Features of exogenous development
of *Trichuris globulosa* (Nematoda, Trichuridae)

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Parasitic nematodes of the genus *Trichuris* Röderer, 1761 are hematophagous helminths, capable of parasitizing many different hosts including humans. The domestic and wild ruminants are hosts of several *Trichuris* species, with *Trichuris globulosa* (Linstow, 1901) one of the most prevalent parasites found in cattle. The exogenous stages of the helminth's life cycle develop in the outer environment, and their activity and survival depend on the abiotic factors. Thus the aim of the work was to determine the influence of temperature on the rate and success of development of infectious eggs of *T. globulosa* in laboratory culture considering their morphological and metric changes. The results of experimental studies showed that the embryogenesis of *T. globulosa* eggs, cultured in laboratory conditions and obtained from gonads of female nematodes, occurs in six stages regardless of the temperature regime. At the same time, the rate of transition from one stage to another, the term for the formation of infectious eggs and their viability directly depends on the temperature of the external environment. At the optimal temperature for the development of *T. globulosa* eggs, 25 °C, 76.3% of eggs reached the mobile larva stage. A decrease in temperature to 20 °C and an increase to 30° C led to an increase in the egg mortality of up to 26% and 32%, respectively, and the viability of eggs decreased. Under such temperature conditions, 74% and 68% of eggs of *Trichuris* reached the mobile larva stage. With an increase in the culture temperature, the time of embryogenesis decreased and amounted to 56 days at 20 °C, 48 days at 25 °C, and 32 days at 30 °C. Depending on the temperature regime, the zygote stage lasted from the release of eggs from the gonads of female nematodes to 12 days, the stage of blastomere formation from 4 to 12 days, the stage of the bean-shaped embryo from 8 to 24 days, the stage of the tadpole embryo from 16 to 36 days, the stage of larval formation lasted from 16 to 48 days, the stage of mobile larva from days 20 to 56. The formation of the infectious *T. globulosa* egg from a non-infectious one is shown in an experiment to include the metric changes confirmed by metric indicators. Mature eggs with a mobile larva are shorter and wider, with shorter and narrower plugs and a thinner shell than eggs at the zygote stage. The obtained research results on the influence of temperature on the exogenous development of *Trichuris globulosa* will make it possible to predict the epizootic situation of trichurosis in animal husbandry, as well as take timely measures to arrest the embryonic stages of nematode development in the external environment.

Keywords: trichurosis; helminth egg; embryogenesis; temperature factor; metric indicators.

Introduction

Gastrointestinal nematodes cause common and very important from an economic point of view diseases of both domestic and wild animals around the world (Little, 2011; van der Voort et al., 2013; Ananta et al., 2014; Marskole et al., 2016; Susana et al., 2019). Among them, the most significant are zoonotic helminthiases which affect humans by the same stages of the pathogen that live in the animal bodies (Bawm & Htun, 2015; Boyko & Brygadyrenko, 2017, 2019, 2020). Such dangerous parasites include nematodes of the genus *Trichuris* Röderer, 1761, hematophagous helminths which have a significant distribution and species diversity (Ghai et al., 2014; Susana et al., 2019). Most of them are considered specific for certain taxonomic host groups (Callejón et al., 2015). Trichurosis of cattle is recorded everywhere (Gul & Tak, 2016; Gunathilaka et al., 2018; Regea, 2019; Winarso et al., 2019). *Trichuris globulosa* Linstow, 1901 is one of the most common species of *Trichuris* that parasitize cattle (Keyyu et al., 2003; Pfukenyi & Mukaratirwa, 2013). This pathogenic species is found in ruminants of the Azores Islands, Great Britain, Cyprus, Turkey, France, Germany, Italian, Poland, Spain, Bhutan, Nigeria, where the incidence in cattle varies from 0.09% to 14.5% (Jimenez et al., 2010; Tshering & Dorji, 2013; De Jong et al., 2014; Odeniran et al., 2016; Susana et al., 2019).

This prevalence, according to researchers, is associated with the biological characteristics of parasites. Moreover, the most resistant to unfavourable factors is the exogenous stage of development, namely the *Trichuris* eggs (Pramasudha et al., 2015). Thus, the egg shell is multi-layered, provides mechanical protection, and also is resistant to chemicals. As a consequence, mature eggs of *Trichuris* can remain viable in the soil for a long time, which ensures the wide geographical distribution of the parasite (Roepstorff & Murrell, 1997; Anvari-Tafti et al., 2013; Tudor, 2015). At the same time, the survival of nematode eggs depends on several factors such as climatic conditions, vegetation cover, seasonal air temperatures, fluctuations of soil moisture, and exposure to sunlight (Storey & Phillips, 1985; Pecson et al., 2007; Maya et al., 2010). Adaptation to a parasitic lifestyle is accompanied by the appearance of special adaptations in helminths and their biological cycle to preserve the species. It is carried out, in most cases, in the external environment, to which the exogenous stages of parasite development adapt by the emergence of resistance to the effects of various environmental factors (Blaxter & Koutsovoulos, 2015; Boyko et al., 2016; Al-Albudi & Mosaab, 2017). Hence, helminth eggs whose embryonic development occurs in the external environment are more resistant to unfavourable factors than eggs that are already infectious in the external environment and partly develop in the body of the host. The resistance to the effects of environmental factors is extremely necessary for all exogenous development stages, since they undergo embryogenesis outside the host organism, and they also wait for rather a long term before entering the body of a new host (Knoop, 2012; Fataliev, 2013; Stroehlein et al., 2017; Charlier et al., 2018).

A number of researchers have studied the features of embryonic development in nematodes of the genus *Trichuris* obtained from pigs, dogs, domestic and wild ruminants, and humans, from the stage of a fertilized egg containing an embryonic disc to the formation of an infectious egg. Their data show that for each individual species, the terms of egg development in the external environment, viability, stages of development and optimal conditions are quite different (Skriabyn et al., 1957; Fataliev, 2013; Yevstafieva et al., 2016; Yevstafieva et al., 2019). Therefore, it is very important to know the influence of changes in the external conditions surrounding the host on the biological characteristics of the exogenous stages of development of parasites, since seasonal changes in natural conditions lead to the parasites' adaptive responses so that the maximum number of eggs would become infectious to the host.

The aim of the present work is to establish the influence of temperature on the development and formation of infectious eggs of *T. globulosa* taking into account their morphological and metric changes.

Materials and methods

The nematodes were collected in the complete helminthological dissection of the large intestine obtained from young cattle (Skriabyn, 1928). The species of *Trichuris* was identified using a key (Skrjabin et al., 1957). In order to study the biological characteristics of parasitic *T. globulosa* nematodes in laboratory conditions, eggs were isolated from the gonads of female *Trichuris*.

Each separately obtained egg culture was placed in a Petri dish and cultivated in a thermostat at different temperatures (20, 25, 30 °C) until the formation of a mobile larva in egg. In total, th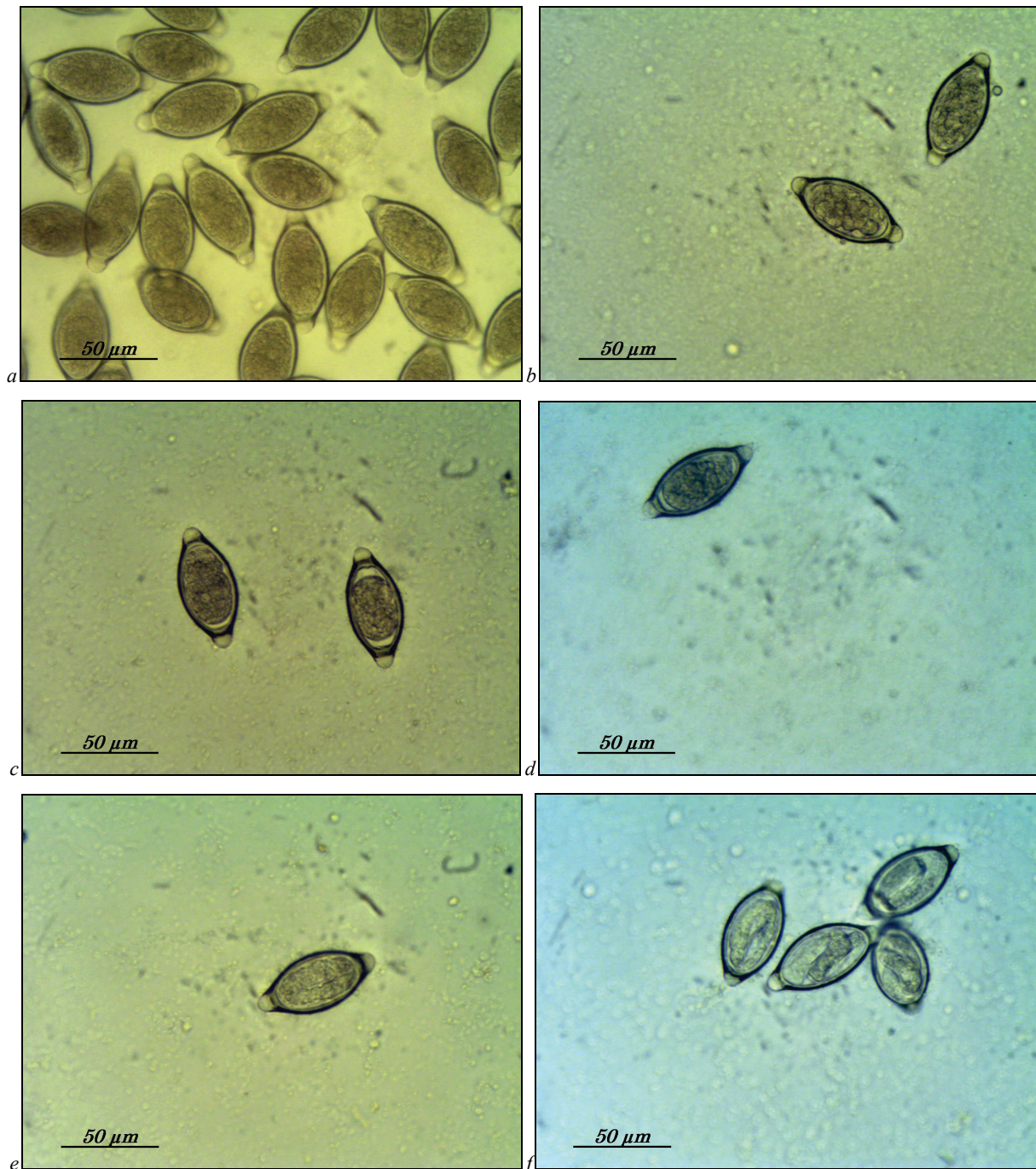ree Petri dishes with cultures of *Trichuris* eggs were prepared. They were examined every four days, 100 eggs were chosen in each culture and their level of development was rated according to the following stages: zygote, blastomere formation, bean-shaped embryo, tadpole-shaped embryo, larva and mobile larva formation. Dead eggs and eggs that stopped developing were also counted.

The metric parameters of *T. globulosa* eggs (length and width of egg; length and width of egg plug; eggshell thickness) during cultivation were studied using the ImageJ for Windows® software (version 2.00) in interactive mode with a ×10, ×40, ×100 objective and a ×10 photo eyepiece. To calibrate the image analyzer, ruled scale of ocular micrometer was adjusted to the scale of stage micrometer included in MikroMed microscope kit. Microphotography was performed using a digital camera attached to a MikroMed 5 Mpix microscope (China).

Standard deviation (SD) and average values (x) were calculated. Significance of difference between average values in the studied *T. globulosa* eggs was established using one-way analysis of variance and F-test for 95% confidence level.

Results

In the embryogenesis of *T. globulosa* nematodes obtained from cattle, six stages can be distinguished: zygote, blastomere formation, bean-shaped embryo, tadpole-shaped embryo, larva and mobile larva formation. The last stage is infectious to animals. The zygote stage corresponded to the fertilized egg, in which its contents were evenly distributed (Fig. 1a).

Subsequently, blastomere formation was noted in the egg (Fig. 1b). At this stage, the presence of larger blastomeres was first noted, which became smaller due to multiplying. Further development was accompanied by a change in the general shape of the embryo, which resembled the shape of a bean. The embryo thickened to the middle and detached from the membrane at egg poles (Fig. 1c). The next stage was characterized by the formation of a tadpole embryo which gradually, unevenly elongated (Fig. 1d). In the next developmental stage, a larva formed with a body filled with a granular mass (Fig. 1e). At this stage, the larva remained motionless. The larva then greatly grew in length, its body became denser and lighter (Fig. 1f). The larva actively moved inside the vitelline membrane. The exogenous development of *T. globulosa* eggs, namely, the rate of transition from one stage to another, duration of the formation of infectious eggs and their viability, directly depended on the temperature of the external environment. The higher the cultivation temperature was, the shorter the stages of development were, and the faster the formation of infectious larvae occurred. At the same time, the number of viable eggs formed in nematodes did not depend on the rate of embryogenesis. At a temperature of 20 °C, the formation of the maximum number of infectious larvae (74.0 ± 2.6%) occurred in 56 days. The death of *Trichuris* eggs was at the level of 26.0 ± 2.6% (Table 1).

The zygote stage lasted from the isolation of eggs from the gonads of female *Trichuris* to 12 days, with their ratio gradually decreasing from 100% to 50.0 ± 1.0%. Until day 20, the number of eggs at this stage decreased to 6.0 ± 3.6%, and on day 24, no eggs with zygotes were detected. The stage of blastomere formation lasted from the 4th to 12th days of cultivation, when the largest number of such eggs formed, from 13.0 ± 2.0% to 26.3 ± 2.0%. From the 20th to 32nd days the number of eggs at this stage decreased to 3.3 ± 1.5%. The formation of the maximum number of eggs (53.3 ± 3.0%) with the stage of bean-like embryo lasted from the 12th to 24th days. Subsequently, their number decreased and the minimum ratios (9.7 ± 2.5%) were recorded on day 44 of cultivation. The stage of formation of a tadpole embryo began from day 24, where 11.0 ± 1.0% of eggs were found at this stage. The maximum number of such eggs formed on the 36th day of cultivation (40.7 ± 1.1%), and by the 48th day only 17.0 ± 2.0% of the eggs contained a tadpole embryo. From the 32nd day of cultivation, the formation of larvae was recorded in 4.0 ± 1.0% of the eggs, and from the 44th day, they became mobile and the eggs became infectious.

Table 1

Periods of exogenous development of eggs and their viability
in laboratory conditions were assessed at a temperature of 20 °C (x ± SD, n = 3)

Duration of cultivation, day	Stage of development, %						
	zygote	blastomere formation	bean-like embryo	tadpole embryo	larva formation	mobile larva	egg death
before cultivation	100	–	–	–	–	–	–
4	87.0 ± 2.0	13.0 ± 2.0	–	–	–	–	–
8	62.3 ± 2.3	18.7 ± 3.0	–	–	–	–	19.0 ± 2.0
12	50.0 ± 1.0	26.3 ± 2.0	2.0 ± 1.0	–	–	–	21.7 ± 1.1
16	23.7 ± 2.5	23.3 ± 1.5	29.3 ± 0.5	–	–	–	23.7 ± 1.5
20	6.0 ± 3.6	17.0 ± 2.0	52.7 ± 1.1	–	–	–	24.3 ± 2.5
24	–	9.7 ± 0.5	53.3 ± 3.0	11.0 ± 1.0	–	–	26.0 ± 2.6
28	–	6.7 ± 0.5	38.3 ± 1.1	29.0 ± 3.6	–	–	26.0 ± 2.6
32	–	3.3 ± 1.5	30.3 ± 0.5	36.3 ± 2.0	4.0 ± 1.0	–	26.0 ± 2.6
36	–	–	24.0 ± 2.0	40.7 ± 1.1	9.3 ± 1.5	–	26.0 ± 2.6
40	–	–	19.0 ± 1.0	35.7 ± 3.2	19.3 ± 1.5	–	26.0 ± 2.6
44	–	–	9.7 ± 2.5	34.0 ± 1.0	24.3 ± 0.5	6.0 ± 1.0	26.0 ± 2.6
48	–	–	–	17.0 ± 2.0	31.3 ± 2.0	25.7 ± 1.5	26.0 ± 2.6
52	–	–	–	–	15.0 ± 2.0	59.0 ± 1.0	26.0 ± 2.6
56	–	–	–	–	–	74.0 ± 2.6	26.0 ± 2.6

Note: they were examined every four days, 100 eggs were chosen in each culture and their level of development was rated according to the following stages: zygote, blastomere formation, bean-shaped embryo, tadpole-shaped embryo, larva and mobile larva formation, egg death.

Fig. 1. Stages of *Trichuris globulosa* embryogenesis: *a* – zygote; *b* – blastomere formation; *c* – bean-shaped embryo; *d* – tadpole-shaped embryo; *e* – larva formation; *f* – mobile larva formation

The temperature regime at 25 °C was found to be the most favourable for the exogenous development of *T. globulosa*. That cultivation temperature was accompanied by formation of the maximum ratio of infectious eggs, 76.3 ± 1.5%, and their death was 23.7 ± 1.5%. The embryogenesis period was shorter than at 20 °C and amounted to 48 days (Table 2).

The embryogenesis proceeded faster, thence the timing of each stage was also shorter. The zygote stage lasted from days 1 to 8, when more than 50% of the eggs were still at that stage. The stage of blastomere formation lasted from days 4 to 12, and the amount of egg formation increased from 25.3 ± 3.0% to 50.7 ± 3.7%. From the 16th day of cultivation, the ratio of eggs with blastomeres gradually decreased to 5.0 ± 3.0%, and from the 28th day, such eggs were not recorded. The stage of bean-like embryo lasted from the 12th day (15.3 ± 4.0%) to the 20th day (49.3 ± 2.0%). A small number of eggs at that stage of development (from 41.0 ± 2.0% to 4.3 ± 3.5%) were detected up to the 36th day. The stage of the tadpole-shaped embryo was found on the 20th day, when 18.6 ± 2.0% of eggs were found. Subsequently, by the 28th day of cultivation, the maxi-

mum ratio of such eggs formed, 40.0 ± 1.0%, and by the 40th day there were 3.7 ± 2.0% of such eggs. The stage of larva formation lasted from the 28th to the 40th day, when the maximum ratio of eggs was 44.3 ± 3.0%. On day 44, only 10.7 ± 1.5% of the eggs contained an immobile larva. The formation of infectious eggs was recorded from the 36th to the 48th day, and their ratio gradually increased from 5.7 ± 3.5% to 76.3 ± 1.5%.

At a temperature of 30 °C, the period of development of *T. globulosa* eggs was the shortest (up to 32 days), but at the same time their survival rate was the lowest and amounted to 68.0 ± 2.6%. The ratio of egg death during embryogenesis was the highest, 32.0 ± 2.6% (Table 3).

The zygote stage lasted from the moment of isolation of eggs from the gonads of female *Trichuris* and up to day 4, when the egg ratio gradually decreased from 100% to 25.7 ± 2.5%. During days 8–12, their ratio was minimal and ranged from 19.0 ± 1.0% to 7.0 ± 2.0%. The stage of blastomere formation lasted from days 4 to 8 of cultivation, when the largest ratio of eggs formed, from 74.3 ± 2.5% to 44.3 ± 2.5%. On the 16th day of egg cultivation, only 9.7 ± 3.5% remained at that stage.

Table 2
Periods of exogenous development of eggs and their viability in laboratory conditions were assessed at a temperature of 25 °C (x ± SD, n = 3)

Duration of cultivation, day	Stage of development, %						
	zygote	blastomere formation	bean-like embryo	tadpole embryo	larva formation	mobile larva	egg death
before cultivation	100	–	–	–	–	–	–
4	74.7 ± 3.0	25.3 ± 3.0	–	–	–	–	–
8	42.3 ± 1.5	44.3 ± 3.5	–	–	–	–	13.3 ± 2.0
12	17.3 ± 2.0	50.7 ± 3.7	15.3 ± 4.0	–	–	–	16.7 ± 0.5
16	6.3 ± 2.5	28.0 ± 1.7	47.3 ± 2.0	–	–	–	18.3 ± 1.5
20	–	8.3 ± 2.5	49.3 ± 2.0	18.7 ± 2.0	–	–	23.7 ± 1.5
24	–	5.0 ± 3.0	41.0 ± 2.0	30.3 ± 2.5	–	–	23.7 ± 1.5
28	–	–	26.0 ± 1.7	40.0 ± 1.0	10.3 ± 1.5	–	23.7 ± 1.5
32	–	–	20.3 ± 2.5	38.3 ± 0.5	17.7 ± 1.5	–	23.7 ± 1.5
36	–	–	4.3 ± 3.5	32.7 ± 2.0	33.7 ± 1.1	5.7 ± 3.5	23.7 ± 1.5
40	–	–	–	3.7 ± 2.0	44.3 ± 3.0	28.3 ± 0.5	23.7 ± 1.5
44	–	–	–	–	10.7 ± 1.5	65.7 ± 1.5	23.7 ± 1.5
48	–	–	–	–	–	76.3 ± 1.5	23.7 ± 1.5

Note: see Table 1.

Table 3
Periods of exogenous development of eggs and their viability in laboratory conditions were assessed at a temperature of 30 °C (x ± SD, n = 3)

Duration of cultivation, day	Stage of development, %						
	zygote	blastomere formation	bean-like embryo	tadpole embryo	larva formation	mobile larva	egg death
before cultivation	100	–	–	–	–	–	–
4	25.7 ± 2.5	74.3 ± 2.5	–	–	–	–	–
8	19.0 ± 1.0	44.3 ± 2.5	20.7 ± 1.5	–	–	–	16.0 ± 2.0
12	7.0 ± 2.0	26.0 ± 2.6	45.7 ± 3.2	–	–	–	21.3 ± 2.5
16	–	9.7 ± 3.5	28.3 ± 1.1	30.0 ± 3.0	7.7 ± 3.5	–	24.3 ± 3.0
20	–	–	3.0 ± 1.7	40.3 ± 4.5	22.0 ± 1.0	2.7	32.0 ± 2.6
24	–	–	–	5.3 ± 2.5	39.0 ± 2.6	23.7 ± 2.8	32.0 ± 2.6
28	–	–	–	–	7.3 ± 3.0	60.7 ± 5.6	32.0 ± 2.6
32	–	–	–	–	–	68.0 ± 2.6	32.0 ± 2.6

Note: see Table 1.

The stage of the bean-like embryo with the formation of the maximum number of eggs (45.7 ± 3.2%) lasted from days 8 to 12 . Subsequently, their number decreased and the minimum values (3.0 ± 1.7%) were recorded on the 20th day of cultivation. The formation of a tadpole embryo in eggs was recorded from the 16th day (30.0 ± 3.0%), and their maximum number was detected on the 20th day of cultivation (40.3 ± 4.5%). From the 16th day of cultivation, 7.7 ± 3.5% of eggs were recorded at the stage of larval formation. On the 24th day, their maximum number was observed, 39.0 ± 2.6%. Mobile larvae were found in eggs starting from the 20th day (2.7%), their maximum ratio formed up to the 32nd day (68.0 ± 2.6%). At all temperature regimes, the death of eggs during embryogenesis was mainly accompanied by an increase in the porosity of the egg shell, as a result of which the embryo became aerated and air bubbles formed in the embryo (Fig. 2). Subsequently, the eggs stopped developing and gradually lysed.
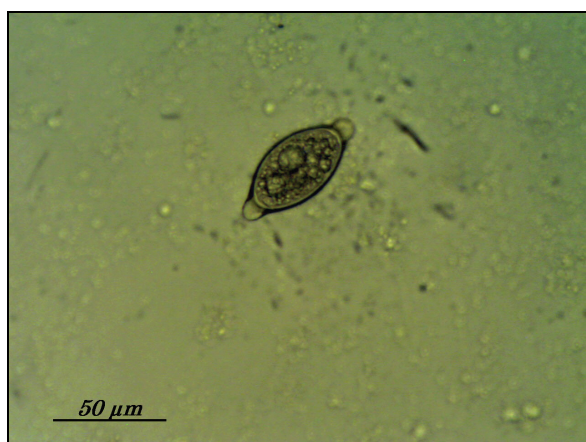
Fig. 2. Features of *Trichuris globulosa* of egg death in embryogenesis

The growth and development of *T. globulosa* eggs during embryogenesis were accompanied by changing metric parameters. Thus, in infectious eggs, a significant decrease in length by 3.2% (62.8 ± 1.6 µm, P < 0.01, Fig. 3a) and an increase in width by 7.7% (30.0 ± 0.7 µm, P < 0.001,

Fig. 3b) were revealed in eggs at the zygote stage (64.9 ± 1.6 and 27.7 ± 0.8 µm, respectively). Also, in eggs of *Trichuris* at the stage of developing mobile larva, the egg plug length decreased by 19.3% (7.1 ± 0.7 µm, P < 0.001, Fig. 3c) and the egg plug width decreased by 15.9% (9.0 ± 0.6 µm, P < 0.001, Fig. 3d), the shell thickness also reduced by 31.6% (1.3 ± 0.1 µm, P < 0.001, Fig. 3e).

Thus, the exogenous development of *T. globulosa* parasitic nematodes obtained from cattle is accompanied by certain stages, each of which is necessary for the formation of an infectious agent capable of infecting a susceptible host. The timing of development and the degree of survival of the parasite depends on the temperature factor.

Discussion

According to scientific studies, *Trichuris* infections are common, and some species are also pathogens of dangerous zooanthroponotic diseases of animals and humans (Bawm & Htun, 2015; Boyko & Brygadyrenko, 2016, 2019). *Trichuris globulosa* is recorded in ruminants in many countries of the world, where the incidence can be 14.5% (De Jong et al., 2014; Odeniran et al., 2016; Susana et al., 2019). Temperature, relative humidity, and pH of the environment are the main abiotic factors affecting the survival of parasites in the environment, and as a consequence the degree of their prevalence. This is because nematodes of the genus *Trichuris* are characterized by a host-soil-host cycle. At the same time, they need an exogenous period for the maturation of infectious eggs, which are the most resistant to adverse environmental factors (Anvari-Tafti et al., 2013; Pramasudha et al., 2015; Tudor, 2015).

We found that the exogenous development of *T. globulosa* isolated from cattle depends on temperature. Moreover, with an increase in the temperature regime, the development of invasive eggs occurs faster. A lower temperature slows down the embryogenesis process. The optimal temperature for the formation of the largest number of viable mature eggs with a mobile larva (76.3 ± 1.5%) was 25 °C. At a temperature of 20 °C, fewer infectious eggs formed (74.0 ± 2.6%). An increase in temperature to 30 °C led to the death of a larger number of eggs, and the ratio of eggs that became infectious was 68.0 ± 2.6%. The data obtained confirm the studies which show that the life of organisms, including parasitic ones, is deter-

mined by temperature more than by any factor of the external environment. This is due to the fact that all organisms are built of chemical components and all life processes occur on the basis of chemical reactions, subject to the laws of thermodynamics.
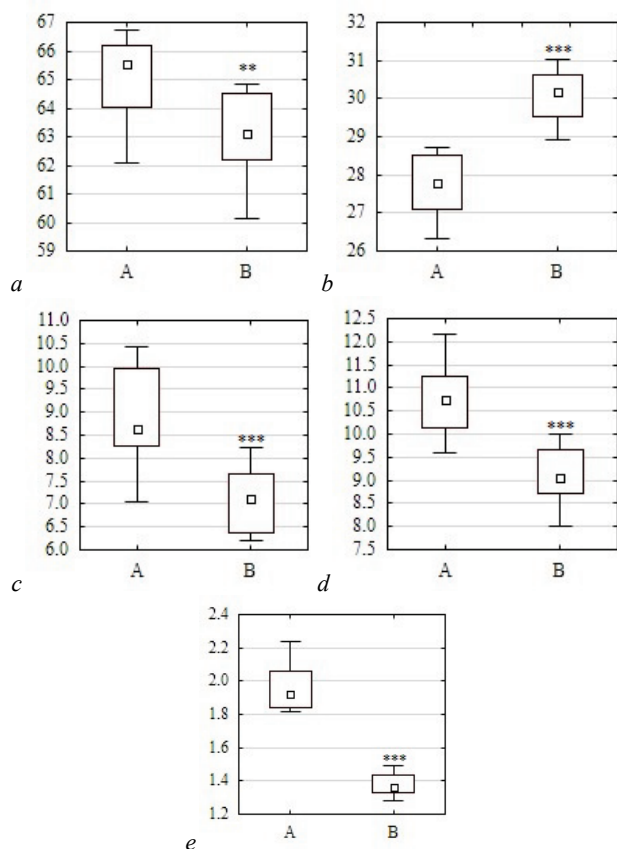
Fig. 3. The indicators of growth and development of *Trichuris globulosa* eggs in laboratory conditions at a temperature of 25 °C: *a* – egg length, *b* – egg width, *c* – egg plug length, *d* – egg plug width, *e* – eggshell thickness (μm); *A* – zygote stage, *B* – stage of mobile larva formation; ** – P < 0.01, *** – P < 0.001 compared to values of egg parameters at zygote stage; the small square in the centre corresponds to the median, the lower and upper borders of the large rectangular correspond to the first and the third quartiles, respectively, vertical line segments, directed up and down from the rectangular, correspond to minimum and maximum values (n = 10)

Temperature affects not only the rate of chemical reactions, but is also a prerequisite for the structural rearrangement of proteins, phase displacement of fats, and changes in the structure of water. For all organisms there is a vital temperature zone, within which their vital activity is carried out and fits into certain boundaries. The lower limit of the active life of parasitic organisms is limited, first of all, by drop-liquid water, the constant flow of which is the processes of assimilation and dissimilation. Therefore, the crystallization of water serves as a critical threshold for their life. The upper temperature limit is characterized by thermal coagulation of proteins. The eggs of parasites are more resistant to low temperatures than to high ones, due to the many direct and indirect adaptations developed in the process of evolution. That is, low temperatures slow down metabolic processes inside the egg and the development of the larva, but the viability of the egg is preserved (El-Hamouri et al., 1994; Pecson et al., 2007).

Our studies have established that temperature also affects the rate of embryogenesis of *T. globulosa*. With an increase in temperature, the rate of development of *Trichuris* eggs of increases. The shortest term of exogenous development was observed at a cultivation temperature of 30 °C, the infectious larvae formed on the 32nd day. At a temperature of 25 °C, the development of eggs slightly slowed down and amounted to 48 days. The longest exogenous development of *T. globulosa* was recorded at a temperature of 20 °C, the period of formation of infectious eggs was 56 days. Similar data are described by the authors who show that the time of development of *T. trichiura* eggs decreases with increasing tempera-

ture. Moreover, the upper limit of the egg survival temperature is at 37–38 °C (Beer, 1976; Bundy & Cooper, 1989; Pullan et al., 2011). In study of the embryonic development of *T. skrjabini* isolated from sheep, it was found that at 27 °C eggs reached the infectious stage at 51 days, and their viability was 80% (Yevstafieva et al., 2018).

In the experimental cultivation of eggs of parasitic *T. globulosa* isolated from cattle, we identified 6 morphologically different stages of embryogenesis, namely zygote, formation of blastomeres, bean-shaped embryo, tadpole-shaped embryo, formation of larva and mobile larva. The duration of each stage was different depending on the temperature regime. The higher the temperature, the less time it took for the transition from one stage of egg development to another. Thus, the zygote stage lasted from the moment the eggs were isolated from the gonads of female *Trichuris* and up to 4 days (at a temperature of 30 °C), to 8 days (25 °C), to 12 days (20 °C). The maximum formation of blastomeres occurred depending on temperature (30, 25, 20 °C) at 4–8, 4–12, and 4–16 days, and the stage of the bean-like embryo at 8–12, 12–20 and 12–24 days, respectively.

The stage of the tadpole-shaped embryo was the shortest at 30 °C and lasted from days 16–20 of cultivation. At temperatures of 25 and 20 °C, this stage began at a later date than at a temperature of 30 °C, and lasted, respectively, from days 20 to 28 and from days 24 to 36 of cultivation. The same tendency was noted in the timing of the stages of larval and mobile larva formation. At 30 °C, these stages lasted from days 16–24 and 20–32, respectively. At a temperature of 25 °C, these stages of development of *Trichuris* eggs lasted from days 28–40 and 36–48, and at a temperature of 20 °C, from days 32–48 and 44–56, respectively. The same data on certain stages in the exogenous development of *Trichuris* were already noted in our previous works, where the peculiarities of *T. suis* and *T. skrjabini* embryogenesis were studied. Moreover, the duration of each identified stage and the degree of egg survival in the process of their development differed significantly for each species (Yevstafieva et al., 2015, 2018).

We also established the metric changes in *T. globulosa* eggs during embryogenesis. The formation of a mobile larva was accompanied by a decrease in egg length (by 3.2%, P < 0.01), the length and width of the egg plug (by 19.3% and 15.9%, P < 0.001), an increase in the egg width (by 7.7%, P < 0.001), and a decrease in the eggshell thickness (by 31.6%, P < 0.001) in comparison with similar indicators in eggs at the zygote stage. Such changes may indicate that the stages of development differ not only in morphological features, but also in metric indicators.

The obtained results of study on the influence of temperature on the exogenous development of *T. globulosa* will make it possible to predict the epizootic situation of trichurosis in animal husbandry, as well as to take timely measures against the embryonic nematode development in the external environment. The environment contaminated with parasite eggs represents the main risk to human and animal health in the case of this disease. Preventing initial contamination and studying the factors that cause egg death in the environment are an important way to avoid contamination of humans and animals.

Conclusion

It is shown that the exogenous development of *T. globulosa* Linstow, 1901 parasitic nematodes in laboratory conditions occurs in 6 stages: zygote, blastomere formation, bean-shaped embryo, tadpole-shaped embryo, larva and mobile larva formation. The duration of embryogenesis, the term for each stage of development, and the degree of egg survival and the ratio of infectious eggs depend on the temperature factor. The cultivation temperature of 25 °C turned out to be optimal for the formation of the largest number of viable mature eggs with a mobile larva (76.3 ± 1.5%). Temperatures at the level of 20 and 30 °C led to a decrease in the viability of eggs during embryogenesis, and the proportion of formed infectious eggs did not exceed 74.0 ± 2.6% and 68.0 ± 2.6%. The development time of *T. globulosa* eggs gradually decreased with increasing temperature. At 30 °C, embryogenesis was the shortest and amounted to 32 days. At 25 °C, the egg development period was longer, occurring in 48 days. At 20 °C, the formation of the maximum number of infectious eggs at the stage of mobile larvae lasted up to 56 days. Morphological changes in *T. globulosa* eggs were accompanied by significant metric changes. Eggs at the stage of mobile larva were shorter and wider, had shorter and narrower plugs, as well as a thinner shell than eggs at the zygote stage.

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