STUDIES ON THE BIOLOGY OF *Cooperia punctata* (v. LINSTOW, 1907) RANSOM, 1907, (NEMATODA: TRICHOSTRONGYLIDAE) IN THE DOMESTIC RABBIT, *Oryctolagus cuniculus* L.

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STUDIES ON THE BIOLOGY OF *Cooperia punctata* (v. LINSTOW, 1907) RANSOM, 1907, (NEMATODA: TRICHOSTRONGYLIDAE) IN THE DOMESTIC RABBIT, *Oryctolagus cuniculus* L.

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I. INTRODUCTION

Pure science, in all its forms, owes its existence and continuance to the ubiquitous pursuit of knowledge, is based on ethical research for the accumulation of facts and represents the solid foundation on which the biological as well as other sciences are built. Veterinary parasitology, a basic biological science, is sustained and advanced by the acquisition of knowledge through diligent experimentation and objective observation. Aware of the complexities and intricacies associated with problems involving pure science research, the writer undertook the study of the biology of Cooperia punctata in the domestic rabbit. In doing so, basic information and practical experience in host-parasite relationships, helminthological techniques, taxonomic systems, morphological structures, nematode life histories and biological relationships of organisms were acquired.

The bulk of the research in modern veterinary parasitology has been conducted from the applied research standpoint with little emphasis being given to basic studies. Research on host-parasite relationships has been motivated largely by the desire to prevent or to cure clinical parasitism in domestic livestock rather than to add to the fund of basic information constituting the science. A better understanding of the intimate associations between the hosts and parasites as well as the inter-relationships of the environment to these biological phenomena will be necessary before effective control and prevention can be established.

Several species of trichostrongylid nematodes of the genus Cooperia

are parasites of domestic and wild ruminants and most of these have limited geographic distribution and host range. In the United States, eight species are known to occur in the small intestine of cattle and sheep, namely: Cooperia bisonis, C. curticei, C. mcmasteri, C. oncophora, C. pectinata, C. punctata, C. spatulata and C. zurnabada. Limited information is available concerning the biology of some of these nematodes and much of what is known has been accrued during the past decade. The life history of the four better known species, namely: C. curticei in sheep and C. oncophora, C. pectinata and C. punctata in cattle, has been determined, on an experimental basis, but none has been verified by subsequent research. Little is known about the ecology and range of potential hosts and only general information is available concerning the viability and infectivity of the third stage larvae of these nematodes. Even less is known about the other species, namely: C. bisonis, C. mcmasteri, C. spatulata and C. zurnabada in cattle, except for a few isolated reports concerned with geographic distribution and host range.

The taxonomy of the genus Cooperia is still subject to some controversy and several systems of classification have been advocated by various writers for the family-group. The classification scheme, accepted for purposes of this thesis, has been proposed by Yamaguti (1961) who determined the taxonomic position of Cooperia to be as follows:

Class:	Nematoda
Order:	Strongylidea Diesing, 1851
Family:	Trichostrongylidae Leiper, 1912
Subfamily:	Trichostrongylinae Leiper, 1908
Genus:	Cooperia Ransom, 1907
Type:	Cooperia curticei (Railliet, 1893)

The use of small laboratory animals in the study of some aspects of the biology of parasitic nematodes of domestic animals offers many technical as well as economic advantages. Very little work has been done in determining what laboratory animals may serve as potential hosts for species in the genus Cooperia. Alicata (1958) and Wood and Hansen (1960), on the basis of a limited number of experiments, reported that the domestic rabbit could be used as an experimental host for C. punctata. However, no research has been done to determine how efficiently these animals can be used as hosts or what effects, if any, might be exerted on the parasite or host as a result of this unnatural relationship.

With the paucity of information regarding the biology of this parasite in the domestic rabbit and because of the potential advantages of having a suitable experimental host available for basic research, a series of experiments was undertaken to determine the extent of the host-parasite relationship, including the life history, of C. punctata in the domestic rabbit.

The objectives of these experiments were:

1. To establish a patent infection of C. punctata in the domestic rabbit.
2. To determine the adaptability and compatability of this parasite to the rabbit.
3. To obtain data on the parasite-host relationships for comparison with those in the natural host.
4. To study and describe the life history stages in the rabbit.
5. To describe and illustrate any deviations in gross morphology of the parasite.

II. HISTORICAL REVIEW

The Taxonomy of the Genus *Cooperia*

A review of the taxonomic history of a nematode species gives an insight to the numerous problems constantly confronting the systematic nematologist and also, an understanding of the importance of taxonomy to the worker engaged in other problems concerning the species. The laborious process of examining large numbers of new or related specimens, of re-examining type specimens of described species in view of new taxonomic findings, of unraveling the confusion due to morphological variation and of perusing diligently the voluminous literature, are only a few of the difficulties involved in the establishment of a classification and the determination or identification of a species. Whatever stability that has been acquired in the systematics of helminths is the result of years of labor by many taxonomists who were devoted to the principle and task of broadening our concepts in respect to reality.

Nematode parasites of the family Trichostrongylidae Leiper, 1912, inhabit the digestive tract of mammals, birds, reptiles, and amphibians. They have been known to parasitologists since 1800 when Zeder described *Strongylus retortaeformis* from the intestinal tract of hares. The nematode parasites usually were placed in the genus *Strongylus* but as the number of species increased it soon became apparent that a more complex system of classification was necessary to bring order out of confusion.

In 1905, Looss created the genus Trichostrongylus, transferred to it the species Strongylus retortaeformis, S. instabilis and S. probolurus, assigned T. retortaeformis as the type species and described Trichostrongylus vitrinus sp. nov. from the small intestine of sheep. Leiper (1908) created a sub-family Trichostrongylinae in his new family Metastrongylidae, for the small intestinal bursate nematodes which were without a buccal capsule. This sub-family included the genera Trichostrongylus, Haemonchus, Nematodirus, Cooperia and Ostertagia. In 1912, he reorganized the trichostrongylid nematodes under a newly created family Trichostrongylidae and retained the genus Trichostrongylus as the type genus for this family and for the sub-family Trichostronglinae. The number of species has continued to increase and at present there are over 400 in the Trichostrongylidae. Many species, however, are incompletely or inadequately described. Because of their small size and simple structure, morphological characteristics are not only limited but often inadequate for establishing valid species and even genera in the Trichostrongylidae.

According to Ransom (1911) and Skrjabin, Shikhobalova and Shul'ts (1954), Strongylus radiatus Rudolphi, 1803, is a synonym for Cooperia oncophora (Railliet, 1898). This appears to be the first report of an original Strongylus species that was transferred to the genus Cooperia. Yamaguti (1961), however, listed, without comment, the same species, Strongylus radiatus Rudolphi, 1803, as a synonym for Bunostomum phlebotomum (Railliet in Rizzo, 1900).

Curtice (1890) reported a nematode from the small intestine of sheep as Strongylus ventricosus Rudolphi, 1809. Giles (1892) concluded that this was a new species and named it S. curticii. This trivial name

was emendated to S. curticei by Railliet (1893).

Harker (1893) described but neglected to name a nematode from the abomasum of the ox. Stödter (1901) named this parasite Strongylus harkeri and included in his paper a discussion of S. curticei Giles, 1892, from the abomasum and S. oncophorus Railliet, 1900, from the small intestine of ruminant animals. These references are incorrect listings, however, since Railliet had emended S. curticei in 1893, and renamed S. ventricosus Rudolphi of Schneider, 1866, S. oncophorus in 1898.

Schnyder (1906) referred to a Strongylus sp. that was collected from the intestine of cattle and later in an abstract (1907) called it S. punctatus v. Linstow. Ransom (1911) included both of these as synonyms of Cooperia punctata (Linstow, 1907) Ransom, 1907. Vrijburg (1907) described Strongyloides bovis from the intestinal tract of cattle in Deli-Sumatra. Ransom (1911) included S. bovis Vrijburg, 1907, as a synonym of Cooperia punctata (Linstow, 1907) Ransom, 1907, on the basis of the description and illustrations in the original paper.

Ransom (1907) in considering the nematode parasites in the alimentary tract of cattle, sheep, and other ruminants, erected the genera Ostertagia, Cooperia and Nematodirus. The genus Cooperia was established for those nematodes having an anterior cephalic cuticular swelling, a lyre shaped dorsal ray, and no gubernaculum. Four species were listed from ruminants: Cooperia curticii (Giles, 1892) (=S. ventricosus Curtice, 1890) was the type species, Cooperia punctata (Schnyder, 1907) and C. oncophora (Railliet, 1898) were included in the genus and C. pectinata was described from cattle in Texas. He indicated that C. pectinata could be identical with S. ventricosus.

Railliet and Henry (1909) described Cooperia alata from the intestine of a macaque monkey, and the brief description indicated that it possessed a gubernaculum. These workers corrected the species name of Cooperia curticii (Giles, 1892) Ransom, 1907, to Cooperia curticei (Railliet, 1893).

Ransom (1911) reviewed the classification of the nematode parasites from the alimentary tract of cattle, sheep and other ruminants. In the genus Cooperia he listed the following species: Cooperia curticei (Railliet, 1893) Railliet and Henry, 1909 (type species); C. punctata (Linstow, 1907) Ransom, 1907; C. pectinata Ransom, 1907 and C. oncophora (Railliet, 1898) Ransom, 1907. He concluded that Strongylus ventricosus Rudolphi of Curtice, 1809, was synonymous with C. curticei and that S. ventricosus Rudolphi of Schneider, 1866, had been renamed Strongylus oncophorus Railliet, 1898. He suppressed Strongylus sp. Harker, 1893, and placed it, in part, in synonymy with Ostertagia ostertagi, stating that at least one of the figures included in the original text had been drawn from a male O. ostertagi.

Travassos (1914) described the species Cooperia brasiliensis from the ox, Bos taurus, and (1915) C. macieli from the stomach of the tropical armadillo, Dasypus novemcinctus. He (1921a) described C. elegans from the small intestine of the South American squirrel monkey, Saimiri sciurea, and C. falsa from the stomach of the broad banded armadillo, Cabassous unicinctus. Travassos (1921b) revised the genus and listed six species.

Fiebiger (1923) referred Strongylus harkeri Stödter, 1901, to the genus Cooperia. Cram (1925) described C. bisonis from the intestine of the buffalo, Bison bison, and considered it to be closely related

to C. oncophora except for a large linguiform vulvar process, a larger egg and a difference in the origin of ventral branches of the dorsal ray.

Yorke and Maplestone (1926) listed seven species of Cooperia in their systematic treatise on nematode parasites of vertebrates: C. curticei (Railliet, 1893); C. alata Railliet and Henry, 1909; C. bisonis Cram, 1925; C. macieli (Travassos, 1915); C. oncophora (Railliet, 1898); C. pectinata Ransom, 1907 and C. punctata (Linstow in Schnyder, 1907).

Hung (1926a) described C. fuelleborni from the small intestine of the waterbuck, Kobus ellipsiprymnus. Skrjabin, et al. (1954) included a wider host range for this species: sheep, Ovis aries; ox, Bos taurus; and blesbok, Damaliscus albifrons.

Gomez (1928) described the lesions in the small intestine of a carabao, Bubalus bubalis, caused by an underscribed Cooperia sp. This parasite caused the formation of nodules in the mucosa of the duodenum and jejunum which resulted in functional impairment of the intestine. Schwartz (1928) described this parasite and named it Cooperia nodulosa. In this review of the genus, Schwartz suppressed C. elegans and C. falsa because of doubtful generic affinities and considered C. fuelleborni Hung, 1926, as a synonym of C. curticei. Skrjabin and Orlov (1934), in Skrjabin, Shikhobalova and Shul'ts (1954); Travassos (1937) and Yamaguti (1961) do not support Schwartz and recognize the validity of C. fuelleborni Hung, 1926.

Baylis (1929) described two species, C. fieldingi and C. nicolli, from the intestinal tract of Australian cattle. The length and characteristics of the spicules were the important structures for establishing these species.

LeRoux (1930) stated that C. punctata (Linstow, 1907) Ransom, 1907, and C. fieldingi Baylis, 1929, were probably identical. He collected C. punctata from the small intestine of the African buffalo, Syncerus caffer, and C. curticei from the small intestine of several animals, namely: roan antelope, Hippotragus equinus; sable antelope, Hippotragus niger; springbuck, Antidorcas marsupialis; black wildebeest, Connochaetes gnu; waterbuck, Kobus ellipsiprymnus; lechwe antelope, Kobus leche; blesbok, Damaliscus albifrons; gemsbuck, Oryx gazella and Cooperia sp. nov. from the bushbuck, Tragelaphus scriptus.

Antipin (1931) described C. surnabada from the small intestine of cattle in Azerbaidzhan. There seems to be some confusion as to the proper spelling of the trivial name. It appears in the literature as C. zurnabada as well as C. surnabada. Skrjabin, et al., and Yamaguti both list the species as C. zurnabada.

Mönnig (1931) described Cooperia fuelleborni var. hungi from the digestive tract of the waterbuck, Kobus ellipsiprymnus and C. serrata and C. antidorca from the intestinal tract of the springbuck, Antidorcas marsupialis. He included as new records for sheep, the species: C. serrata, C. antidorca, C. fuelleborni, and C. fuelleborni var. hungi and emended C. fuelleborni var. hungi Mönnig, 1931, as C. hungi Mönnig, 1931.

Dikmans (1932) considered C. nicolli Baylis, 1929, as a synonym of C. pectinata Ransom, 1907, after comparing specimens from the two collections. He (1935) agreed with the LeRoux (1930) supposition and synonymized C. fieldingi Baylis, 1929, and C. punctata Ransom, 1907. He stated that the length of spicules of males of Cooperia sp. varied extensively and that this characteristic alone could not be used to differentiate species.

Mönnig (1932a) described C. africana from the small intestine of an eland, Taurotragus oryx, in Kenya; C. hamiltoni from the small intestine of an impala, Aepyceros melampus, and C. neitzi from the small intestine of a kudo (spiral horned antelope), Strepsiceros strepsiceros in Transvaal. He (1932b) reported C. hungi from the impala, C. neitzi from the kudo, C. verrucosa and C. africana from the eland and C. serrata from the springbuck. He established experimentally in the domestic sheep the following parasites of antelopes: C. hungi, C. neitzi, C. nicolli, and C. serrata.

Sprehn (1932) listed, without comment, Cooperia brasiliensis Travassos, 1914, as a synonym of C. punctata (Linstow, 1907).

Gordon (1932) described C. mcmasteri from the small intestine of a calf in Australia. This species is very similar to C. oncophora. LeRoux (1936) considered C. mcmasteri to be identical with C. zurnabada but Travassos (1937), Skrjabin, et al. (1954) and Yamaguti (1961) regarded it as a valid species. Allen and Becklund (1958) reviewed the literature concerning C. mcmasteri and C. zurnabada without further clarifying the situation.

Daubney (1933) suggested that C. nicolli and C. pectinata were synonymous and erected the genus Cooperioides for those species which although closely related to Cooperia differed basically in the structure of the bursa, and included Cooperia antidorca Mönnig, 1931, in the new genus. He questioned the validity of C. nodulosa and C. serrata because of the morphology of the dorsal ray. The presence of small prebursal papillae (supposedly not to be a characteristic of Cooperia) and the low number (10 to 12) of cuticular pectinate lines. Mönnig (1933) transferred C. hamiltoni Mönnig, 1932, to the genus Cooperioides Daubney, 1933.

Travassos (1935) erected the genus Macielia which was readily distinguished from the genus Cooperia on the basis of the accessory bursal membrane, the non-lyrate dorsal ray, and the distinctive bursal ray and spicule characteristics. He transferred C. macieli to the genus Macielia as the type species and included C. falsa in the new genus. Travassos (1937) listed four species for this genus.

Leiper (1935) described Cooperia okapi collected from the digestive tract of an okapi, Okapia johnstoni, in the Zoological Gardens of London. Berghe and Vuylsteke (1937) described C. okapiae from an okapi in Africa. Baer (1950) studied specimens of the parasites described by both Leiper and Berghe and Vuylsteke and concluded that the species were identical and suppressed C. okapiae. Yamaguti (1961), however, listed C. okapiae Berghe et Vuylsteke, 1937, as a distinct species and questioned its synonymy with C. okapi Leiper, 1935.

LeRoux (1936) erected the genus Schwartziella and included Cooperia nodulosa Schwartz, 1928, and C. serrata Mönnig, 1931, in the new genus. Species in the genus Schwartziella differ from cooperids because they have no telamon and have distinctive spicule and bursal ray characteristics. Schwartziella nodulosa (Schwartz, 1928) was designated type species and S. serrata (Mönnig, 1931) was considered closely related to, if not identical with, the type species. LeRoux corrected Daubney (1933) by stating that cervical and prebursal papillae were described for Cooperia sp., especially for C. zurnabada and also for C. curticei, C. oncophora, C. pectinata, C. punctata, C. hungi, and C. neitzi.

Chen (1937) described Cooperia laterouniformis from the small intestine of the buffalo, Bubalus bubalis, in China.

Skrjabin, et al. (1954) credit Skrjabin and Shul'ts (1937) for the establishment of the tribe Cooperiea (subfamily Trichostrongylinae) and for the inclusion of genera, the species of which were characterized by the presence of a cuticular vesicle at the anterior end. The genera, Cooperia Ransom, 1907, Trichohelix Ortlepp, 1922 and Travassostrongylus Orlov, 1933, were included in the tribe.

Travassos (1937) revised the genus Cooperia and enumerated 22 species. He recognized C. oncophora, C. punctata, C. pectinata, C. fülleborni, C. hungi, C. verrucosa, C. neitzi, C. africana, and C. zurnabada as being very similar to the type species, C. curticei; suppressed C. harkeri Fiebiger, 1923, as a synonym of C. oncophora and C. bisonis as a synonym of C. oncophora; transferred C. alata Railliet and Henry, 1909, to the genus Pithecostrongylus Lubimov, 1930, and recognized the transfer of C. elegans Travassos, 1921, to the genus Molineus of Travassos and Darriba (1929); acknowledged Dikmans' (1935) note suppressing C. fieldingi Baylis, 1929, as a synonym of C. punctata, but placed C. fieldingi in synonymy with C. curticei; considered C. nicolli to be identical with C. pectinata but again ignored Dikmans' (1932 and 1935) notes synonymizing C. nicolli and C. pectinata; and referred to his genus Paracooperia (1935) to which he transferred C. serrata and C. nodulosa.

The genus Paracooperia was erected by Travassos (1935) and species were characterized by an anterior cuticular swelling, small cervical papillae, an anterior fingerlike vulvar convexity and distinctive bursal ray and spicule morphology. Skrjabin, et al. (1954) rejected the genus Schwartziella LeRoux, 1936, as a junior synonym of Paracooperia Travassos, 1935.

Baylis, (1938a) agreed with Dikmans (1932, 1935) that Cooperia nicolli Baylis, 1929, was a synonym of C. pectinata Ransom, 1907, and C. fieldingi Baylis, 1929, was synonymous with C. punctata Ransom, 1907. He indicated that Travassos (1937) was not justified in treating C. fieldingi as a synonym of C. curticei and concluded that C. mcmasteri, Gordon, 1932, and C. oncophora (Railliet, 1898) were valid species.

Baylis (1938b) described Cooperia spatulata from the small intestine of cattle and sheep in Australia and the Federation of Malaya. Becklund (1958) recovered this parasite from cattle in southern Georgia. Mönnig (1939) described C. yoshidai from the small intestine of the reedbuck, Redunca arundinum, in South Africa. The cuticle of this parasite has approximately eight longitudinal ridges which surround the body with broad spiral windings, an unusual character for any cooperid species. Burdjanadze and Tschotschischvili (1942) described C. svanetica from the small intestine of cattle in the Georgian region of Russia. Ault (1943) reported C. zurnabada from the intestinal tract of cattle in Argentina.

Brito Gutteres (1947) described four species of Cooperia from domestic and wild animals in the Belgian Congo: C. borgesi from cattle, sheep, and the reedbuck, Redunca redunca; C. hippotragusi from cattle, roan antelope, Hippotragus equinus, reedbuck, and oribi, Ourebia ourebi; C. minor from cattle; and C. reduncai from the reedbuck.

Skrjabin, et al. (1952), as outlined in Skrjabin, Shikhobalova and Shul'ts (1954), reorganized the family Trichostrongylidae Leiper, 1912; superfamily Trichostrongyloidea Cram, 1927. In this reference, the Trichostrongylidae, consisting of 71 genera and more than 350

species, was subdivided into 15 subfamilies and 18 tribes. The tribe Cooperiea was elevated to the status of a subfamily, Cooperiinae, and included a number of genera which earlier were included in the subfamily Trichostrongylinae, namely: Cooperia, Cooperioides, Macielia, Paracooperia, Travassostrongylus, and Hyostrongylus. Skrjabin, et al. accepted, in part, the classification systems proposed by Cram (1927) and Travassos (1937).

The subfamily Cooperiinae Skrjabin and Shikhobalova, 1952, is characterized by nematodes with a cuticular thickening at the anterior end; cuticle with transverse striations anteriorly and longitudinal lines or ridges throughout length; mouth cavity absent or rudimentary and cervical papillae absent or weakly developed. The subfamily is divided into three tribes: a) Cooperiea Skrjabin and Shul'ts, 1937, with three genera (Cooperia, Cooperioides, and Paracooperia) characterized by the absence of a gubernaculum and telamon; b) Hyostrongylea Skrjabin and Shikhobalova, 1952, with three genera (Hyostrongylus, Macielia, and Travassostrongylus) characterized by the presence of gubernaculum and telamon; c) Trichohelicea Skrjabin and Shikhobalova, 1952, with a single genus (Trichohelix) characterized by the presence of a gubernaculum and the absence of a telamon.

Diagnosis of the tribe Cooperiea according to Skrjabin and Shul'ts (1937): Cooperiinae with symmetrical head vesicle. Prebursal papillae present or absent. Spicules short, equal, complex, split at distal ends, often with serrated edge. Gubernaculum or telamon absent. Vulva in posterior one-fourth of body. Parasites of digestive tract of ruminants.

Diagnosis of the genus Cooperia Ransom, 1907, according to Skrjabin et al. (1954):

Anterior end narrow, without clearly defined lips, papillae poorly developed. Mouth cavity small. Cuticle at anterior end inflated to form vesicle. Cervical papillae very small. Body cuticle with transverse striations anteriorly and 14-16 longitudinal lines throughout length.

Skrjabin, et al., (1954), recognized 22 species in the genus Cooperia: C. neitzi Mönnig, 1932; C. spatulata Baylis, 1938; C. fieldingi Baylis, 1928; C. yoshidai Mönnig, 1939; C. laterouniformis Chen, 1937; C. punctata (Linstow in Schnyder, 1907) Ransom, 1907; C. africana Mönnig, 1932; C. zurnabada Antipin, 1931; C. verrucosa Mönnig, 1932; C. curticei (Giles, 1892); C. fuelleborni Hung, 1926; C. bisonis Cram, 1925; C. hungi Mönnig, 1931; C. pectinate Ransom, 1907; C. mcmasteri Gordon, 1932; C. okapi Leiper, 1935; C. oncophora (Railliet, 1898); C. svanetica (Burdzhanadze and Tschotschischvili, 1942); C. borgesi Gutteres, 1947; C. hippotragusi Gutteres, 1947; C. minor Gutteres, 1947 and C. reduncai Gutteres, 1947.

They retained C. fieldingi but did not clarify their reasons. They stated that the spicules of C. fieldingi were similar to those of C. curticei, however, the spicules that were figured in the text were similar to C. punctata. These workers acknowledged Dikmans (1935) in the bibliography along with other non-Russian references but they made no comments on any of the foreign reports that were concerned with this species.

Wu (1958) described Cooperia erschovi from the pancreas of cattle in China. He stated that it closely resembled C. pectinata, but differed in that the spicules had curved striations instead of straight ones; the shape and structure of the genital cone was different; the branching of the dorsal ray was not the same; and the habitat of the adult worm was the pancreas rather than the intestine of cattle.

Mamedov (1960) reported observing C. punctata embedded in the parenchyma of the pancreas of Zebu cattle in Azerbaidzhan. Jansen (1961) listed the trichostrongylids of domestic mammals and fowl in

in the Netherlands and discussed the classification of these parasites. He did not accept the subfamily Cooperiinae Skrjabin and Shikhobalova, 1952, but included the genera Cooperia, Cooperioides and Paracooperia in the subfamily Trichostrongylinae Leiper, 1908.

Yamaguti (1961) presented a systematic treatment of all the known parasitic nematodes in the world. The parasites were divided first according to the primary divisions of their host animals and then classified by differential keys into orders, families, and genera. A diagnosis is presented for each of these taxons and a list of species is given for each genus.

Yamaguti emphasized the Order in his taxonomic system and included 10 families, 33 subfamilies, and 246 genera in the order Strongylidea Diesing, 1851, and 16 subfamilies, 113 genera, and over 480 species in the family Trichostrongylidae Leiper, 1912. He considered the genus Cooperia in the subfamily Trichostrongylinae Leiper, 1908. The key for classification of species, developed by Skrjabin, et al. (1954) was accepted by Yamaguti.

He listed 21 species in the genus Cooperia: C. curticei (Railliet, 1893); C. africana Mönnig, 1932; C. borgesi Brito Gutterres, 1947; C. fülleborni Hung, 1926; C. hippotragusi Brito Gutterres, 1947; C. hungi Mönnig, 1931; C. laterouniformis Chen, 1937; C. mcmasteri Gordon, 1932; C. minor Brito Gutterres, 1947; C. neitzi Mönnig, 1932; C. okapi Leiper, 1935; C. okapiae Berghe et Vuylsteke, 1937; C. oncophora (Railliet, 1898); C. pectinata Ransom, 1907; C. punctata (v. Linstow in Schynder, 1907); C. reduncai Brito Gutterres, 1947; C. spatulata Baylis, 1938; C. svanetica Burjanadze et Tchotchischwili, 1942; C. verrucosa Mönnig, 1939; C. yoshidai Mönnig, 1939; C. zurnabada Antipin, 1931.

This species list differs from the one accepted by Skrjabin, et al. (1954) in the suppression of C. fieldingi and C. bisonis as synonyms of C. punctata and C. oncophora respectively. In addition, he retained C. okapiae as a valid species even though Skrjabin, et al., suppressed it as a synonym of C. okapi. The species Cooperia ershovi Wu, 1958, was not included in the genus.

In retrospect, it is this writer's opinion that Dougherty's (1944) complaint "Various names of groups . . . are habitually or generally associated in the literature with incorrect dates or authorities" is valid. The problem is greater than expressed, however, for species descriptions were often based on superficial characters, taxonomic schemes were developed on whims and characterizations of a species were written without adequate consideration of available literature.

It is obvious, on the basis of this discussion, that the position of Cooperia Ransom, 1907, in the taxonomy of trichostrongylid nematodes is not yet stabilized because there appears to be at least three logical systems of classification, Travassos (1937), Skrjabin, Shikhobalova and Shul'ts (1954) and Yamaguti (1961), in the current literature which include this genus. No attempt by taxonomists has been made to unify these concepts as an acceptable taxonomic scheme. As species descriptions are made more complete, as life cycles are unraveled and as the fund of knowledge concerned with the biology of nematode parasites of ruminants is increased, only then will the classification of the trichostrongyles be placed on a firmer basis and the validity of species stabilized.

The Incidence and Pathology of Cooperia punctata

Species in the genus Cooperia are among the more important parasites

of the small intestine and are capable of producing clinical disease and even death in ruminant animals. This dissertation is based on an investigation of one of the species in this genus.

There are at least eight species of Cooperia that have been collected from domestic ruminants in the United States, namely: C. curticei which occurs primarily in sheep; while C. bisonis, C. mcmasteri, C. oncophora, C. pectinata, C. punctata, C. spatulata, and C. zurnabada occur primarily in cattle. A wealth of information is available which is concerned with host preference, incidence and geographical distribution of these species, however, only those reports pertaining to C. punctata will be emphasized in this discussion.

Dikmans (1939) reported that C. punctata had a higher incidence and a wider geographic distribution in the United States than any of the other species of Cooperia. Shorb (1940) listed C. punctata as a common intestinal nematode of cattle in the northern states and Porter (1942) observed that it was the most prevalent nematode parasite of cattle in the Southeastern States. Cooperrider, et al. (1948) reported that 84 per cent of the calves from 14 locations in Oklahoma were infected with Cooperia species while Bell, et al. (1959) stated that the incidence was similar for cattle in Texas. Andrews, et al. (1953) noted that C. punctata was the dominant species in cattle in the Southeastern United States and reported extreme infections in two dairy calves in which 350,000 and 450,000 mature C. punctata were collected. Andrews and Maldonado (1941) determined 54 per cent of the Puerto Rican cattle to be infected with C. punctata while Alicata (1960) reported this species to be the most important helminth in beef and dairy calves in Hawaii.

Buckley (1933) collected C. punctata from the small intestine of the

domestic pig, Sus scrofa, in southern Rhodesia. Shorb (1940) included the domestic sheep as a natural host in the United States and Porter (1953) concluded that more adults of this species would develop in calves than in lambs when these hosts were exposed to similar numbers of infective larvae. Gilmore and Allen (1960) identified C. punctata from the small intestine of 72 per cent of the pronghorn antelope, Antilocapra americana, examined in New Mexico. Ash (1961) recovered nature nematodes from the small intestine of axis deer, Cervis axis, in Hawaii, and concluded that under field conditions, deer can serve as reservoir hosts as a source of infections in cattle.

There are numerous reports in the literature concerned with the helminthoses of the domestic ruminants. In many instances, the cooperids have been implicated in the complex of field cases of severe parasitism in cattle and sheep. Since other parasites were present in these infections, it is difficult to determine the extent of the detrimental effects directly traceable to Cooperia species. In this discussion, those reports that have to do with the pathology attributable to C. punctata will be related. In a few instances, descriptions of the lesions and symptoms of infections associated with other species will be given because they are of value in clarifying and amplifying the disease potential of species in the genus.

Cooperia punctata inhabits the digestive tract of cattle, sometimes sheep, and is located primarily in the anterior part of the small intestine. Ransom (1920) reported that this parasite occurred in the lumen of the abomasum and in the upper part of the small intestine and that mature worms were sometimes embedded in the intestinal mucosa. Hung (1926b) observed that the parasite was capable of producing a necrotic

enteritis and concluded that mature worms were able to penetrate deeply into the mucosa where they probably fed on blood. Bailey (1949) concluded, on the basis of experimental infections with this parasite, that it was pathogenic to calves and described clinical symptoms of diarrhea anorexia, dehydration, and progressive emaciation leading to death. Gross lesions, observed in a young calf that died from the infection, were confined largely to the duodenum and consisted of catarrhal inflammatory areas with fibrinonecrotic exudates and hemorrhages and thickening of the intestinal wall. Microscopically, leucocytic and serous infiltrations of the intestinal wall were evident, especially in the submucosa, and localized degeneration with marked fragmentation of the mucosa was noted. Worms were found principally in contact with the surface of the mucous membrane but in one calf, they had penetrated to the serosa. No evidence of anemia was observed in any of the infected calves and no evidence of age resistance was observed in calves to 12 months of age.

Porter, et al. (1956) observed that C. punctata infections were sometimes detrimental to cattle. They concluded, on the basis of experimental infections, that scouring, loss of appetite, weakness and death could result in severe cases but that anemia did not occur in the experimentally infected animals. Lesions consisted of numerous petechial and ecchymotic hemorrhages of the mucosa, a thickened intestinal wall, a greyish-white appearance of the surface of the mucous membrane, and a white or yellowish, cheesy accumulation on the surface of the mucosa for the entire length of the small intestine. Herlick (1962) reported that in infections with C. punctata in calves, serum phosphorus, blood glucose, and serum protein begin to decline about the seventh day after infection. He determined that there was a drastic drop in the albumin-globulin ratio

after the fourteenth day because of a fall in the albumin fraction and a corresponding rise in the alpha and gamma globulin fractions.

Roberts, et al. (1952) studied the epidemiology of parasitic gastroenteritis of pastured cattle in Australia and observed that infections with Cooperia species were built up in the host much more rapidly and at a younger age than with the other species of intestinal strongyle nematodes. They concluded that heavy infections of Cooperia species in cattle could occur at any time of the year.

Spedding (1954) concluded that the injurious effects of subclinical parasitic infections of sheep were due to a depression of the appetite (up to 8 per cent) and a depression of digestive efficiency (up to 10 per cent) of the host. He concluded that these factors limit the potential live weight gain in animals regardless of the quality of feed offered. Andrews (1938) determined that C. curticei infections in lambs caused a decrease in the ability of the host to convert feed into body weight gain.

Edgar (1936) reported on an infection of a goat from which 30,000 mature C. curticei were recovered from the first 30 feet of the small intestine. He described scattered irregular hemorrhagic areas on the mucosa and concluded that infections with this parasite may be lethal.

It is apparent from these reports that C. punctata and other Cooperia species are pathogenic and are capable of producing clinical disease and even death in domestic animals. More work is necessary, however, to describe the cause and extent of the pathogenic effects since the apparent limited lesions do not fully account for the symptoms and pathology produced in the host.

The Domestic Rabbit as an Experimental Host for Ruminant Nematodes

Host specificity, although one of the fundamental phenomena in the host-parasite relationship, is still very poorly understood and little work has been done to determine how or why parasites become established on or in particular species of hosts. Numerous reports of apparently abnormal or unusual host relations, both natural and experimental, have been published and most are concerned with superficial conditions such as the degree of development or the type of behavior of parasites in the different hosts. As a result of these investigations, a large variety of animals have been found to be potential experimental hosts for many parasites but only a few have been infected with nematodes important in veterinary medicine. Those laboratory animals currently being used as experimental hosts for animal parasites include: The domestic rabbit, Oryctolagus cuniculus; the guinea pig, Cavia porcellus; the golden hamster, Mesocricetus auratus; the laboratory mouse, Mus musculus and the mongolian gerbil, Meriones unguiculatus.

The rabbit has been used as an experimental host for a large number of metazoan and protozoan parasites, however, only selected references which include the rabbit as an experimental host for ruminant nematode parasites will be reviewed in this discussion.

Ortlepp (1939) was able to infect rabbits with third stage larvae of Trichostrongylus colubriformis which had been cultured from eggs in sheep fecal pellets. He infected sheep also with larvae of this parasite which had been cultured from eggs in fecal pellets of infected rabbits. In the study of the bovine filariid, Setaria cervi, Williams (1955) successfully implanted sexually mature females in the peritoneal cavity of

rabbits and demonstrated microfilariae in the blood of these animals after 56 hours.

Several workers have determined that components of the diet may influence the development of the larval stages of a nematode parasite, from another host, in the small intestine of the rabbit. Rohrbacher (1957) concluded that fresh green feed, fed as a supplement, restricted the development but not the establishment of Trichostrongylus axei in California White rabbits to a greater extent than did a standard commercial pelleted feed. In another investigation, Rohrbacker, et al. (1958) completed experiments to determine the effect of milk in the diet of rabbits on the development of T. axei and T. colubriformis and concluded that a significantly smaller percentage of T. axei and T. colubriformis were recovered from unweaned rabbits than from weaned rabbits 18 days after infection.

Alicata (1958) reported the development of Cooperia punctata in young (4 to 8 week old) domestic rabbits that had been fed infective larvae cultured from eggs in feces of naturally infected young cattle. He concluded that the rabbit was not a very satisfactory host for C. punctata because the uteri of mature females recovered from the small intestine of the rabbits, examined at necropsy 16 days post-inoculation, contained mostly unsegmented eggs. The infected rabbits had free access to a complete commercial pelleted feed.

Wood (1958) and Wood and Hansen (1960) were able to infect experimentally domestic rabbits with larvae of species in several genera of nematodes which had been cultured from the feces of a naturally infected steer and of an experimentally infected lamb. They concluded that the species, namely: Cooperia curticei, C. punctata, Haemonchus contortus, Ostertagia circumcincta and Trichostrongylus colubriformis, could

develop to sexual maturity in the rabbit.

Unusual or abnormal host-parasite relationships resulting from experimental conditions are of interest to the helminthologist, especially when small laboratory animals are used, because of the technical and economic advantages offered for studies on the biology of nematodes normally occurring in domestic animals.

III. MATERIAL AND METHODS

Each of two worm-free, 10 week old, Holstein calves, purchased from the Dairy Department, Oklahoma State University, was infected experimentally with more than 75,000 third stage larvae of Cooperia punctata which were kindly supplied by the late Dr. George E. Cauthen, Texas Agricultural Experiment Station, Angleton, Texas. The larvae were administered in water, as a drench, in a single dose. These infected animals were used to provide a continuous source of feces containing cooperid eggs which were used for the culture of larvae. The calves were kept in litter-free stalls, cleaned every morning, and were given daily a limited amount of commercial grain ration with an abundant supply of prairie hay. The grain ration contained crude protein, 10 per cent; crude fat, 3 per cent; crude fiber, 12 per cent; salt, 2 per cent; cane molasses, linseed oil meal, vitamin A and D oil and calcium carbonate, as well as the sulfate salts of iron, manganese, copper, cobalt, potassium and zinc. Clean water was available at all times.

Male and female, worm-free rabbits, 6 to 10 weeks old, were procured from local suppliers and were used as experimental hosts to study some aspects of the biology, including the life cycle, of C. punctata. Three domestic breeds were represented: New Zealand White, New Zealand Red and Dutch Belted. Each rabbit was isolated and was kept on wire mesh in a stainless steel rabbit cage, 22 inches wide, 20 inches deep, and 15 inches high. The wire mesh floor was kept free of fecal material. The paper lining of each cage pan was changed daily and the entire cage

was sterilized with flowing steam approximately every 2 weeks. To check the development of eggs in feces on the mesh floor of cages of infected rabbits, dried feces, removed during the daily cleaning process, were examined occasionally for nematode eggs and those eggs that were observed usually were distorted by drying. There was no evidence that accidental or extraneous infections with C. punctata or any other nematode occurred during the experiments.

Each rabbit was fed twice daily but received at each feeding a restricted amount of a commercial pelleted feed with no supplemental green feed. The pelleted feed contained protein, 15 per cent; fat, 2 per cent; fiber, 12 per cent; nitrogen-free extract, 48 per cent; steamed bone meal, 1 per cent; calcium carbonate, 1 per cent; salt, 0.5 per cent; vitamin A feeding oil, D-activated plant sterol, riboflavin, calcium pantothenate, niacin, choline chloride, vitamin B-12 and antibiotic feed supplement as well as trace minerals: manganese oxide, ferrous carbonate, copper hydroxide, potassium hydroxide and calcium sterate. Clean water was available at all times.

The number of infective third stage C. punctata larvae given to each rabbit used in the experiment varied from 4,000 to over one million. In most instances, the larvae were administered in water, as a drench, in a single dose; however, in several instances, multiple daily doses were given during a period of 1 week.

A method to determine the number of larvae per volume of fluid was devised in order to calculate the size of the infective dose given to each of the experimental rabbits. This was done by a dilution counting technique in which the Stoll pipette was used to draw a 0.15 ml aliquot sample from a well mixed water-larva suspension. This sample was dispersed

in 5 ml of distilled water in a watch glass and the larvae were counted with the aid of a stereoscopic microscope. Larvae were inactivated by heating the water in a watch glass to facilitate counting when large numbers were involved. The number of larvae in the aliquot sample was then multiplied by the quotient of the division of 0.15 into the number of milliliters of the water-larva suspension to get the total number of larvae present in the suspension.

A standard dropping pipette was used to administer the water-suspended dose of larvae. Each rabbit was restrained on its back while being held against the operator's body with his left forearm and with the rabbit's head cradled in the operator's left hand. The pipette tip was placed gently in the mouth, the fluid containing larvae was administered slowly and the rabbit swallowed promptly without gagging. Rabbits of various ages differed in their response to this method of dose administration. Young rabbits, 6 to 8 weeks old, did not object to the tip of the pipette entering the mouth nor did they hesitate in swallowing the liquid. However, older rabbits had a tendency, at first, to bite at the pipette and to fight the tip but in a short time became passive to the technique although some were reluctant at times to swallow promptly. Care had to be used to see that the rabbits swallowed the fluid for it was observed that they could hold a considerable amount of water in their mouths. It was found that the swallowing reflex in rabbits could be initiated in one of two ways: by pushing down on the dorsum of the tongue with the tip of the pipette or by pushing against the palate in such a way as to slowly raise the head to an upward, backward position. After the larval dose had been administered, clean tapwater was given orally by pipette to each rabbit. No uncontrollable difficulty was encountered

in this dosing procedure and as far as it could be determined, the dose was administered in every case.

Third stage larvae were obtained by culturing eggs in feces from one of the two calves with pure infections of Cooperia punctata. The larvae collected from these cultures were used for the preliminary experiments concerned with the determination of the infectivity and adaptability of C. punctata to rabbits and for experiments concerned with the determination of the life cycle developmental stages of this parasite in rabbits. To maintain a constant supply of larvae for the successive rabbit infections, third stage larvae were obtained by culturing eggs in fecal pellets from rabbits with pure infections with C. punctata. The Cauthen sphagnum moss culture technique was modified by using a 0.1 per cent sodium carbonate solution to dilute the feces prior to mixing it with the moss. Dr. J. H. Whitlock, New York Veterinary College, was first to advocate the use of this solution to reduce fungus growth in cultures when moss was used.

Fecal material was collected several times every week from each of the infected rabbits and was added to a sufficient quantity of the sodium carbonate solution to make a thin paste-like fluid which was then thoroughly mixed with dry sphagnum moss. Rabbit fecal collections and cultures were kept separate at all times from the calf fecal collections and cultures. The fecal pellets from rabbits that were observed to have been contaminated with urine were washed in running tapwater before mixing with the sodium carbonate solution. This was done because urine is supposed to inhibit the development of nematode eggs. All cultures were maintained at 27 C for 12 days. These conditions were nearest the optimum as well as the most convenient for the development of the maximum

number of larvae.

A sufficient quantity of moss was mixed with the feces to control odor and bacterial growth and to provide a suitable substrate for larval development. The feces-moss mixture was placed in large moist chamber dishes for culturing. These were covered by glass squares which were supported by applicator sticks to prevent the formation of a water seal resulting from water condensation and to allow for the circulation of air.

The amount of moisture available in the chamber appeared to be a critical factor in the culture process. Cultures which had an excess of water accumulating on the dish wall and glass cover contained a greater number of larvae of a free-living nematode, Rhabditis species, and a more luxurient growth of fungi, as contaminants, than those cultures with a lower moisture content. While the drier cultures had fewer contaminants, they contained a lesser number of infective larvae as compared with moister cultures. The most successful cultures were those that contained sufficient moisture to wet the sphagnum moss without an excess to accumulate on the sides or cover of the culture dish. The surface material in such cultures remained comparatively dry during most of the culture period. An abundant fungal growth was considered to be undesirable in the culture mass because of manipulation procedures although no adverse effect was observed on the development or number of larvae recovered from those cultures that were contaminated.

In the initial phases of the investigation, fecal cultures were baermannized in filtered ditch water, 22 C. However, it was determined later that tapwater, 38 C, not only could be substituted without deleterious effect on the larvae but possibly increased the relative number

of larvae that developed and consequently, this was used in all subsequent Baermann preparations. The first withdrawal of water bearing larvae from a Baermann funnel was made 4 to 10 hours after the culture mass had been introduced and a second withdrawal was taken 4 to 6 hours later. Larvae were collected and concentrated in stender dishes by cooling the water to approximately 10 to 15 C to inactivate the larvae. After they had settled, the excess water was siphoned off. When large amounts of particulate matter from sphagnum moss were in suspension, an alternate method of collecting and concentrating the larvae was used. The suspended matter in such collections was allowed to settle. The dish and contents were exposed then to a moderate light and heat source, the free-swimming larvae would tend to clump to one side of the dish and the infective larvae, free from debris, could be siphoned off with a pipette without disturbing the particulate matter.

The sodium nitrate simple flotation technique was used in the examination of feces for the routine demonstration of cooperid eggs and in determining the termination of the prepatent period and extent of the patent period of infections. This procedure involved the comminution of feces in water, sieving the mixture through a single layer of cheesecloth and allowing the insoluble debris and eggs in the filtrate to settle. After the supernatant fluid was decanted, the sediment was mixed with a sufficient quantity of saturated sodium nitrate solution to fill each of two plastic vials, 5/8 inch in diameter, and 2 inches in length. A 22 mm square coverslip was placed on each filled vial so that it was in contact with the mixture for a minimum of 10 minutes. The coverslip was removed, oriented on a glass slide and the preparation examined microscopically at 100 magnifications to detect eggs.

The fecal pellets of all rabbits used in these experiments were examined repeatedly for the presence of nematode eggs prior to exposing the rabbits to infection. In the determination of the prepatent period, fecal material was examined several times during the first 8 days to intercept any extraneous infection or contamination. Beginning on the eighth day and thereafter, fecal pellets were examined twice daily for eggs and this procedure continued until the prepatent period was determined.

Necropsies were performed on all rabbits used in this investigation. Euthanasia was accomplished by subjecting each rabbit to a continuing 110-volt electrical charge. This procedure produced immediate death and permitted examination of the undisturbed digestive tract.

During the necropsy, the small intestine was handled quickly and carefully to reduce the possibility of mechanical translocation of parasites. The movement of intestinal contents between sections was further reduced by tying off the small intestine at three locations: At the terminal end of the pylorus of the stomach, and at the ends of both the first and second 18 inches of the small intestine. Immediately after the removal of the stomach and entire small intestine, a complete parasitological examination was made of these organs using the sedimentation-decanting technique. The contents of the large intestine and cecum were collected, fixed in hot 10 per cent formol-saline solution and transferred to specimen jars. These collections have not been examined for parasites because of the character and bulk of the individual samples.

The washings of the stomach and the designated sections of the small intestine were kept separate throughout the subsequent procedures of the examination. This material was fixed in hot 10 per cent formol-

saline solution and was transferred to specimen jars. When these collections were examined, the sex and stage of development of each worm, as well as the total number of worms, were determined and were recorded for each rabbit. In a few instances, the numbers of worms in the collections were so large that they had to be sampled by the aliquot method instead of being counted individually, to determine the totals. This was accomplished by the extrapolation of the results obtained in aliquot samples drawn from the collections. The efficacy of the collecting methods used in the sedimentation-decanting technique was determined by the pepsin-hydrochloric acid digestion process described by Herlick (1956). To do this, the stomach and the entire small intestine of most of the experimental rabbits were digested by this technique. The viscera-pepsin-hydrochloric acid preparations were kept at 37 C for 12 to 18 hours, after which the digestant was examined, by dilution and decantation, for the various stages of the parasite.

A total of 18 hosts was used in two experiments to determine the characteristic life cycle developmental stages of Cooperia punctata in the domestic rabbit. In one experiment, 12 worm-free rabbits, 6 to 7 weeks old, were infected to determine the development of the parasite during the first 18 days of infection. Each of the rabbits was infected on the same day and one rabbit was sacrificed daily on each of the first 6 days after administration of the larvae and one rabbit was sacrificed on alternate days for the subsequent 12 days. In the second experiment, six worm-free rabbits, 6 to 7 weeks old, were infected. Individual rabbits were sacrificed and examined at intervals, namely: the second, fourth, sixth, tenth, fourteenth, and eighteenth day after the administration of the larvae.

Each rabbit used in these two experiments was maintained in the manner previously described. The number of infective third stage C. punctata larvae given to each rabbit was approximately 100,000. The larvae were administered in water, as a drench, in a single dose.

Measurements were made on eggs of the nematodes collected from both calf and rabbit feces and on third stage infective larvae collected from both calf and rabbit fecal cultures and compared with measurements reported by other workers. The eggs that were measured were obtained on slide preparations using the sodium nitrate flotation technique. Sufficient debris was present between the coverslip and slide to prevent distortion of eggs by pressure. Strict randomization could not be used in obtaining these measurements because care had to be taken to measure only those eggs that were in a horizontal position. Measurements of third stage larvae were made promptly on heat killed specimens, unstained and suspended in distilled water under supported coverslips. All eggs were measured microscopically at 430 magnifications and all third stage larvae were measured at both 100 and 430 magnifications. All measurements were made with the aid of a calibrated ocular micrometer.

The lengths of the fourth stage larvae, juvenile and adult worms were determined with the aid of a Trisimplex microprojector. To obtain these measurements, the various developmental and adult stages were mounted on a slide in formol-saline solution under supported coverslips, the slides were placed on a microprojector and the image of each was projected onto a sheet of paper. A pencil line was drawn along the longitudinal axis of the image of each of the worms. The length of this line was determined by the use of a strip of paper. This result was

compared then to a calibrated scale, in millimeters, to secure the length of the worm. The use of this technique allowed the measurement of worms fixed in any position.

Microphotographs of lacto-phenol mounts were made with a Zeiss photomicroscope, were recorded on Afga Isopan IFF, 35 mm film and were enlarged on Kodabromide F-5, single weight photographic paper.

IV. LIFE HISTORY OF *Cooperia punctata* (v. LINSTOW, 1907) RANSOM, 1907

Most of the species of strongyle nematodes of domestic ruminants inhabit the digestive tract and have a direct life history or one in which no intermediate host is necessary for the completion of the cycle. In such a life history, the definitive host is usually exposed to infection via the oral route. The infective larvae gain entrance to the digestive tract with the ingestion of contaminated food and water, usually of pasture origin. The growth of most larval strongyle nematodes within the host is characterized by a succession of two molts, with alternating growth periods, and the terminal or fifth stage juvenile which matures.

According to Stewart (1954), the eggs of *C. punctata* were usually in the 8 to 16 cell stage when passed in the feces. They were thin shelled, transparent, with sides more or less parallel, and had an average length of 79 microns, a width of 36 microns and a form index, the ratio of length to width, of 43. Under optimum conditions, the 8 to 16 cell embryos developed into larvae by progressive division and differentiation of cells. The eggs hatched and the free-feeding, free-living larval stages had a characteristic developmental cycle outside of the host. Cultured at room temperature, the eggs hatched in approximately 20 hours.

The first stage rhabditiform larva was approximately 0.32 mm long

when hatched and almost doubled its length before the first molt. The first ecdysis, preceded by a short period of lethargy, occurred about 30 hours after hatching. The second stage rhabditiform larva was similar to the first but larger, average 0.62 mm in length. The larva fed actively, became quiescent immediately prior to the second ecdysis and molted to become a third stage larva. This larva differed from the two preceding ones in that it was infective and was resistant to environmental conditions. This infective third stage larva had a filariform esophagus, retained the cuticula of the previous molt as a so-called protective sheath ("ensheathed larva") and had an average length of 0.80 mm. Under optimum conditions the infective larval stage was reached 75 to 96 hours after the eggs were deposited in the feces of the host.

Such larvae when ingested continue their development in the host and Stewart followed their progress by recovering them periodically from the digestive tract. Some of the ingested third stage larvae, recovered in the abomasal contents of calves on the day following infection, had cast the loose sheath of the second molt ("exsheathed larva") and averaged 0.77 mm in length. On the second day following infection, third stage larvae were found only in the small intestine; they were all exsheathed and apparently had fed on the contents of the intestine. The larvae increased very little in length during the parasitic period of the third stage. One has to assume from the information given that the third ecdysis usually occurred while the larvae were in the crypts between the villi of the anterior part of the small intestine. Sexual differentiation became apparent four days following infection, after the worms had completed the third ecdysis and were in the fourth stage of development; the males averaged 1.4 mm and the females, 1.6 mm in

length. The posterior end of the larval male was shorter and thicker than that of the larval female and the genital primordium of the female was located nearer to the posterior end than that of the male. On the sixth day after infection, the length of the fourth stage male usually had increased to 2.6 mm and the female to 3.2 mm. The spicular mass and the bursal rays could be distinguished in the expanded posterior end of the male but the seminal vesicle and the testis were not well differentiated from the other body organs. The cuticle of the male terminated beyond the posterior primordial bursal expansion as a clear, spike-like tail. Most of the organs of the female genital system were easily recognized during this stage. The vulva was distinct and was located approximately 0.55 mm anterior to the anus, but both of the muscular ovejectors, including the sphincters, could be distinguished in only some of the specimens and together were approximately 0.23 mm in length.

Most of the worms completed the fourth ecdysis to become juvenile worms by the eighth day following infection. The juvenile males averaged 4.1 mm and the juvenile females, 5.4 mm in length. The genital systems of both sexes were well differentiated at this stage. Each of the spicules of the males averaged 0.17 mm in length, was not chitinized but had some of the characteristic folds and concavities peculiar to these structures in the mature worm. The genital cone and the rays of the bursa were differentiated. The vulva of the juvenile female was approximately 0.95 mm anterior to the anus. The ovejectors were well developed; the average length of both of the muscular parts, including sphincters, was 0.33 mm. About one-half of the body diameter of the female worm at the level of the vulva may be taken up by the width of the ovejector.

The young worms reach maturity rapidly. On the tenth day after

infection, the male worms averaged 6.2 mm and the females, 8.0 mm in length. Each of the spicules of the male was fully developed, was well chitinized and averaged 0.17 mm in length. A number of fully developed eggs were present in the uteri of some females.

Sexually mature worms (females with fully developed eggs) were collected by Stewart from calves as soon as 12 days after infection; the adult males averaged 6.65 mm and the adult females, 8.4 mm in length. The length of the esophagus in mature worms of both sexes averaged 0.38 mm. Other average measurements for organs in the adult female worm were: Anus to tip of tail, 0.18 mm; vulva to anus, 1.69 mm and length of both muscular ovejectors together with sphincters, 0.39 mm. The length of each of the paired spicules of the mature male was 0.18 mm.

According to Bailey (1949), the prepatent period (defined on page 45) for C. punctata infections in calves, based on experimental infections, ranged from 11 to 16 days, average 12 days, with the duration of the patent period (defined on page 60) as long as 9 months.

V. EXPERIMENTS INVOLVING SOME ASPECTS OF THE BIOLOGY OF *Cooperia punctata* INFECTIONS IN THE DOMESTIC RABBIT

A reciprocal relationship, the adaptation of the parasite to the host with concomitant adjustment of the host, is fundamental to successful parasitism. The adaptive capabilities of most animal parasites are governed by the interaction of various ecologic and physiologic factors which limit the potential host range. In normal host species it may be assumed that the parasite usually encounters near optimum conditions for maintenance and perpetuation but in an abnormal host or in a new adaptation, such conditions may not exist. Under the latter circumstances, the parasite may be affected by an inestimable number of inherent host factors, the effects of which may determine the degree of acceptability and susceptability of the host to the parasite. The demonstration of these effects, that is, the natural resistance of the host to the parasite, would be a measure of the degree of compatability of the host-parasite relationship. In most instances, this degree of compatibility can be ascertained without having to define or determine the causes of the host effects that are involved.

A maladjustment of the host-parasite relationship may hinder or retard the development of the larval or juvenile stages, may limit the number of worms that eventually establish, may affect the reproductive ability of the mature female worm, may restrict the distribution or even cause the elimination of all or part of the worms in the host.

Since specific nematode species vary in their ability to establish

successfully in different hosts within species and in different species, one purpose of this investigation was to determine the adaptability of Cooperia punctata to the domestic rabbit without attempting to define those physiological factors involved or to describe those pathological effects that developed within the host as a result of the relationship. Consequently, morphological characteristics of various stages of the parasite were determined by commonly used laboratory techniques and were compared with like stages from a normal host.

The preparasitic larval stages of C. punctata from experimental calf infections were described in detail by Stewart (1954) and the eggs of this species, also from calf infections, were illustrated by both Stewart (1954) and Shorb (1940). No attempt has been made by other workers to verify the accuracy of these determinations or to describe the eggs or developmental stages of this parasite from the sheep, a normal host, or other potential hosts. In order to gain a more comprehensive picture relative to the variation in the life cycle stages, the gross characteristics of eggs of C. punctata, collected from feces of calves and rabbits, experimentally infected, and of the third stage infective larvae, cultured in feces of these experimental hosts, were studied in detail.

Egg Characteristics

According to Shorb (1940), the egg of C. punctata has a thin shell surrounding a light brown cellular mass which is usually in the 16 cell stage when passed in the feces. The poles of the egg are bluntly rounded and similar. The sides are nearly straight and parallel. The average length is 76.6 microns, the width 32.2 microns and the form index, 42.

The eggs collected from experimental calf and successive rabbit

infections during this investigation did not differ significantly from the gross characteristics described by Shorb. Measurements and other data on eggs collected from these infections are presented in Table I.

TABLE I

MEASUREMENTS AND FORM INDEXES OF EGGS OF Cooperia punctata, FROM AN EXPERIMENTAL CALF INFECTION AND SUCCESSIVE RABBIT INFECTIONS*

Egg Source	Number Eggs Measured	Average Length**	Average Width**	Form Index
Calf, Experimental	100	74.2 (67.3--90.0)	36.3 (33.0--42.5)	49
Rabbits, First Passage	100	73.9 (68.1--81.7)	34.2 (31.6--37.7)	46
Rabbits, Second Passage	100	73.3 (65.9--81.7)	35.4 (33.6--40.9)	48
Rabbits, Third Passage	50	72.9 (62.4--82.2)	35.2 (31.8--38.6)	48
Rabbits, Fourth Passage	100	73.1 (68.1--80.6)	36.1 (34.1--39.7)	49
Rabbits, Average	350	73.4 (62.4--82.2)	34.7 (31.6--40.9)	47

*All measurements are in microns.

**Figures in parentheses indicate the range of measurements.

On the basis of 100 eggs from fresh feces of calves, the average length was 74.2 microns, the width, 36.3 microns and the form index, 49 and for 350 eggs from successive rabbit infections, the average length was 73.4 microns, the width, 34.7 microns and the form index, 47.

Pseudo-rumination or coprophagy is a normal physiological function in rabbits and is thought to result in improved digestion and in an increased supply of the vitamin B complex. It has been suggested that since the muscles of the stomach of the rabbit have little power of contraction, coprophagy provides a constant supply of fiber, the bulk of which helps to maintain the tone of these muscles. According to Ostler (1961), two types of fecal pellets are normally produced in the lower digestive tract of rabbits. Small soft pellets are formed at night and are normally ingested, the rabbit picking them from the anus as they are released. The more abundant hard pellets are formed both during the day and night and are not usually ingested.

Because all of the rabbits in these experiments were hungry constantly, the coprophagic habit became less selective but more intensified and the rabbits ate not only the soft but also the hard fecal pellets during both day and night. It was obvious that some cooperid eggs were ingested and possibly even reingested in the pellets from the infected rabbits. Consequently, it was necessary to determine what detrimental effects, if any, additional passages through the digestive tract of the rabbit had on these eggs. It was concluded that most of the eggs, whether or not they had been ingested by the rabbit, were viable since they could be cultured and normal infective larvae developed. This conclusion was based on experiments in which worm-free rabbits, 6 to 7 weeks old, were fed normal C. punctata eggs collected from calf feces. It was determined that viable eggs were passed in the stool of these rabbits in 8 to 10 hours. When cultured in distilled water at 27 C, 78 to 84 per cent of these eggs larvated and hatched.

Average daily egg production in the worm populations of rabbit

infections was not determined. The restricted dietary regime, the intensified, non-selected coprophagy and the fact that cooperid eggs could pass through the alimentary tract unchanged, produced effects which interfered with the random distribution of eggs in fecal pellets.

Third Stage Larval Characteristics

The ensheathed, infective larvae of C. punctata can be identified by characteristics of relative length and width measurements, anterior and posterior body structures and length and shape of the tail of the sheath. Measurements were made, for comparison purposes, of the length and width and of some anatomical parts of third stage larvae collected during this investigation. Average measurements of larvae obtained from successive rabbit infections were compared with the average measurements of larvae from normal hosts reported by other workers as well as those obtained from experimental infections in calves used in this study. These data are summarized in Table II.

Results based on these data show that the average length of infective larvae from rabbit sources was longer than those obtained for the experimental calves used in this investigation and for bovine sources reported by other workers. These data have not been subjected to statistical analysis because of the limited numbers involved.

The structural and behavioral development of a parasite may be acted on by physiological and immunological factors which are inherent in the host. The effect of these conditions may influence the growth pattern of the parasite and may result in morphological abnormalities or irregularities which can be demonstrated by using acceptable laboratory or experimental techniques. To reiterate, the consequences of this host-parasite interaction may be reflected by changes such as: (a) an

TABLE II

MEASUREMENTS OF INFECTIVE THIRD STAGE LARVAE OF Cooperia punctata FROM EXPERIMENTAL INFECTIONS IN RABBITS AND CATTLE*

Source of Larvae	Number of Larvae Measured	Average Length Including Sheath**	Average Width of Body**	Average Length of Esophagus	Average Length Anus to End of Body	Average Length of Sheath Beyond Larval Body**
Rabbits, First Passage	50	830.4 (750.0-980.0)	22.7	--	--	60.1
Rabbits, Second Passage	50	826.3 (760.0-880.0)	24.9	162.2	72.8	58.5
Rabbits, Fourth Passage	50	829.3 (770.0-895.0)	25.0	168.0	69.7	59.5
Cattle, Experimental, (1963)	50	798.1 (734.0-850.0)	24.6	161.9	69.1	58.1
Cattle, Stewart, (1954)	100	798.0 (738.0-889.0)	--	159.0	71.0	65.0
Cattle, Keith, (1953)	--	(666.0-866.0)	(22.0-23.0)	--	--	(47.0-71.0)
Cattle, Hansen Shivnani, (1956)	--	814.0 (760.0-865.0)	--	157.0	65.0	55.0

* All measurements are in microns.

**Figures in parentheses indicate the range of measurements.

inhibition of larval development, (b) an increase in the length of the prepatent period, (c) a decrease in the size of the mature worm, (d) a smaller number of mature worms established in the host, (e) a decrease in the length of the patent period and (f) the development of abnormal morphological characteristics. Other subtle changes no doubt occur which are not easily recognized or which may be difficult to demonstrate on the basis of present methods.

Prepatent period

The length of time between the invasion of the host by an infective larva and recovery from that host of some new stage of the parasite is referred to as the prepatent period of infection. During this interval, the rate of development of the larval or juvenile stages may depend on specific environmental conditions. Any variation from the normal growth pattern should be reflected in the length of the prepatent period. It is apparent that variations in the length of the prepatent period can be used as one of the means for determining the compatibility of the parasite and the potential host.

The prepatent period for infection in rabbits was determined by fecal examination, using the sodium nitrate flotation procedure, and it was considered to be terminated when at least one normal or immature egg was observed in the stool. The prepatent period for C. punctata was based on experimental infections of 47 domestic rabbits, irrespective of age, sex or breed of host and larval source or size of larval dose.

Rabbits that were used in this part of the investigation were given infective larvae from either calf or rabbit sources. The initial rabbit infections were established after administration of third stage larvae that were cultured from eggs in feces of an experimentally infected calf.

Thereafter, each successive passage was established after administration of third stage larvae that were cultured from eggs in feces of rabbits of the previous infection.

The results of experiments to determine the influence of successive rabbit infections on the length of the prepatent period are presented in Table III.

TABLE III

RESULTS OF EXPERIMENTS TO DETERMINE THE INFLUENCE OF SUCCESSIVE RABBIT INFECTIONS ON THE LENGTH OF THE PREPATENT PERIOD

Larval Source	Male Rabbits		Female Rabbits		Total Number of Hosts	Prepatent Period* All Hosts (Days)*
	Number of Hosts	Prepatent Period Average (Days)*	Number of Hosts	Prepatent Period Average (Days)*		
Calves, Experimental	9	13 (11--15)	11	13 (11--15)	20	13 (11--15)
Rabbits, First Passage	7	13 (12--14)	3	13 (13--14)	10	13 (12--14)
Rabbits, Second Passage	5	14 (12--16)	4	13 (11--13)	9	13 (11--16)
Rabbits, Third Passage	-	--	3	13 (12--13)	3	13 (12--13)
Rabbits, Third Fourth Passage	4	13 (12--14)	1	13 (13)	5	13 (12--14)
Totals or Averages	25	13 (11--16)	22	13 (11--15)	47	13 (11--16)

*Figures in parentheses indicate the range of the prepatent period.

It is shown from these data that the source of the infective larvae whether from calves or successive rabbit infections had no effect on the

course of the development of the worms in the rabbit host. The length of the average prepatent period was unusually constant, 13 days, range 11 to 16 days.

Data from the infections in the same 47 rabbits were used to show the influence of breed of the host on the length of the prepatent period. These results are presented in Table IV.

TABLE IV

RESULTS OF EXPERIMENTS TO DETERMINE THE INFLUENCE OF BREED OF THE HOST ON THE LENGTH OF THE PREPATENT PERIOD

Larval Source	Male Rabbits		Female Rabbits		Total Number of Hosts	Prepatent Period All Hosts (Days)*
	Number of Hosts	Prepatent Period Average (Days)*	Number of Hosts	Prepatent Period Average (Days)*		
Dutch Belted	5	14 (13--15)	7	14 (12--15)	12	14 (12--15)
New Zealand White	15	13 (11--16)	9	13 (11--14)	24	13 (11--16)
New Zealand Red	5	12 (11--14)	6	12 (11--13)	11	12 (11--14)
Totals or Averages	25	13 (11--16)	22	13 (11--15)	47	13 (11--16)

*Figures in parentheses indicate the range of the prepatent period.

The results of these experiments show that the prepatent period for infections, regardless of breed, do not range widely nor differ significantly from the average of 13 days in either male or female rabbits.

The data obtained from the 47 experimental infections in rabbits were used also to determine whether age of the host had an influence on the

length of the prepatent period. These data are summarized in Table V.

TABLE V

RESULTS OF EXPERIMENTS TO DETERMINE THE INFLUENCE OF AGE OF THE HOST ON THE LENGTH OF THE PREPATENT PERIOD

Age Group	Male Rabbits		Female Rabbits		Total Number of Hosts	Prepatent Period All Hosts (Days)*
	Number of Hosts	Prepatent Period Average (Days)*	Number of Hosts	Prepatent Period Average (Days)*		
6 to 8 weeks	13	13 (11--15)	14	13 (11--15)	27	13 (11--15)
9 to 12 weeks	12	13 (12--16)	5	13 (13)	17	13 (12--16)
3 to 6 months	--	--	3	15 (14--15)	3	15 (14--15)
Totals or Averages	25	13 (11--16)	22	13 (11--15)	47	13 (11--16)

*Figures in parentheses indicate the range of the prepatent period.

Results show that age of the host has little influence on the development of C. punctata in rabbits under 12 weeks of age. The three Dutch Belted females, 3 to 6 months old, were considered to be sexually mature when infected. The prepatent period average and range in these rabbits were slightly greater but more data would be necessary to determine their significance.

The size of the larval dose given to each of the rabbits was in the range of 10,000 to over 1 million infective larvae. The prepatent period for infections of 41 of the 47 rabbits under 12 weeks of age, which re-

ceived less than 200,000 larvae each, ranged from 11 to 16 days, average 13 days. The average prepatent period for the 23 males and 18 females of this group was the same for each sex, 13 days, while the prepatent period range for each sex differed only slightly, 11 to 16 days for males and 11 to 15 days for females. The prepatent period for infections in the remaining six rabbits, 8 weeks to 6 months of age, which received over 200,000 larvae each, were determined. These were in the range of 13 to 15 days, average 14.5 days, however, the results were not considered significant because of the low number of individuals in each of the age groups represented. The range of the prepatent period, 11 to 16 days, for C. punctata infections in rabbits compares favorably with the range of 11 to 16 days reported for experimental infections in ten calves by Bailey (1949).

Several additional experiments were done that involved the length of the prepatent period for infections in rabbits. These were not considered a part of the successive infection experiments and were completed to obtain additional data. Only the most important and significant ones will be described.

In one experiment, each of six rabbits, 5 to 10 weeks old, was given infective larvae of species in several genera of gastro-intestinal nematodes which commonly occur in cattle. These rabbits were fed a commercial pelleted feed ad libitum instead of the restricted amount given to the rabbits used in the successive infection experiments. Several different species of nematodes were collected at necropsy, namely: Trichostrongylus axei, T. colubriformis, Nematodirus sp. and C. punctata. The prepatent period for only C. punctata was determined and no data were obtained concerning the prepatent period of the other species. The length of this

period for C. punctata infections ranged from 15 to 18 days, average 17 days. This average was significantly longer than the average, 13 days, obtained from the experimental infections in the 47 rabbits. This variation of the life cycle may be an indication of the natural resistance of the domestic rabbit, on a normal diet, to infection with C. punctata or to the effects, directly or indirectly, of the competition of the other nematode species. The average prepatent period obtained from these infections agree with the 17 day prepatent period for experimental infections of C. punctata in rabbits reported by Wood and Hansen (1960). However, these workers based their report on only one rabbit infection and no information was given for the other rabbit infections included in their preliminary studies.

In another experiment, an adult female New Zealand White rabbit, estimated age 18 months, was given approximately one million infective C. punctata larvae cultured from eggs in feces of an experimentally infected calf. This rabbit had been on a restricted diet for at least 3 months prior to infection. Viable cooperid eggs were not observed in fecal flotation preparations until 26 days after the rabbit was exposed to infection. The extended prepatent period possibly resulted from an inhibition of larval development due to an innate age resistance in the mature rabbit. Apparently this age resistance was not influenced by the effects of the restricted diet. This is merely an opinion based on conjecture, however, since there are numerous other possibilities.

It is obvious from these experiments that the domestic rabbit remains susceptible to infection with C. punctata while being maintained on full feed or after attaining 18 months of age. In each instance, however, the length of the prepatent period was longer than the average

obtained from the other experimental rabbits which were maintained on a restricted diet.

It is apparent that the rabbit, kept and maintained under controlled conditions, is a suitable host for the propagation of C. punctata. This is indicated by the fact that this parasite develops to sexual maturity in a period of time comparable to that required in the normal host.

Ratios and Distribution of Worms in the Rabbit Host

The development of a parasite to sexual maturity in an abnormal host is not prima facie evidence that the host-parasite association is completely optimal. Degrees of compatibility between the host and the parasite do occur but no satisfactory criterion for these determinations has been established that is applicable to all situations. It was assumed that further experiments could be completed to secure additional information concerning the degree of compatibility between the rabbit host and C. punctata. Experiments were undertaken to demonstrate the ratio of the number of infective larvae administered to the number of parasites that established and matured; to determine the distribution of the nematodes in the alimentary tract; to compare the average size of adult worms with those from a normal host; and to compare the length of the patent period with that in the normal host.

In one of these experiments, 16 domestic rabbits were infected with C. punctata larvae collected from either calf or rabbit sources. This was done to determine the percentage of the infective dose recovered as worms and to demonstrate the distribution of the worm population within the alimentary tract. The rabbits were examined and parasites collected 13 to 19 days after infection. These results are summarized in Tables VI and VII.

TABLE VI

TOTAL NUMBER OF MATURE Cooperia punctata AND THE PERCENTAGE OF THE INFECTIVE DOSE RECOVERED FROM SOME EXPERIMENTALLY INFECTED RABBITS AFTER A LIMITED PERIOD OF PATENCY

Rabbit Number	Breed and Sex*	Size Larval Dose	Source Larval Dose**	Number Male Worms	Number Female Worms	Total Number Worms	Percentage Infective Dose	Age at Necropsy (Weeks)§
12a-2	NZR-F	105,600	C	7	3	10	0.01	8.5(18)
38	NZW--	123,000	C	14	47	61	0.05	11.0(19)
11-1	NZW-M	104,700	C	84	80	164	0.16	9.5(16)
12b-3	NZR-M	98,100	C	100	102	202	0.21	8.0(18)
39	NZW-F	135,000	C	233	322	555	0.41	11.0(19)
10a-2	NZR-F	103,400	C	465	500	965	0.94	8.0(14)
R-2	NZW-M	10,372	RP-3&4	54	49	103	1.00	8.0(13)
R-3	NZW-M	10,900	RP-3&4	52	59	111	1.02	8.0(13)
51	NZR-F	100,000	RP-2	448	732	1180	1.18	13.0(19)
12-1	NZR-F	101,700	C	817	792	1609	1.57	9.0(18)
41	NZW-M	90,000	C	1013	1177	2190	2.43	11.0(16)
73	NZR-F	85,000	RP-4-C	1159	1479	2638	3.10	10.0(15)
R-1	NZW-F	10,450	RP-3&4	149	230	379	3.62	8.0(13)
69	NZW-M	32,300	RP-4	542	852	1394	4.31	17.5(15)
72	NZR-M	85,000	RP-4-C	3553	3750	7303	8.60	10.0(15)
10-1	NZW-M	106,500	C	4934	4929	9863	9.20	9.0(14)

* NZR and NZW indicate New Zealand Red and New Zealand White breeds while M and F indicate male and female. The age of rabbits represented was in the range 5.5 to 14.0 weeks, average 7.6 weeks.

**C indicates calf sources. RP- indicates rabbit passage and the associated number indicates the particular passage. RP-4-C indicates larval source was from calves that were infected with larvae from the fourth rabbit passage.

§ Figures in parentheses indicate the number of days from the administration of the infective dose to necropsy.

TABLE VII

DISTRIBUTION AND THE SEX OF THE WORMS IN THE ARBITRARY DIVISIONS OF THE SMALL INTESTINE OF SOME EXPERIMENTALLY INFECTED RABBITS

Rabbit	Small Intestine											
	First 18 Inches			Second 18 Inches			Remainder					
Number	Males	Females	Per Cent of Total	Males	Females	Per Cent of Total	Males	Females	Per Cent of Total	Total Number Males	Total Number Females	Total Number Worms
12a-2	—	—	--	--	--	--	7	3	100.0	7	3	10
38	—	7	11.4	—	3	4.9	14	37	83.7	14	47	61
R-2	48	46	91.3	4	1	4.9	2	2	3.8	54	49	103
R-3	12	21	29.7	14	7	18.9	26	31	51.4	52	59	111
11-1	4	2	3.6	11	5	9.7	69	73	86.6	84	80	164
12b-3	13	13	12.9	20	16	17.8	67	73	69.3	100	102	202
R-1	98	140	62.8	23	44	17.7	28	46	19.5	149	230	379
39	120	199	57.5	32	37	12.4	81	86	30.1	233	322	555
10a-2	45	117	16.8	227	199	44.1	193	184	39.1	465	500	965
51	157	343	42.4	255	316	48.4	36	73	9.2	448	732	1180
69	202	436	45.8	182	203	27.6	158	213	26.6	542	852	1394
12-1	32	74	6.6	82	96	11.1	703	622	82.3	817	792	1609
41	392	425	37.3	174	308	22.0	447	444	40.7	1013	1177	2190
73	224	362	22.2	609	776	52.5	326	341	25.3	1159	1479	2638
72	288	568	11.7	25	43	0.9	3240	3139	87.3	3553	3750	7303
10-1	317	373	7.0	2393	2485	49.5	2224	2071	43.5	4934	4929	9863

Results based on these data show that all worms collected were mature and that no immature stages were recovered. The number of worms collected from all rabbit infections varied from 10 to 9863, average 1796, while the percentage of the infective dose recovered ranged from 0.01 to 9.20 per cent, average 2.52 per cent. The relative number of C. punctata that developed appeared to be affected by the sex of the host but not by the size or the source of the larval dose. The percentage of worms recovered in eight male rabbits ranged from 0.16 to 9.20 per cent, average 3.37 per cent and for 7 females, 0.10 to 3.62 per cent, average 1.55 per cent. The infectivity of C. punctata in rabbits did not appear to increase when the infection was completed in successive passages. Bailey (1949) reported that the percentage of the infective dose recovered as worms, without reference to sex of the worms, in three experimentally infected calves, was 44 to 70 per cent, average 58 per cent. It appears that the domestic rabbit has a more limited susceptibility to the establishment of C. punctata as compared to a calf but this is difficult to assess for the rabbits were not only controlled as to environmental conditions but also as to the nature and amount of food given to them. Furthermore, other factors such as the anatomy of the digestive tract, the composition and pH of digestive secretions and the absence of a conditioning process on the infective larvae, which occurs in the ruminant animal, no doubt produced effects.

Data from the infections in the same 16 rabbits were used to demonstrate the distribution of the worms in three arbitrary divisions of the small intestine. The total number of male and female worms, was determined from washings collected from each of the intestinal divisions. Washings from the large intestine and cecum were also collected but these have not been examined for nematodes. These data are presented in Table VII.

It is obvious from these data that during the initial stages of patency nematodes are distributed throughout the small intestine. There is no indication that any one part of the intestine was consistently preferred as a habitat, rather, the distribution of the worms appeared to be an arbitrary one with no apparent trends demonstrated. The causes for this pattern of distribution can only be conjectured.

From these data, it would appear that there is some difference in the distribution of the worm population in the intestine of the rabbit as compared with that of the normal host. Bailey (1949) collected mature C. punctata from experimentally infected calves and, on the basis of their distribution as related to the intestinal lesions, concluded that they were more numerous in the first 30 feet as compared, for instance, to the remaining 80 to 90 feet of the small intestine.

Average Measurements of Mature Male and Female Cooperia punctata

Since the rabbit is an abnormal host for C. punctata, and since the digestive tract is much different from that of the ruminant, it is conceivable that the normal growth pattern as well as the gross morphology of the worms could be influenced by various factors associated with this unusual habitat. It was thought that evidence of any adverse effect would probably be indicated by the relative average size of the parasites. Consequently, data were collected from worms from rabbits to be compared with similar data obtained from other sources. Body measurements were made on mature worms collected from the small intestines of a succession of rabbits that were infected with larvae cultured each time from previous rabbit infections. These data are presented in Table VIII.

It is apparent from the data that the average body measurements are surprisingly constant for male and female worms collected from all

TABLE VIII

AVERAGE MEASUREMENTS OF BODY STRUCTURES OF MATURE Cooperia punctata OBTAINED FROM SUCCESSIVE RABBIT INFECTIONS

Rabbit Passage**	Source of Larvae**	Number of Worms Measured		Male*				Female*			
		Males	Females	Body Length	Body Width	Length Dorsal Ray	Length Spicules	Body Length	Body Width	Vulva to Tip of Tail	Anus to Tip of Tail
RP-1	Calves	102	141	6.02	0.081	0.092	0.142	7.29	0.083	1.57	0.147
RP-2	RP-1	33	51	6.06	0.085	0.095	0.148	7.59	0.092	1.72	0.153
RP-3	RP-2	38	48	5.80	0.087	0.095	0.145	7.26	0.090	1.67	0.155
RP-4	RP-3	62	62	5.82	0.086	0.092	0.146	7.10	0.090	1.56	0.151
RP-5	RP-4	20	20	6.16	0.084	0.093	0.144	7.51	0.090	1.58	0.157
Average Measurements All Infections ***		255	322	5.97 (5.44-6.32)	0.085 (0.073-0.096)	0.093 (0.084-0.100)	0.145 (0.136-0.156)	7.35 (6.41-7.93)	0.089 (0.075-0.093)	1.62 (1.30-1.76)	0.153 (0.133-0.157)

* All measurements are in millimeters.

** RP indicates rabbit passage and the associated number indicates the particular passage.

***Figures in parentheses indicate the range of measurements.

infections. These results indicate that the degree of compatibility between host and parasite was not influenced by successive passages in rabbits.

In order to determine if the average worm size attainable in the rabbit host differs from the average worm size in the natural host, measurements were compared with those of worms that were taken from naturally infected calves in Oklahoma and with those that have been reported in the literature by other workers. These data are summarized in Table IX.

It is apparent that worm measurements obtained from natural calf infections in Oklahoma compared favorably with those reported from calves by Stewart (1954). Consequently, these data were used as a basis for comparison to the average measurements obtained from nematodes from rabbits. When these data were compared, it was evident that the average body lengths of C. punctata from rabbit infections were consistently shorter than those from calf infections. Body measurements of C. punctata in other hosts were not available.

The reasons for size differences of a nematode species in a particular host can only be conjectured; however, Read (1951), and others, observed a "crowding effect" in tapeworm infections in which the size of the worm was inversely proportional to the number of worms present. They assumed that the limiting growth factor was possibly attributable to a component of the diet or to a lack of oxygen tension in the small intestine. Little is known about the existence of such physiological phenomena in nematode infections. To determine whether such a condition possibly existed in rabbit infections, specimens of mature C. punctata were collected from light, moderate, or heavy infections and were measured. These data are presented in Table X.

TABLE IX

AVERAGE BODY MEASUREMENTS OF MATURE *Cooperia punctata* FROM CALF INFECTIONS AS REPORTED IN THE LITERATURE COMPARED TO THOSE OBTAINED FROM NATURAL CALF INFECTIONS IN OKLAHOMA AND FROM EXPERIMENTAL RABBIT INFECTIONS

Reference	Male*				Female*			
	Body Length **	Body Width **	Length Dorsal Ray**	Length Spicules **	Body Length **	Body Width **	Vulva to Tip of Tail**	Anus to Tip of Tail**
Successive Rabbit Infections	5.97 (5.44-6.32)	0.085 (0.073-0.096)	0.093 (0.084-0.100)	0.145 (0.136-0.156)	7.35 (6.41-7.93)	0.089 (0.075-0.093)	1.62 (1.30-1.76)	0.153 (0.133-0.157)
Natural Calf Infections Oklahoma	6.60 (6.00-7.27)	0.089 (0.080-0.100)	0.101 (0.093-0.123)	0.157 (0.143-0.168)	8.20 (7.20-9.20)	0.095 (0.085-0.100)	1.77 (1.45-2.12)	0.159 (0.140-0.200)
Stewart (1954)	6.65	--	--	0.180	8.40	--	1.87	0.180
Ransom (1911)	(4.70-5.90)	(0.070-0.100)	(0.070-0.075)	(0.120-0.150)	(5.70-7.50)	(0.065-0.075)	(1.00-1.50)	(0.135-0.160)
Sprehn (1932)	(4.70-8.00)	(0.070-0.142)	(0.064-0.071)	(0.120-0.199)	(5.70-11.00)	(0.065-0.200)	(-- --)	(0.135-0.260)
Skrjabin, et al. (1954)	(5.00-9.00)	(0.080-0.140)	(0.060-0.071)	(0.125-0.145)	(5.70-10.00)	(0.200)	(0.989-1.500)	(0.135-0.260)

* All measurements are in millimeters.

**Figures in parentheses indicate the range of measurements.

TABLE X

MEASUREMENTS OF MATURE *Cooperia punctata* COLLECTED FROM LIGHT, MODERATE AND HEAVY INFECTIONS OF RABBITS

Degree of Infection	Rabbit Number	Breed **	Sex §	Size Larval Dose	Number Nematodes Recovered	Number Nematodes Measured Each Sex	Average Length Mature Nematodes*			
							Male		Female	
							Average	Range	Average	Range
Light Infections	R-2	NZW	M	10,370	103	10	5.96	5.53-6.38	7.45	6.77-8.08
	R-3	NZW	M	10,900	111	10	5.71	5.00-6.40	7.08	6.60-7.50
	R-1	NZW	F	10,450	379	10	5.75	5.07-6.48	6.91	6.35-7.63
	39	NZW	F	135,000	555	10	6.24	5.80-6.60	7.34	6.88-7.75
	Totals or Averages				287	40	5.92	5.35-6.47	7.20	6.65-7.49
Moderate Infections	41	NZW	M	90,000	2190	10	6.36	5.92-6.62	7.95	7.15-8.50
	69	NZW	M	32,000	1394	10	6.20	5.56-6.75	7.45	6.76-8.13
	73	NZR	F	85,000	2638	10	5.71	5.27-6.46	6.78	6.03-7.26
	12-1	NZR	F	101,700	1610	10	5.68	5.08-6.30	6.92	6.55-7.34
	Totals or Averages				1958	40	5.99	5.46-6.53	7.28	6.62-7.81
Heavy Infections	72	NZR	M	85,000	7323	10	5.78	5.34-6.43	6.91	6.28-7.40
	10-1	NZW	M	106,500	9869	10	6.01	5.20-7.78	7.29	6.80-8.00
	Totals or Averages				8596	20	5.90	5.27-7.11	7.10	6.54-7.70

* All measurements are in millimeters.

**NZR and NZW indicate New Zealand Red and New Zealand White Breeds.

§ M and F indicate male and female.

The interpretation of these figures indicated that no similar phenomenon existed in C. punctata infections in rabbits. Bailey (1949), Porter, et al. (1956) and others, have indicated that this parasite did not feed on blood because anemia was not observed in experimentally infected natural hosts. Since they are not blood suckers, they must take their nourishment from the tissues of the intestinal tract or from the intestinal contents. From the similarity of length measurements obtained from nematodes taken from rabbit hosts, regardless of degree of infection, it appears that the components of the intestinal contents were not a factor in limiting the size of the mature worm. Indirect evidence, therefore, indicates that C. punctata is a tissue feeding parasite.

Patent and Post-patent periods

The duration of the patent period of infection can be used as a criterion for determining the compatibility of a host-parasite relationship. In most instances, C. punctata, males and females, persisted in the rabbit host even after their presence could not be detected by routine laboratory methods. However, the techniques used measured only the reproductive period of the parasites and in reality only that of the females. The absence of eggs in feces for 3 successive days, based on at least two examinations daily, was the criterion used to determine the end of the patent period of infection. Data associated with some aspects of the patent period for C. punctata infections in rabbits are summarized in Table XI.

It is shown that the patent period for infections ranged from 3 to 38 weeks, average 12 weeks. Furthermore, the length of the patent period appeared to be affected by the sex of the host but not by the age or breed of the rabbit or by the source or size of the larval dose. The patent

TABLE XI

MISCELLANEOUS DATA CONCERNED WITH SOME ASPECTS OF THE PATENT PERIOD FOR *Cooperia punctata* INFECTIONS IN RABBITS

Rabbit Number	Breed and Sex*	Age When Dosed (Weeks)	Size Larval Dose	Source of Larvae **	Prepatent Period (Days)	Patent Period (Weeks)	End of Patent Period to Necropsy (Weeks)§	Age at Necropsy (Weeks)	Distribution of Worms in Small Intestine					
									First 18 Inches		Second 18 Inches		Remainder	
									Male	Female	Male	Female	Male	Female
59	NZW-M	13	52,000	RP-2	15	3.0	-- (4)	18.5	--	4	--	--	--	--
66	NZW-M	11	20,400	RP-2	14	3.5	2.0(124)	19.0	23	98	--	3	--	--
70	NZR-F	7	25,300	RP-3	12	4.5	2.5(9)	15.5	1	7	1	--	--	--
58	NZW-M	12	70,000	RP-2	12	4.5	2.5(9)	21.0	--	7	--	2	--	--
46	NZW-F	8	47,000	RP-1	13	6.0	13.0(-)	23.0	--	--	--	--	--	--
65	NZW-F	9	25,500	RP-2	13	6.0	1.0(50)	18.5	7	43	--	--	--	--
64	NZR-F	9	46,500	RP-3	12	6.0	4.5(8)	21.5	1	2	--	4	1	--
40	NZW-M	9	43,000	RP-1	14	7.0	9.0(-)	26.0	--	--	--	--	--	--
61	NZR-F	8	45,000	RP-2	11	7.0	3.5(1)	20.0	--	1	--	--	--	--
67	NZW-F	11	22,300	RP-3	13	8.0	1.0(10)	22.0	1	7	--	2	--	--
42	NZW-M	9	120,000	C	13	9.0	0.5(1)	21.0	--	1	--	--	--	--
60	NZW-M	6	43,000	RP-2	15	9.5	2.0(2)	19.0	--	2	--	--	--	--
36	DB-F	7	1,000,000	C	15	10.0	-- (88)	19.5	29	44	8	6	--	1
34	DB-M	7	168,000	C	14	10.0	-- (56)	19.0	10	35	2	4	--	5
32	DB-F	18	160,000	C	14	10.0	4.0(-)	30.0	--	--	--	--	--	--
31	DB-F	28	460,000	C	15	10.5	-- (36)	40.5	8	21	--	4	--	3
62	NZR-M	9	47,700	RP-2	16	10.5	1.0(14)	20.5	--	8	4	2	--	--
49	NZR-F	14	60,000	RP-2	14	11.0	3.0(5)	30.0	--	5	--	--	--	--
48	NZR-M	10	57,000	RP-1	13	13.0	-- (18)	23.5	--	12	1	5	--	--
43	NZW-M	6	65,000	RP-1	14	14.0	2.0(4)	24.0	--	4	--	--	--	--
50	NZR-M	13	104,000	RP-2	13	14.5	1.5(20)	30.0	1	13	--	6	--	--
44	NZW-F	6	50,000	RP-1	13	16.0	-- (3)	24.0	--	1	--	2	--	--
33	DB-M	6	130,000	C	14	18.0	-- (13)	26.0	2	10	--	1	--	--
37	DB-M	7	500,000	C	13	18.0	-- (41)	27.0	11	25	1	3	--	1
47	NZW-M	8	47,000	RP-1	12	23.0	2.0(7)	35.0	1	3	--	2	--	1
45	NZW-M	10	58,000	RP-1	14	24.0	2.0(9)	39.0	2	7	--	--	--	--
63	NZR-M	9	43,800	RP-3	12	38.0	2.0(2)	50.5	--	1	--	1	--	--

* NZR, NZW, and DB indicate New Zealand Red, New Zealand White and Dutch Belted breeds respectively, while M and F indicate male and female.

**C indicates calf sources. RP indicates rabbit passage and the associated number indicates the particular passage.

§ Figures in parentheses indicate the number of worms recovered at necropsy.

period in male rabbits ranged from 3 to 38 weeks, average 14 weeks and for females, 5 to 16 weeks, average 9 weeks.

It is evident from the data that C. punctata, at the end of the patent period, is distributed with the larger number at the anterior end and the smaller number at the posterior end of the small intestine. This is quite different from the distribution at the end of the prepatent period and shows that a differential elimination of nematodes from some parts of the intestine occurred after patency.

The post-patent period of infection refers to the length of time the parasite can persist in the host after its presence can no longer be detected by usual laboratory methods. The length of this period can be established only by the examination, at necropsy, of a number of hosts to determine the presence or absence of nematodes. It is evident from the data presented that the post-patent period ranged from 1 to 13 weeks, average 3 weeks. The lack of egg production in mature females may be due to the effects of a developing host resistance, to the unfavorable influence of specific dietary components or possibly, to the establishment of a period of senescence of the parasites.

VI. OBSERVATIONS ON THE DEVELOPMENT OF THE VARIOUS AGES OF IMMATURE STAGES AND THE MATURE, *Cooperia punctata*, IN RABBIT INFECTIONS

Cooperia punctata, an economically important nematode parasite of domestic ruminants, has been collected from the small intestine of cattle and sheep in all sections of the United States. The results of numerous investigations, concerned with the epizootiology of this parasite, are available but there is only one report that includes detailed descriptions of the characteristic life cycle stages, based on specimens from the calf. Domestic rabbits have been infected with this parasite but no descriptions of the life cycle stages nor any other details of infections have been recorded previous to this investigation.

This part of the investigation was undertaken to determine the developmental cycle of *C. punctata* in the domestic rabbit, to describe the life cycle stages based on specimens from this host and to compare the descriptions of parasitic stages with those reported from the calf.

To obtain the various parasitic stages, experimentally infected rabbits were sacrificed and examined at various intervals of time subsequent to their initial infection. The details of methods and procedures are described in the chapter on materials and methods. The course of infection for securing these life cycle stages was limited to the period of 1 to 18 days.

After a 24 hour period of infection, both ensheathed and exsheathed third stage larvae were distributed throughout the entire digestive tract

of the rabbit. The average length of the ensheathed larvae, collected at random from all parts of the intestine, was 0.81 mm. These specimens appeared to be similar anatomically to those administered to the host. No differences were observed in the morphology of the exsheathed larvae examined from the various parts of the digestive tract except that the average length measurement for those from the stomach, 0.76 mm, was shorter than the average length, 0.82 mm, for those collected from the small intestine.

After a 48 hour period of infection, all of the larvae had completed the second ecdysis. About 30 per cent of the larvae were in the stomach and the remainder were not distributed throughout the small intestine but were confined to the posterior half. The average length for third stage larvae ranged from 0.88 mm, for those in the stomach, to 0.99 mm, for those in the small intestine. The genital primordia and intestinal cells of these larvae were apparent as were the anal and excretory pores and the excretory gland cell. The tail ended bluntly and was bent dorsally in the manner which is characteristic for exsheathed, third stage larvae of this species.

After the third day of infection, most of the larvae had completed the third ecdysis, were in the fourth larval stage of development and were located throughout the small intestine. A few third stage larvae were collected from the stomach while the majority were distributed throughout the small intestine. Approximately 80 per cent of the fourth stage larvae were confined to the posterior half of the intestine while the ramainder were distributed in the anterior half. The third stage larvae in the stomach and intestine averaged 0.88 mm and 1.15 mm in length respectively, while the fourth stage larvae in the intestine

averaged 1.53 mm in length. Sexual differentiation was not apparent during this phase of development of the fourth stage larvae although the genital primordium of some specimens had undergone some growth and appeared to consist of a double row of cells.

After the fourth day of infection, most of the larvae were in the fourth stage of development and were located throughout the small intestine but a few were still in the third stage and were distributed in the stomach and uniformily throughout the small intestine. About 60 per cent of the fourth stage larvae were distributed in the posterior half of the small intestine with the remainder confined to the anterior portion. The average length for third stage larvae ranged from 0.84 mm, for those in the stomach, to 1.1 mm, for those in the small intestine. The fourth stage larvae which had just developed and were not differentiated as to sex were the smallest, average 1.4 mm, while the average length of fourth stage larvae, males and females, was 2.1 mm and 2.2 mm respectively. Sexual differences for larvae in the fourth stage were shown by tail characteristics. An enlargement, present in the region of the anus, made the posterior end of the male in this stage appear to be short and thick, while in the female, the tail tapered to a point and was transversely striated in the manner characteristic of the mature female. The parts of the genital primordium were not readily distinguished in either sex; however, the spicular area in male larvae was evident and in some larval females, a cluster of epithelial cells extended from either end of the central mass of the primordium. Invagination of the cuticle, proximal to the central part of the genital primordium, was observed in some and indicated the position of the developing vulva. The musculature of the esophagus of both males and females during the fourth larval stage appeared to consist of three linear parts, a characteristic of the esophagus

of the mature worm. The excretory pore was evident during this stage and was located on the ventral, median surface of the body immediately anterior to the end of the bulb of the esophagus.

After the fifth day of infection, all larvae were in the fourth stage of development, were distributed throughout the small intestine and some were in the process of molting to become fifth stage larvae. Approximately 98 per cent of the larvae were distributed in the posterior half of the small intestine with the remainder in the anterior part. No larvae were recovered from the stomach during this period. The average length for all larvae, 2.1 mm for males and 2.2 mm for females, regardless of degree of development, had not increased during the previous 24 hour period. In most of the numerous specimens examined, considerable variation was observed in the stage of development of the enlarged posterior end of the larval male. In this region, the bursa and bursal rays were partially differentiated but species characteristics were not yet evident. The spicular mass was apparent but the structure of the spicules could not be distinguished. In some individuals, the spicular sheath and cloacal structures were detectable. The expanded posterior end of the fourth stage larval male appears to develop in a postero-ventral direction and the cuticle terminates beyond this area as a clear spike-like tail. The structures of the female genital system were easily defined in most of the specimens examined. The margins of the vulvar opening were beginning to thicken, the outline of the vagina was apparent and was contiguous with the vulva, and both of the muscular ovejectors, including the spincters, could be recognized; however, the limits of the non-muscular part of the ovejectors could not always be distinguished. Transverse cuticular striations were seen at the extreme anterior end of the body of some of the

individuals of both sexes. The three parts of the musculature of the esophagus were more distinct than previously noted. More than 50 per cent of the larvae of both sexes from the posterior half of the small intestine were atypical in morphology. The reasons for these deformities were not determined but apparently were the result of some adverse effect exerted on the parasites in this habitat.

After the sixth day of infection, all worms were still in the fourth stage of development. Approximately 96 per cent of the larvae were located in the posterior half of the small intestine with most of the remainder distributed in the anterior part, while a few were in the stomach. The average length of the fourth stage larval males was 2.4 mm and for the fourth stage larval females, 2.6 mm. The fourth stage larval males were not uniform in the degree of development, a few had just completed the third ecdysis with little differentiation of reproductive structures while many had partially formed spicules and well developed bursal lobes and bursal rays. Most of the latter larvae were in the process of molting to become fifth stage larvae. In these specimens, the outline of the genital cone also was evident. The longitudinal cuticular markings could be seen on the body of the developing juvenile male through the loose cuticle of the fourth stage. All of the parts of the genital system of the fourth stage larval female could be differentiated, however, the primordia of the uterus and the ovary were both short and the lumen of the vagina did not connect with the lumen of the muscular ovejectors. Transverse cuticular striations of the body of the nematodes were apparent at the extreme anterior end and a slight bulging was observed in this area of some of the larvae examined. Many of the fourth stage female larvae were in the process of completing the fourth molt as was evident by the loosened cuticle

that enclosed the juvenile worm.

After the eighth day of infection, more than two-thirds of the worms had completed the fourth molt to the fifth stage of development while the remainder were still in the fourth stage. Approximately 99 per cent of the nematodes, fourth stage larvae and fifth stage juveniles, were confined to the posterior half of the small intestine while the remainder were distributed in the anterior portion. No fourth stage larvae or juvenile worms were recovered from the stomach during this period. The average length of the fourth stage larvae, 2.5 mm for males and 2.6 mm for females, had not increased even though they were 2 days older. However, after the fourth ecdysis, which occurred between the sixth and eighth day, the fifth stage juveniles apparently grew rapidly, the average length of juvenile males was 3.68 mm and of juvenile females, 3.97 mm. The testis of the juvenile males was short, the spicules had the characteristic shape but were not completely cuticularized and the lobes of the bursa and the bursal rays were well formed but not completely developed as to size. The genital cone appeared to be more prominent than in the fourth stage and had the characteristic shape of that of the mature male. In the juvenile female, the lumen of the vagina opened into the lumen of the muscular ovejectors, a lumen was apparent in the enlarged uterus and germ cells, en masse, could be distinguished in the ovaries near the contiguous ends of the uteri. The excretory pore, in both sexes, was situated on the ventral, median surface of the body immediately posterior to the end of the bulb of the esophagus. The characteristic vesicle of the head region along with the transverse striations was well developed. The pattern of the longitudinal cuticular lines or ridges, 14 to 16 in number, was similar to that observed in mature worms but was less distinct. More

than one fourth of the juvenile worms, both sexes, in the posterior half of the intestine were altered grossly in shape with evidence of degeneration of some internal structures.

After the tenth day of infection, all worms collected from rabbits were approaching sexual maturity. About 65 per cent were located in the posterior half of the small intestine with most of the remainder distributed throughout the anterior half, while a few juvenile worms were in the stomach. The period of the greatest growth in the fifth stage males and females occurred between the eighth and tenth day during which time the worms almost doubled in size; average length for males was 4.85 mm and for females, 6.15 mm. The size of the bursa of the males had increased to the proportions of mature specimens and the color of the spicules had deepened as a result of further cuticularization. The degree of development of the female reproductive system varied considerably but the uteri of most of the larger females were filled with well developed, full-sized eggs containing a nonsegmented mass. These eggs were positioned so that the lateral margins of the shells were in apposition and the longitudinal axes were set at an oblique angle to the transverse section of the worm. In several females, eggs containing nonsegmented masses were observed also in the lumen of the muscular ovejectors. Paired esophageal glands had developed and were located at the extreme anterior end of the esophagus in the region of the cephalic vesicle in both males and females. The ducts of these glands appeared to open at the junction of the buccal cavity and esophagus.

After the twelfth day of infection, the worm population consisted of fourth stage larvae, juvenile stages and sexually mature males and females. Approximately 67 per cent of the worm population was distributed throughout

the posterior portion of the small intestine with the remainder in the anterior half except for a few juvenile worms in the stomach. Some of the fourth stage larvae were in the process of completing the final molt and males averaged 2.4 mm and females, 1.95 mm in length. The persistence of the fourth stage larvae to the twelfth day after infection is unusual and was not reported for calf infections. Also, the size relationship, in which the males were larger than the females, is not consistent with that which was observed for other stages. The reasons for these unusual conditions were not determined. The average length of mature males and females was 5.62 and 6.72 mm respectively. The spicules of the mature males were still not completely cuticularized. Eggs with segmented masses were present in the uteri of the female worms located in the small intestine but eggs were not observed in the feces of this rabbit; however, a prepatent period of 11 days was recorded for one experimental rabbit in the course of this investigation. A few sexually mature worms, males and females, were collected from the stomach and it is unlikely that mechanical translocation occurred. Cooperia punctata has been reported by Ransom (1911), Skrjabin, et al. (1954) and LaPage (1956), to develop occasionally in the abomasum of cattle.

After the fourteenth day of infection, most of the worms in the intestine were sexually mature and some were gravid; however, a few juveniles were located in the anterior half of the small intestine. About 44 per cent of the worms were distributed throughout the posterior half of the small intestine with most of the remainder in the anterior half except for a few mature worms in the stomach. The average length, based on a few specimens, was 2.4 mm for juvenile males and 2.0 mm for juvenile females. The reasons or causes for this unusual size relationship, males

larger than females, were not determined. Sexually mature males and females averaged 5.8 mm and 7.0 mm in length, respectively, which, on the basis of comparison with mature specimens collected on previous days, shows that they increased in size after maturity was attained. The spicules of the male worms were completely cuticularized. The uteri of the females contained numerous typical eggs except that they showed different degrees of embryo segmentation, from zygotes to the eight cell stage. The average prepatent period for infections, based on numerous experimental infections in rabbits, was 13 days. The morphological characteristics of sexually mature worms have been described adequately by Ransom, Skrjabin and Yamaguti and since the morphology of C. punctata from the rabbit compares favorably with this parasite in the calf, a description of the mature stage is not included.

After the sixteenth day of infection, all worms collected from the small intestine were sexually mature. Although the majority of them were in the posterior half of the intestine, 87 per cent, as compared to the anterior half, 13 per cent, the total number present was small as compared to the previous infections. No worms were recovered from the stomach during this period. The average length was 5.3 mm and 6.2 mm for males and females respectively, and these measurements were significantly shorter than those of the fourteenth day. The low infection percentage and the smaller size of the sexually mature worms may have been due to an individual difference in resistance of the rabbit.

After the eighteenth day of infection, all of the worms were sexually mature. Approximately 82 per cent of the worms were in the posterior half of the intestine while the remainder were in the anterior half except for a few mature worms in the stomach. The average length for males and females

was 5.7 mm and 6.8 mm respectively. The alimentary tracts of two rabbits were examined during this period, and from the results obtained it is evident that there was a significant difference in the number of worms recovered: One rabbit had 10 mature worms in the intestine while the other had more than 1600. The large difference in the number of worms that established in these hosts may be due to individual variation to infection, to natural or acquired resistence or possibly to one or more of the numerous conditions associated with any host-parasite relationship.

Discussion

According to the life history of C. punctata in the calf as determined by Stewart, the third stage larvae were exsheathed when recovered from the small intestine of a susceptible calf 24 hours after infection; these larvae passed through the third ecdysis to the fourth larval stage by the end of the fourth day; sex of the larvae could be determined at this stage, the body of the male was enlarged at the region of the anus while the body of the female tapered posteriorly to a sharp point; the fourth ecdysis occurred during the seventh day after which the juvenile worms developed and grew rapidly. Eggs were formed in the uteri by the tenth day and mature worms were collected from the small intestine of calves 12 days after infection. No one has attempted to verify Stewart's work on the life cycle of C. punctata in calves.

One purpose of this investigation was to compare the developmental cycle and descriptions of the life cycle stages of C. punctata in the domestic rabbit with those reported for the calf. These experiments were carried out in greater detail and with more attention given to the descriptions of the morphological changes in the developing parasite than were shown in the work by Stewart. A larger number of animals was used

to determine or to verify the sequence of development of the parasite and the cycle was covered for a period of 18 days instead of 12 days.

Results based on the experiments reported here show that the life history of C. punctata in the domestic rabbit is similar to the cycle as reported in the calf. The following conditions were comparable in calf and rabbit infections: Most of the infective larvae in the stomach and small intestine were exsheathed 24 hours after infection; the third ecdysis occurred between the third and fifth days after which the structures of the genital system of both the male and female were distinguishable; the fourth ecdysis occurred between the sixth and eighth day and was followed by a period of rapid growth in both sexes; sexual maturity was evident by the end of the tenth day and normal sized eggs with non-segmented contents were developed in the uteri of many of the females; sexually mature males and females were collected from the stomach on the twelfth day of infection; the maximum length for worms of both sexes were recorded on the fourteenth day of infection and the average prepatent period was 13 days.

Developmental and morphological characteristics of C. punctata that were observed during this investigation and which have not been reported for experimental infections in calves include: The excretory pore, in the developmental and mature stages, was conspicuous and was situated on the ventral, median surface of the body at the posterior end of the bulb of the esophagus; the musculature of the esophagus showed three distinct areas in individuals of both sexes from the fourth larval stage to maturity; the posterior end of the fourth stage larval male developed in a postero-ventral direction; transverse cuticular striations, but no evidence of a cephalic vesicle, were developed at the extreme end of some

fourth stage larvae after the fifth day of infection; the lumen of the vagina was observed to be contiguous with the lumen of the muscular ovejectors in juvenile females after the eighth day while the characteristic vesicle of the head region was evident in both sexes during this stage and a pair of esophageal glands was observed, after the tenth day of infection, at the extreme anterior end of the esophagus in the region of the cephalic vesicle.

Developmental characteristics of the nematode in rabbits which differed from those reported in the calf included: The atypical body shape of fourth stage larval males and females, with evidence of degeneration of some internal structures, recovered from the small intestine on the fifth and eighth days; the persistence of fourth stage larval males and females, in the small intestine, to the twelfth day of infection; the unusual size relationship in fourth stage larvae and juvenile worms, in which males were larger than females, on the twelfth and fourteenth days; the distribution of developing larval stages, juvenile and mature worms in the stomach and throughout the intestine and, in most instances, with the majority distributed in the posterior half of the small intestine; an increase in the size of the mature worm after sexual maturity and the smaller average body length of sexually mature C. punctata obtained from patent rabbit infections.

To show graphically the growth of the various developmental stages of C. punctata in rabbits, a growth curve was constructed and was based on measurements of worms collected from rabbits 1 to 14 days after infection. Such a growth curve has not been reported previously. This curve is plotted in Figure 1.

It is obvious from this curve that exsheathed third stage larvae did

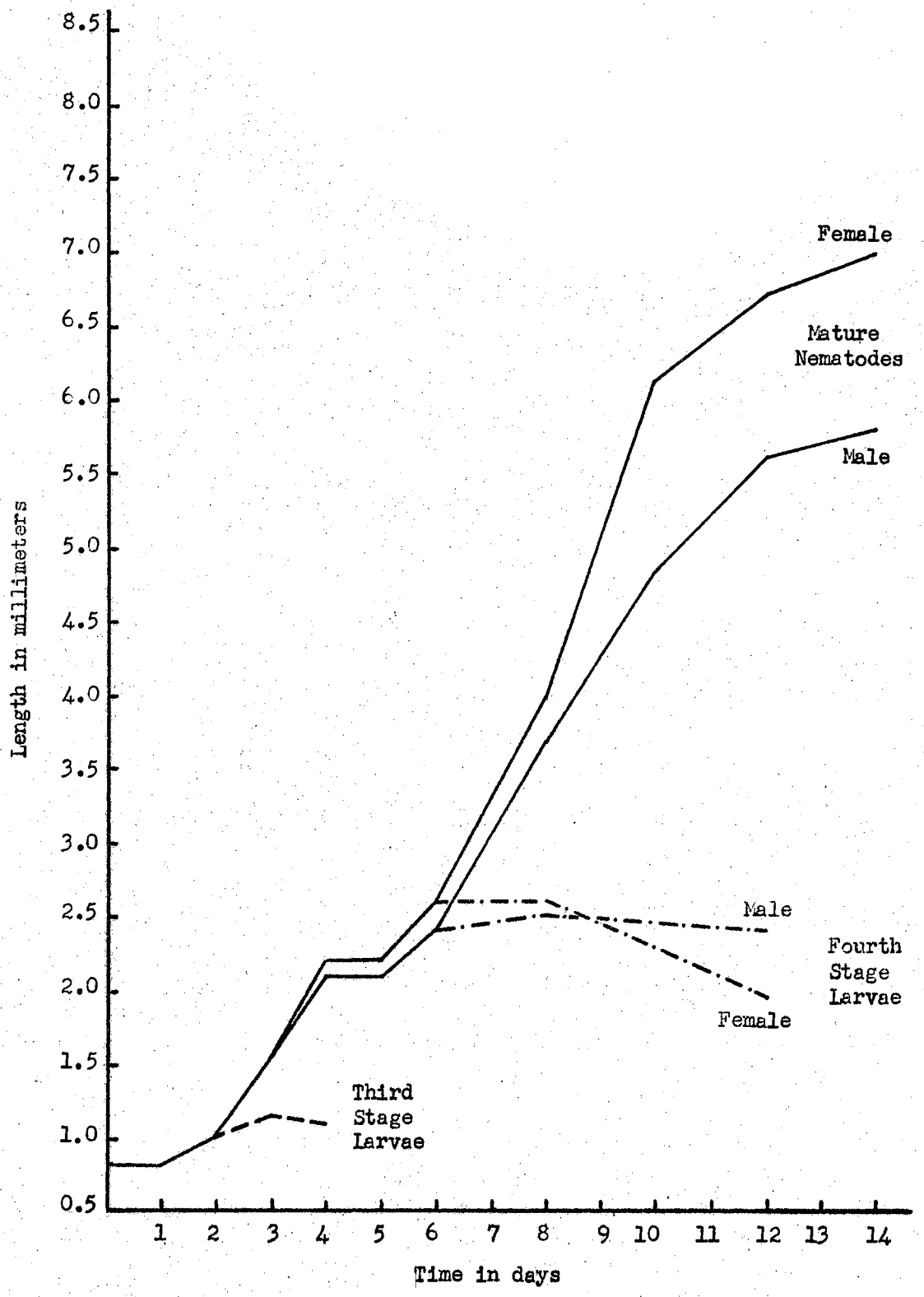


Figure 1. Growth curve of the developmental stages of Cooperia punctata in the domestic rabbit.

not grow very much during the first 48 hours. The growth of the fourth stage larvae occurred immediately after the third ecdysis and was followed by a period of lethargy, with no appreciable growth, which preceded the fourth ecdysis. Fourth stage larvae molted to the juvenile stage during the period of the sixth to the twelfth day with most of the individuals completing the transformation in the initial part of the period. The persistence of the fourth stage larvae to the twelfth day of infection has not been reported for calf infections. An explanation for the extensive delay that may be associated with the transformation from one developing stage to another was suggested by Sommerville (1960). He concluded that retarded development of nematode species often occurs prior to or following an ecdysis when the worms are most likely to be adversely affected by unfavorable features of the internal environment.

Comparison of the growth curves of the parasitic stages of C. punctata in the calf and in the domestic rabbit is presented in Figure 2. Length measurements reported by Stewart were used to construct the growth curve for stages from the calf source. It is obvious, when the curves are compared, that the growth of the parasite is similar in both hosts; however, the average length of the mature worms of both sexes were significantly larger for worms from calf infections. The reasons for this size difference, as stated previously, were not determined.

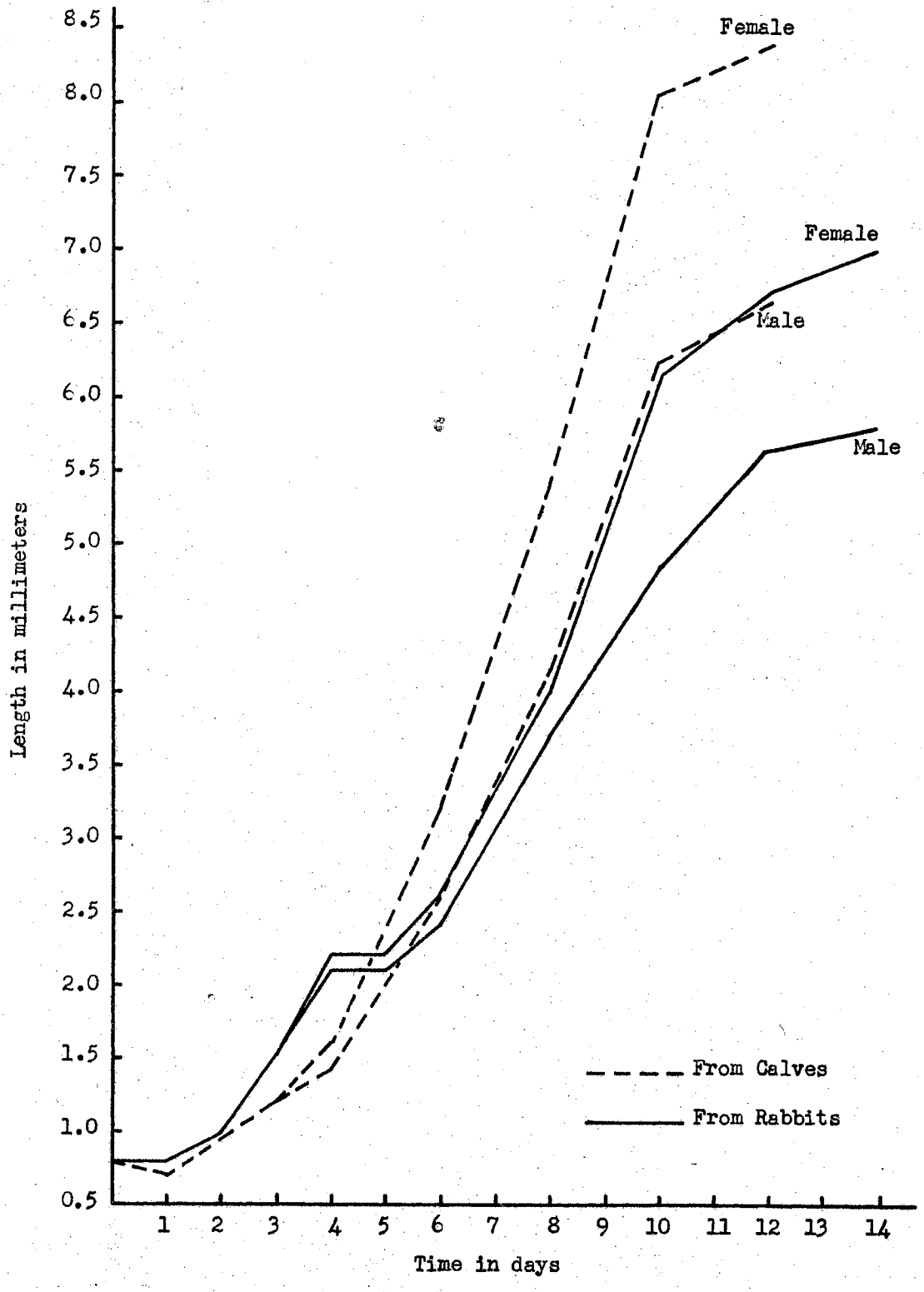


Figure 2. Comparison of growth curves of Cooperia punctata in the calf and in the domestic rabbit.

VII. OBSERVATIONS ON THE GROSS MORPHOLOGY OF *Cooperia punctata* COLLECTED FROM RABBIT HOSTS

According to Ransom (1911), species in the genus *Cooperia* have relatively broad, symmetrical anterior ends, the cuticula of which appears to be bulbous or expanded and marked by transverse striations. In *C. punctata*, this cephalic vesicle is well developed, is characteristic for the species, and is illustrated in Figures 3 and 4.

Various degrees of apparent deformities of the cephalic vesicle were observed in a number of mature worms collected from the small intestine of experimental rabbits. The percentage of worms that was involved in each collection varied considerably as did the degree and type of the deformity. These misshapen cephalic vesicles may have resulted from abnormal development or from the methods used in preserving the worms. Nevertheless, several live worms with deformed cephalic vesicles were seen in some of the samples collected; this observation eliminates manipulation as a factor. Cephalic deformation has not been reported previously for *C. punctata* or for any of the *Cooperia* species. The circumstances involved in the development of these cephalic anomalies are not understood but may be the result of natural or acquired resistance of the host. Several examples of distortions are illustrated in Figures 5 to 10.

Ransom (1907) reported that the various species of the genus *Cooperia* did not possess cervical papillae. Skrjabin, et al. (1954) stated that species in the genus *Cooperia* had small, scarcely noticeable papillae, situated either at the level of the esophagus or immediately posterior to

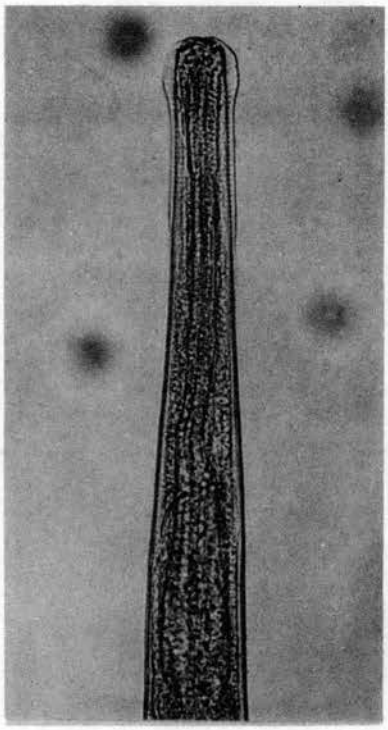
Figure 3. Anterior end of body, Cooperia punctata, showing the characteristic cephalic vesicle, the three parts of the esophagus and the location of the nerve ring.

Figure 4. Anterior end of body, Cooperia punctata, greatly enlarged, showing the characteristic cephalic vesicle, the first two parts and anterior end of third part of the esophagus to near the level of the nerve ring.

Figure 5. Deformed cephalic vesicle showing a slight enlargement of the extreme anterior end.

Figure 6. Deformed cephalic vesicle showing a decided enlargement of the anterior end to near the middle of the striated region.

Figure 7. Deformed cephalic vesicle showing an extensive asymmetrical enlargement of the anterior end including most of the striated region.



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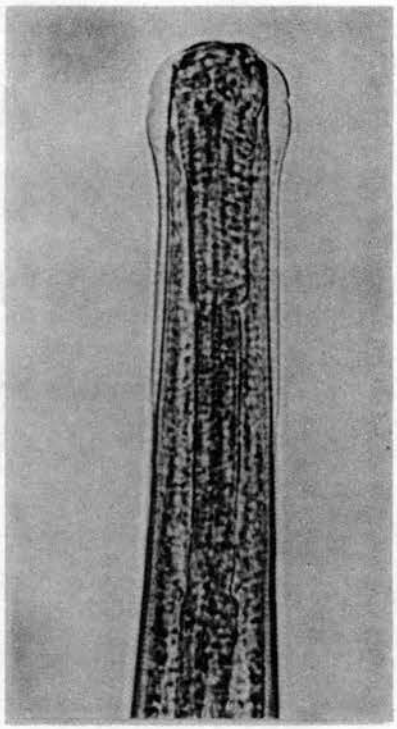

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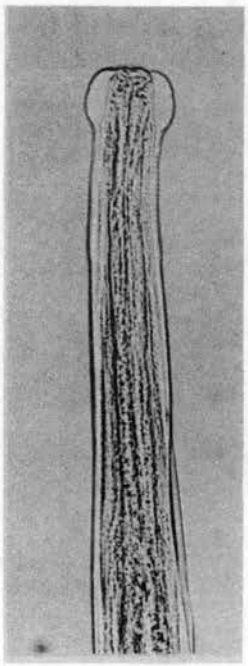

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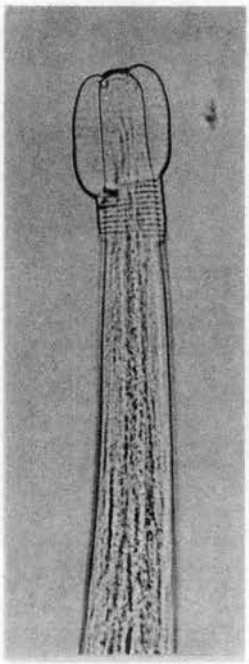

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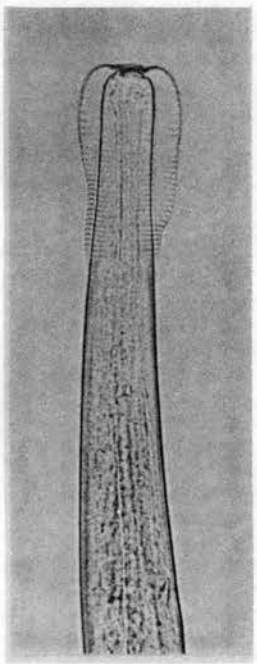

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it. Yamaguti (1961) in his diagnosis of the genus Cooperia noted that small cervical papillae were located at the level of the middle of the esophagus. Although Skrjabin, et al. and Yamaguti referred to the presence of cervical papillae as characteristic of species in the genus Cooperia, LeRoux (1936) stated that poorly defined cervical papillae were known to occur in C. punctata but he did not comment on them nor did he give any basis for his statement. Although several other writers alluded to their presence, no description of the cervical papillae of C. punctata is available. During the course of this investigation, a small, poorly defined structure, interpreted to be a cervical papilla was observed in a shallow, cuticular depression located on the ventral, medial surface of the body at the proximal end of the distal quarter of the esophageal region in both male and female mature worms. The minute, unpaired papilla was not always evident, but the cuticular depression was apparent in most of the numerous specimens examined. Several nerve fibers were observed to extend from the narrow ventral part of the nerve ring to the center of the depression in which the minute papilla was located. These observations do not agree with those made by Chitwood and Chitwood (1931) who indicated that cervical papillae, or deirids, of nematodes, in general, normally occur in pairs, are situated near the nerve ring, and in all cases, are innervated by the lateral longitudinal nerves. More information will be necessary to clarify the status of cervical papillae in C. punctata. The location of this minute papilla is illustrated in Figure 11.

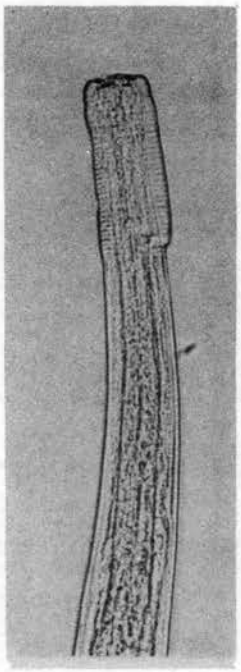
Ransom (1911) was the only writer to describe the shape and dimensions of the esophagus of the mature worm. In a brief discussion, he stated:

Figure 8. Deformed cephalic vesicle showing a slight asymmetrical enlargement of the striated region.

Figure 9. Deformed cephalic vesicle showing an anterior enlargement in which the esophagus appears to be affected.

Figure 10. Deformed cephalic vesicle showing an extreme asymmetrical enlargement of the anterior and terminal end, the striated region and the adjacent cuticle of the body.

Figure 11. Posterior part of the esophagus and anterior end of the intestine. The location of the minute papilla is indicated by A while B indicates the location of the excretory pore leading to the excretory duct.



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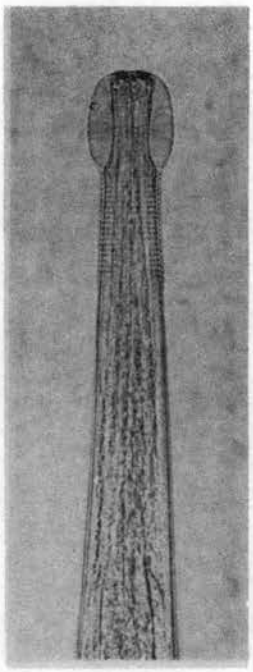

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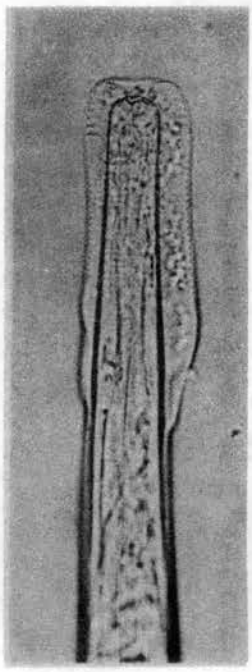

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The esophagus is about 20 microns in diameter at its beginning, is then reduced in size for a short distance, after which it gradually increases to a maximum of 30 to 35 microns at its posterior end.

In sexually mature C. punctata of both sexes, collected from calf and rabbit infections in the present investigation, the musculature of the esophagus appeared to consist of three distinct linear parts. The anterior terminal part was expanded and contiguous with the cuticle; diameter 0.026 mm. It tapered gradually posteriorly to the level of the middle of the striated part of the cephalic cuticular inflation where it narrowed abruptly; diameter, 0.017 mm. The second or middle part of the esophagus was approximately of uniform diameter to a level of the middle of the esophageal length; diameter, 0.016 mm. The third or posterior part of the esophagus gradually increased in diameter posteriorly to the maximum width near the terminal end; diameter, 0.032 mm. Lines of demarcation between areas are distinct. In reality, both the expanded anterior part and the narrow mid-section of the esophagus are sub-equal in length and comprised approximately one half of the total length. The characteristic structure of the esophagus was obvious in the fourth stage larvae as early as the fourth day after infection. The three parts were well developed and differentiated in juvenile worms on the eighth day.

The length of the esophagus of both sexes of mature worms collected from rabbit infections were very similar: for males, 0.315 to 0.390 mm, average 0.335 mm, and for females, 0.315 to 0.450 mm, average 0.343 mm. The nerve ring surrounded the posterior part of the esophagus, 0.08 mm from the terminal end.

VIII. SUMMARY AND CONCLUSIONS

The taxonomic history of the genus Cooperia Ransom, 1907, is reviewed and discussed. The systematic position of the trichostrongylid nematodes has not been stabilized and no taxonomic system for them has been universally accepted. It is apparent that the lack of harmony which persists in the various proposed classification schemes is the result of the insistence of the proponents to emphasize particular and divergent taxa in the development of their respective systems. All of these workers agree on the species group name but, of the three logical systems that may be used, each has a different taxon emphasis in the family group as well as in the higher taxon categories. No attempt has been made to unify these concepts as an acceptable taxonomic scheme.

A series of experiments was completed to determine the biology of Cooperia punctata, an intestinal nematode parasite of ruminant animals, in the domestic rabbit, Oryctolagus cuniculus. It was observed that viable C. punctata eggs were passed in the feces of infected rabbits and that the gross characteristics of eggs did not differ significantly from those described from the calf, the normal host. The characteristics of the infective third stage larvae, obtained from moss cultures of eggs from rabbit feces, were comparable to those reported from the calf host except for the length measurements which were consistently larger than those obtained from calf sources.

Results of data indicate that the rabbit, when kept on a restricted diet, is a suitable host for the propagation of C. punctata and that the prepatent period, range 11 to 16 days, average 13 days, was comparable to the prepatent period in calf infections, range 11 to 16 days, average 12 days. The length of the prepatent period was not influenced by the source of the infective larvae, by the size of the larval dose or by the sex and breed of the rabbit. Age of the host appeared to be one of the factors that influenced susceptibility; most of the rabbits infected were under 12 weeks of age while the age range for all rabbits was 5 weeks to 6 months. Other rabbits were infected while being maintained on full feed and in one instance, after attaining 18 months of age, however, in each of these infections, the prepatent period was longer than that obtained in rabbits on the restricted diet.

The total number of nematodes collected from each rabbit infection varied from 10 to 9863, average 1796, while the percentage of the infective dose recovered as mature nematodes from these infections ranged from 0.01 to 9.20 per cent, average 2.52 per cent. The relative number of nematodes that developed in the alimentary tract appeared to be affected by the sex but not the breed of the host nor by the size or source of the larval dose; the percentage of worms recovered from male and female rabbits averaged 3.39 and 1.55 per cent respectively. The infectivity of C. punctata in rabbits did not appear to increase when infection was completed for five successive passages.

The distribution of nematodes in the small intestine was determined for all rabbit infections. During the prepatent period, the majority of the developing larval stages, juvenile and mature worms was distributed in the posterior half of the small intestine with the remainder in

the anterior half and stomach. During the initial stages of patency, the nematodes were distributed throughout the small intestine with no one part consistently preferred as a habitat; while during the latter stages of patency, they were distributed with the larger number at the anterior end and the smaller number at the posterior end of the intestine.

Average body measurements were determined and were unusually constant for male and female nematodes recovered from all rabbit infections. These results indicate that the degree of compatibility between host and parasite was not influenced by successive passages in rabbits.

The average size for male and females did not appear to be influenced by the total number of nematodes, whether small or large, present in the alimentary tract. Cooperia punctata are not considered to be blood suckers, consequently, their nourishment must be derived from the tissues of the intestinal tract or from the intestinal contents. Since the measurements of the length of the nematodes recovered from rabbits were so similar, regardless of the degree of infection, it appears that the components of the intestinal contents were not a factor in limiting the size of the mature worm, and this indicates, indirectly, that C. punctata is a tissue feeding parasite.

The length of the patent period in rabbits, irrespective of age, sex, breed of host or source and size of the larval dose, ranged from 3 to 38 weeks, average 12 weeks. The length of the patent period appeared to be affected by sex but not by the age or breed of the host or by the source and size of the larval dose. The patent period in male rabbits ranged from 3 to 38 weeks, average, 14 weeks, and in female rabbits, 5 to 16 weeks, average 9 weeks.

The post patent period of infection was established by examination at necropsy and in 27 rabbit infections, ranged from 1 to 13 weeks, average 3 weeks.

The life history of Cooperia punctata has been completed, under laboratory conditions, in the domestic rabbit. When the rabbit is kept on a restricted diet, the cycle is comparable to that reported for the calf by Stewart. The following developmental and morphological characteristics were found to be similar in both calf and rabbit infections: Infective third stage larvae exsheathed within 24 to 48 hours in the stomach and small intestine; the third ecdysis of these larvae occurred in the small intestine between the third and fifth day and primordial structures of the sex organs were distinguishable in some larvae on the fourth day after infection; sexual dimorphism was evident at this stage and sexes differed in characteristics of tail structures, an enlargement in the region of the anus in the male and no enlargement but a tapered, pointed tail in the female; most of the worms had completed the fourth ecdysis by the end of the eighth day; there were numerous well developed eggs containing nonsegmented masses in the uteri of most females after 10 days and in a few instances, eggs with nonsegmented masses in the lumen of the muscular ovejectors; sexual maturity was reached in 11 to 14 days after infection and the prepatent period average, based on numerous rabbit infections, was 13 days. Developmental and morphological characteristics not reported heretofore included: A conspicuous excretory pore, in the developmental and mature stages, situated on the ventral, median surface of the body at the posterior end of the bulb of the esophagus; an esophagus consisting of three distinct areas in the fourth larval stage to maturity; a postero-ventral

expansion at the terminal end of fourth stage larval males; transverse cuticular striations at the anterior end of the body, but no developed cephalic vesicle, as early as the fifth day of infection in some fourth stage larvae; a characteristic cephalic vesicle well formed in both sexes beginning on the eighth day in the juvenile stage; a pair of esophageal glands evident in the region of the cephalic vesicle beginning on the tenth day in the mature worm and a small, poorly defined structure, in a shallow, cuticular depression located on the ventral, median surface of the body at the proximal end of the distal quarter of the esophageal region in both male and female mature worms, interpreted to be a cervical papilla.

Developmental and morphological characteristics which differed from those taken from calves included: The atypical body shape of fourth stage larval males and females, with evidence of degeneration of some internal structures, recovered from the small intestine on the fifth and eighth days respectively; the persistence of fourth stage larval males and females in the small intestine to the twelfth day of infection; the distribution of developing larval stages, juvenile and mature worms, in the stomach and throughout the intestine and, in most instances, with the majority distributed in the posterior half of the small intestine; an increase in the size of the mature worm after sexual maturity and the average size of mature male and female worms was never equal to that reported from calves, the normal host.

The growth pattern for the various developmental stages of C. punctata, based on length measurements of worms collected from rabbits 1 to 14 days after infection, was determined and plotted. Such a growth curve had not been reported previously for this species.

Various degrees of apparent deformities of the cephalic vesicle were observed in a number of the mature worms recovered from the small intestine of the experimental rabbits.

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