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## START




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DIFFERENTIATION OF EGGS OF VARIOUS GENERA OF NEMATODES PARASITIC IN DOMESTIC RUMINANTS IN THE UNITED STATES ${ }^{1}$

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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 lost 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--produec 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. Dingnostic methods. such as culturing eggs and developing larvae to the infective stage, as has been done by Dikmans and Andrews (9) ${ }^{2}$ and Andrews ( 1 ) and killing an animal from a flock or herd and examining the internal organs for worms, have iso 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.

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Furthermore, all species of heiminths do not have free-living larval stages. The second method amnot be resorted to when material is sent from the field. When only preserved fecal material is a railable. a diagnosis as to helminth species present must be based on morphology of the eggs.
Ransom (6) gave the dimensions of eggs of nematodes parasitic in the alimentary tract of ruminants reported to 1911, the year in which bis paper was written. However, he did not attempt to separate the different genera of nematodes on the basis of the size and appenrance of their eggs. Mönnig ( $\overline{0}$ ). Clunies Ross and Gordon (2), and Trechorn and Stewart (4) gave measurements of eggs of nematodes parasitic in domestic ruminants but made no attempt to diflementinte these args on morphologieal grounds. Wood ( ${ }^{\prime}$ ) emphasized the limitation of size alone as a basis of sperific diagnosis.

In view of the foregoing lacts it was considered advisuble to examinne this problem in detail in order to determine whether the cugs of nematodes parasitic in ruminants present suffeciently comstanf morphological and size differences to permit a remsonably acmurate diagnosis.

## materials and methods

Eggs were obtained from adnlt female worms of (1) sincep, killed at the abattoir of the Animal llusbmatre Division. Burena of Amimal Industry, Agricultural Rescarch Center. Beltsinille. Md.; © 2 ruminunts killed at an abattoir in Baltimore, Md ; and (3) ruminants killed in the labore'ous of the Zoological Division, Agricultural Researel: Center. Esgs of nematodes not commonly found in the easampart of the United States were obtained from preserved specimens.

Adult rorms, free from detritus. were teased apart in physiohogienl suline to btain the eqgs. In order fo prerent distortion, if few picces of broken cover ghass were placed an the slide as a support in order to prevent the cover ghass from pressing on the exges in the proparation. This precaution was found necessary because the apparent size and shape of the eggs change if the cover yhase is allowed to press on them. This difficulty was also encountered in using the Stoll techmique of egg counting. Distortion of this type was mot noticed in salt- or shgrarflotation preparations, because enough materinl to keep the cover glass from pressing on the cergs was raised with the eqgs. . As the water eraporated from the preparations, it was repaced by distilled water. The stage of development of eggs in the feces was determined by examining fresis feces from experimental amimals at Belterille. These eggs were separated by emulsifying the feces in a saturated solution of common sait or in a 40 -percent sugur solution. It was lound that the eggs floated better in the salt solution than in the surar 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 beiner made of a $4-\mathrm{mm}$. objective and a $10 \times$ ocular. A $2-\mathrm{mm}$. oil-immersion less was used for measuring the thickness of we 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 terms "shell" is couffined to the firm part that is not affected by plasmolvzing 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 hrdroxide. Following treatment. the cells roll along the bottom within the eggsheli when the position of the egg changes. This sel staine lightly with Sudan III. The quants of this material differs somewhat in egas of different species. depending on the relative size of the embryo. If the embroo almost fills the

 brane, 4, the gel surfounding the embryo, $t$; and the embryo, $f$.
sheil, as in eggs of ('orperia. this liquid is not esident at first. but if the mbry is withurawn from the shell, as in egers of Nematedirn. the ged is noticenble. The semipermenble membraue is party whble in both chloroform nod artificial gastric juice. It was noticed that the membrane berame thimer after treament with choroform, artifind gastric juice, alcohol, or ether.

In the thin-walled eggs the cogshell consists of thee layers. The outermost hayer (fig. i, a) an all eggs studied except those with mam:nillations is very thin and transparent, baving a limiting membraue which is seen with difficulty when a $4-\mathrm{mm}$. objective is used. This layer is not dissolved when the eggs are placed in cold 10 -percent or saturated potassium bydroxide, in chloroform, or in absolute alcohol, for 24 hours: neither js this membranc 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 dissolv $d$ 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 bydroxide. Tt is the third layer that gives the shell most of its firmness, the first and second layers being soft and nonresistant to pressure of the cover glass. When bydrochloric acid is used to dissolve the third layer, the first and second hayers 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 hrdrochloric 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 yellow, whereas the second layer is usually colorless. These two layers have definite limiting membrames, easily seen with a $4-\mathrm{mm}$. objective, in contrast to the first laver, 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 slated that the shells of the eggs of Cooperio pectinata. Ostertagia mentulata, Trichostrongylus colubriformis ( $T$. instabilis), and T, axet (T, extenuahus) consisted of three or perhaps four membranes, namely, an outer membrane $A$, a medial nuembrane $B$, and an inner membrane $C+D$. and that the shell of the egg of Nematodirus spahigrr 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 Zawndowsky and coworkers for $N^{T}$. spathiger, but differs from their interpretation for' ('ouperia pectinata, Ostertagia mentulata, Trichostrongphas colubriformis, and Trichostrongylus arei. A comparative study of figure 1 and a figure presented by Zawadowsky and coworkers ( $\delta, p .46$ ) for the egg of Trichostrongylus colubriformis shows alearly 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 Zawndowky and coworkers, the third layer with membrane B , and the fourth layer with membrane $\mathrm{C}+\mathrm{D}$.

## measurements and appearance of eggs of various genera

Measurements of eggs of nematodes from domestic ruminants in the United States, their appearance in fresh leces, their form index, and the measurements of the shells nee given in table 1. A comparison of the eggs on the busis 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 grenter than twice the width the form index is less than 50 , and in eggs with a length less thno lwice the width the form index is more than 00 . 'The rounder the egg, the nearer the form index approaches 100 .


Figere ? - - begys of various genera of nematodes parasitic in domestic ruminants in the 'nited States: 1 nad $\leq$, Gonghlonema sph., from the uteri of worms preserved in alcohen; $x^{4}, \overline{7}$, and 4 , Cooperia sppe, from both fresh and preserved worms; $i$, Skrjabincma sp., from the uterus of adult worm; $B$, Strongyloides sp.e, from feces by salt fotaliou; 9, 10, and 1t, Oenophagostomum spp., from uteri of both fresh and preserved remale worms: $1 z$ and 13 , Bunostomum, sppp, from freshly collected worms; $1 / 4$ and 10 . Ostertugiu spp.. (exeept for $O$. marshalli) fron fresh worms; 16 , Seouscaris sp, from a preserved worm; $\hat{i}$, Tricharis spe, from a freshly obtained warm; 1s and 19 , Capillaria spp., from fresh and preserved worms; 20 , Synfamhs sp, from a preserved specimen; $z 1$, Chabertia sp., from a fresh specimen: 22, Ostertuifia marshtalli, from the uterns of a preserved specimen; $23,24,2 \overline{0}$, and 20, , Trichostrongphes Sph, from fresh and preserved specimens; 27 and 25 , Haemonchas spp., from fresh and preserved specimens; \$9, 90 , $\$ 1$, and 82 , Nematodirus spp.: from fresh and preserved specimene.

Tabbif 1. Data on eggs of nematodes parasatic in domestic ruminants of the I'nited States


| 6. ostertayi | Cutue | pro... | 20 | 110 | 74 | 78.3 | 3.2 | 4 | 38 | 40. 1 | 1. 6 | 511 | 42 | 51.0 |  | . 9 | 3 | da. |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0. murshalli | Sheep | Preservad | 100 | 217 | 17 S | 103.8 | 4.7 | 100 | 78 | 83.5 | 2.6 | 54 | .37 | 45.8 | 3.8 | 14.9 | 7. 7 | do |
| Skrjabinema ovis. | Gouts | do | 100 | 3 t | 17 | 51.6 | 1. 5 | 35 | 27 | 30.9 | 1.0 | 68 |  |  |  | 32.8 | 3.5 2.6 |  |
| Ntrongyloites pupillosiss | Sheep | Fresh | 50 | 05 | 52 | 0.5 | 3.3 | 36 | 31 | 3 | 1.1 | 67 | ${ }_{4}^{18}$ | 60.1 57.1 | 2.15 | 2.1 +5 | 2.6 .0 | Yermiform |
| Sunyamas larynuets | Comis | Preserved | 100 | 45 | 78 | 85.7 | 2.3 | 56 | 4 | 19, 7 |  |  |  |  |  |  | 3.6 | embryo. |
| Trichostronuplus axei | Shered. | Fresh... | 52 | 02 | 79 | 81. 6 | 3. 5 | 4 | 31 | 316.0 | 2. 1 | 60 | 36 | 58.2 | 2.1 | 9 | 3.6 1.3 | cedl Morıla. |
| 7. capricola | Gionts . | Preserved | 43 | 4 S | 74 | 86.5 | 4.6 | 11 | 38 | 40.9 | 1.6 | 51 | 38 | 47.2 | 3. | 9 | 1.3 | Morida. |
| 7. colubriformis | Sheep | Fresh | 8 S | 101 | 79 | 87.5 | 5.11 | 17 | 33 | 43.9 | 1.7 | 5 S | 40 | 50, 3 | 38 | () | 1.3 | do. |
| T. vilrints | do | . do. | 100. | 118 | 93 | 108.2 | 1.7 | 62 | 41 | 45.0 | 2.3 | 53 | 37 | 13.7 | 3.3 | 9 | 1.3 |  |
| Trichutis onis | 10. | do. | 100 | 711 | 71 | 71.7 | 1.7 | 311 | 31 | 348 | 1.8 | 52 | 10 | 4 H \% | 21 |  | 5. 6 | 10:cmils. |

Females of these species are indistinguishable from one another sines

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, Shrjabinema ovis, 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 cggs 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 distinguisbed 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 $S$. onis 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 oris 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 bave mammillotions, 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 rarious 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. bave slightly thicker shells than those of the three genera just mentioned. Eggs of Bunostomuin 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 Desophagostomum 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 nid in recognizing the eggs and larvae of the rarious genera of nematodes occurring in the feces of domestic ruminants in the [nited States.

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

1. Eggs containing fully developed vermiform embryos................ $\mathbf{2}$

Eggs not containing fally developed vermiform embryos.-......... $\quad 3$
2. Eggs with thin walls, nonoperculate Strongyloides
Eges with thick walls $\{3.6 \mu\}_{\text {, }}$ with opercohm at each end Sirongyloides
3. Eggs with pluglike structure at poles
..- Gongylonema
Eggs without pluglike structurt at poles
4. More than $60 \mu$ in length

Less than $60 \mu$ in length.
5. Alore than $130 \mu$ in length 6
Less than $130 \mu$ in length

7. Shell with mammillations. . Nematodirus

Shell without mammillatious. . . . . . . . . 8
8. Eggs flattened on one sirfe. . . . . Syngamus

Eggs rounded
leoascaris
9. Efgs less than $60 \mu$ long, thattened on une side .... Skeascaris

Fogs more than $60 \mu$ long, either not fattened or fattencd on both skides 10
10. Combined thickness of the second and third layers of eggsheli, less
than $1 \mu$ -

Combined thickness of the second and third layers of egyshell, more
than $1 \mu$. . ...-
12. Sides nearly parallel ..... 12
Sides curved
Ostertagia texcept O. marshalli 13. Combined thickness of second and third layers of eggshell, his ..... 14Combined thickness of second and third layers of eggshell, more than1.50
15
14. Freshly passed egy with 24 cells or less: cells dark

## SUMMARY

The eggs of various species of nematodes representing 15 generas parasitic in the alimentary tract ol domestic rummants 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, thickuess of shell. pigment in cells. and stage of development. These characteristics are presented in table form, and a kes has been formulated which will assist in the identifation of the various types. A discussion of the morphologs of the egg is also given.

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