

MORPHOMETRICAL STUDIES ON SOME EGGS OF GASTROINTESTINAL NEMATODES FROM SHEEP

D. INDRE, GH. DĂRĂBUȘ, I. OPRESCU, S. MORARIU, NARCISA MEDERLE,
M.S. ILIE, IONELA HOTEA, K. IMRE, A. BALINT, DENISA SORESCU,
MIRELA IMRE

Faculty of Veterinary Medicine Timisoara, Department of Parasitology, 300645,
Calea Aradului No. 119, Timisoara, Romania
E-mail: indredinu1983@yahoo.com

Summary

Our objective was to quantify the diagnostic method based on morphological aspects and dimensions of eggs, compared with existing data in specialized literature. These eggs were collected from four adult female sheep flocks slaughtered in two individuals. Species determination was made based on identification keys played Soulsby (10), Euzeby (9), and Dunn (8). Of these eggs were made measurements with microscope Leica DM-2500.

Key words: eggs, gastrointestinal nematode, sheep

Trichostrongylids implications in the product of large economic losses through lower yields, reduced growth rate, low quality wool and especially milk, is that lately, more and more studies appear in this area (1, 2, 10, 11).

Diagnosis methods, regarding the differentiation by genera/species of gastrointestinal nematodes of sheep, by counting eggs that their morphological study is difficult or almost impossible. These diagnosis methods are largely related to approximate values and relatively inaccurate (12, 13).

The most common are eggs of the genera: *Haemonchus* spp, *Ostertagia* spp, *Teladorsagia* spp, *Trichostrongylus* spp, *Oesophagostomum* spp, *Chabertia* spp, *Cooperia* spp *Bunostomum* spp *Gaigeria* spp.

Many eggs of gastrointestinal nematodes are morphologically similar, precise measurements and calculations could play an important role for their differentiation. Their difference is very difficult to achieve, in addition to the morphologically distinct, such as *Trichocephalus* spp., *Nematodirus* spp. and *Stroglyoides papillosus* (12, 13).

Description of each genus/species must be accompanied by a series of morphological measurements on several populations of eggs, filled with different biostatistics parameters. Of these we mention: average, standard deviation, standard error, minimum, maximum or some features of the measurements: length, width and shape index (6, 13).

This study aimed to quantify the diagnostic method based on morphological matters, egg size compared with existing data in literature.

Materials and methods

This study was done in the discipline of Parasitology and Parasitological Diseases of the Faculty of Veterinary Medicine of Timisoara. Research was conducted during September (2009) and March (2010).

To conduct this study was required two stages:

First, four sheep were slaughtered in two private herds. One sheep was part of Turcana race and race three of the pan, with ages between 2 and 4 years. Gastrointestinal mass after processing, adult nematodes were collected, which were identified by the keys of determination described Soulsby (10), Euzaby (9), and Dunn (8). After identification of females and males of various species of gastrointestinal nematodes, were taken ten females from each corpse taken in the study, which have been triturate. Of triturated were made between the blade and slide preparations, clarified by lactofenol. To appeal at this stage to be identified with certainty identified new species eggs. There performed ten measurements of eggs from adult females, each species identified. In the second phase were randomly collected faecal samples from 10 sheep from each flock directly from the rectum. Highlighting the eggs was done by microscopic examination of preparations by the flotation method (Willis). Ten eggs were examined, which were assessed as the length, width. At this stage, highlighting eggs certainly not made light of their similar morphology. Egg shape was determined by microscopic examination between slide and slide preparation. Lamella size was a little special, it helps to clarify the characteristics of the eggs, eventually helping to image quality. Egg length and width were measured using a microscope Leica DM-2500.

Results and discussions

Overall, the gastrointestinal mass derived from the five bodies of sheep, adult nematodes were extracted, which, based on determination keys, identified the following species of gastrointestinal nematodes: *Nematodirus filicollis*, *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Bunostomum trigonocephalum*. By microscopic examination of preparations, the eggs appear as formations, oval, ellipsoidal, symmetrical, some light brown, some yellow and even black. Within them, can see some very dark blastomeres in six or eight.

Based on measurements of the eggs of gastrointestinal nematodes, one can say that the samples examined were identified following species: *Nematodirus filicollis*, *Trichostrongylus colubriformis*, *Haemonchus contortus* and *Bunostomum trigonocephalum*. Analyzing the morphological aspects of *Haemonchus contortus* eggs, most corresponded with those given by other authors (fig. 1).



Fig. 1. *Haemonchus contortus*-egg (ob.x40)



Fig. 2. *Nematodirus filicollis*-egg (ob.x40)

In the literature, we know the following information with reference to the characteristics of *Haemonchus contortus* eggs: egg has an average length of 74 μ m, with a minimum length of up to 95 μ m and 62 μ m and 44 μ m (35-60 μ m) wide. They are regular, large, ellipsoidal, slightly flattened at the poles and almost filled blastomerele whole egg (12, 14). In our study, the egg has a minimum length of 91 μ m and a length of 101 μ m, with an average of 94.7 μ m.

Maximum width was 62 μ m, and the minimum was 52 μ m, with an average of 58.3 μ m (tab.1). These dimensions have found level exceeded the average length of about 20 μ m and 14 μ m width with size aprovimativ species *Haemonchus contortus* described Thienpont et al. (12) (fig. 1).

Haemonchus contortus egg was oval shape, with equal poles, edges mombate and morula not fully filled cavity of the egg.

Table 1

Biostatistics parameters of *Haemonchus contortus* eggs

EGG SHAPE	COLO R	STATISTICAL INDEX	EGG LENGTH (μ m)	EGG WIDTH (μ m)	LITERATURE (Thienpont 1986)	
					L (μ m)	I (μ m)
Wide ellipsoidal	Dark brown	Average	94.7	58.3	74	44
		Standard error	0.978093	1.145523	-	-
		Standard deviation	3.093003	3.622461	-	-
		Sample variance	9.566667	13.12222	-	-
		Minimum	91	52	62	35
		Maximum	101	62	95	60
		N	10	10		

Legend: L-length;I-width; N-number of eggs examined

Analyzing the morphological characteristics of *Nematodirus filicollis* eggs. Soulsby (10) described a standard size: 130-200x70x90µm. Comparing the size and morphological eggs characteristics of *Nematodirus filicollis* obtained. extracted from adult females, their length varied from 145 to 264µm, with an average of 205.1 micrometres and width of eggs varied from 75 to 161µm, with a 102.7µm (tab. 2).

Following investigations on several samples, the length of this species has exceeded the size of the species *N. filicollis* respectively exceeded the average width of standard size about 20µm and the average length of about 50µm. Eggs were very large, the characteristic form, ovoid, black, and within them was present an morula up of 6-8 blastomere (Fig. 2). They have fine surface shell chitin and within their present 2.8 blastomere large dark (13). It should be noted that eggs prevented from faeces, not adult females, were larger.

Two of them met the larger, corresponding to the size of the species *Nematodirus spathiger*. One had a length of 257µm and 161µm wide width, and one was 264 x 144µm. Eggs are presented as more or less regular, slightly ellipsoidal, slightly colored, such as *N. filicollis*. and *N. spathiger* and *N. helevetianus* colorless or brown *N. battus* (11, 13).

After the eggs *Trichostrongylidae* study, we focused attention to the study *Ancylostomidae* eggs, respectively those of *Bunostomum trionocephalum*.

If eggs *Bunostomum trionocephalum*, literature brings us the following information on their main specifications:

Thienpont, and col. (1986) on *Bunostomum trionocephalum* eggs, reported an average length of about 90µm, with a minimum length of 75µm and 104µm maximum and average width is 51 (45-57µm), the egg is a large part quite large, irregular oval, and are present inside the 4-8 blastomeres as dark spots (13).

Table 2

Biostatistics parameters of *Nematodirus filicollis* eggs

EGG SHAPE	COLOR	STATISTICAL INDEX	EGG LENGTH (µm)	EGG WIDTH (µm)	LITERATURE Soulsby (10)	
					L (µm)	I (µm)
Wide ellipsoidal	Black	Average	205.1	102.7	150	75
		Standard error	12.06045	9.567364	-	-
		Standard deviation	38.13849	30.25466	-	-
		Sample variance	1454.544	915.3444	-	-
		Minimum	145	75	130	70
		Maximum	264	161	200	90
		N	10	10		

Legend: L-length;I-width; N-number of eggs examined

Compared with standard dimensions of literature, we obtained a minimum length of 102 μ m and a length of 115 μ m, with an average of about 106.9 μ m. Their minimum width was 55 μ m and the maximum width of 63 μ m, with an average of 59.3 μ m (fig.3). In this case, the measurements we obtained values were higher, mean length and width that exceeded the standard by about 15 μ m. Egg appearance was characteristic, it has a brownish-black, but noted that inside it was not obviously segmented morula, as depicted in the literature.



Fig. 3. *Bunostomum trigonocephalum*-egg (ob.x40)



Fig. 4. *Trichostrongylus colubriformis*-egg (ob.x40)

The study carried out on eggs of *Trichostrongylus colubriformis*, minimum length obtained was 95 μ m and the maximum length was 125 μ m, with an average of 107.7 μ m. Minimum width of 51 μ m was obtained and the maximum width was 72 μ m, with an average of 59.2 μ m (fig. 4). Corroborating our data, the morphological characteristics of eggs, with those in the literature, some results are inconclusive.

Thienpont et al. (12) found the species *Trichostrongylus colubriformis* (85-90 μ m average), is generally an average length of 87 μ m, with a minimum length of 79 μ m and a length of 101 μ m and the average width of 46 μ m, range from 38-50 μ m. We obtained the dimensions were larger ones, representing a difference of about 20 μ m average length and average width 18 μ m. The eggs were unequal poles and morula not fill the entire cavity of the egg. The eggs of *Trichostrongylus colubriformis*, noted that fresh eggs harvested from adult females are slightly different in terms of morula inside them. The eggs from the faeces, morula is somewhat segmented.

Conclusions

From measurements made on trichostrongilids eggs, that other gastrointestinal nematodes in the samples examined were identified following species: *Nematodirus filicollis*, *Trichostrongylus colubriformis*, *Haemonchus contortus* and *Bunostomum trigonocephalum*.

Appearance of eggs in these preparations can be a criterion for their identification.

In general, we obtained the dimensions are larger than the size of genera / species *Trichostrongylidae* respectively *Ancylostomidae* specified in the literature.

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