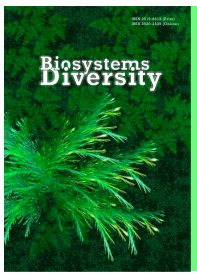

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# Comparative embryonic development of nematodes of the genus *Trichuris* (Nematoda, Trichuridae) obtained from sheep (*Ovis aries*)

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Biological specifics of *Trichuris ovis* Abildgaard, 1795 and *T. globulosa* Linstow, 1901 parasitizing domestic sheep were analyzed enhancing the species identification of these nematode species. The embryonic development of nematodes was timed, survival of embryonic stages was determined taking into account their morphological and metric specifics in laboratory culture. *Trichuris* eggs were isolated from gonads of adult female nematodes collected from caeca of dissected sheep. Then the eggs were cultured at optimal temperature to the formation of eggs with mobile larvae. Six morphologically distinct stages of embryogenesis were established in *T. ovis* and *T. globulosa* nematodes. The protoplast stage lasted from the 1st to the 12th day in *T. ovis* and to the 18th day in *T. globulosa*. Blastomeric formation occurred from the 3rd to the 18th day in *T. ovis* and from the 3rd to the 21st day in *T. globulosa*. Bean-shaped embryos formed from the 6th to the 21st day in *T. ovis*, and from the 9th to the 30th day in *T. globulosa*. Tadpole-like embryos developed from the 12th to the 24th day in *T. ovis* and from the 18th to the 33rd day in *T. globulosa*. Larvae formed in eggs of *T. ovis* from the 18th to the 27th day, and in eggs of *T. globulosa* from the 21st to the 36th day. Mobile larvae formed from the 21st to the 30th day in *T. ovis*, and from the 30th to the 39th day in *T. globulosa*. At 27 °C, mature eggs with mobile larvae developed in 30 days in *T. ovis* and in 39 in *T. globulosa*. The egg survival in laboratory culture was 84.3 ± 4.2 % and 76.3 ± 1.5%, respectively. Developmental changes of metric parameters in *Trichuris* nematode eggs (length and width of eggs, plug length, eggshell thickness) were species-specific.

*Keywords*: trichurosis, *Trichuris ovis*; *Trichuris globulosa*; nematode eggs; embryogenesis; *in vitro*; morphological changes; metric parameters

## Introduction

Nematodes of the genus *Trichuris* Schrank, 1788 are common parasitic pathogens of various wild and domestic animals. For example, they have been observed in the even-toed mammals (argali, roe deer, camels, bulls, boars, pigs), carnivores (foxes, dogs and wolves), and rodents (marmots, gerbils, mice and rats) (Fahmy, 1954; Salaba et al., 2003; Robles et al., 2006; Eichenberger et al., 2018). *Trichuris* pathogens infect humans too (Fincham & Markus, 2001; Kyung-Sun et al., 2009; Manz et al., 2017). There are more than 70 species in this genus, most of which are specific to certain host taxa. However, there are reports of possibility of infections in humans by non-specific nematode species (Dunn et al., 2002).

In domestic ruminants (cattle, sheep, goats), the most common species of this genus are *T. skrjabini* (Baskakov, 1924), *T. ovis* (Abildgaard, 1795) and *T. globulosa* (Linstow, 1901). For example, in Europe the *Trichuris* infection rates in sheep range from 41.8% to 100% (Salaba et al., 2013). In South-Western Turkey, *T. skrjabini* pathogens were found in 74% of examined sheep, *T. ovis* was recorded in 72% of studied animals (Umur & Yukari, 2005). In Nigeria, the most common *Trichuris* species in goats was *T. ovis* (72.5%), while *T. globulosa* was rarer (38.3%) (Nwosu et al., 1996). In certain regions of Sudan, *T. globulosa* was observed only in 0.1% and 0.6% of studied sheep and goats, respectively (Almalaik et al., 2008). The *Trichuris* species parasitizing in sheep in Ukraine are *T. ovis* (most common, abundance index 3.4 specimens), *T. skrjabini* (less common, abundance index 2.2 specimens), and *T. globulosa* (the rarest, abundance index 0.6 specimen) (Yevstafieva et al., 2018). This dominance of certain nematode species over others, even in the same species, is usually linked to their adaptive capabilities and tolerance of unfavourable environmental conditions. The parasitic habits which are known to be species-specific are seen in all its specimens and are secured in its phylogeny. Hence, adaptation is the main vital function, almost overdeveloped in parasites to preserve both individual and species survival. At the same time, the survival of a specimen depends, mostly, on feeding, while species survival is linked to its biological features (McSorley, 2003; Yazwinski & Tucker, 2006; Boyko et al., 2009, 2016; Blaxter & Koutsovoulos, 2015).

The development of *Trichuris* nematodes is direct, without an intermediate host. The main and deciding environmental factors in viability and durability of embryonic stages in these nematodes are the vegetation cover and also the temperature, the moisture of air and soil, landscape forms, rainfall, and other abiotic factors (Thapar & Singh, 1954; Mamedova & Fataliev, 2009). Other researchers observe that the development of infectious eggs of *Trichuris* nematodes in the environment is principally influenced by temperature (Beer, 1788) or soil type (Brown, 1927).

It is known that the embryogenesis of *Trichuris* nematodes occurs in several stages. Some authors recognize the following stages of development of *Trichuris* eggs in the environment: nuclear fusion, first rest period, fission groove formation, second rest period, protoplasm clearing, larva formation, larva in one revolution, larva in two revolutions, formation of infectious larva (Fataliev, 2013). Other researchers note such embryonic periods in *T. trichiura* (Linnaeus, 1771): fertilized egg with two pronuclei, formation of 2 to 10 blastomeres, further cleavage, gastrulation, embryo with a closed blastopore, moving embryo, formed larva with a stylet (Malahov et al., 1984).

There are many studies of embryonic development of *Trichuris* nematodes which infect humans (Bundy & Cooper, 1989; Skriabyn et al., 1957). At the same time, certain aspects of embryonic development in

eggs of *Trichuris* nematodes of sheep are still insufficiently known. There are also rather counterintuitive data on the periods of egg development in nematodes of this genus (Skriabyn et al., 1957). Thus, the aim of the present work is to study the specifics of embryogenesis in nematodes of the species *T. ovis* and *T. globulosa in vitro*, taking into account the morphological and metric changes in the eggs.

## Materials and methods

Nematodes were collected in during helminthological investigation of the large intestine of dead or slaughtered sheep (Skriabyn, 1928). The nematode species were identified using keys (Skriabyn et al., 1957; Ivashkyn et al., 1989). To study the biological specifics of *T. ovis* and *T. globulosa* nematodes in laboratory culture, their eggs were collected from gonads of female nematodes. Each separate culture was placed in a Petri dish and cultured in a thermostat at 27 °C to the point of mobile larva formation. The cultures were examined every three days under a light microscope. The stage of development was identified by morphology of the embryo, also eggs that had stopped developing or were destroyed were counted. Each experiment was performed in triplicate.

Morphometric parameters of *T. ovis* and *T. globulosa* eggs (length and width of egg, length and width of egg plug, eggshell thickness) were studied in cultures, using the software Image J for Windows® (version 2.00) in interactive mode using $10^{\times}$, $40^{\times}$, $100^{\times}$ objective and $10^{\times}$ photo eyepiece. To calibrate the image analyzer, the ruled scale of the ocular micrometer was coincided with the scale of stage micrometer included in the MikroMed microscope kit. Microphotographs were taken using a digital camera of a MikroMed 5 Mpix (China) microscope.

Statistical processing of the experimental results was carried out using Statistica 10 (StatSoft Inc., 2011) software. Standard deviation (SD) and average values (x) were calculated. Significance of difference between average values in the studied egg cultures of *Trichuris* nematodes was established using one-way analysis of variance and F-test for 95% confidence level.

## Results

It was established that embryogenesis of *T. ovis* and *T. globulosa* nematode parasites of sheep occurs in six stages in laboratory culture at 27 °C: protoplast, blastomeric formation, bean-like embryo, tadpole-like embryo, larva formation, and mobile larva formation. These stages are characterized by morphological changes in nematode eggs. At the protoplast stage, egg cytoplasm was filled with large granules which visually merged into one mass (Fig. 1*a*). Blastomeric formation was characterized by the cleavage and formation of two (Fig. 1*b*) and then three and more large cells, and the size of the blastomeres decreased in inverse proportion to their numbers (Fig. 1*c*). The bean-like embryo was morphologically distinct by the development of multicellular bean-shaped embryo in the egg (Fig. 1*d*). Later, the embryo changed into a shape resembling a tadpole, which was hence the tadpole-like embryo stage (Fig. 1*e*).The next stage was characterized by formation of motionless larva, with granular, slightly contoured body (Fig. 1*f*). The embryogenesis of the studied nematodes, regardless of the species, ended in mobile larva formation in the egg. The larva was twisted, completely filled the internal volume of the egg and actively moved under the influence of warmth, and also had a clear morphological structure (Fig. 1*g*).

Although the process of embryogenesis did not differ by morphologic changes in eggs of *T. ovis* and *T. globulosa* nematodes, their periods of development and survival were dissimilar. For example, eggs obtained from *T. ovis* female gonads in vitro at 27 °C developed mobile larvae in 30 days, and their survival was 84.3 ± 4.2% (Table 1).

All of the eggs (100.0%), obtained from female nematode gonads were protoplast. From the third to 12th day of culture, the eggs progressed to the second stage of development, formation and cleaving of blastomeres. First, two blastomeres appeared (44.3 ± 3.1% at the third day), then three and more blastomeres (41.3 ± 1.2% at the sixth day, 41.7 ± 3.5% at the ninth day). From the sixth day, 8.0 ± 1.7% of all eggs were at the bean-like embryo stage. The maximum ratio of eggs on the third stage of development (54.7 ± 6.4 and 57.7 ± 8.1%) was recorded from the 12th to 15th day of culture. At the 12th day of experiment, 5.3 ± 3.5% of *T. ovis* eggs were observed to be developing from bean-like to tadpole-like stage.

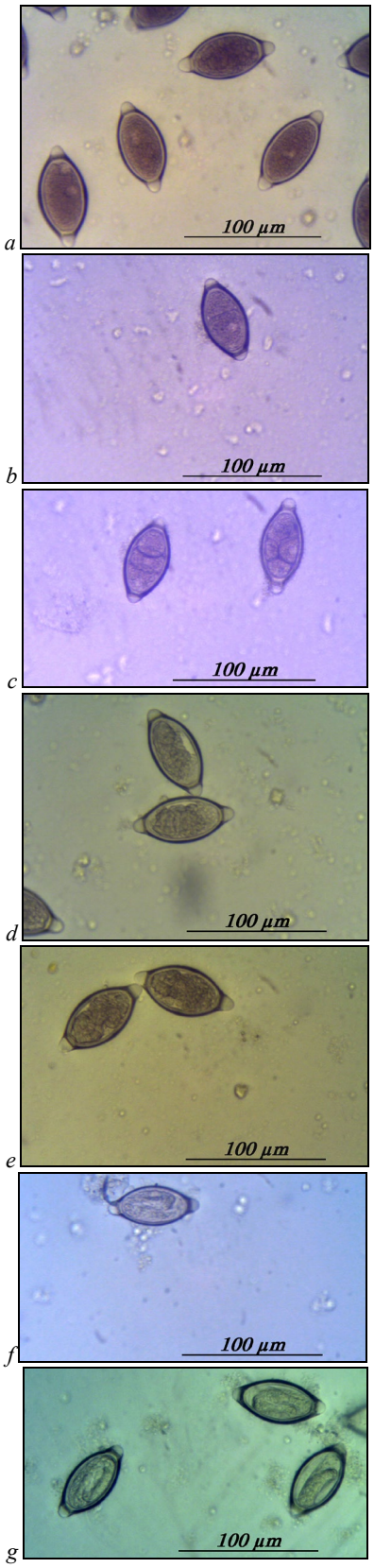


**Fig. 1.** Embryonic stages of development in nematodes of the species *T. ovis* and *T. globulosa* parasitizing domestic sheep: *a* – protoplast, *b* – cleavage and formation of two blastomeres, *c* – cleavage and formation of three and more blastomeres, *d* – formation of bean-like embryo, *e* – formation of tadpole-like embryo, *f* – larva formation, *g* – mobile larva formation

Maximum ratio of eggs at this stage was observed at the 21st day of culture (44.3 ± 4.0%). At the 18th day, 14.0 ± 4.4% of eggs contained formed, though motionless, larvae. The duration of that stage of development was until the 27th day of culture, with maximum ratio (50.3 ± 5.7%) on the 24th day of culture. Formation of mobile larvae started at the 21st day, when they comprised 10.3 ± 2.1% of all eggs. Subsequently, the ratio of eggs containing mobile larvae was 29.3 ± 2.5% at the 24th day, 81.3 ± 4.9% at the 27th day, and 84.3 ± 4.2% at the 30th day of experiment.

Also, 15.7 ± 4.2% of all eggs did not continue developing and died. In the study of the embryonic specifics of *T. globulosa* nematodes, their eggs were observed to mature to the mobile larvae stage more slowly compared to *T. ovis* nematodes. That development occurred in 39 days. At the same time, *T. globulosa* eggs were less viable: only 76.3 ± 1.5% developed into mature eggs, while 23.7 ± 1.5% of eggs did not develop and died (Table 2). The obtained cultures of *T. globulosa* eggs were all at the protoplast stage at the beginning of experiment. Further, the ratio of protoplast egg decreased to 23.7 ± 1.5% at the 18th day. From the 3rd to 21st day of culture, blastomeric formation occurred in the eggs. Maximum ratio of eggs with two blastomeres was observed at the 3rd day (35.3 ± 2.1%), that of eggs with three and more blastomeres at the 6–9th days (39.7–41.3%). The bean-like stage of development was recorded at the 9th (23.0 ± 1.0% of all eggs) to 30th day of culture. Their ratio increased to its maximum at 15th to 18th day (from 52.0 ± 3.0 to 54.0 ± 2.7%). From the 21st to 30th day of experiment, the numbers of eggs containing bean-like embryos gradually decreased with their development to the next stage, the tadpole-like embryo. That stage occurred from the 18th to 33rd days of culture. Maximum ratio of eggs with that embryonic stage was recorded at the 24th to 27th day of culture (39.7–42.3%). The larval formation was observed from the 21st day of experiment (11.3 ± 1.5%) to 43.7 ± 3.5% at the 30th day, the stage was observed till the 36th day. Mobile larva formation occurred in *T. globulosa* eggs from the 30th to 39th day of experiment, the ratio of the eggs with that stage increasing from 5.3 ± 2.1 to 76.3 ± 1.5%.

**Table 1**

Parameters of embryonic development of eggs obtained from *Trichuris ovis* gonads, in experimental culture (x ± SD, n = 100)

| Day of culture | Stage of development, % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | protoplast | blastomere formation | | bean-like embryo | tadpole-like embryo | larva formation | mobile larva formation | arrested development |
| | | 2 | ≥3 | | | | | |
| 1 | 100.00 | – | – | – | – | – | – | – |
| 3 | 25.67 ± 2.08 | 44.33 ± 3.06 | 30.00 ± 1.73 | – | – | – | – | – |
| 6 | 19.67 ± 1.53 | 31.00 ± 2.00 | 41.33 ± 1.15 | 8.00 ± 1.73 | – | – | – | – |
| 9 | 18.33 ± 0.58 | 6.67 ± 2.08 | 41.67 ± 3.51 | 33.33 ± 4.73 | – | – | – | – |
| 12 | 15.67 ± 4.16 | 3.00 ± 1.00 | 21.33 ± 1.53 | 54.67 ± 6.35 | 5.33 ± 3.51 | – | – | – |
| 15 | – | 2.00 ± 1.00 | 12.00 ± 3.00 | 57.67 ± 8.14 | 12.67 ± 7.57 | – | – | 15.67 ± 4.16 |
| 18 | – | – | 3.67 ± 2.08 | 45.33 ± 5.13 | 21.33 ± 1.53 | 14.00 ± 4.36 | – | 15.67 ± 4.16 |
| 21 | – | – | – | 11.33 ± 2.52 | 44.33 ± 4.04 | 18.33 ± 2.08 | 10.33 ± 2.08 | 15.67 ± 4.16 |
| 24 | – | – | – | – | 4.67 ± 2.52 | 50.33 ± 5.69 | 29.33 ± 2.52 | 15.67 ± 4.16 |
| 27 | – | – | – | – | – | 3.00 ± 1.73 | 81.33 ± 4.93 | 15.67 ± 4.16 |
| 30 | – | – | – | – | – | – | 84.33 ± 4.16 | 15.67 ± 4.16 |

**Table 2**

Parameters of embryonic development of eggs obtained from *Trichuris globulosa* gonads, in experimental culture (x ± SD, n = 100)

| Day of culture | Stage of development, % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | protoplast | blastomere formation | | bean-like embryo | tadpole-like embryo | larva formatior | mobile larva formation | arrested development |
| | | 2 | ≥3 | | | | | |
| 1 | 100.00 | – | – | – | – | – | – | – |
| 3 | 48.33 ± 2.08 | 35.33 ± 2.08 | 16.33 ± 2.08 | – | – | – | – | – |
| 6 | 33.00 ± 2.65 | 25.67 ± 2.08 | 41.33 ± 1.53 | – | – | – | – | – |
| 9 | 26.00 ± 1.73 | 11.33 ± 2.52 | 39.67 ± 1.53 | 23.00 ± 1.00 | – | – | – | – |
| 12 | 23.67 ± 1.53 | 4.67 ± 1.53 | 35.67 ± 1.53 | 36.00 ± 2.65 | – | – | – | – |
| 15 | 23.67 ± 1.53 | 2.33 ± 0.58 | 22.00 ± 1.73 | 52.00 ± 3.00 | – | – | – | – |
| 18 | 23.67 ± 1.53 | 1.33 ± 0.58 | 11.00 ± 2.00 | 54.00 ± 2.65 | 10.00 ± 1.00 | – | – | – |
| 21 | – | – | 2.00 ± 1.00 | 31.67 ± 1.53 | 31.33 ± 1.53 | 11.33 ± 1.53 | – | 23.67 ± 1.53 |
| 24 | – | – | – | 21.33 ± 1.53 | 39.67 ± 2.31 | 15.33 ± 0.58 | – | 23.67 ± 1.53 |
| 27 | – | – | – | 11.33 ± 1.15 | 42.33 ± 0.58 | 22.67 ± 2.08 | – | 23.67 ± 1.53 |
| 30 | – | – | – | 2.33 ± 1.53 | 25.00 ± 3.61 | 43.67 ± 3.51 | 5.33 ± 2.08 | 23.67 ± 1.53 |
| 33 | – | – | – | – | 10.67 ± 1.53 | 37.67 ± 1.53 | 28.00 ± 2.00 | 23.67 ± 1.53 |
| 36 | – | – | – | – | – | 15.33 ± 4.51 | 61.00 ± 4.36 | 23.67 ± 1.53 |
| 39 | – | – | – | – | – | – | 76.33 ± 1.53 | 23.67 ± 1.53 |

Morphometric study revealed that the sizes of *T. ovis* and *T. Globulosa* eggs changed during embryonic development, and that there were species-specific differences. The length and width of eggs obtained from *T. ovis* females at protoplast stage were 71.3 ± 1.7 and 32.7 ± 1.8 μm respectively, with egg plug length of 8.8 ± 1.1 μm and egg plug width of 10.7 ± 0.6 μm. Eggshell thickness of those eggs was 3.0 ± 0.2 μm, internal surface area was 1053 ± 82 $\mu m^2$. Mature eggs containing mature larvae were statistically smaller ($P < 0.05$) by 3.4% (68.9 ± 1.3 μm) of egg length (Fig. 2*a*), 16.8% (2.5 ± 0.3 μm) of eggshell thickness (Fig. 2*e*) and 13.7% (7.6 ± 0.4 μm) length of egg plugs (Fig. 2*c*). Simultaneously, egg width increased to 35.1 ± 1.2 μm (by 6.8%, $P < 0.05$) (Fig. 2*b*). There were no statistically significant differences in egg plug width (11.1 ± 0.6 μm) and internal surface area (1107 ± 31 $\mu m^2$) during the studied embryogenesis (Fig. 2*d*, *f*).

In the study of metric parameters of eggs, it was established that their length and width at the protoplast stage were 70.8 ± 3.6 and 38.9 ± 1.6 μm respectively; length and width of egg plug were 3.3 ± 0.5 and 9.1 ± 0.4 μm, eggshell thickness was 4.1 ± 0.3 μm, internal surface area was 1306 ± 51 $\mu m^2$. During embryogenesis, the eggs grew and developed: their length decreased by 4.9% (67.4 ± 3.7 μm, $P < 0.05$) (Fig. 3*a*), width decreased by 9.6% (3.7 ± 0.4 μm, $P < 0.05$) (Fig. 3*e*). Simultaneously, the egg width increased by 4.3% (38.9 ± 1.6 μm, $P < 0.05$) (Fig. 3*b*). During this time, egg plug length and width (3.1 ± 0.1 and 9.4 ± 0.4 μm) (Fig. 3*c*, *d*) and internal surface area (1338 ± 37 $\mu m^2$) (Fig. 3*f*) were not statistically different. These changes point to the species-level biological specifics of *T. ovis* and *T. globulosa*, which also can influence their survival and abilities to adapt to inconstant environmental conditions.

## Discussion

In the course of the present study, new data is collected on the embryogenesis of sheep nematode species *T. ovis* (Abildgaard, 1795) and *T. globulosa* (Linstow, 1901), on eggs obtained from nematode gonads. Knowledge of biological specifics, especially those of human parasites, factors into the regulation of their numbers, more so in the ecosystems experiencing anthropogenic pressure.

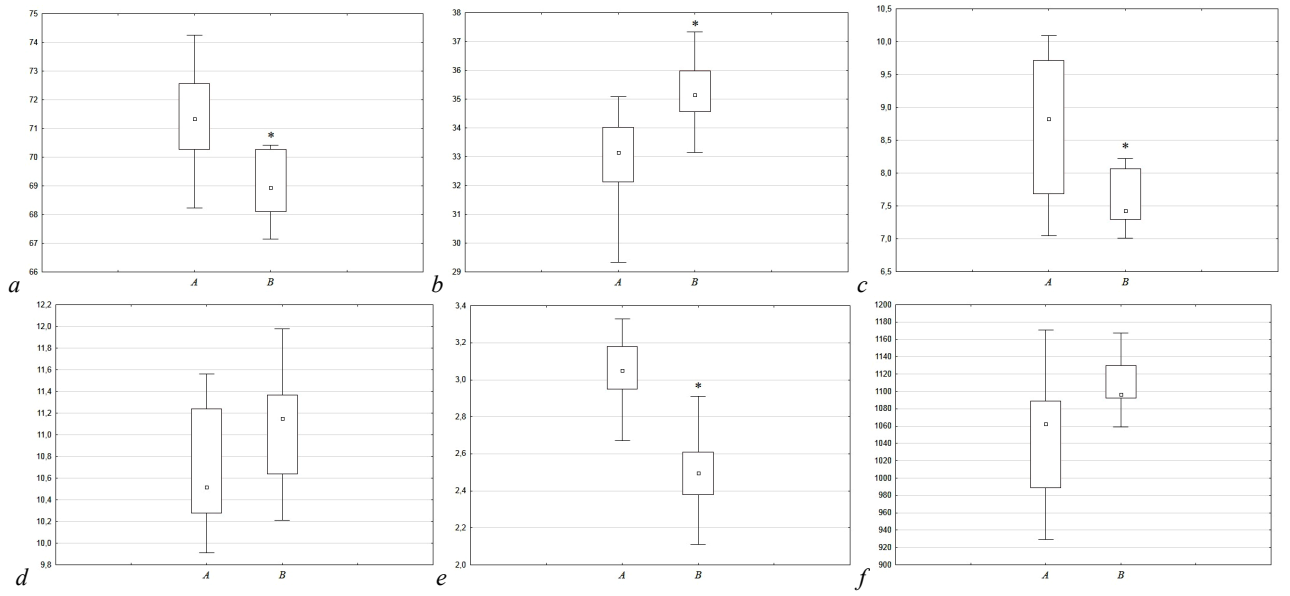


**Fig. 2.** Metric changes in *T. ovis* eggs during embryogenesis: *a* – length, *b* – width, *c* – length of plug, *d* – width of plug, *e* – shell thickness (μm), *f* – internal surface area (μm$^2$); *A* – protoplast, *B* – mobile larva formation; * – P < 0.05 compared to egg parameters at protoplast stage; n = 10

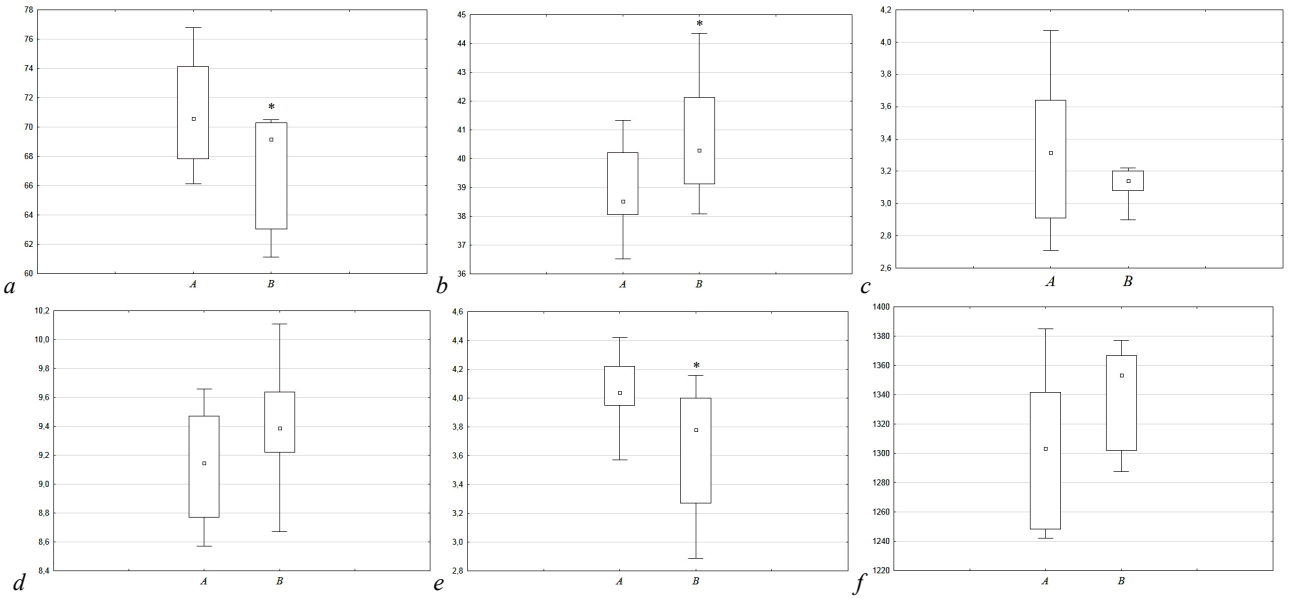


**Fig. 3.** Metric changes in *T. globulosa* eggs during embryogenesis: *a* – length, *b* – width, *c* – length of plug, *d* – width of plug, *e* – shell thickness (μm), *f* – internal surface area (μm$^2$); *A* – protoplast, *B* – mobile larva formation; * – P < 0.05 compared to egg parameters at protoplast stage; n = 10

Also in several cases, such data is taken into account in species or genus identification (Liang et al., 2007; Wong & Candolin, 2015; Zvinorova et al., 2016). According to scientific reports, trichurosis is very common in wild and domestic ruminants. The infection is recorded world-wide, with rates varying in different countries (Salaba et al., 2013; Yaro et al., 2015; Eichenberger et al., 2018). In our opinion, domination of one species over several other *Trichuris* species in a host population is to some degree dependent on the nematode specifics of embryogenesis which occurs outside the host. We observe that *in vitro*, at 27 °C the eggs of *T. ovis* develop faster (in 30 days) and are more viable (84.3 ± 4.2%) compared to the eggs of *T. globulosa* (which develop to mobile larva stage in 39 days with 76.3 ± 1.5% survival). This may be the basis of domination of *T. ovis* over *T. globulosa* in sheep in several regions of Ukraine (Yevstafieva et al., 2018).

Hence, we delineate the main morphological stages of development in eggs of *T. ovis* and *T. globulosa*, which occur in the same way. The embryogenesis in the nematode species, studied at 27 °C, occurs in six stages: protoplast, blastomeric formation, bean-like embryo, tadpole-like embryo, larva formation, mobile larva formation. These stages of development are quite morphologically distinct. Similar stages of development were found for *T. suis* (Schrank, 1788) nematodes obtained from pigs, and *T. skrjabini* (Baskakov 1924) roundworms, obtained from sheep (Yevstafieva et al., 2015, 2018). At the same time, quantitative indicators of changing embryonic stages in the development of *T. ovis* and *T. globulosa* nematodes were different. For example, protoplast stage in *T. ovis* occurs during the 1st to 12th days of culture, and from the 1st to 18th days of culture in *T. globulosa*. At the first day of culture, 100% of all eggs were at the protoplast stage. Blastomeric formation occurred from the 3rd to 18th days of culture in *T. ovis* nematodes, and from the 3rd to 21st days of culture in *T. globulosa*, with maximum ratio of eggs with protoplasts, respectively, 41.7 ± 3.5% to 44.3 ± 3.1% and 35.3 ± 2.1% to 41.3 ± 1.5%. The bean-like embryo developed from the 6th to 21st days of culture in the eggs of *T. ovis*, and from the 9th to 30th days of culture in the eggs of *T. globulosa* (the maximum ratios were respectively 57.7 ± 8.1% and 54.0 ± 2.7%). The tadpole-like embryo occurred in the eggs of *T. ovis* from the 12th to 24th days of culture and in the eggs of *T. globulosa* from the 18th to 33rd days of culture. The maximum ratio of eggs with tadpole-like embryos were,

respectively, 44.3 ± 4.0% and 42.3 ± 0.6%. Larva formation occurred in the eggs of *T. ovis* from the 18th to 27th days of culture, and in the *T. globulosa* eggs from the 21st to 36th days of culture (maximum ratios were 50.3 ± 5.7 and 43.7 ± 3.5%, respectively). Mobile larva formation occurred in the eggs of *T. ovis* from the 21st to 30th day of culture, and in the eggs of *T. globulosa* from the 30th to 39th days of culture (maximum ratios were 84.3 ± 4.2 and 76.3 ± 1.5%). These data can be used in differential diagnostics of the studied *Trichuris* species. Most authors note that nematodes are identified by the morphology of adult specimens, especially the males (Bailey et al., 2009; Ghasemikhah et al., 2011). However, adult nematodes are not always available because they are obtained from dead hosts. In most cases, especially in diagnosing intestinal nematodes, coprologic samples are taken and only eggs are available. Some authors propose keys based on egg identification using various methods such as genetic analysis, electronic microscopy, dyeing and metric parameters (Palmer & McCombe, 1996; Sommer, 1996; Phosuk et al., 2018). Hence we propose new data on morphometric indicators of the eggs of *T. ovis* and *T. globulosa* and their changes in embryogenesis. We found that morphology of eggs obtained from gonads of the studied nematode species were not statistically different. The eggs were different by several metric parameters. For example, length and width of the eggs of *T. ovis* were 71.3 ± 1.7 and 32.7 ± 1.8 μm, and those of *T. globulosa* were 70.8 ± 3.6 and 38.9 ± 1.6 μm, and eggshell thickness was 3.03 ± 0.19 and 4.05 ± 0.25 μm respectively. Length and width of egg plug in *T. ovis* eggs were 8.8 ± 1.1 and 10.7 ± 0.6 μm, and those of *T. globulosa* were 3.3 ± 0.5 and 9.1 ± 0.4 μm, the internal surface areas were, respectively, 1053 ± 82 and 1306 ± 51 $\mu m^2$. At the same time, embryogenesis in both species was characterized by species-specific changes of metric parameters. The mature eggs with mobile larva in *T. ovis* eggs were accompanied by decreasing egg length (by 3.4%, $P < 0.05$), eggshell thickness (by 16.8%, $P < 0.05$), egg plug length (by 13.7%, $P < 0.05$), and increasing egg width (by 4.3%, $P < 0.05$). The eggs of *T. globulosa* grew and developed with decreasing length (by 4.9%, $P < 0.05$), eggshell thickness (by 9.6%, $P < 0.05$), and increasing egg width (by 4.3%, $P < 0.05$). Our data increase the knowledge of biological features of *T. ovis* and *T. globulosa* nematodes.

## Conclusion

The study showed that it is possible to use embryonic specifics (morphometric changes of eggs, periods of development, survival ratio) of nematodes of domestic sheep ( *Ovis aries*) of the genus *Trichuris* Roederer, 1761 in species identification. Six stages of embryonic development are recognized in *Trichuris* eggs: protoplast, blastomeric formation, bean-like embryo, tadpole-like embryo, larva formation and mobile larva formation. The stages are similar in *T. ovis* (Abildgaard, 1795) and *T. globulosa* (Linstow, 1901) nematodes. The differentiating species characters of *T. ovis* and *T. globulosa* are times of development and quantitative parameters of each embryonic stage. At 27 °C *in vitro*, maximum numbers of mature *T. ovis* eggs occur at the 30th day of culture with 84.3% survival. The embryonic development of *T. globulosa* in culture is longer, it takes place in 39 days, with lower survival of 76.3%. Metric changes of the eggs of *T. ovis* and *T. globulosa* during embryogenesis occur dissimilarly, pointing to their species differences. Formation of the mature eggs with mobile larva in *T. ovis* is accompanied by increasing egg width and decreasing eggshell thickness, egg length and egg plug length. In *T. globulosa*, the morphometric characters of eggs during embryonic development are characterized by decreasing length and eggshell thickness, with increasing egg width. These data have an application value in identifying the studied Trichuris species by collected eggs.

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