ovis* fell in *Skrjabinema.* Therefore a comparison with *S. ovis* is not included with his description of these species. If, as the author believes, Monnig's subgeneric characters are not valid, then differences between the latter's species, *alata* and *africana,* and *S. ovis* disappear. However, it may well be that distinct species occur in African antelopes, but these are not recognizable on the basis of the literature to date.

S. rupicaprae Böhm and Gebauer, 1930, from the chamois (*Rupicapra rupicapra*) remains as the only described species, not herein considered, that may be presently recognized. It is clearly characterized by the shape of the female lips but, unfortunately, the male oral structures are not described, and thus the character given greatest weight in this paper cannot be assessed. The species could not be restudied since in personal correspondence Dr. Böhm has informed the author that all his material of *S. rupicaprae* was lost during the occupation of Vienna. Additional collections could not be located.

Setting aside Monnig's two species as requiring confirmation, then outside of North America there are three species reported in the literature; these are *S. rupicaprae* from the chamois, *S. ovis* from the gazelle, goat, sheep, and steinbok, and *S. tarandi* from the reindeer. These, no doubt, are all valid as species, but it would seem to the author that, considering the scarcity of males in many collections, the similarity of females, and the numerous misinterpretations of structure in the earlier reports, it is conceivable that more than one species may be included under *S. ovis*. As has been shown in the present paper, in North America at least, *S. ovis* is not the only species occurring in goats; both *S. ovis* and *S. carprae* are found.

LITERATURE CITED

ALLEN, R. W., BECKLUND, W. W. and GILMORE, R. F. 1956. Parasites of the Barbary sheep. J. Parasitol. 42 (Sect. 2): 19.

BÖHM, L. K. and GEBAUER, O. 1930. Ein neuer Wiederkauer-Oxyuride (Nematoda), *Skrjabinema rupicaprae* aus der Gemse. Ztschr. Parasitenk. 2: 589-594.

MONNIG, H. O., 1932. Wild antelopes as carriers of nematode parasites of domestic ruminants. Part II. 18th Rpt. Dir. Vet. Serv., Dept. Agric., Union South Africa, pp. 152-172.

OLSEN, O. W. and TOLMAN, C. D. 1950. Description of the male of the pinworm, *Skrjabinema parva* Dikmans, 1942 (Nematode: Oxyuroidea) from deer with an emendation of the genus. J. Parasitol. 36: 411-415.

SCHAD, G. A. 1957. Preliminary observations on the life history of the sheep pinworm. J. Parasitol. 43 (Sect. 2): 13.

SKRJABIN, K. I., SHIKHOBALOVA, N. P., and MOZGOVOI, A. A. 1951. [A key to parasitic nematodes.] V. 2. Oxyurata and Ascaridata. 631 pp. Moskva.

SWALES, W. E. 1934. *Skrjabinema oreamni* sp. nov., a nematode parasite of *Oreamnos americanus* and *Rangifer* sp. in Canada. Canad. J. Research 10: 527-531.

Aspiculuris ackerti, n. sp., (Nematoda: Oxyuridae) from the Wood Rats of Arizona*

FRANCIS J. KRUIDENIER and KRISHNA N. MEHRA
Department of Zoology, University of Illinois, Urbana

A species of nematode with the general characteristics of the genus *Aspiculuris* Schulz, 1924, was recovered from a series of neotomid rats examined during a 1954 expedition to the Grand Canyon, Coconimo County, Arizona (see Kruidenier and Peebles, 1958).** Preliminary examination indicated that the oxyurid differs appreciably from other described species of *Aspiculuris*. A differential study of the nematodes was therefore undertaken. The specific *ackerti* is proposed in honor of Dr. J. E. Ackert, Professor Emeritus, Kansas State College.

*This investigation was supported in part by a research grant (E-1319) from the National Institute of Health, Public Health Service. Financial support was also furnished by the University of Illinois Graduate Research Board.

**Gratitude is expressed to Dr. Donald F. Hoffmeister, Director of the Museum of Zoology, University of Illinois who organized the expedition and, with his staff, identified the mammals collected during the expedition. The valued help of the other mammalogists of the expedition is gratefully acknowledged. They included Drs. W. H. Davis (Univrsity of Minnesota, St. Paul) and W. Z. Lidicker (University of California at Berkeley) and Messrs. K. N. Nelson (Chicago, Ill.) and W. Goodpaster (of Cincinnati, Ohio). We also thank Miss A. Boatright, Scientific Illustrator, Zoology Department, Univ. of Ill. for enthusiastic cooperation.

MATERIALS AND METHODS

The organs of the host animals were examined individually and the parasites recovered during the expedition were preserved in separate containers in accordance with tentative identification, host, and the organ from which they were recovered. The nematodes were killed in hot, 70% ethyl alcohol unless field conditions necessitated using the reagent at prevailing temperatures. Specimens were preserved in the alcohol, to which 5% glycerin was added upon the return to Urbana.

A total of 10 mature female and 3 male *Aspiculuris* were recovered from the neotomid rats as follows: 2 *Neotoma albigula* Hartley, 1894, of 12 examined, contained 1 male and 9 female *Aspiculuris;* 1 *N. cinerea acraia* (Elliot, 1904) Hooper, 1940, of 3, contained 2 male and 1 female specimens and none of 10 *N. stephensi* Goldman, 1932 or 3 *N. lepida* Thomas, 1893 were infected. None of the other animals examined during the survey contained specimens of *Aspiculuris*.

The worms were studied unstained, after partial clearing in glycerol. Permanent preparations were made of the anterior (en face) and posterior regions by ringing gelatin-mounted specimens with H & R mounting resin.

DESCRIPTION

The following description of *Aspiculuris ackerti,* n. sp., is based on two mature males and 10 gravid females.

MALE: Length, 4.64 mm. (4.41-4.87 mm.); width, 0.168 mm. (0.162-0.174 mm.), anterior esophagus, 0.267 mm. (0.264-0.271 mm.) x 0.039 mm. (0.039-0.040 mm.); esophageal bulb, 0.150 mm. (0.145-0.155 mm.) x 0.075 mm. (0.071-0.079 mm.); total length of esophagus, 0.417 mm. (0.409-0.426 mm.); distance from anterior end to nerve ring, 0.158 mm.; cuticular vesicle, length, 0.099 mm. (0.097-0.105 mm.); length of tail, 0.259 mm. (0.238-0.280 mm.); caudal alae (with two deep notches each), length, 0.382 mm. with 0.185 mm. to first notch, 0.079 mm. between first and second notches and 0.118 mm. between second notch and end of alae; 11 caudal papillae, 2 preanal, 2 adanal, and 2 postanal pairs closely associated with anus, a single median and 2 postanal pairs.

FEMALE: Length, 5.5-6.8 mm. (6.2 mm.); width, 0.197-0.232 mm. (0.210 mm.); anterior esophagus, 0.290-0.348 mm. (0.317 mm.) x 0.042-0.052 mm. (0.044 mm); esophageal bulb, 0.145-0.171 mm. (0.158 mm.) x 0.068-0-0.087 mm. (0.080 mm.); total length of esophagus, 0.445-0.511 mm. (0.476 mm.); distance from anterior end to nerve ring, 0.155-0.185 mm. (0.168 mm.), to vulva, 2.1-2.5 mm. (2.3 mm.), to lateral cervical alae, 0.037-0.044 mm. (0.040 mm.); cervical alae, 0.522-0.603 mm. (0.561 mm.) x 0.031-0.039 mm. (0.036 mm.) ;length of cuticular vesicle, 0.092-0.132 mm. (0.105 mm); length of tail, 0.232-0.690 mm. (0.445 mm.); uterus (terminal egg) extends 0.232-0.690 mm. (0.445 mm.) from tip of tail; eggs, 0.097-0.106 mm. (0.101 mm.) x 0.039-0.047 mm. (0.044 mm.).

PROPORTIONS, FEMALES: Total length/width, 25-34 (30); total length/total length of esophagus, 12.3-14.2 (13); length of ant. esophagus/width of anterior esophagus, 6.0-8.3 (7.2); total length/length of tail 7.7-9.7 (8.5); total length/distance from anterior end to nerve ring, 32-41.1 (37.3); total length/distance from anterior end to end of cervical alea, 10.4-12.2 (11.5); total length/distance from anterior end to vulva, 2.6-2.8 (2.65); length of esophageal bulb/width of bulb, 1.8-2.1 (1.9); length of eggs/width of eggs, 2.0-2.4 (2.2).

HOSTS: *Neotoma albigula*—U. of Ill. Museum #11650
N. cinerea—U. of Ill. Museum #11688
HABITAT: Intestine
LOCALITY: Coconino County, Arizona
HOLOTYPE: U.S. Nat'l Mus. Helm. Coll. #56163

A uterine loop extends characteristically well beyond the anus into the tail of the female (Fig. 2). The excretory pore is very difficult to see. We believe that it lies at the approximate level of the posterior margin of the esophageal bulb.

The lateral alae are very slender. The caudal alae are so deeply notched

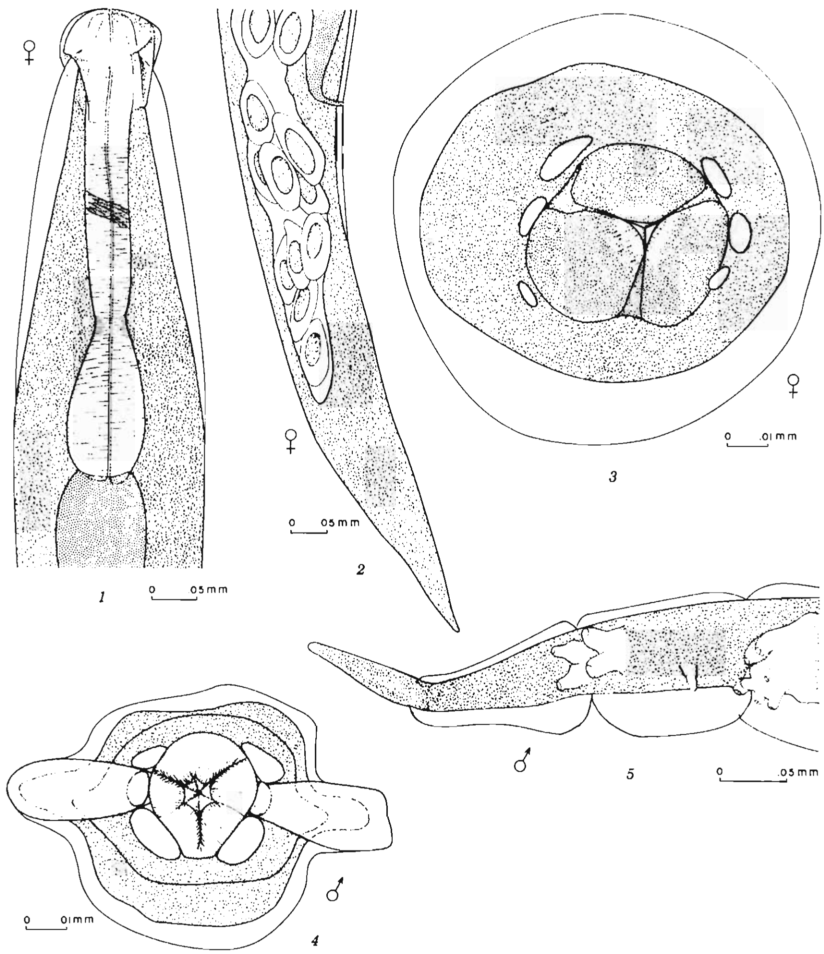


Figure 1. Anterior end showing esophageal bulb and cervical alae.
Figure 2. Posterior end showing posterior uterine loop.
Figure 3. Anterior end (*en face*) showing the three undivided lips of the female.
Figure 4. Anterior end (*en face*) showing the divided lips of the male.
Figure 5. Posterior end showing the terminal caudal alae and the posterior papillae of the male.

that they could be interpreted as 3 bilateral pairs of alae in alignment along the posterior ends of the males (Fig. 5). The tails of the males extend distinctly beyond the posteriormost margins of the terminal alar units.

The lips (Figs. 3 & 4) differ in the male and female specimens. The females possess three distinct, undivided lips, one is dorsal and 2 are subventral. Although the males also possess one dorsal and 2 subventral lips, each lip is distinctly subdivided into two equal portions so that a superficial appearance of 6 lips in 3 groups is imparted. The secondary divisions are not as distinct as the interlabial clefts.

Discussion

Nine species of *Aspiculuris* have been described previously. *A. ackerti* differs from each of these in the details of the actual and proportional dimensions of its various organs. It can be most readily distinguished, however, on the basis of gross morphological features. Thus it differs from *A. caviellae* Freitas et al., 1937, in the possession of a distinct cephalic cuticular inflation which is absent from the latter. The gradual posterior attenuation of its cervical alae (Fig. 1) distinguishes it from all species except *A. asiatica* Schultz, 1927, which it resembles most closely. The cervical alae of the other species (namely, *A. dinniki* Schultz, 1927, *A. americana* Erickson, 1938, *A. lahorica* Akhtar, 1955, *A. pakistanika* Akhtar, 1955, *A. tetraptera* Nitzsch, 1821, *A. schulzi* Popov and Nazarovo, 1930, and *A. kazakistanika* Nazarova and Sveschinikova, 1930), possess an abruptly recurved or sickle-shaped posterior margin. The caudal alae of *A. ackerti* terminate an appreciable distance from the tip of the tail while these structures of *A. asiatica* were specifically described as extending around the tip of the tail (Schultz, 1927).

The division of the dorsal and both subventral lips of the males of *A. ackerti* is unique. Chitwood and Chitwood (1950: pp. 59-60) note a similar division of only the dorsal lip of *A. tetraptera* and do not record sexual dimorphism for this character. The divisions in our specimens are distinct in the male and no tendency toward the separation can be seen in the female specimens studied by us.

Literature Cited

Akhtar, S. A. 1955. On nematode parasites of rats and mice of Lahore, with some remarks on the genus *Aspiculuris*, Schultz, 1924, and two new species of the genus. Pak. Jour. Sci. Res. 7:104-11.

Chitwood, B. G. and M. B. Chitwood. 1950. An introduction to Nematology. Section 1, Anatomy pp. 59-60. Monumental Printing Co., Baltimore, Md.

Erickson, A. B. 1938. Parasites of some Minnesota Cricetidae and Zapodidae, with a host catalogue of helminth parasites of native American mice. Amer. Midl. Nat. 20: 575-589.

Nazarova, IÛ. A. and N. M. Sveschinikova. 1930. Zum Stadium parasitischer Würmer der Nager der Autonomen Kasakschen. Vestnik Mikrobiol. Epimediol. i Parazitol Saratova. 9: 101-104.

Nitzsch, C. L. 1821. Ascaris. Allg. Encycl. d. Wissensch. u. Künste (Ersch and Gruber). Leipzig, 6: 44.

Popov, N. P. and IÛ. A. Nazarova. 1930. Neue Art parasitischer Würmer aus der Gattung Oxyuridae bei Mäusen. Vestnik Mikrobiol. Epimediol. i Parazitol Saratova. 9: 105-108.

Schultz, R., Ed. 1927. On the genus *Aspiculuris* Schultz, 1924 and two new species of it—*A. dinniki* and *A. asiatica*. Ann. Trop. Med. and Parasitol. 21: 267-275.

Teixeira de Freitas, J. F.; Herman Lent e J. Lins de Almeida. 1937. Paquena contribuicao ao estudo da fauna helminthologica da Argentina. Inst. Oswaldo Cruz Mem. 32: 195-209.

Teixeira de Freitas, J. F.; Herman Lent e J. Lins de Almeida. 1938. *Aspiculuris caviellae* a new name for *Aspiculuris schulzi* Freitas, Lent and Almeida, 1937, preoccupied. Proc. Helm. Soc. Wash. 5: 65.

Studies on *Xiphinema* spp. (Nematoda: Dorylaimoidea) from Aligarh (North India), with Comments on the Genus *Longidorus* Micoletzky, 1922

M. RAFIQ SIDDIQI*

It is for the first time that an account is being published of the so-called dagger nematodes, *Xiphinema* spp. from India. Loos (1949) reported five species of these nematodes from Ceylon, only one of which is found in Aligarh. These nematodes mostly attack valuable fruit trees and as, in many instances, they occur in enormous numbers, it is not unlikely that the dagger nematode situation is quite serious in this country. In all, five species of the genus *Xiphinema* Cobb, 1913, of which three are new, are described. This includes the description of two forms whose present position is still uncertain because they share some of the diagnostic characters of the genus *Longidorus* Micoletzky, 1922.

Xiphinema brevicaudatum Schuurmans Stekhoven, 1951 (Fig. 1, A-D)

MEASUREMENTS: 10 females: Length = 3.5-3.89 mm.; a = 125-138; b = 11.3-14.8; c = 87.5-109.4; V = $^{5.4-4.7}49.4\text{-}53.5\%^{4.8-5.4}$; spear = 65-68 microns; spear extension = 41-45.5 microns.

8 larvae: Length = 1.803-2.615 mm.; a = 95-112; b = 8.3-11.4; c = 51.5-68.2; spear = 49-57 microns; spear extension = 23-42 microns.

FEMALE (Holotype): Length = 3.55 mm.; a = 135; b = 14; c = 91; V = $^{5}50.1\%^{5.4}$.

Body long and slender, assuming a spiral form when the worm is killed by gradual heat, and tapering towards both the extremities. Anteriorly the neck expands and forms a knob-shaped head which is 9.5 microns in diameter. Two narrow lateral fields originate from the anterior end of the oesophagus as narrow streaks which later expand to become 2/9th and 2/7th as wide as the corresponding body diameter at the base of the neck and middle of the body respectively. There are two series of lateral pores arranged in two lines in each lateral field throughout the major portion of the body. These pores appear to lead into large hypodermal pouches arranged in a serial order beneath the lateral fields.

Body cuticle very finely striated, appearing in two layers, being thickest at the anterior end of the neck and the caudal terminus. Longitudinal striae also present. An *en face* view shows the usual sixteen labial papillae arranged in two circlets, namely, an inner of six and an outer of ten. Amphid apertures very minute and exceedingly difficult to see. Large amphids appear to encircle the head. Buccal spear long and attenuated, measuring 68 microns in length and joined posteriorly with its extension which is 42 microns in length. The latter bears distinct basal swellings in the form of flanges which are one-fifth as wide as the corresponding body diameter. Guiding ring situated at a distance of four labial diameters from the anterior end and approximately at about the level of the middle of the spear.

Oesophagus comprises two parts, the anterior slender portion which is 4.5 microns in diameter and a cylindrical posterior bulb, 64 microns long by 12 microns wide, containing oesophageal gland nuclei of which the nucleus of the dorsal oesophageal gland is distinct. Nerve ring situated posterior to the

*Department of Zoology, Aligarh Muslim University, Aligarh (U. P.), India.
The author is thankful to Dr. M. A. Basir under whose guidance this work was done.

base of the spear extension. Oesophago-intestinal cells form a well developed, bluntly rounded, conoid cardia which projects into the lumen of the anterior end of the intestine.

Vulva a slit-like aperture, situated at the middle of the body. Before leading into the lumen of the uteri, the vagina extends into two short, blunt diverticulae. Uterine chamber 25 microns long, with a prominent lumen. Uteri highly extensile, joined to their corresponding oviducts by a small, muscular valvular apparatus. Ovaries symmetrical, reflexed; oöcytes arranged in single file except for a short region of multiplication where they form double rows. Anterior ovary on the left and posterior on the right side of the intestine.

Intestine filled with large-sized food globules. Prerectum 8 per cent of the total body length. Rectum short, about one anal body diameter in length, opening outside through a conspicuous anus. Tail elongate-conoid, with a broadly rounded terminus. Caudal pores of the lateral fields probably three although not clearly seen.

Larvae: Body assumes a spiral form when killed by gradual heat. Lip region similar to that of female. Spear extension bears distinct basal flanges. A second spear invariably present in the anterior slender part of the oesophagus. Tail elongate-conoid.

Holotype: Female collected on November 1, 1957; slide deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

Paratypes: 9 females and 8 larvae collected from soil about roots of sugarcane; other data same as for holotype.

Host: Collected from soil around roots of sugarcane, *Saccharum officinarum* L.

Locality: Aligarh (U. P.), India.

Diagnosis and relationship: *Xiphinema* with a long slender body, with basal flanges on spear extension, and with spear guiding ring located much behind the lip region. This species is distinctive because of long, attenuated body; a well set off, knob-like head; spear guiding ring located near middle of the spear; location of vulva at middle of the body; and an elongate-conoid tail with obtuse terminus.

Xiphinema brevicaudatum is at once distinguished from all the known species of the genus *Xiphinema* by its knob-like head and anteriorly placed guiding ring. With these characters together with a slender body and large amphids it comes near to *Longidorus elongatus* (de Man) Thorne and Swanger, 1936, from which it differs in the more posteriorly located guiding ring and the presence of distinct flanges on the base of the spear extension.

The original description of *X. brevicaudatum* by Schuurmans Stekhoven (1951) is limited to a larval specimen which was collected around *Ageratum* roots in Belgian Congo.

Xiphinema indicum n. sp. (Fig. 1, E-G)

Measurements: 15 females: Length = 1.983-2.241 mm.; a = 50.8-60.5; b = 5.5-6.8; c = 24.5-30.9; V = 30.3-33.3%; spear = 101-106 microns; spear extension = 57-61 microns.

Larvae: Length = 0.75-1.371 mm.; a = 31.2-43; b = 3.4-4.2; c = 10.7-18.2; spear = 54-90 microns; spear extension = 39-51 microns.

Female (Holotype): Length = 2.19 mm.; a = 57.6; b = 6.8; c = 26.7; $V = {}^{6.8}30.3\%^{10.2}$.

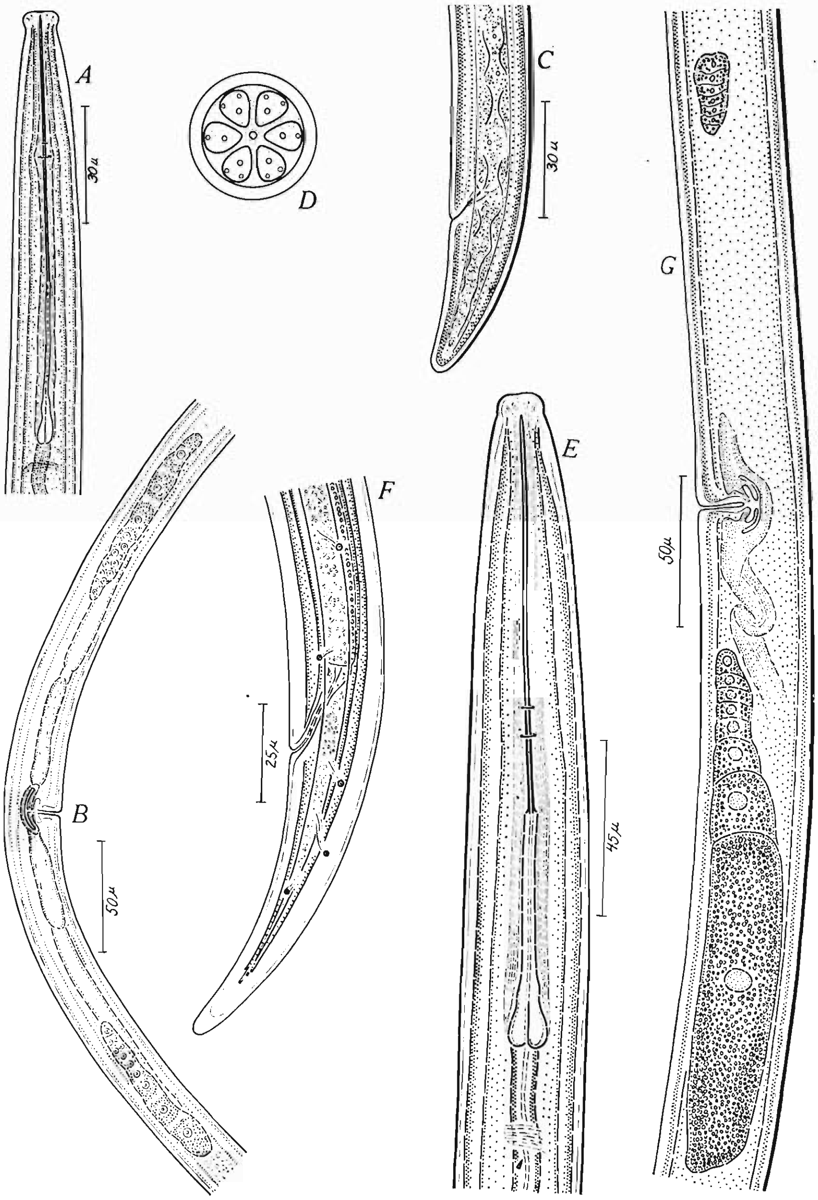


Figure 1. A-D—*Xiphinema brevicaudatum*. A. Anterior end of female. B. Reproductive region of female. C. Posterior end of female. D. *En face* view. E-G—*Xiphinema indicum*. E. Oesophageal region of female. F. Tail end of female. G. Reproductive region of female.

Body elongate, slender, attaining its maximum width at the region of vulva and tapering anteriorly to lip region which is marked off from the neck contour, and posteriorly to an elongate, evenly tapering tail. When killed the worm assumes a slightly arcuate position. Amphids crescent-shaped, located at the base of lateral lips. Lip region 11 microns in diameter and 5 microns high bearing six papillae in inner and ten in outer circlet. Lateral fields three-elevenths of body-width at mid-body region and bearing a double series of lateral pores. Posteriorly the former narrow considerably behind the level of the anus and end slightly anterior to the caudal terminus. Cuticle finely striated. It is thickest at the anterior end of the neck, at the region of the vulva and the tail terminus.

Spear long and slender, 105 microns in length. At the junction with its extension it splits up to give a firm attachment to the former. Spear extension 58 microns long bearing at its base large-sized flanges which are 10 microns wide. Due to slightly protruding position of the spear the two guiding rings are set at 27 microns apart, the posterior one encircling the anterior end of the spear extension. Normally they lie closer and slightly anterior to the proximal end of the spear, as shown in Fig. 1E.

About 25 microns behind the base of the spear extension and lying embedded in the wall of the anterior portion of the oesophagus is a triangular cuticularized piece resembling the apex of the spear. The same structure has also been found in X. *americanum* and X. *basiri*. No function has yet been assigned to it. As its position in the adult oesophagus is a constant feature it is presumed to be associated with formation of a new spear whenever the older one is shed or lost.

Nerve ring located 67 microns behind the base of the spear extension. Oesophageal bulb 80 microns long by 19 microns wide. Nucleus of the dorsal oesophageal gland distinct. Cardia conoid, not well developed. Anterior end of the intestine with a pronounced lumen. Intestinal cells packed with food granules.

Vulva a transverse slit, one-third the corresponding body diameter in width. From it, at right angles to the ventral surface of the body, a thick-walled vagina with cuticularized inner lining leads into the lumen of the uterine chamber. Before opening into the latter, the lumen of the vagina extends length-wise to the body for a short distance thus forming two symmetrical, short diverticulae. Details of the distal ends of the uteri and their oviducts obscure. Ovaries asymmetrical, reflexed. Anterior ovary rudimentary, 28 microns in length posterior one enormously developed being 176 microns long. In paratypes too the anterior ovary is never normal.

Prerectum conspicuously marked off from the intestine, 17 per cent of the total body length. Rectum 26 microns long, opening outside through a conspicuous anus. Tail elongate, slightly arcuate, evenly tapering, measuring 82 microns in length. It bears only three pairs of caudal pores which are arranged as illustrated (Fig. 1, F). Terminus of the tail hemispheroidal, smoothly rounded.

MALE: Unknown.

LARVAE: Lip region and body similar to that of female. A developing spear which is always larger than the functioning one is present in the oesophageal wall. Tail is proportionately longer than that of the adult female.

HOLOTYPE: Female collected on December 19, 1957; slide deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

PARATYPES: Hundreds of females collected about roots of *Grewia asiatica* L.; other data same as for holotype.

TYPE HOST: Collected from soil around roots of *Grewia asiatica* L.

TYPE LOCALITY: Aligarh (U. P.), India.

HOSTS AND GEOGRAPHICAL DISTRIBUTION: In Aligarh only the above-mentioned host has been found to be attacked by this parasite. However, specimens were also recovered by the author from soil samples collected around coffee roots in Ossoor Estate, Hasan District, south India in November, 1957. These samples were kindly sent by Dr. P. Soma Sekhar of the Coffee Research Station, Balehonnur, South India.

DIAGNOSIS AND RELATIONSHIP: *Xiphinema* with the above measurements and general description. It can easily be distinguished from other species of the genus by the anteriorly placed vulva, didelphic condition of the gonads, presence of a triangular, cuticularized piece in the anterior slender portion of the oesophagus, a uterine chamber and elongate, slightly arcuate, tapering tail with three pairs of caudal pores.

X. indicum comes closest to *X. insigne* Loos, 1949, but can be distinguished by having a slightly longer spear and only three pairs of caudal pores as compared to seven for the latter. It also resembles *X. ensiculiferum* (Cobb, 1893) Thorne, 1937, and *X. krugi* Lordello, 1955, but can be distinguished from them by its elongate, ventrally arcuate tail.

Xiphinema americanum Cobb, 1913 (Fig. 2, A-G)

MEASUREMENTS: 15 females: Length = 1.55-1.73 mm.; a = 41.5-45.5; b = 5-6.7; c = 45.5-48.1; V = 50.5-53.6%.

1 male: Length = 1.52 mm.; a = 51.5; b = 5.7; c = 46; T = 40%.

FEMALE (Neotype): Length = 1.622 mm.; a = 45; b = 5.8; c = 47.7; V = $^{16.8}53\%^{19.4}$.

Body cylindrical with tapering ends, assumes a spiral form when relaxed in hot water. Lateral fields one-fourth as wide as body diameter at the middle of the worm. Lateral pores not in definite lines. Lip region slightly set off from the body contour. An *enface* view shows six similar, perfectly amalgamated lips bearing an inner circlet of six and an outer of ten papillae. The papillae of the outer circlet are arranged in two definite levels (Chitwood and Chitwood, 1950) forming an inner circlet of four and an outer of six. The two lateral lips do not bear any such projections as described by Tarjan (1956). Amphids opening at the base of lateral lips through crescentic apertures, each measuring 4.5 microns wide.

Spear 79 microns in length, with a 45.5 microns long extension whose basal flanges measure 9 microns across. Double guiding rings, 18.5 microns apart, situated near the junction of the spear and its extension. A small, triangular, cuticularized structure present in the wall of the anterior slender portion of the oesophagus near its middle. Nerve ring about 40 microns posterior to base of the spear extension. Cardia large, conoid. Intestinal cells packed with food granules.

Vulva a transverse slit, situated near the middle of the body, leading into a thick-walled vagina which is two-fifths the vulvar body diameter long. Uteri long and highly extensile. Ovaries roughly symmetrical, reflexed, with oöcytes arranged in single file. Uterine egg measuring 157 microns by 27 microns. Prerectum not clearly distinguishable. Rectum short, about half the anal body diameter long. Tail conoid, with caudal pores as illustrated (Fig. 2, E).

MALE: Body similar to that of female. Spear 74 microns long; its extension measuring 47 microns in length. Nerve ring 20 microns behind the base of the spear extension. Cardia conoid, protruding into the lumen of the anterior end of the intestine.

Testes paired; the anterior 132 microns long, outstretched, the posterior 114 microns in length, directed towards the tail. Just behind the cap-cell the spermatogonia are not distinctly marked out but beyond it they occur in double rows for a short distance and then come to lie in multiple lines.

Besides a pair of adanal papillae there are six ventromedian supplementary papillae extending within a distance equal to 4 spicula lengths from the anus. They are spaced as illustrated (Fig. 2, F). Each papilla is distinctly elevated and bears a nerve ending. Copulatory musculature powerfully developed. Strongly built spicula 36 microns long, sharply curved in the middle. Each is re-enforced with a stout accessory piece which is joined posteriorly with its distal end and anteriorly with the proximal end of its ventral prong (Fig. 2, G). Protractors of the spicules prominent. Prerectum indistinct. Tail dorsally convex-conoid, with two pairs of caudal pores.

NEOTYPE: Female, collected around roots of citrus on March 18, 1957; slide deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

PARATYPES: Hundreds of females collected about roots of citrus trees and one male collected in March, 1957; other data same as for neotype.

HOSTS AND GEOGRAPHICAL DISTRIBUTION: *X. americanum* enjoys a wide range of distribution in U. S. A. Tarjan (1956) reported it as one of the most common phytonematode species of Rhode Island. Meyl (1954) reported this species from Ischia Island, Italy. Loos (1949) described only female specimens which were collected only once from soil of a coconut estate near Kurunegala, Ceylon. The author has collected specimens from at least ten districts of Uttar Pradesh, a North Indian state. Following hosts have been recorded from India: *Citrus limon* (L.) Burm., *C. aurantium* L., *C. sinensis* (L.) Osbeck, *Grewia asiatica* L., and *Magnifera indica* L.

DIAGNOSIS AND RELATIONSHIP: *Xiphinema* with the above measurements and general description. Distinctive because of its short body which becomes spirally coiled when the worm is killed by gradual heat, the length of the spear, position of the vulva and dorsally convex-conoid tail.

Present specimens appear to be identical with those described by Cobb (1913), Loos (1949) and Tarjan (1956). The male specimen described here is apparently smaller in size than that of Cobb (1913) and Oregon specimens of Tarjan (1956). However, it conforms very closely to the Rhode Island specimens described by Tarjan (l. c.) although there are certain differences, as for example, in the form and size of spicules and arrangement of the supplementary papillae—the characters which vary within this species as pointed out by Tarjan (l. c.).

Xiphinema basiri n. sp.* (Fig. 3, A-H)

MEASUREMENTS: 35 females (averages given within brackets): Length = 2.65-3.437 mm. (3.1 mm.); a = 57.3-71.9 (61.7); b = 6.4-8 (7.6); c = 61.8-80 (69); V = 49.8-52.6% (50.7%); spear = 111-125 microns (119 microns); spear extension = 57-63 microns (60.6 microns).

1 male: Length = 3.027 mm.; a = 70.3; b = 6.8; c = 64.3; T = ?

*Named after Dr. M. A. Basir.

Female (Holotype): Length = 2.76 mm.; a = 61.3; b = 7.1; c = 67.3; V = $^{9.2}50.1\%^{12.6}$.

Body long and cylindrical, regularly tapering towards the extremities. When killed by gradual heat the worm assumes a spiral form in which the posterior half is mainly curved and the anterior becomes slightly arcuate.

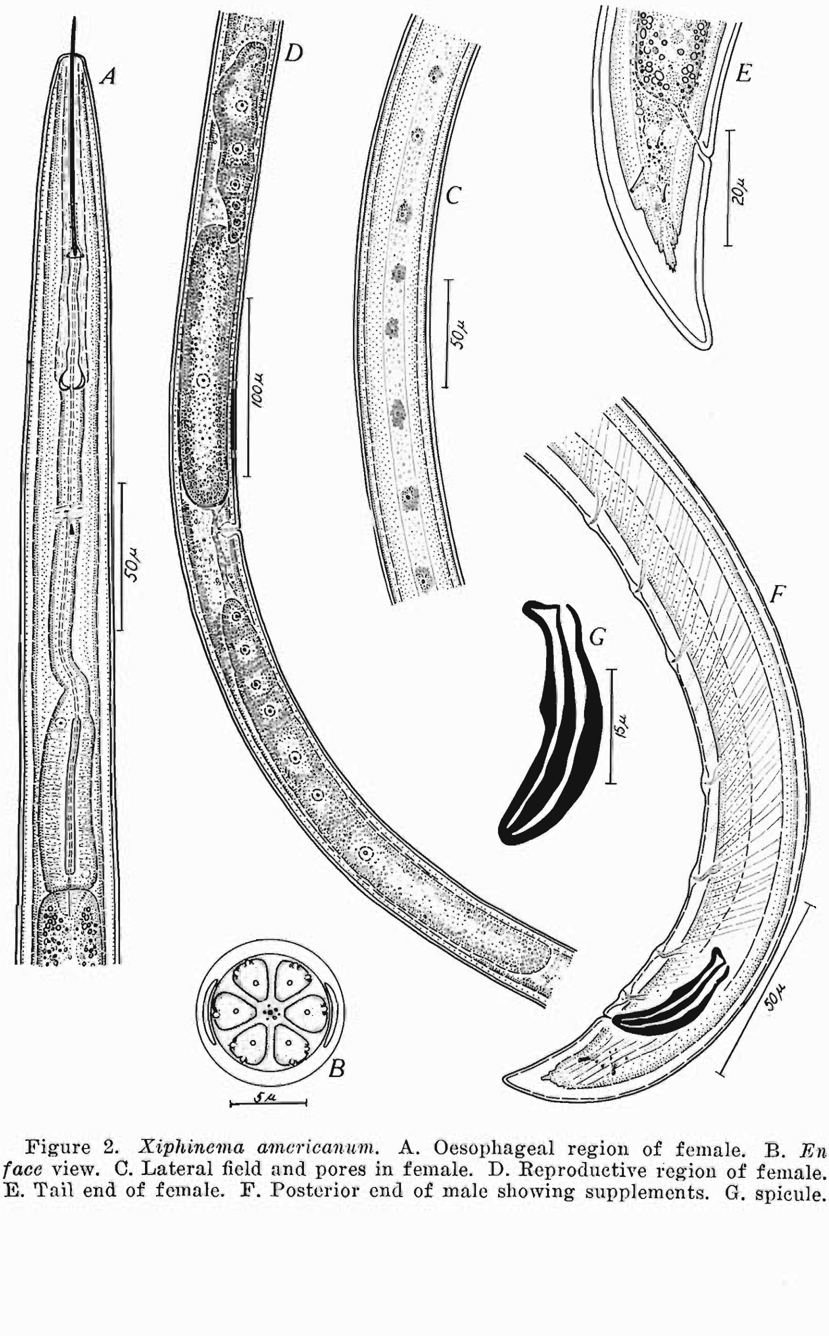


Figure 2. *Xiphinema americanum*. A. Oesophageal region of female. B. *En face* view. C. Lateral field and pores in female. D. Reproductive region of female. E. Tail end of female. F. Posterior end of male showing supplements. G. spicule.

Lip region only slightly marked off from the body. Anterior end somewhat truncated. Amphids stirrup-shaped, three-fifths of the labial diameter. An *en face* view shows a large central oral opening bounded by six similar, amalgamated lips bearing the usual six papillae in inner and ten in outer circlet. Lateral fields approximately one-fourth of the body diameter. Double series of lateral pores present throughout the body except in the anterior region where they are serially arranged. Dorsal and ventral series of pores present near the anterior end of the body.

Spear or odontostylet 112 microns long, with a 60 microns long extension which bears powerful flanges at its base. Two guiding rings, 22 microns apart, located near the junction of the spear with its extension. Nerve ring at 47 microns posterior to base of spear extension. A small triangular cuticularized piece resembling the apex of the spear is present at about the level of the beginning of its posterior third. Spear in larvae is shed with its guiding rings and a new larger spear which develops in the walls of the anterior slender portion of the oesophagus takes its position (Fig. 3, G.&H.). Oesophageal bulb contains a large nucleus of the dorsal oesophageal gland and the usual pairs of anterior and posterior submedian gland nuclei. Cardia large, conoid, protruding into the pronounced lumen of the anterior end of the intestine. Intestinal cells packed with granules of various sizes.

Vulva a depressed transverse slit, one-third the vulvar body diameter in width. Vagina thick-walled, leading into two short diverticulae. Powerful muscles, similar to those described by Lordello (1955) for *X. krugi*, are attached to vulva and vagina. Uterine chamber is 49 microns long, with thick muscular walls and enclosing a prominent lumen. Uteri long, with muscular walls. Ovaries symmetrical, reflexed; oöcytes mostly arranged in double rows. One egg in a paratype female measured 178 microns by 35 microns.

Prerectum 12.6 per cent of the total body length, marked off from the intestine by a lesser diameter and fewer granules in the cells. Rectum slightly longer than the anal body diameter, opening outside through a conspicuous anus. Tail sub-digitate with four pairs of caudal pores and finely, radially striated cuticle.

MALE (Allotype): Body similar to that of female. Spear 125 microns in length, with an extension measuring 63 microns.

Supplements consist of a pair of ventro-submedian papillae and a ventromedian series of four which are regularly spaced, the first beginning at about one-and-a-half spicula length from anus. Copulatory musculature strongly developed. Spicules similar, measuring 60 microns in length. They are distinctly cephalated and ventrally arcuate, a sharp curvature occurring at the middle. Each spicule is strengthened with two accessory rod-like pieces which are joined with its distal end and lie freely between the two prongs. The ventral piece is bifurcated at its distal half and measures 55 microns in length, the dorsal one is a solid piece measuring 33 microns long (the details of the spicules were examined while the worm was alive). Protractor and retractor muscles of the spicula very distinct.

Prerectum clearly marked off from intestine, 10.9 per cent of the total body length. Tail similar to that of female, with four pairs of caudal pores.

LARVAE: Length = 0.79-2.51 mm.; a = 39-57; b = 3.7-6; c = 17.5-44.8; spear 47 = 107 microns.

Body becomes acruate on death. A developing spear, larger than the functioning one, is always present in the anterior slender portion of the oesophagus. Tail cylindrical, tapering to a bluntly rounded terminus.

HOLOTYPE: Female collected on April 8, 1957; slide deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

ALLOTYPE: Male collected on April 10, 1957 from soil about roots of *Citrus sinensis* (L.) Osbeck; other data same as for holotype.

PARATYPES: Hundreds of females; other data same as for holotype.

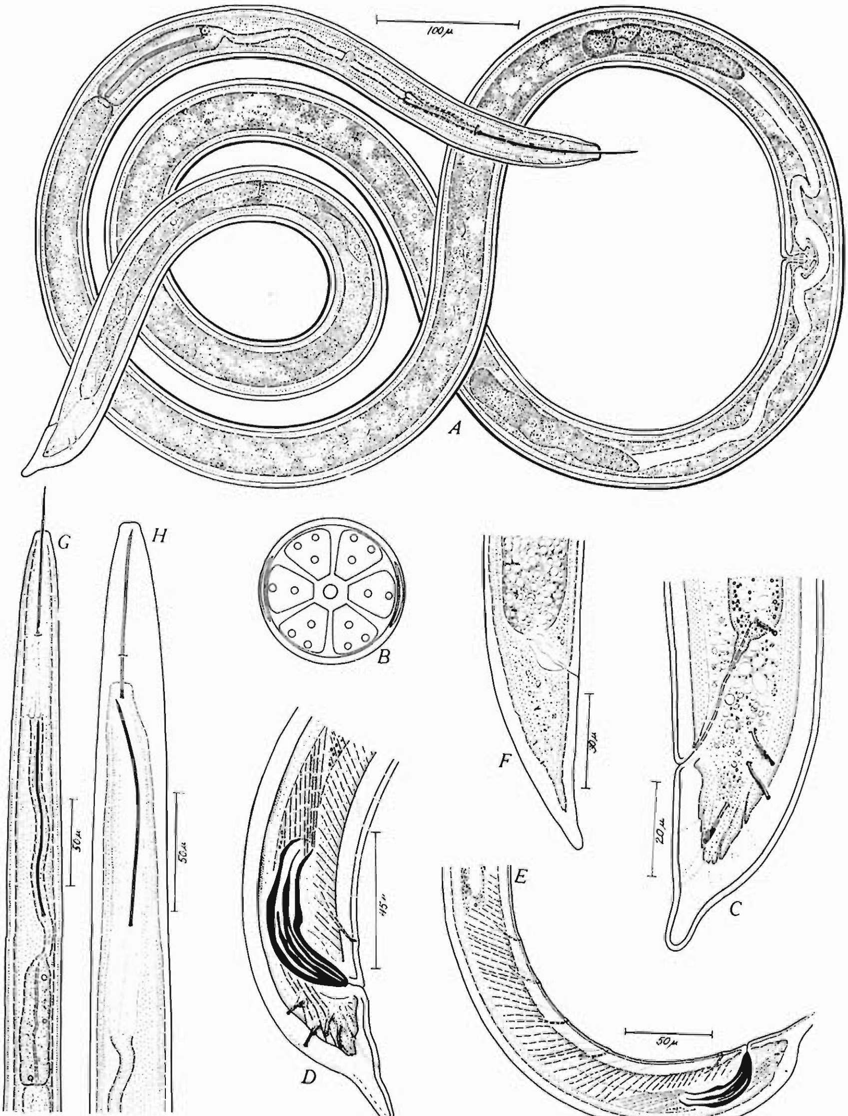


Figure 3. *Xiphinema basiri*. A. Female. B. *En face* view. C. Tail end of female. D. Tail end of male. E. Posterior end of male showing supplements. F. Larval tail showing anlage of spicules. G. Oesophageal region of larva showing fully developed spear in oesophagus. H. Anterior end of moulting larva.

TYPE HOST: *Citrus sinensis* (L.) Osbeck.

TYPE LOCALITY: Aligarh (U. P.), India.

HABIT, HABITAT AND GEOGRAPHICAL DISTRIBUTION: Ectoparasitically feeding on roots of oranges, *Citrus sinensis* (L.) Osbeck. Lives in soil around roots and forms colonies of large numbers of females and larvae in various stages of development. Specimens have also been collected from soil about roots of oranges in Jhansi (U. P.), a town about 300 miles south of Aligarh. It is assumed that this parasite is fairly well distributed in U. P.

DIAGNOSIS AND RELATIONSHIP: *Xiphinema* with the above measurements and genral description. It can easily be recognized by its body length, 2.65-3.437 mm.; spear measuring 111-125 microns in length; vulva located just at the middle of the body; presence of a uterine chamber; ovaries with most of the oöcytes arranged in double rows; a sub-digitate tail; four submedian supplements and size and structure of the spicules.

X. basiri is most closely related to *X. index* Thorne and Allen, 1950, and *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939. It is distinguished from *X. index* by a slightly marked off lip region, a longer spear, a more posterior position of the vulva, presence of a uterine chamber and large guiding pieces and musculature of the spicula. It differs from *X. diversicaudatum* in having a shorter spear, more posterior position of vulva, greater width of the body, greater length of the neck, the smaller ratio of the tail with the body, presence of only four pairs of male caudal pores as compared with six for *diversicaudatum* and a smaller length of the body.

It has also some affinities with *X. mammilatum* Schuurmans Stekhoven and Teunissen, 1938, because of its body length and digitate tail but can easily be distinguished by having a shorter spear and more posteriorly located vulva.

Xiphinema citri n. sp. (Fg. 4, A-G)

MEASUREMENTS: 8 females: Length = 6.73-7.448 mm.; a = 122-142; b = 12-14; c = 183-197; V = 43.3-44.8%; spear = 128-139 micron; spear extension = 70-75 microns.

FEMALE (Holotype): Length = 6.8 mm.; a = 141; b = 13.6; c = 183; $V = {}^{3.8}43.3\%^{4.4}$.

Body almost cylindrical, only slightly tapering at both ends, becoming spirally coiled when the animal is killed by gradual heat. Lip region smoothly rounded, set off from the neck, measuring 18 microns wide by 5.5 microns high. Behind the lip region the two lateral fields begin as narrow strips formed by two lines which regularly widen out to become one-eighth of the body diameter at the base of the neck. The fields become one-fifth as wide as the body diameter on the middle of the worm. They attain their maximum width at the posterior end of the body. There is a series of lateral hypodermal pouches which open to the exterior through pores which correspond in number with them. At the anterior end these pores are arranged in one line but behind the oesophageal region the openings are irregularly displaced and appear to form a double series. The first pore from the anterior end is situated 24 microns behind the lip region.

An *en face* view shows an inner circlet of six and an outer of ten labial papillae. The papillae of the outer circlet are arranged in two different levels; those close to amphid apertures lie nearer to the base of the lips (Fig. 4, D). Amphid apertures very minute and difficult to see. Amphids only 7 microns wide, not encircling the head completely (Fig. 4, E).

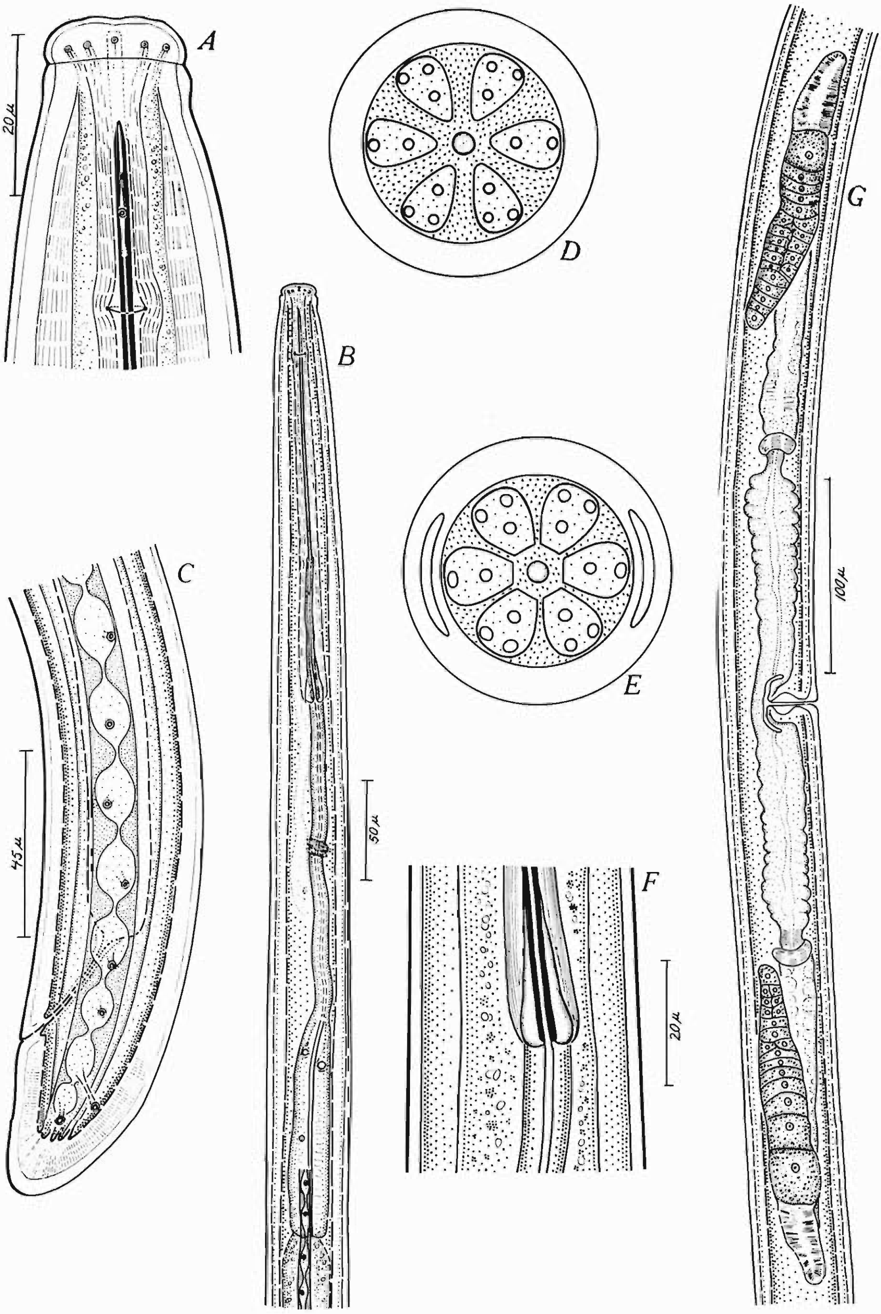


Figure 4. *Xiphinema citri.* A. Anterior end of female. B. Oesophageal region of female. C. Posterior end of female. D. *En face* view. E. Amphids at the base of the lip region. F. Flanged base of spear extension. G. Reproductive region of female.

Odontostylet 133 microns in length; its extension measuring 72 microns long, with distinct basal flanges at its base (Fig. 4, F). A new spear is formed in the walls of the anterior slender portion of the oesophagus. Spear guiding ring located two cephalic diameters posterior to the anterior extremity. Anterior slender portion of the oesophagus a narrow tube, enveloped by nerve ring at slightly anterior to its middle. Oesophageal bulb cylindroid, 112 microns long by 2 microns wide. Nucleus of the dorsal oesophageal gland prominent. No distinct cardia present. Intestine with a large lumen at its anterior end. Intestinal cells packed with food granules of uniform size.

Genital organs consist of a transverse, slit-like vulva located anterior to middle of the body, an extensible vagina leading nearly half-way in the body into a uterine chamber with a well-marked lumen, two uteri with highly extensile walls and a pair of oviducts and ovaries. A muscular valve is present at the junction of each uterus with its oviduct. Walls of the oviducts also extensile. Ovaries similar and reflexed.

Prerectum about 400 microns long. Rectum almost equal to the anal body diameter, opening outside through a conspicuous anus. Tail dorsally convex-conoid with two pairs of lateral hypodermal pouches and their corresponding pores. Caudal terminus obtuse, smoothly rounded.

HOLOTYPE: Female collected on August 7, 1957; slide deposited with the Zoolozy Museum, Aligarh Muslim University, Aligarh (U. P.), India. Male: unknown.

PARATYPES: 7 Females and 5 larvae; other data same as for holotype.

TYPE HOST: Collected from soil about roots of *Citrus limon* (L.) Burm.

TYPE LOCALITY: Aligarh (U. P.), India.

DIAGNOSIS AND RELATIONSHIP: *Xiphinema* because of slender body, amphids not encircling the head completely and presence of flanges on the base of the spear extension. The species is distinctive because of the greatly attenuated body, a well set off lip region, a long spear with its guiding ring located near its apex, position of the vulva and a short, dorsally convex-conoid tail.

In its large size of the body *X. citri* resembles *X. cylindricaudatum* Schuurmans Stekhoven & Teunissen, 1938, but can easily be distinguished by its extremely slender body, anteriorly located nerve ring, smaller length of the oesophagus, more anteriorly placed vulva and a short, dorsally convex-conoid tail.

GENUS *Longidorus* Micoletzky, 1922

The characters by which the genus *Longidorus* Micoletzky, 1922, is differentiated from its nearest relative *Xiphinema* Cobb, 1913, are the following:

1) Body long and greatly attenuated.
2) Amphids abnormally large, practically encircling the head; amphid aperature very minute and difficult to see.
3) Spear greatly attenuated, with guiding ring located near its apex.
4) Spear extension without basal swellings or flanges.

The species described here as *Xiphinema brevicaudatum* is close to *Longidorus* in having (1) a long, attenuated body, (2) large amphids which envelop the head and (3) a greatly attenuated spear with forwardly placed guiding ring. Similarly *X. citri* has a long, attenuated body and a slender spear with guiding ring located near its apex. But, due to the fact that both of these forms possess flanges or basal swellings on the spear extension, these have been grouped under *Xiphinema*.

Further findings of such forms as *X. brevicaudatum* and *X. citri* might help in either erecting a new genus lying intermediate between *Longidorus* and *Xiphinema* or even challenging the validity of the former.

LITERATURE CITED

CHITWOOD, B. G. and M. B. CHITWOOD. 1950. An introduction to nematology. Section 1. Monumental Printing Co., Baltimore, Maryland.

COBB, N. A. 1913. New nematode genera found inhabiting freshwater and non-brackish soils. Jour. Wash. Acad. Sci. 3: 432-444.

LOOS, C. A. 1949. Notes on free-living and plant-parasitic nematodes of Ceylon—No. 5. Jour. Zool. Soc. India. 1: 23-29.

LORDELLO, LUIZ GONZAGA E. 1955. *Xiphinema krugi* n. sp. (Nematoda, Dorylaimidae) from Brazil with a key to the species of *Xiphinema*. Proc. Helm. Soc. Wash. 22: 16-21.

MEYL, A. H. 1954. Die bisher in italien gefundenen fgreilebenden Erd-und Süsswasser-Nematoden. Archiv. Zoologico Italiano. 39: 161-265.

MICOLETZKY, H. 1922. Die freilebenden Erd-Nematoden. Archiv. f. Naturgesch. Berlin (1921). 87A: 1-650, figs. 1-55n.

————. 1927. Neue und seltene freilebende Nematoden aus dem Wolgagebiet (Kama). Zool. Anz. 73: 113-123.

SCHUURMANS STEKHOVEN, J. H., and R. J. H. TEUNISSEN, 1938. Exploration du Parc National Albert, Mission G. F. de Witte. Nématodes libres terrestres. Inst. des Parcs Nationaux du Congo Belge. Bruxelles. 22: 229 pp.

SCHUURMANS STEKHOVEN, J. H. 1951. Nématodes saprozoaires et libres du Congo Belge. Mém. Inst. Royal Sci. Nat. Belgique, 39: 1-79.

TARJAN, A. C. 1956. Known and suspected plant-parasitic nematodes of Rhode Island II. *Xiphinema americanum* with notes on *Tylencholaimus brevicaudatus* n. comb. Proc. Helm. Soc. Wash. 23: 88-92.

THORNE, G. and M. W. Allen. 1950. *Paratylenchus hamatus* n. sp. and *Xiphinema index* n. sp., two nematodes associated with fig roots, with a note on *Paratylenchus anceps* Cobb. Proc. Helm. Soc. Wash. 17: 27-35.

THORNE, G. 1939. A monograph of the nematodes of the superfamily Dorylaimoidea. Capita Zoologica 8: 1-261.

Errata

In the paper by Hwang (Proc. Helm. Soc. Wash. 26(1): 47-50. 1959) the specific name of the quill mite is misspelled in the title, the c and t having been transposed. The correct spelling as used in the text is *S. bipectinatus*. On page 48 the plate was printed upside down resulting in the misidentification from the legend. To correct read "right" in place of left and "center and left" in place of center and right.

Report of the Brayton H. Ransom Memorial Trust Fund

FUNDS ON HAND, Jan. 1, 1958		$1,821.79
RECEIPTS:	Interest rec'd in 1958	60.59
	Contribution to principal	5.00
DISBURSEMENTS:	Grant to Helminthological Society of Washington	10.00
BALANCE ON HAND, Dec. 31, 1958		$1,877.38

A. O. FOSTER
Secretary-Treasurer

New Book

CHRSTIE, J. R. **Plant Parasitic Nematodes, Their Bionomics and Control**—This book summarizes the available information on life cycles, habitats, plant hosts, nature of injury, distribution, and control of the nematodes known to injure plants. Taxonomy and morphology are not included. Emphasis is on control. Published by the Florida Experiment Station (address: The Editor), Gainesville, Florida. 1959, 256 pp. $3.75 domestic, $4.00 foreign (tax and mailing included).

MINUTES

Three hundred fifty-seventh through the three hundred sixty-fourth meetings

357th meeting: Maloney Chemistry Laboratory, Catholic University of America, Washington, D. C., 22 October, 1958. Buhrer reported Society is exempted from paying Federal Income Tax. Paper presented: The VIth International Congresses of Tropical Medicine and Malaria, by Beye; the July 1958 Colloquim of the International Commission on Zoological Nomenclature, by Wharton; the 1958 World's Poultry Congress, by Wehr; Impressions of Veterinary Science in U.S.S.R., by Enzie.

358th meeting: Log Lodge, Agricultural Research Center, Beltsville, Md., 21 November, 1958. Nominating committee presented following slate of officers: M. A. Stirewalt, President; G. W. Luttermoser, Vice-President; E. M. Buhrer, Corresponding Secretary, and D. R. Lincicome, Recording Secretary. A. L. Taylor elected to Editorial Committee for 1959. C. M. Herman appointed as representative to Washington Acad. Science. Affiliation with American Society of Parasitologists tabled. Grace S. Cobb appointed Assistant Corresponding Secretary-Treasurer. Papers presented: Impressions of Veterinary Science in U.S.S.R. (part 2), by Enzie; Cephalic structure of *Haemonchus,* by Douvres; Treatment of dog demodectic mange, by Colglazier; Materials to replace charcoal in fecal cultures, by Shorb; effect of parasite exposure and grain supplementation in Hereford yearlings on fescue pasture, by Vegors, Ciordia, Bizzel and Baird; Axenic culture of *Entamoeba invadens,* by Diamond; Infection of Sheep and Goats with Coccidia, by Leek.

359th meeting: McMahon Hall, Catholic University, Washington, D. C., 17 December, 1958. Slate of officers presented at 358th meeting elected. Papers presented: Lower threshold for immunity to *Nippostrongylus* in rats, by Hurley; Transfer of immunity to *Nippostrongylus* from mother to young, by Langenkamp; Parasitic barnacles and Charles Darwin, by Reishman; New wildlife disease journal published on microcards, by Herman; Chigger pathogenic to black duck, by Clark.

360th meeting: Naval Medical Research Institute, 21 January, 1959. Annual Editorial Board report read by Foster. Affiliation with the American Society of Parasitologists voted with provision that society can not comply with Art. VI, Sec. 4 of the American Soc. Parasitologists' By-Laws. Papers presented: Development of avian malaria exoerythrocytic stages in tissue culture, a motion picture by Huff, Pipkin, Weathersby and Jensen; Interference Microscope study of living exoerythrocytic stages of *Plasmodium fallax,* motion picture by Weathersby, Jensen and Shiroishi; Biophysical properties of isolated CHR and cercarial agglutinating factor from human antiserum to *Schistosoma mansoni,* by Evans and Stirewalt; Trypanosome growth-factor reduction by starvation, by Lincicome and Hinnant; motion picture on Middle East Hydatid Disease, by Pipkin.

361st meeting: Home Economics building, University of Maryland, College Park, 18 February, 1959. Announcement made of receipt of honorary doctor's degree by McIntosh from University of Miami. Annual report of treasurer read. Following action of Executive Committee read: voted $30.00 as contribution to Washington Acad. Sci.; approved increase in size of *Proceedings* to 96 pp. for the next 2 issues; increased subsidy to corresponding secretary's office to $500.00. Herman reported activity of Wash. Acad. Sci. during Science and Architecture Day Celebration. Papers presented: Reactions of the Krebs cycle in infective larvae of *Strongyloides papillosus*, by Costello; Filarial infection in gray squirrels, by Price; Tactic responses of *Nippostrongylus* infective larvae, by Parker and Haley; Single oral treatment with systematic insecticide of parakeet mange, by Yunker; Gold chloride and picric acid iodine as nematode stains, by Hasbrouck.

362nd meeting: Wilson Hall, Nat. Institutes of Health, Bethesda, 17 March, 1959. $83.00 transferred from General Fund to Publication Fund. Papers presented: Parasitology evolves, by Andrews; Liver glycogen and lipid in parasitic infections, by Mercado and von Brand; congenital transmission of toxoplasmosis, by Remington; Quinine and metabolism of malaria parasites, by Schellenberg; Low concentration of sodium pentachlorophenate effect on *Australorbis* fecundity and eggs, by Olivier.

363rd meeting: School of Hygiene and Public Health, Johns-Hopkins Univ., Baltimore, 17 April, 1959. Approved motion whenever minimum number of 25 copies of any issue of *Proceedings* is reached, editor is authorized to reprint 200 copies provided cost doesn't exceed $300.00. Approved motion setting cost of back issues of *Proceedings* (5 yrs. or more old) at $4.00 except to agencies ($3.75). Motion passed to contribute $35.00 for the annual picnic meeting to be held on 23 May 1959. Papers presented: Kinetics of glucose uptake by *Hymenolepis*, by Phifer; Mechanism of infection of *Aedes aegypti* by *Plasmodium gallinaceum*, by Howard; Kinetics or urea permeation in shark helminths, by Simmons; comparative hibernation mechanisms in *Culex pipiens* and *C. fatigans*, by Tekle. Demonstrations on echinococcosis by Schiller and electron microscope micrographs of *Hymenolepsis* and *Schistosoma* by Rothman and Inatomi followed presentation of papers.

364th meeting: Annual picnic at Log Lodge, U. S. Dept. Agriculture Research Center, Beltsville, Md., 23 May 1959.

The following were elected to membership at the meetings indicated: 357th—F. Dao D.; R. Davis; V. F. Flyger; G. D. Griffin; E. J. Hugghins; S. H. Lee; R. H. Mulney; O. Maugn; Liang-Yu Wu; A. Ostretkar; D. Mettrick; J. C. Frandsen; R. I. Anderson; B. J. Jaskoski; 358th—R. J. Boisvenue; E. R. Hasbrouck; F. Shafiiee; 359th—A. Di Edwardo; H. T. Stren; Mohammad Rafiq Siddiqi; 360th—F. N. Katz; D. Jensen; D. F. Marchbank; A. C. Pipkin; A. B. Weathersby; 361st—B. P. Marapao; L. A. Terzian; T. Shiroishi; E. J. L. Soulsby; A. R. Maggenti; P. Schroeder; 362nd—H. K. Beye; M. N. Lunde; M. Ichinohe; P. A. McMahon; G. Wertheim; Seuti Inatomi; M. H. Satti; 363rd—S. Krassner; G. L. Hoffman; H. L. Ching; E. H. Sadun; 364th—G. N. Johri, E. S. Robinson, N. Tolgay; O. Vargas.

D. R. LINICOME
Recording Secretary

CONTENTS, VOLUME 26

PAGE

BABERO, BERT B. AND JACQUELINE R. SHEPPERSON. On the Occurrence of Gnathostomes in Georgia, U. S. A. ... 53

BRAYTON H. RANSOM MEMORIAL TRUST FUND ... 164

BROWN, GEORGIANNA L. Three New Species of the Genus *Paratylenchus* from Canada (Nematoda: Criconematidae) ... 1

CAVENESS, FIELDS E. *Trophurus minnesotensis* (Caveness, 1958), n. comb. ... 64

CAVENESS, FIELDS E. AND J. E. BOSHER. On the Misuse of the Term "Incisure" ... 124

CHENG, THOMAS C. The Histology of the Prostate Mass in the Genus *Acanthatrium* (Trematoda: Lecithodendrium) ... 111

CHRISTIE, J. R. Plant Parasitic Nematodes, Their Bionomics and Control ... 164

COLGLAZIER, MERLE E., EDWARD H. WILKENS, AND DAVID K. CHESTER. Action of Piperazine Against Mixed Infections of *Ancylostoma caninum* and *Uncinaria stenocephala* in Dogs ... 8

DALY, EDWARD F. A Study of the Intestinal Helminths of the Southern Crow (*Corvus brachyrhynchos paulus*) in Virginia ... 66

ERRATA—HWANG, JOSEPH C. ... 163

FISCHTHAL, JACOB H. AND ROBERT E. KUNTZ. Trematode Parasites of Fishes from Egypt. Part I. *Basidiodiscus ectorchis*, n. gen., n. sp., and *Sandonia sudanensis* McClelland, 1957 (*Paramphistomidae*) ... 32

GODEBERG, AARON AND JOHN T. LUCKER. Survival on Pasture of Larvae of Gastrointestinal Nematodes of Cattle. II. Spring Contamination 37

HARGIS, JR., WILLIAM J. Systematic Notes on the Monogenetic Trematodes ... 14

HERLICH, HARRY. Experimental Infections of Cattle with the Stomach Worms, *Ostertagia ostertagi* and *Trichostrongylus axei* ... 97

HIRSCHMANN, HEDWIG. Histological Studies on the Anterior Region of *Heterodera glycines* and *Hoplolaimus tylenchiformis* (*Nematoda*, Tylenchida) ... 73

HURLEY, FRANCIS J. Immunization Threshold in Laboratory Rats Given a Small Initial Infection of *Nippostrongylus muris* ... 91

HWANG, JOSEPH C. Case Reports of the Quill Mite, *Syringophilus bipectinatus*, in Poultry ... 47, 163

KRUIDENIER, FRANCIS J. AND KRISHNA N. MEHRA. *Aspiculuris ackerti*, n. sp., (Nematoda: Oxyuridae) from the Wood Rats of Arizona 147

LAUTENSCHLAGER, EDWARD W. Meningeal Tumors of the Newt Associated with Trematode Infection of the Brain 11

LOOS, CLIVE A. Symptom Expression of Fusarium Wilt Disease of the Gros Michel Banana in the Presence of *Radopholus similis* (Cobb, 1893) Thorne, 1949 and *Meloidogyne incognita acrita* Chitwood, 1949 103

LUCKER, JOHN T. AND RALPH F. HONESS The Infective Larva of *Nematodirella longispiculata antilocaprae* (Nematoda: Trichostrongylidae) 43

MINUTES, Three hundred fifty-seventh Through the three hundred sixty-fourth Meetings 165

NEW BOOK—See Christie, J. R. 164

RAUSCH, ROBERT. Studies on the Helminth Fauna of Alaska. XXXV. On the Identity of Certain Cestodes (Taeniidae) from Foxes 125

ROBINSON, TREVOR AND A. L. NEAL. The Influence of Certain Mineral Elements on Emergence of Golden Nematode Larvae 60

SCHAD, G. A. A Revision of the North American Species of the Genus *Skrjabinema* (Nematoda: Oxyuroidea) 138

SIDDIQI, M. RAFIQ. Studies on *Xiphinema* spp. (Nematoda: Dorylaimoidea) from Aligarh (North India), with Comments on the Genus *Longidorus* Micoletsy, 1922 151

TAYLOR, DONALD P. The Male of *Scutellonema brachyurum* (Steiner, 1938) Andrassy, 1958 51

THOMAS, H. A. On *Criconemoides xenoplax* Raski, with Special Reference to its Biology under Laboratory Conditions 55

TROMBA, F. G. A Technique for Oral Infection of Earthworms 65

TURNER, JAMES H. Experimental Strongyloidiasis in Sheep and Goats. III. An Attempt to Induce Passive Immunity in Lambs; Changes in Serum Proteins after Infection; and Effect of Immune Serum on Infective Larvae 114

VAN GUNDY, S. D. The Life History of *Hemicycliophora arenaria* Raski (Nematoda: Criconematidae) 67

WEBER, THOMAS B. AND JOHN T. LUCKER. Immunity Against the Cattle Lungworm: Resistance Resulting from Initial Infection with Small Numbers of Larvae 132

MEMBERS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The following membership list, arranged geographically, includes life, resident, and non-resident members, as defined in Art. 3 of the Constitution (Vol. 13, No. 1 of the Proceedings).

Alabama
Cairns, E. J.
Herlich, H.
Hopper, B. E.
Lesser, E.
Porter, D. A.
Price, E. W.
Rohrbacher, G. H., Jr.
Smith, W. N.
Thorson, R. E.

Alaska
Rausch, R.

Arizona
O'Bannon, J. H.
Reynolds, H. W.

California
Allen, M. W.
Allen, P. H.
Ayoub, S. M.
Baker, N. F.
Chitwood, B. G.
Dao.D., F.
Dougherty, E. C.
Golden, A. M.
Golden, Thelma S.
Hart, W. H.
Heyneman, D.
Ichikawa, S. T.
Larson J. E.
Lear, B.
Lider, L. A.
Lownsbery, B. F.
Lownsbery, Joyce W.
Mankau, R.
McBeth, C. W.
Raski, D. J.
Sher, S. A.
Van Gundy, S. D.
Voge, Marietta
Youngson, C. R.

Canal Zone
Grocott, R. G.

Colorado
LaMotte, L. C., Jr.
Olsen, O. W.
Rubin, R.
Stabler, R. M.

Connecticut
Bray, R. L.
Miller, P. M.
Penner, L. R.
Pistey, W. R.

Delaware
Fielding, M. J.

District of Columbia
Anderson, R. I.
Ballard, Ethyl L.
Barron, C. N.
Briscoe, M. S.
Bruce, J. I., Jr.
Buchheit, J. R.
Durbin, C. G.
Duxbury, R. E.
Fox, R. C.
Gould, D. J.
Harry, H. W.
Hurley, F. J.
Kenworthy, F. T.
Lincicome, D. R.
Mollari, M.
Needy, C. F.
Ostdiek, J. L.
Sarles, M. P.
Seitner, P. G.
Slie, Rita B.
Taylor, C. R.
Watson, R. L., Jr.
Young, Viola M.

Florida
Birchfield, W.
Brothers, S. L.
Christie, J. R.
Esser, R. P.
Feldmesser, J.
Hannon, C. I.
Hunter, G. W., III
Kincaid, R. R.
Overman, Amegda J.
Perry, V. G.
Rau, G. J.
Short, R. B.
Sledge, E. B.
Tarjan, A. C.
Thames, W. H., Jr.
Whitton, G. M., Jr.

Georgia
Babero, B. B.
Bogitsh, B. J.
Denton, J. F.
Good, J. M., Jr.
Kagan, I. G.
Steele, A. E.
Stewart, T. B.

Hawaii
Alicata, J. E.
Goto, S.

Illinois
Bauman, P. M.
Elishewitz, H.
Garoian, G.
Jaskoski, B. J.
Jeske, H. A.
Krueger, H. J.
Kruidenier, F. J.
Levine, N. D.
Linford, M. B.
Otto, G. F.
Thomas, L. J.
Walton, A. C.

Indiana
Dunagan, T. T.
Ehrenford, F. A.
Shumard, R. F.

Iowa
Hsü, H. F.

Kansas
Ackert, J. E.
Ameel, D. J.
Hansen, M. F.

Kentucky
Baxter, L. W., Jr.
Chapman, R. A.
Valleau, W. D.

Louisiana
Cordray, D. R.
Cross, H. F.
Hollis, J. P., Jr.
Martin, W. J.
Mayhew, R. L.
Newsom, L. D.
Wehunt, E. J.
Whitlock, L. S.

Maine
Meyer, M. C.

Maryland
Anastos, G.
Andrews, J. M.
Andrews, J. S.
Bang, F. B.
Becklund, W. W.
Borsos, T.
vonBrand, T.
Buhrer, Edna M.
Cheng, T. C.
Chitwood, May B.
Clark, G. M.
Clifford, C. M., Jr.
Coatney, G. R.
Cobb, Grace S.
Coleman, E.
Colglazier, M. L.
Daly, E. F.
DeWitt, W. B.
Diamond, L. S.
Doss, Mildred A.
Douvres, F. W.
Elbl, Alena
Erickson, D. G.
Enzie, F. D.
Evans, A. S.
Farr, Marion M.
Flyger, V. F.
Foster, A. O.
Freytag, R. E.
Gardiner, J. L.
Goldberg, A.
Grayson, Margaret A.
Habermann, R. T.
Haley, A. J.
Hasbrouck, E. R.
Hawkins, Naomi
Herman, C. M.
Hill, C. H.
Huff, C. G.
Humphrey, Judith M.
Hwang, J. C.
Jacobs, L.
Jahnes, W. G.
Jenkins, W. R.
Johnson, H. W.
Jones, Myrna F.
Kates, K. C.
LaRue, G. R.
Leek, R. G.
Lotze, J. C.
Lucker, J. T.
Lund, E. E.
Luttermoser, G. W.
McIntosh, A.
McLoughlin, D. K.
Newton, W. L.
Olivier, L. J.
Phillips, B. P.
Price, D. L.
Read, C. P.
Reardon, Lucy V.
Rohde, R. A.
Rothman, A. H.
Rozeboom, L. E.
Schiller, E. L.
Schindler, A. F.
Schwartz, B.
Shafiee, F.
Shorb, D. A.
Sonenshine, D.
Sonnenberg, Bessie
Spindler, L. A.
Spruyt, F. J.
Stirewalt, Margaret A.
Taylor, A. L.
Tromba, F. G.
Turner, J. H.
Underwood, P. C.
Vegors, H. H.
Wehr, E. E.
Weinstein, P. P.
Wharton, G. W.
Wilkens, E. H.
Wilson, G. I.
Wright, W. H.
Yunker, C. E.

Massachusetts
Boyd, Elizabeth M.
Riser, N. W.

Michigan
Clark, D. T.
Cook, Alice R.
Cowan, A. B.
DeGiusti, D.
Dikmans, G.

Minnesota
Taylor, D. P.

Mississippi
Knight, R. A.

Missouri
Santmyer, P. H.

Montana
Jellison, W. L.
Senger, C. M.

Nebraska
Coil, W. H.
Kelley, G. W., Jr.
Manter, H. W.
Pritchard, Mary H.
Schuster, M. L.

New Jersey
Goble, F. C.
Haenseler, C. M.
Lynch, J. E.
McCoy, E. E., Jr.
Tiner, J. D.

New Mexico
Allen, R. W.
Locke, L. N.
Massey, C. L.
Samson, K. S.

New York
Betz, D. O., Jr.
Braun, A. J.
Dropkin, V. H.
Fischthal, J. H.
Friedman, W.
Granek, I.
Ingalls, J. W., Jr.
Mai, W. F.
Neal, A. L.
Spaeth, F. W.
Stoll, N. R.
Stunkard, H. W.
Teodorovic, S.
Wilson, W. D.

North Carolina
Burton, P. R.
Cort, W. W.
Drolsom, P. N.
Garriss, H. R.
Hirschmann, Hedwig
Ruehle, J. L.
Sasser, J. N.

Ohio
Etges, F. J.
Harwood, P. D.

Oklahoma
Lee, S. H.
Smith, P. E.

Oregon
Jensen, H. J.
Macy, R. W.
Smithson, Harriet R.

Pennsylvania
Bloom, J. R.
Gilford, J. H.
Graham, G. L.
Martin, H. M.
Meinkoth, N. A.
Uricchio, W. A.

Puerto Rico
Ayala-Almodovar, A.
Jachowski, L. A., Jr.
Oliver-Gonzales, J.
Peebles, C. R.
Roman, J.
Steiner, G.

South Carolina
Dery, D. W.
Graham, T. W.
Holdeman, Q. L.

South Dakota
Caveness, F. E.
Hugghins, E. J.

Texas
Atkins, J. G.
Cauthen, G. E.
Drummond, R. O.
Machmer, J. H.
Norton, D. C.
Thomas, H. A.
Turk, R. D.

Utah
Frandsen, J. C.
Griffin, G. D.
Grundmann, A. W.

Vermont
Chute, R. M.

Virginia
Barnard, Maye Hansen
Bartsch, P.
Byrd, M. A.
Elsea, J. R.
Freund, Frances E.
Hargis, W. J., Jr.
Lautenschlager, E. W.
Riggin, G. T., Jr.
Somerville, A. M., Jr.

Washington
Apt, W. J.
Courtney, W D.

West Virginia
Hall, J. E.

Wisconsin
Standifer, Marie A.
Thorne, G.
White, L. V.

Wyoming
Honess, R. F.

Australia
Sprent, J. F. A.

Austria
Wieser, W.

Belgium
DeConinck, L.

Brazil
DeBona, Arlette
Franco, E.
Lordello, L. G. E.

Canada
Anderson, R. C.
Baker, A. D.
Bosher, J. E.
Brown, Georgiana L.
Mountain, W. B.
Orchard, W. R.
Schad, G. A.
Welch, H. E.
Wu, Liang-Yu

Chile
Isaias Tagle, V.

Cuba
Kouri, P.
Pineda, Felicitas

Egypt
Oteifa, B. A.

England
Yeh Liang-Sheng

France
Dollfus, R. Ph.

Germany
Meyl, A. H.

Ghana
Edwards, E. E.
Thomas, J. D.

India
Basir, M. A.

Israel
Minz, G

Japan
Morris, J. A.
Schneider, M.D.
Walton, B. C.
Yamaguti, S.
Yokogawa, M.

Malaya
Traub, R.

Mexico
Caballero y C., E.
Winter, H. A.

Pakistan
Timm, R. W.

Panama
Loos, C. A.

Peru
Gonzales-Mugaburu, L.

Philippines
DeJesus, Z.
Velasquez, Carmen C.
Yogore, M. G., Jr.

Scotland
Mabbott, T. W.

Southern Rhodesia
Mettrick, D.

Switzerland
Baer, J. G.
Dubois, G.
McMullen, D. B.

Taiwan
Kuntz, R. E.

Venezuela
Hemerick, G. A.

CONTENTS

PAGE

BRAYTON H. RANSOM MEMORIAL TRUST FUND 164

CAVENESS, FIELDS E. AND J. E. BOSHER. On the Misuse of the Term "Incisure" 124

CHENG, THOMAS C. The Histology of the Prostate Mass in the Genus *Acanthatrium* (Trematoda: Lecithodendrium) 111

CHRISTIE, J. R. Plant Parasitic Nematodes, Their Bionomics and Control 164

ERRATA—HWANG, JOSEPH C. 163

HERLICH, HARRY. Experimental Infections of Cattle with the Stomach Worms, *Ostertagia ostertagi* and *Trichostrongylus axei* 97

HIRSCHMANN, HEDWIG. Histological Studies on the Anterior Region of *Heterodera glycines* and *Hoplolaimus tylenchiformis* (Nematoda, Tylenchida) 73

HURLEY, FRANCIS J. Immunization Threshold in Laboratory Rats Given a Small Initial Infection of *Nippostrongylus muris* 91

KRUIDENIER, FRANCIS J. AND KRISHNA N. MEHRA. *Aspiculuris ackerti*, n. sp., (Nematoda: Oxyuridae) from the Wood Rats of Arizona 147

LOOS, CLIVE A. Symptom Expression of Fusarium Wilt Disease of the Gros Michel Banana in the Presence of *Radopholus similis* (Cobb, 1893) Thorne, 1949 and *Meloidogyne incognita acrita* Chitwood, 1949 103

MINUTES, Three hundred fifty-seventh Through the three hundred sixty-fourth Meetings 165

NEW BOOK—See Christie, J. R. 164

RAUSCH, ROBERT. Studies on the Helminth Fauna of Alaska. XXXV. On the Identity of Certain Cestodes (Taeniidae) from Foxes............ 125

SCHAD, G. A. A Revision of the North American Species of the Genus *Skrjabinema* (Nematoda: Oxyuroidea) 138

SIDDIQI, M. RAFIQ. Studies on *Xiphinema* spp. (Nematoda: Dorylaimoidea) from Aligarh (North India), with Comments on the Genus *Longidorus* Micoletsy, 1922 151

TURNER, JAMES H. Experimental Strongyloidiasis in Sheep and Goats. III. An Attempt to Induce Passive Immunity in Lambs; Changes in Serium Proteins after Infection; and Effect of Immune Serum on Infective Larvae 114

WEBER, THOMAS B. AND JOHN T. LUCKER. Immunity Against the Cattle Lungworm: Resistance Resulting from Initial Infection with Small Numbers of Larvae 132