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UNITED STATES DEPARTMENT OF AGRICULTURE WASHINGTON, D. C.

# DIFFERENTIATION OF EGGS OF VARIOUS GENERA OF NEMATODES PARASITIC IN DOMESTIC RUMINANTS IN THE UNITED STATES

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#### INTRODUCTION

Domestic animals commonly harbor different species of helminths in their alimentary and respiratory tracts. The presence of these internal parasites may be determined by finding the eggs or larvae in the feces of the hosts. In some cases specific identity may be easily determined by the morphology of the eggs or larvae. In other cases the parasites present in the host are much more difficult to identify because the eggs or larvae of closely related species are morphologically similar

Specific diagnosis of common helminthic infections of dogs by fecal examination for eggs and larvae is relatively simple because the hookworms, tapeworms, whipworms, and ascarids-parasites ordinarily harbored by these carnivores-produce eggs and larvae which can be readily differentiated as to species. Cattle, sheep, and goats, however, are often infested with different species of roundworms, many of them producing eggs so similar in size and shape that a specific diagnosis on the basis of eggs present has not usually been attempted. Diagnostic methods, such as culturing eggs and developing larvae to the infective stage, as has been done by Dikmans and Andrews  $(3)^2$  and Andrews (1)and killing an animal from a flock or herd and examining the internal organs for worms, have also been suggested as a means of establishing definite diagnosis.

Although both of these methods are of value, they have obvious limitations. The first method depends for success on a knowledge of the appearance of the infective larvae and is, at best, time consuming.

<sup>3</sup> Submitted for publication February 3, 1939. <sup>4</sup> Halle numbers in parentheses refer to Literature Curd. p. 10.

Furthermore, all species of helminths do not have free-living larval stages. The second method cannot be resorted to when material is sent from the field. When only preserved fecal material is available. a diagnosis as to helminth species present must be based on morphology of the eggs.

Ranson (6) gave the dimensions of eggs of nematodes parasitic in the alimentary tract of ruminants reported to 1911, the year in which his paper was written. However, he did not attempt to separate the different genera of nematodes on the basis of the size and appearance of their eggs. Mönnig ( $\delta$ ). Clunies Ross and Gordon (2), and Freeborn and Stewart (4) gave measurements of eggs of nematodes parasitic in domestic ruminants but made no attempt to differentiate these eggs on morphological grounds. Wood (7) emphasized the limitation of size alone as a basis of specific diagnosis.

In view of the foregoing facts it was considered advisable to examine this problem in detail in order to determine whether the eggs of nematodes parasitic in runnants present sufficiently constant morphological and size differences to permit a reasonably accurate diagnosis.

#### MATERIALS AND METHODS

Eggs were obtained from adult female worms of (1) sheep killed at the abattoir of the Animal Husbandry Division. Bureau of Animal Industry, Agricultural Research Center, Beltsville, Md.; (2) ruminants killed at an abattoir in Baltimore, Md.; and (3) ruminants killed in the laboratory of the Zoological Division, Agricultural Research Center. Eggs of nematodes not commonly found in the eastern part of the United States were obtained from preserved specimens.

Adult worms, free from detritus, were teased apart in physiological saline to obtain the eggs. In order to prevent distortion, a few pieces of broken cover glass were placed on the slide as a support in order to prevent the cover glass from pressing on the eggs in the preparation. This precaution was found necessary because the apparent size and shape of the eggs change if the cover glass is allowed to press on them. This difficulty was also encountered in using the Stoll technique of egg counting. Distortion of this type was not noticed in salt- or sugarflotation preparations, because enough material to keep the cover glass from pressing on the eggs was raised with the eggs. As the water evaporated from the preparations, it was replaced by distilled water. The stage of development of eggs in the feces was determined by examining fresh feces from experimental animals at Beltsville. These eggs were separated by emulsifying the feces in a saturated solution of common salt or in a 40-percent sugar solution. It was found that the eggs floated better in the salt solution than in the sugar solution. but they were more readily plasmolyzed by the former.

In most cases 100 eggs of each species were measured. When this number was not obtainable and when eggs were so morphologically characteristic that they could not be confused with those of other species, a smaller number were measured. Measurements were taken with an ocular micrometer, use being made of a 4-mm, objective and a  $10 \times \text{ocular}$ . A 2-mm, oil-immersion lens was used for measuring the thickness of the shells, the thickness being checked by camera lucida drawings.

#### DESCRIPTION OF THE EGGS

Eggs (fig. 1) in fresh feces consist of a shell lined with a semipermeable membrane. The term "shell" is confined to the firm part that is not affected by plasmolyzing agents. This shell and membrane may enclose one or more cells or a fully developed vermiform embryo. The embryo is suspended in a transparent gel (fig. 1, e), which is evident when the eggs are rolled about. The cells never change their position with relation to the shell. This gel becomes liquid when treated with potassium hydroxide. Following treatment, the cells roll along the bottom within the eggshell when the position of the egg changes. This gel stains lightly with Sudan III. The quanty of this material differs somewhat in eggs of different species, depending on the relative size of the embryo. If the embryo almost fills the



FIGURE 1. Egg showing the three layers of the shell, a, b, and c, the egg membrane,  $d_i$  the gel surrounding the embryo,  $e_i$  and the embryo,  $f_i$ .

shell, as in eggs of *Cooperia*, this liquid is not evident at first, but if the embryo is withdrawn from the shell, as in eggs of *Nematodirus*, the gel is noticeable. The semipermeable membrane is partly soluble in both chloroform and artificial gastric juice. It was noticed that the membrane became thinner after treatment with chloroform, artificial gastric juice, alcohol, or other.

In the thin-walled eggs the eggshell consists of three layers. The outermost layer (fig. 1, a) in all eggs studied except those with maminillations is very thin and transparent, having a limiting membrane which is seen with difficulty when a 4-mm, objective is used. This layer is not dissolved when the eggs are placed in cold 10-percent or saturated potassium hydroxide, in chloroform, or in absolute alcohol, for 24 hours; neither is this membrane dissolved when the eggs are

placed in boiling 10-percent potassium hydroxide for 20 minutes. This first layer is dissolved in saturated hydrochloric acid but is more resistant than the third layer. The second layer (fig. 1, b) is dissolved in cold 10-percent potassium hydroxide within 24 hours and in hot 10-percent potassium hydroxide within 20 minutes. It is not soluble in hydrochloric acid, alcohol, or chloroform. The third layer (fig. 1, c) is dissolved in concentrated hydrochloric acid but not in potassium hydroxide. It is the third layer that gives the shell most of its firmness, the first and second lavers being soft and nonresistant to pressure of the cover glass. When hydrochloric acid is used to dissolve the third layer, the first and second layers spread out and the eggs become rounded under pressure of the cover glass. If the egg is first treated with hot potassium hydroxide to dissolve the second layer, and is then treated with saturated hydrochloric acid to dissolve the third layer, the first layer spreads rapidly to about twice its original diameter and then slowly dissolves. In thin-walled eggs the third layer is vellow, whereas the second layer is usually colorless. These two layers have definite limiting membranes, easily seen with a 4-mm. objective, in contrast to the first layer, which is clearly visible in most eggs only with the aid of an oil-immersion lens.

These findings with reference to the structure of the shells of the eggs studied confirm in part the conclusions of Zawadowsky and coworkers (8, 9). They stated that the shells of the eggs of Cooperio pectinata, Ostertagia mentulata, Trichostrongylus colubriformis (T. instabilis), and T. axei (T. extenuatus) consisted of three or perhaps four membranes, namely, an outer membrane A, a medial membrane B, and an inner membrane C+D, and that the shell of the egg of Nematodirus spathiger consisted of four well-defined membranes. In the study of all the species reported in this bulletin, the interpretation of the structure of the shells agrees with the interpretation of Zawadowsky and coworkers for N. spathiger, but differs from their interpretation for Cooperia pectinata, Ostertagia mentulata, Trichostrongylus colubriformis, and Trichostrongylus axei. A comparative study of figure 1 and a figure presented by Zawadowsky and coworkers (8, p. 46) for the egg of Trichostrongylus colubriformis shows clearly this difference of interpretation. The first layer in figure 1 does not appear to have been observed by Zawadowsky and coworkers for T. colubriformis. The second layer in figure 1 corresponds with membrane A in the figure of Zawadowky and coworkers, the third layer with membrane B, and the fourth layer with membrane C+D.

## MEASUREMENTS AND APPEARANCE OF EGGS OF VARIOUS GENERA

Measurements of eggs of nematodes from domestic ruminants in the United States, their appearance in fresh feces, their form index, and the measurements of the shells are given in table 1. A comparison of the eggs on the basis of appearance is shown in figure 2. The form index used in the table is a means of recording the relative thickness of the eggs. It is obtained by dividing the width of each egg by the length and multiplying the result by 100. In eggs with a length twice the width the form index is 50, in eggs with a length greater than twice the width the form index is less than 50, and in eggs with a length less than twice the width the form index is more than 50. The rounder the egg, the nearer the form index approaches 100.



FIGURE 2. -- Eggs of various genera of nematodes parasitic in domestic ruminants in the United States: I and 2, Gongylonema spp., from the uteri of worms preserved in alcohol; 3, 4, 5, and 6, Cooperia spp., from the uteri of worms; served worms; 7, Skrjabinema sp., from the uterus of adult worm; 8, Skrongyloides sp., from feees by salt flotation; 9, 10, and 11, Oesophagostomum spp., from uteri of both fresh and preserved female worms: 12 and 13, Bunostomum spp., from freshly collected worms; 14 and 15, Ostertagia spp., (except for 0, marshalli) from fresh worms; 16, Neouscaris sp., from a preserved worm; 17, Trickaris sp., from a freshly obtained worm; 18 and 19, Capillaria sp., from fresh and preserved worms; 20, Syngamus sp., from a preserved specimen; 21, Chabertia sp., from a fresh specimen : 22, Ostertagia marshalli, from the uterus of a preserved specimen; 23, 24, 25, and 26, Trichostrongylus spp., from fresh and preserved specimens; 27 and 25, Haemonchus spp., from fresh and preserved specimens; 27 and 25, Haemonchus spp., from fresh and preserved specimens; 29, 50, 51, and 52, Nematodirus spp., from fresh and preserved specimens.

		-		I	length	of egg	s	١	Vidth	of egg	5		Forn	index		Thick of sl	iness tell		
Genus and species	Host	Condition of material	Eggs measured	Maxmum	Minimum	Meetin	standard deviation	Visvinum	Minimum	Mean	Standard deviation	Broadest	Narrowest	Меан	Standard deviation	Combined thick- ness of second and third layers	Total thickness	Stage of de- velopment of eggs in fresh feces	Remarks
Bunostomum phlebotomum	- Cattle	Fresh	Nn m ber 100	Mi- crons 101	Mi- crons 88	Mi- crons 96, 5	Mi- cronx 3: 4	Mi- crons 56	Mi- crons 47	Mi- crons 50, 3	Mi- crons 1.3	Mi- crons 59	Mi- crons 47	M1- crons 52.2	Mi- crons 2. 1	Mi- erons 1 5	Mi- crons 2.0	8 to 16 cells	Cells larker than in
B. trigonoce phalum Capillaria toris C. brevipes Chabertia oclua Cooperia curticei	Sheep Cattle Sheep do do	do Preserved Fresh do do	100 61 5 100 100	97 54 53 100 82	82 41 49 83 70	80, 2 17, 6 51, 2 90, 9 76, 8	$   \begin{array}{c}     3, 3 \\     2, 1 \\     2, 8 \\     2, 7   \end{array} $	57 25 26 59 11	17 21 21 47 35	51.0 22-3 25.0 53.2 -37.7	1.9 3 2.0 9	69 58 51 67 55	50 10 47 51 45	$\begin{array}{c} 57.\ 2\\ 46.\ 7\\ 48.\ 8\\ 58.\ 5\\ 19.\ 3\end{array}$	$     \begin{array}{c}       1.0 \\       3.6 \\       3.3 \\       2.0 \\     \end{array} $	1.5 2.1 9	2 0 2.3 3.5 3.5 1.4	t to 2 cells do Morula do	Cells pale, almost lack
C. onchophora C. onchophora C. pectinata C. punctata C. punctata Gongylonema pulchenm	do. Cattle do. Slicep Cattle Slicep	do do Presorved Fresh do Preserved	108 100 17 100 25 100	92 95 80 85 83 61	74 74 67 67 69 52	83. 2 85. 7 72. 7 76. 6 76. 6 58, 0	3.2     3.6     3.4     3.7     3.7     2.6	44 44 38 38 - 34 34	36 36 31 29 26	39, 7 39, 4 34, 5 35, 0 32, 2 31, 6	2.4 1.7 1.5 1.5 1.6 1.5	$58 \\ 52 \\ 55 \\ 54 \\ 48 \\ 62$	43 37 40 38 35 46	50.5 15.9 47.9 45.9 42.0 54.6	$     \begin{array}{r}       3.7 \\       2.7 \\       3.7 \\       3.3 \\       2.8 \\       3.0 \\       \end{array} $	9 .9 .9 .9 .9 2.8	$1.4 \\ 1.4 \\ 1.4 \\ 1.4 \\ 1.4 \\ 1.4 \\ 3.6 \\ 3.6 \\ 1.1 \\ 1.4 \\ 3.6 \\ 1.1 \\ 1.4 $	do do do do Vermiform	Do. Do. Do. Do. Do.
G verrucosum Haemonchus contortus Haemonchus contortus H. similis Nematodirns abnormalis.	Cattle Sheep Cattle do Sheep	do Fresh do Preserved do	100 100 100 96 47	49 82 92 82 223	44 65 72 64 178	$\begin{array}{r} 47.\ 6\\ 74.\ 0\\ 81.\ 7\\ 70.\ 8\\ 211.\ 6\end{array}$	1.0 2.6 3.6 3.8 4.6	29 46 47 49 107	26 39 39 39 39 91	$\begin{array}{c} 28.4\\ 43.4\\ 43.8\\ 44.2\\ 96.3\end{array}$	1, 4 1, 6 2, 1 -1, 8	64 70 61 75 56	55 50 11 50 40	$\begin{array}{c} 60,4\\ 58,8\\ 53,7\\ 62,6\\ 45,6\end{array}$	$\begin{array}{c} 2, 1 \\ 4, 0 \\ 3, 5 \\ 4, 7 \\ 3, 2 \end{array}$	2.8 1.5 1.5 1.5 2.1	3.6 1.9 1.9 1.9 2.8	do Morula do do 1 to 8 cells	Cells withdrawn from
N. ülicollis N. helvelianus N. spathiger Neonscaris vitulorum Oesophayostomum columbi-	do Cattle Sheep Cattle Sheep	Fresh Preserved Fresh Preserved Fresh	81 76 100 100 100	194 233 230 93 88	149 184 181 69 74	$\begin{array}{c} 171.0\\212.0\\200.2\\80.0\\79.3\end{array}$	7.8 4.9 10,7 3.9 3.1	107 110 107 77 54	74 - 84 - 91 - 62 - 45	87. 5 06. 7 98. 3 67. 8 48. 8	5, 1 2, 1 1, 0 2, 0 1, 9	60 53 58 100 71	48 41 43 69 51	50, 8 15, 7 40, 2 85, 0 61, 8	$2.7 \\ 2.7 \\ 3.3 \\ 7.4 \\ 3.6 \\$	3.2 3.0 3.8 1.9	4.1 3.5 5.0 7.7 2.4	do do do l cell f to 16 cells	Do. Do. Do. Do.
anum. O, radiatum O, venulosum Ostertagia circumcincta and O, trifurcuta. <sup>1</sup>	Cattle Goats Sheep	do Preserved Fresh	100 127 100	98 105 103	75 85 85	85, 8 92, 7 94, 0	3, 9 3, 8 3, 9	51 39 56	46 47 44	49, 2 52, 2 48, 2	1.8 1.5 2.5	72 67 62	50 15 43	57, 6 56, 8 51, 7	3.6 3.6 3.9	1.9 1.9 .9	2.4 2.4 1.3	_do do Morula	a da Arabitationes de la composición Arabitationes de la composición

# TABLE 1. Data on eggs of nematodes parasitic in domestic ruminants of the United States

C. osterlagi D. marshalli	Cattle Sheep	do Preserved	- â0 100	00 217	74 178	78.5 193.8	3.2 4.7	44 100	38 78	40, 1 88: 5	$\begin{bmatrix} 1.6\\ 2.6 \end{bmatrix}$	50 54	42 37	51, () 45, 8	1.3 3.8	.9	1.3 7.7	do do
Skrjabinema ovis Strongyloides pupillosus	Gonts Sheep	do Fresh	100 50	.36 65	47 52	51, 6 58, 3	1.5 3.3	35 36	27 31	30, 9 33, 2	1.0 1.4	68 67	53 -18	60. 1 57. 1	$\frac{2.1}{1.5}$	<sup>3</sup> 2, 8 2, 1 5	$3,5 \\ 2,6 \\ .9$	Vermiform
Syngamus laryngeus Trichostrongylus axei T, capricola T, colubriformis T, vitrinus. Trichuris ovis	Goats Sheep Goats Sheep do do	Preserved Fresh Preserved Fresh do do	100 52 43 86 100 100	95 92 98 101 118 79	78 79 79 79 93 70	85. 7 84. 6 86. 8 87. 5 103. 2 74. 7	2.3 3.6 4.6 5.0 1.7 1.7	56 41 41 47 52 30	44 31 38 39 41 31	49, 7 36, 0 40, 9 43, 9 45, 0 34, 8	$ \begin{array}{c} 1. \ 6\\ 2. \ 1\\ 1. \ 6\\ 1. \ 7\\ 2. \ 3\\ 1. \ 8 \end{array} $	65 50 54 58 53 52	51 36 38 40 37 10	$58. 2 \\ 42. 6 \\ 47. 2 \\ 50. 3 \\ 43. 7 \\ 46. 7$	2.3 3.5 3.5 3.7 2	.9 .9 .9 .9	3.6 1.3 1.3 1.3 1.3 1.3 5.6	embryo. 1 cell Morula to do to 2 cells

Females of these species are indistinguishable from one another
 Sides.
 Ends.

DIFFERENTIATION OF EGGS

-1

It is evident from the data in table 1 that the eggs of Nematodirus spp. and of Ostertagia marshalli, which are much larger than other strongyle eggs found in the feces of domestic ruminants, and the eggs of Strongyloides papillosus, Skrjabinema ouis, Gongylonema spp., and Capillaria spp., which are much smaller than the others, may be separated from the eggs of other species on the basis of size alone. The eggs of Nematodirus spp. and O. marshalli resemble one another in size, as do also those of the other four genera mentioned. In order to separate these eggs from one another, criteria other than size must be used. The eggs of O. marshalli may be separated from those of Nematodirus spp. on the basis of shape and the stage of development of the embryo in eggs present in freshly passed feces. The eggs of Strongyloide, and Gongylonema spp. may be separated from other eggs resembling them in size by the fact that the former contain fully developed embryos when passed in feces. The eggs of Gongylonema are thick walled, possess opercula, and are easily distinguished from thinwalled nonoperculate Strongyloides eggs. The eggs of Capillaria spp. are easily distinguished from other eggs of the same size by the presence of pluglike structures at each pole. They resemble the eggs of Trichuris but are much smaller and differ sufficiently in shape to be easily distinguished from them. The eggs of S, or is differ from others in a group of the same size by being flattened on one side and rounded on the other. Of the remaining genera, the eggs of Syngamus laryngeus resemble those of Skrjabinema on's by being flattened on one side, but the former are larger and are provided with mammillations which serve to distinguish them from the eggs of the latter species. The eggs of Neoascaris ritulorum also have mammillations, but they are larger, rounder, and have thicker shells than those of Syngamus laryngeus.

The eggs of the other genera dealt with in this bulletin are similar to one another in most respects, but they may be distinguished by differences in size, shape, color, thickness of shells, and stage of development. The eggs of the various species of *Cooperia*, *Ostertagia* (with the exception of *O. marshalli*) and of *Trichostrongylus* have very thin shells and can be separated from one another by difference in shape and variations of color in the pigment of cells. The eggs of *Bunostomum* spp. and *Haemonchus* spp. have slightly thicker shells than those of the three genera just mentioned. Eggs of *Bunostomum* spp. are larger, have straighter sides, darker cell pigment, and are found in the feces in a slightly earlier stage of development than those of *Haemonchus* spp. The eggs of *Chabertia* and *Oesophagostomum* spp. may be distinguished from all others as well as from each other by the thickness of their shells and by the stage of development in fresh feces.

The following key will aid in recognizing the eggs and larvae of the various genera of nematodes occurring in the feces of domestic ruminants in the United States.

# Key to eggs of nematodes occurring in domestic ruminants in the United States

1,	Eggs containing fully developed vermiform embryos
-	Eggs not containing fully developed vermiform embryos
2.	Eggs with thin walls, nonoperculate
	Eggs with thick walls $(3.6\mu)$ , with operculum at each and Congregation
- 3.	Eggs with pluglike structure at poles
	Eggs without pluglike structure at poles
4.	More than 60 <sub>a</sub> in length
	Less than 60µ in length.
5.	More than 130µ in length
	Less than $130\mu$ in length $0$
б.	Shell greatly thickened at sides
	Shell not thickened at sides
7.	Shell with mammillations
	Shell without mammillations
- 8.	Eggs flattened on one side
	Eggs rounded
- 9.	Eggs less than 60g long, flattened on one side
	Eggs more than 60a long, either not flattened or flattened on both Skrjaoinema
10.	Combined thickness of the second and third layers of organized in 10
	than $1\mu_{-}$
	Combined thickness of the second and third burgs of organized
	than $1\mu_{\pm}$
11	Eggs tapering toward one or both ands
	Eggs not tapering toward ends
12.	Sides nearly parallel
	Sides curved () (operio
13.	Combined thickness of second and third layers of orgeneil a
	Combined thickness of second and third layers of aggined, $1.0\mu$ . 14
	1.5µ
14.	Freshly passed egg with 24 cells or less calls dark
	Freshly passed egg with 24 cells or more cells valiantal.
15	Combined thickness of second and third layars of orresholt 10 Haemonchus
	$\Lambda$ = $\Lambda$
	Combined thickness of second and third layers of energy and and third layers of energy and the second and third layers of energy and the second and third layers of energy and the second
	Chaberlia

#### SUMMARY

The eggs of various species of neuratodes representing 15 genera parasitic in the alimentary tract of domestic ruminants were studied. It was found that the eggs of these species may be distinguished from one another when fresh feces are examined. The criteria for differentiation are size, shape, characteristics of shell, thickness of shell, pigment in cells, and stage of development. These characteristics are presented in table form, and a key has been formulated which will assist in the identification of the various types. A discussion of the morphology of the egg is also given.

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