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NOTES ON PARASITIC NEMATODES, INCLUDING DESCRIPTIONS OF NEW GENERA AND SPECIES, AND OBSERVATIONS ON LIFE HISTORIES.

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I. THE GENERA TRICHOSTRONGYLUS, OSTERTAGIA, N. G., COOPERIA, N. G., AND NEMATODIRUS, N. G., WITH BRIEF DESCRIPTIONS OF NEW SPECIES.

In addition to the larger, more or less readily recognizable species of strongyloid nematodes occurring in the alimentary canal of ruminants, there are numerous small forms which, on account of the imperfect state of our knowledge concerning them, have received special attention in the course of an investigation of the nematodes of American ruminants. A study of a large number of specimens from various localities has shown that over a dozen species of these small strongyles are present in this country, some of which have been identified as species already described, and others as new species. They are discussed only very briefly in the present paper, but in a forthcoming article they will be described more fully.

Genus TRICHOSTRONGYLUS Looss, 1905.

Before Looss (1905) issued his paper on *Trichostrongylus*, the small strongyles referred to above, so far as concerned the various species then known, were all grouped in the genus *Strongylus*. From this genus Looss separated the species *Strongylus retortaeformis*, *S. instabilis*, and *S. probolurus*, and placed them in a new genus *Trichostrongylus*, which included also the species *Trichostrongylus vitrinus*, newly described by Looss. With the exception of *Trichostrongylus retortaeformis*, the type of the genus, which occurs in rabbits, all of the species mentioned occur in ruminants, and some of them also in other animals, including man.

The species of *Trichostrongylus* listed by Looss have all been found in ruminants in this country except *T. retortaeformis*, which has been collected in this country only from a domesticated rabbit, and but once. Two additional species of *Trichostrongylus* have been found in America.

Trichostrongylus extenuatus (Railliet, 1898) = *Strongylus gracilis* McFadyen, 1897, not Leuckart, 1842.

This species, originally described from specimens collected from the fourth stomach of cattle in England, has been collected in this country from sheep, goats, cattle, and various other ruminants.

Trichostrongylus capricola, n. sp., from goats and sheep, United States.

This species is of about the same size as *T. instabilis*, but is readily distinguished from the latter by the fact that the latero-ventral ray of the bursa is noticeably thicker than any of the other rays, whereas in *T. instabilis* the externo-lateral ray is usually thicker than the others. The dorsal ray proper is also quite different from that of the latter species, its branches being nearly equal in length to the stem. In *T. instabilis* the branches are very short and the stem relatively long. The spicules are of about the same size in both species, but differ in that their points are smooth in the new species, while in *T. instabilis* they are barbed.

Three other genera formerly included in the genus *Strongylus* are represented by the lesser strongyles of American ruminants. These are here proposed for the first time.

Genus OSTERTAGIA, n. g.

Type species: *Strongylus ostertagi* Stiles, 1892, from cattle, Europe and America.

Important characters of this genus are the presence of cervical and prebursal papillæ; the two ventral rays of the bursa close together, with their tips in relation to a more or less conspicuous indentation of the edge of the bursa; the presence of an accessory bursal membrane attached to the dorsal end of the body near the dorsal side, inside the bursa; accessory piece of the spicules present or absent, inconspicuous if present.

Besides *Ostertagia ostertagi*, other species of this genus occurring in this country are as follows:

Ostertagia circumcincta (Stadelmann, 1894).

Ostertagia trifurcata, n. sp. From the fourth stomach of sheep and goats, United States.

Ostertagia trifurcata may be distinguished from *O. circumcincta*, which it usually accompanies, by the shorter spicules (150 to 180 μ), which are divided lengthwise in the distal portion into three processes, namely, two shorter, acutely pointed processes and one longer, thicker process truncated at the end. In *O. circumcincta* the spicules measure 280 to 320 μ in length and are divided distally into but two processes. The female of *O. trifurcata* has not been identified.

Ostertagia marshalli, n. sp. From the fourth stomach of sheep, Montana.

This species has a large bursa 550 to 690 μ in width when spread out, with a long, slender dorsal ray (280 to 400 μ long) bifurcated 200 to 280 μ from its origin. The accessory bursal membrane is unusually conspicuous (65 to 75 μ long by 70 μ wide). The spicules are rather delicate in structure, 250 to 280 μ long, split lengthwise in the posterior one-fourth to form three terminal processes, two of which are slender; the other, less conspicuous, is shorter, broader, and thinner. Accessory piece absent. The eggs of this species are very large, 160 to 200 μ long by 75 to 100 μ wide, with a double shell; the outer shell is very thin and delicate, the inner slightly thicker.

Ostertagia occidentalis, n. sp. From the fourth stomach of sheep, Montana.

This species has been found in company with the preceding species. The male is somewhat larger (12 to 16 mm. long) than the male of *O. marshalli* (10 to 13 mm. long), and the bursa is also larger (650 to 740 μ wide). The most conspicuous difference is in the spicules and in the presence of an accessory piece. In *O. occidentalis* the spicules are very strongly built, 290 to 320 μ long, divided lengthwise, beginning a short distance posterior of the middle, into three prominent terminal processes. The female of this species has not been identified.

Genus COOPERIA, n. g.

Type species: *Strongylus curticii* Giles, 1892, from sheep, United States.

This genus is characterized especially by its relatively thick head, the cuticle of which is annulated and often forms a vesicular swelling; by the absence of cervical and prebursal papillæ; by the peculiar conspicuous beaded appearance of the cuticle along the longitudinal lines; by the wide separation of the tip of the ventro-ventral ray of the bursa from the tip of the latero-ventral ray; by the peculiar branching of the dorsal ray into two prominent main branches curved to form a lyre-shaped or horseshoe-shaped structure, each with a branch which originates near the junction of the main branch with the stem and extends ventrally in a vesicular swelling on the inner surface of the bursa; and by the absence of an accessory piece.

Besides *Cooperia curticii*, the following species of this genus have been collected from American ruminants:

Cooperia punctata (Schnyder, 1907).

Cooperia oncophora (Railliet, 1898).

Cooperia pectinata, n. sp. From cattle, Texas.

Cooperia pectinata may be identical with *Strongylus ventricosus* Rudolphi, from *Cervus elaphus*, but it is impossible to determine this

point until the type specimens of Rudolphi's species can be restudied. It may be readily distinguished from *C. curticii* and *C. punctata*, with which it is most likely to be confused, by the fact that the middle portion of the spicules has a prominent ventrally projecting curved edge with well-marked corrugations at right angles to the border, giving the latter a toothed or pectinated appearance, while the terminal portion of the spicules is relatively very slender. Furthermore, the spicules are larger, measuring 240 to 280 μ in length. In *C. curticii* the middle portion of the spicules forms a ventrally projecting edge, but this is less conspicuous than in *C. pectinata*, and the corrugations are less marked. The spicules of *C. curticii* are also smaller, having a length of but 135 to 145 μ . In *C. punctata* the spicules measure only 120 to 150 μ in length and are without corrugations.

Genus NEMATODIRUS, n. g.

Type species: *Nematodirus filicollis* (Rudolphi, 1802), from the small intestines of sheep and other ruminants, Europe and America.

In this genus the ventro-ventral and latero-ventral rays of the bursa are close together, as are the medio-lateral and postero-lateral rays, the tip of the externo-lateral ray being about midway between the tips of the ventral rays and those of the medio-lateral and postero-lateral rays. Instead of one unpaired dorsal ray, as is usual in strongyles, there are two dorsal rays. The spicules are long and filiform, without an accessory piece. The eggs are large with rather thick shells, and the embryo develops to the ensheathed stage, or nearly to the ensheathed stage, before hatching. One species of this genus, *Nematodirus filicollis* (= *Strongylus filicollis* Rudolphi), has been found to be a very common parasite in the various ruminants of this country.

II. OBSERVATIONS ON THE LIFE HISTORY OF STRONGYLOIDES LONGUS.—INFECTION THROUGH THE MOUTH AND THROUGH THE SKIN.

It has recently been demonstrated by different investigators that infection with certain nematodes parasitic in the intestine may take place by the penetration of the larvæ through the skin. In some cases it has been shown that the larvæ enter the hair follicles, reach the lymphatics and blood vessels, are carried to the heart, then through the pulmonary vessels to the lungs, where they enter the air sacs, and then migrate up the trachea, and finally down the esophagus into the stomach and intestine.

Looss and others have proved the occurrence of infection with hookworms in this way, and Van Durme (1902) observed that the larvæ of a species of *Strongyloides* found in an African chimpanzee readily penetrated the skin of guinea pigs. Whether the larvæ might finally reach the intestine and there undergo further development was, however, not determined. Recently Gonder (1907, Arb. a. d. k.

Gsdnhtsamte., v. 25) has demonstrated that the larvæ of *Strongyloides longus* readily penetrate the skin of dogs. In his experiments, however, intestinal infection did not result, and the larvæ were not traced further than the deeper layers of the skin.

The common occurrence of *Strongyloides longus* (Grassi & Segré, 1887) in sheep at the Experiment Station of this Bureau, Bethesda, Md., has afforded an opportunity of testing the possibility of infection with this species through the skin, and a few experiments bearing on this point and on other questions in the life history have been carried out.

Some fresh feces from a sheep, which was killed September 15, 1906, and found to be heavily infested with *Strongyloides*, were placed in a covered glass vessel and kept at the temperature of the laboratory. At this time the eggs of the parasites, present in the feces in great numbers, were nearly all unhatched, but contained active vermiform embryos.

September 16 the eggs had hatched and the culture contained a multitude of free rhabditiform embryos, not yet developed to the final larval stage. The following day some of this culture was fed to a rabbit. About two weeks and a half later (October 5) this rabbit was killed and the stomach and small intestine examined. No *Strongyloides* were found.

September 19 rabbit No. 2038 was fed some of the culture. October 24 the feces of this rabbit were examined microscopically, but no eggs of *Strongyloides* were found, nor were any worms of this species found when the rabbit was examined post-mortem December 15.

September 22 rabbit No. 2037 was fed some of the culture, and this feeding was repeated September 24, 25, and 26. October 24 the feces of this rabbit were examined and eggs of *Strongyloides* were found. The following day the rabbit was killed and two adult *Strongyloides* were found in the small intestine.

September 22 a small quantity of the culture of sheep feces was placed on the skin of rabbit No. 2039 and kept moist for one hour. The rabbit was kept under restraint during this time in order to avoid the possibility of getting any of the material into his mouth, and at the end of the hour 95 per cent alcohol was applied to kill any of the larvæ which might be remaining on the skin. Additional applications of larvæ were made September 24, 25, and 26 under similar conditions. October 24 eggs of *Strongyloides* were found in the feces of this rabbit, and eight adult worms were collected from the small intestine October 26, when the animal was killed and examined post-mortem.

The worms collected from these rabbits were carefully compared with specimens from sheep, and they appeared in every respect similar and unquestionably of the same species.

The possibility of infection from sources other than the culture used was considered, but as other rabbits, raised at the Bureau Experiment Station like those used in the foregoing experiments and kept under

similar conditions after being brought to the laboratory, were free from infection with *Strongyloides*, ten or twelve having been examined with special reference to the presence of this parasite, it seems safe to conclude that the rabbits in the experiments became infected from the culture of sheep feces which they received.

The probable explanation of the failure of infection in the two rabbits which were fed two and four days, respectively, after the feces were taken from the infested sheep, is that the larvæ had not yet developed to their final ensheathed stage, and hence were not infectious.

These experiments show that *Strongyloides longus* is transmissible from sheep to rabbits, which may become infected either by swallowing larvæ which have developed in the feces of infested sheep, or by the penetration of larvæ through the skin. At ordinary room temperature the larvæ in feces from infested sheep develop to the infectious stage in about a week, and after being swallowed by rabbits or entering the body through the skin reach maturity within a month. The occurrence of infection in rabbits both by the entrance of embryos through the mouth and by their penetration through the skin indicates that sheep may become infected in a similar manner.

III. NOTES ON THE LIFE HISTORY OF TRICHOSTRONGYLUS RETORTÆFORMIS.

In the course of an examination of the feces of rabbits in connection with the experiments described in the preceding article, in order to determine whether the rabbits bred at the Experiment Station of this Bureau at Bethesda, Md., were infested with *Strongyloides longus*, the eggs of a nematode were found, which were apparently those of some strongyle. These eggs were thin-shelled, measured 85 to 90 μ long by 45 to 50 μ wide, and contained embryos in the morula stage. The rabbit in whose feces these eggs were found was being used in an experiment by another division of this Bureau, and was unfortunately destroyed without a post-mortem examination for parasites, so that the worms producing the eggs were not obtained. A culture, however, had been made of the feces on November 1, 1906.

November 15, 1906, ensheathed embryos of a strongyloid worm were noticed on the inner surface of the bottle containing the culture, and on December 26, 1906, some of this culture was fed to rabbit No. 2046.

January 22, 1907, the feces of rabbit No. 2046 were examined, with negative results.

February 8, 1907, another fecal examination was made and eggs similar to those passed by the original rabbit were found.

May 25, 1907, rabbit No. 2046 died and was examined post-mortem. Several males and females of the species *Trichostrongylus retortæformis* were found in the small intestine.

The obvious conclusions from this experiment are that the original

rabbit from whose feces the culture was made was infested with *T. retortaeformis*, that the eggs found in the feces belonged to this species, that embryos hatched out and developed to the ensheathed stage within two weeks after the passage of the eggs from the intestine of the host, and that the embryos swallowed by the second rabbit developed to egg-producing maturity in the intestine in about a month after infection.

Although the possibility that the second rabbit became infested from some other source is not absolutely excluded, the fact that of ten or twelve other rabbits kept under the same conditions as rabbit No. 2046 none showed any infection with *T. retortaeformis* is additional evidence that the conclusions expressed above are correct.

The results of this experiment confirm those of Railliet (1889)¹, who succeeded in infecting a rabbit by feeding with the embryos of the parasite in question, and thus first established the fact that this species develops directly without an intermediate host.

The ability of the embryos of *T. retortaeformis* to survive for a long period of time under favorable conditions is witnessed by the fact that many of the embryos in the culture made from the feces passed November 1, 1906, were still alive at the date of the last examination, September 13, 1907, having survived more than ten months.

¹ Railliet. 1889.—Développement expérimental du *Strongylus strigosus* Duj. et du *Strongylus retortaeformis* Zeder. <Bull. Soc. zool. de France, Par., v. 14(10), déc., pp. 375-377.

Approved:

JAMES WILSON,
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