Vestnik zoologii, **50**(2): 173–178, 2016
DOI 10.1515/vzoo-2016-0020

DE GRUYTER OPEN

UDC 59.616.995.132.6:59.087

AN INVESTIGATION OF EMBRYO AND EGGSHELL DEVELOPMENT IN *TRICHURIS SUIS* (NEMATODA, TRICHURIDAE0 UNDER LABORATORY CONDITIONS

V. A. Yevstafieva[1], I. D. Yuskiv[2], V. V. Melnychuk[1]

[1]*Poltava State Agrarian Academy*
Skovorody st., 1/3, Poltava, 36003 Ukraine
E-mail: evstva@ukr.net
[2]*S. Z. Gzhytskiy National Veterinary and Biotech University of Lviv*
Pekarska st., 50, Lviv, 79010 Ukraine

An Investigation of Embryo and Eggshell Development in *Trichuris suis* (Nematoda, Trichuridae) under Laboratory Conditions. Yevstafieva, V. A., Yuskiv, I. D., Melnychuk ,V. V. — Peculiarities of embryogenesis morphology and biometric parameters of *Trichuris suis* Schrank, 1788 eggs sampled from different organic substrates are described. The eggs of *T. suis* under laboratory conditions at a temperature of 27 °C reach the infectious stage in 40 days and pass through seven stages of embryogenesis. The study revealed significant differences in growth and development of eggs obtained from the nematode gonads and the faeces of infected animals (*Sus scrofa domesticus* Linnaeus, 1758), according to length and width of eggs and eggshell plugs. The excreted *T. suis* eggs *were shown to be better adapted* to environment (survivability 96.6 ± 0.33 %), than the eggs obtained from the gonads of female nematodes (survivability 89.3 ± 0.33 %).

K e y w o r d s: *Trichuris suis* eggs, embryogenesis, morphological and biometric characteristics.

Introduction

Trichocephalosis caused by representatives of Trichuridae (Nematoda) is one of the most widely distributed nematoses of gastrointestinal tract of domestic pigs (*Sus scrofa* dom.), as scientific reports testify worldwide. The infection is accompanied by delayed animal growth and development, reduced performance, metabolic disturbances, immunosuppression, and sometimes death (Safiullin, 1991; Stybel, 2004; Nejsum et al., 2009; Yevstafieva, 2011).

The survivability of the whipworm, *Trichuris suis* Schrank, 1788, is closely connected with its biology. The environment plays a significant role in the development of the nematode eggs up to the infectious stage of the parasite, ensuring the parasite's capacity for adaptation, morphological changes, and improving survival (Beer, 1973 a, b; Nasonova, 1975).

It was proven that after one hour after excretion of the eggs into environment, the protoplast fills the whole egg cavity and in 14–21 hr, fusion of the male and female nuclei of the protoplast results in the zygote. In two to four days, two unequal blastomeres (larger and smaller) are formed in the nucleus of whipworm egg and begin to fragment. On the seventh-eighth day there are 16–24 blastomeres lesser in size. The embryo becomes bean-like on 15–16th day. The next, tadpole stage of development, happens on 18–20th day. On 25–28th day, there is a mobile larva in the egg. The larvae do not leave the eggshell until the egg is swallowed by the definitive host (Olekhnovich, Yatusevich 1994, 2001).

In a number of studies (Nasonova, 1974; Toluzarova, 1982), several factors were shown to influence *T. suis* egg development. These are primarily temperature and humidity. In natural opportune conditions, *T. suis* eggs become infectious in 44–54 days. On pigsty floors, the eggs develop in 38–43 days.

Hence, studies on the morpho-biological characters of *T. suis* eggs at different stages of development, taking into account their metrical characteristics and environmental conditions, allow for better understanding the life aspects and adaptability traits of this parasitic organism.

Material and methods

Research was carried out in 2014–2015 at the Laboratory of Parasitology and Veterinary-Sanitary Expertise of the Department of Veterinary Medicine of Poltava State Agrarian Academy. Partly it was also conducted at Pathomorphological Department of the Regional State Laboratory of Veterinary Medicine, Poltava, Ukraine.

Eggs of *T. suis* were sampled according to Kotelnikov-Khrenov method (Kotelnikov, 1984) from different biological substrates: female whipworm gonads (Eg); faeces of infected pigs (Ef). The eggs were cultured to the infectious stage in a thermostat at 27 °C for 30–45 days. The culture was examined under a light microscope (×100, ×150) in five days intervals. Each experiment was performed in triplicate. Form, structure, length, width of the eggs, length and width of the egg plugs and the eggshell thickness were measured.

To measure metrical characteristics of collected *T. suis* eggs, ImageJ for Windows® (version 2.00) software was used in interactive mode using 16× objective and 10× photo eyepiece. To calibrate the image analyzer, ruled scale of ocular micrometer was coincided with the scale of stage micrometer included in MikroMed microscope kit. Microphotographs were taken using a 3Mpix digital camera of MikroMed (China) microscope. Statistical processing of the experimental results was carried out using Student t-test (Lapach et al., 2001).

Results and discussion

In laboratory conditions at 27 °C the mobile larvae completed their development in *T. suis* eggs on the 40th day regardless of sampling method. In embryogenesis, seven stages of development were identified: protoplast, two blastomeres, blastomere cleavage, bean-like embryo, tadpole embryo, developing larva, and mobile larva. Development and survivability of *T. suis eggs sampled from the nematode gonads was significantly different from that of eggs collected from faeces of infected pigs* (table 1).

Thus, at the beginning of cultivation, 100 % of collected whipworm eggs were at the protoplast stage of development (fig. 1). Culture of eggs obtained from the nematode gonads also contained unformed eggs (fig. 1, *a*).

On 5th and 10th days of culture, 53.0 ± 0.58 % and 31.7 ± 0.88 % of Eg eggs contained two blastomeres respectively; 29.0 ± 0.58 % and 56.0 ± 0.58 % Eg contained *three or more small blastomeres* (fig. 2, *a*); 18.0 ± 0.58 % and 12.3 ± 0.67 % Eg *were protoplastic.* The Ef

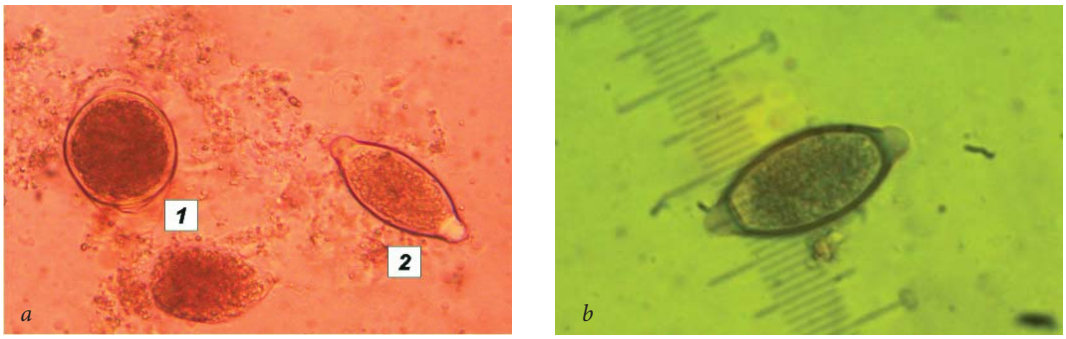

Fig. 1. Eggs of *Trichuris suis* at the protoplast stage of development: *a* — sampled from nematode gonads: *1* — unformed, *2* — formed (×350); *b* — sampled from faeces of sick pigs (×450).

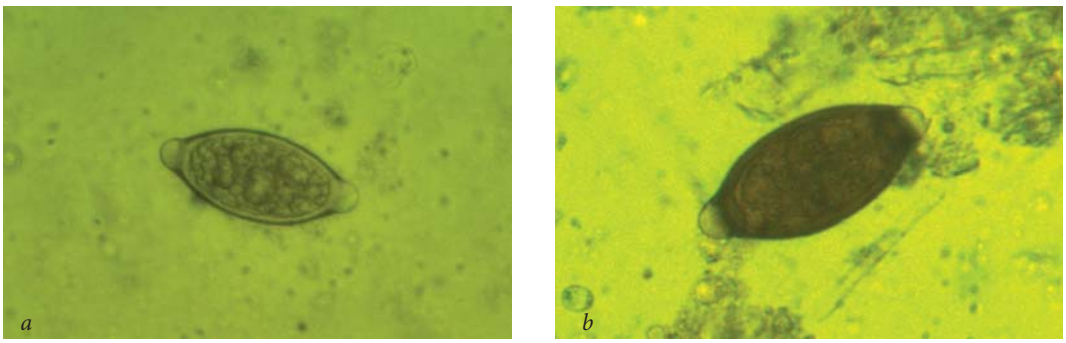

Fig. 2. Eggs of *Trichuris suis* at blastomere cleavage stage of development: *a* — sampled from nematode gonads, 15th day of cultivation (×400); *b* — sampled from faeces of infected pigs, 10th day of cultivation (×500).

Table 1. Developmental characters of cultured *Trichuris suis* eggs obtained from different organic substrates, M ± m (n = 200)

Day of cultivation	Egg type	Stage of egg development, %							
		proto-plast	two blasto-meres	blasto-mere cleavage	bean-like embryo	tadpole embryo	developing larva	mobile larva	end of develop-ment
Before culti-vation	Eg	100.0	–	–	–	–	–	–	–
	Ef	100.0	–	–	–	–	–	–	–
5th	Eg	18.0 ± 0.58	53.0 ± 0.58	29.0 ± 0.58	–	–	–	–	–
	Ef	15.0 ± 0.57	58.0 ± 0.57	27.0 ± 1.00	–	–	–	–	–
10th	Eg	12.3 ± 0.67	31.7 ± 0.88	56.0 ± 0.58	–	–	–	–	–
	Ef	3.3 ± 0.33	9.3 ± 0.33	87.3 ± 0.33	–	–	–	–	–
15th	Eg	–	11.3 ± 0.67	68.0 ± 0.58	10.0 ± 0.58	–	–	–	10.7 ± 0.33
	Ef	–	–	24.0 ± 0.57	77.6 ± 0.33	–	–	–	3.3 ± 0.33
20th	Eg	–	–	15.7 ± 0.88	35.0 ± 0.58	21.7 ± 1.20	17.0 ± 0.58	–	10.7 ± 0.33
	Ef	–	–	4.0 ± 0.57	38.0 ± 0.57	72.6 ± 0.33	–	–	3.3 ± 0.33
25th	Eg	–	–	–	35.0 ± 0.58	23.0 ± 1.0	57.3±0,88	2.3 ± 0.33	10.7 ± 0.33
	Ef	–	–	–	2.3 ± 0.33	38.0 ± 0.57	43.3 ± 1.20	9.6 ± 0.88	3.3 ± 0.33
30th	Eg	–	–	–	–	2.0 ± 0.58	21.7 ± 0.88	65.7 ± 0.33	10.7 ± 0.33
	Ef	–	–	–	–	2.3 ± 0.33	10.0 ± 0.57	85.3 ± 0.88	3.3 ± 0.33
35th	Eg	–	–	–	–	–	10.7 ± 0.88	78.7 ± 0.67	10.7 ± 0.33
	Ef	–	–	–	–	–	1.3 ± 0.33	95.3 ± 0.33	3.3± 0.33
40th	Eg	–	–	–	–	–	–	89.3 ± 0.33	10.7 ± 0.33
	Ef	–	–	–	–	–	–	96.6 ± 0.33	3.3± 0.33
45th	Eg	–	–	–	–	–	–	89.3 ± 0.33	10.7 ± 0.33
	Ef	–	–	–	–	–	–	96.6 ± 0.33	3.3± 0.33

embryogenesis was more intense: on 5th and 10th days, 58.0 ± 0.57 % and 9.3 ± 0.33 % of eggs contained two blastomeres; 27.0 ± 1.00 % and 87.3 ± 0.33 % *had three or more small blastomeres* (fig. 2, *b*); 15.0 ± 0.57 % and 3.3 ± 0.33 % were protoplastic.

On the 15th day of experiment, further development of *T. suis* eggs was detected: 10.0 ± 0.58 % Eg (fig. 3, *a*) and 77.6 ± 0.33 % Ef (fig. 3, *b*) eggs contained bean-like embryos. On 20–*25th days of cultivation,* 35.0 ± 0.58 % Eg eggs contained bean-like embryos, 21.7 ± 1.20 *and* 23.0 ± 1.00 %, respectively, were at tadpole embryo stage, and 17.0 ± 0.58 and 57.3 ± 0.88 %, respectively, were developing larvae (fig. 4, *a*). At the same time, on 20–30th days of cultivation, the percentage of Ef eggs with bean-like embryos gradually decreased from 38.0 ± 0.57 to 2.33 ± 0.33 % while the percentage of Ef eggs with tadpole embryos grew to 72.6 ± 0.33 % on 20th day, Ef eggs with developing larvae (fig. 4, *b*) constituted 43.3 ± 1.20 % of all Ef eggs on 25th day.

On 30–40th days of cultivation, the percentage of formed infectious Eg eggs of *T. suis containing mobile larvae* (fig. 5, *a*) gradually increased from 65.7 ± 0.33 to 89.3 ± 0.33 %. At the same time, the percentage of infectious Ef eggs (fig. 5, *b*) was much higher, increasing from 85.3 ± 0.88 to 96.6 ± 0.33 %. Only 10.7 ± 0.33 % Eg and 3.3 ± 0.33 % Ef terminated their development and deteriorated.

During cultivation of *T. suis* eggs, their morphometric characters were determined. The results are shown in tables 2 and 3.

On the 10th day of Eg cultivation (table 1), the eggshell thickness reduced by 11.76 % (3.0 ± 0.11 μm, $p < 0.01$) relative to characters before cultivation (3.4 ± 0.10 μm). Later, on the 20th day of experiment, morphometric characters of egg length, egg plug length and eggshell thickness changed more significantly. For example, egg length increased by 5.52 % (68.9 ± 0.41 μm, $p < 0.001$), plug length increased by 7.14 % (8.4 ± 0.25 μm, $p <$

0.05), eggshell thickness reduced by 14.71 % (2.9 ± 0.10 μm, $p < 0.001$). On 30th day of cultivation, characters of Eg egg length continued to increase significantly by 6.47 % (69.6 ± 0.56 μm, $p < 0.001$) Eg plug length grew by 21.21 % (9.9 ± 0.35 μm, $p < 0.001$), Eg egg length increased by 6.08 % (32.9 ± 0.30 μm, $p < 0.001$) relative to same characters before cultivation. On the 40th day all morphometric egg characters did not change, aside from length that increased by 6.59 % (69.7 ± 0.46 μm, $p < 0.001$). Width of whipworm eggs have not changed significantly during cultivation.

Morphometric studies of embryogenesis of *T. suis* eggs obtained from whipworm gonads and cultured experimentally revealed that the growth and development of eggs lasted 40 days at 27 °C. On 10–20th days of embryogenesis, eggshell began to thin. On 20–40th day, egg length increased, on 20–30th plug grew, on 30th day egg width did.

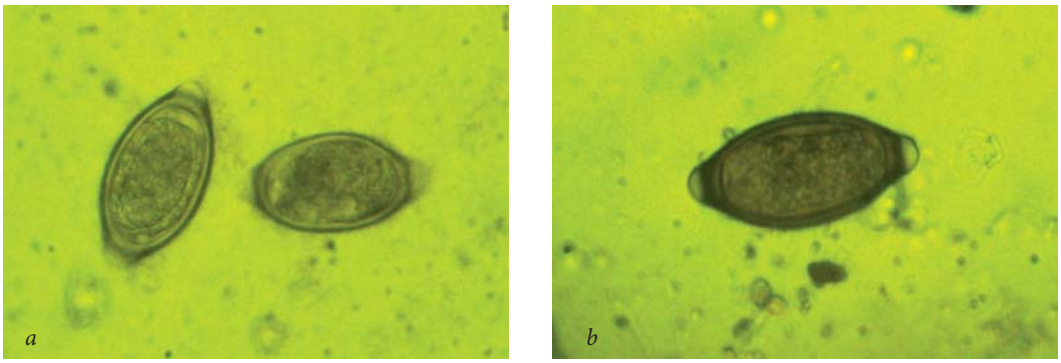

Fig. 3. Eggs of *Trichuris suis* at bean-like embryo stage of development: *a* — sampled from nematode gonads, 25th day of cultivation (×400); *b* — sampled from faeces of infected pigs, 20th day of cultivation (×450).

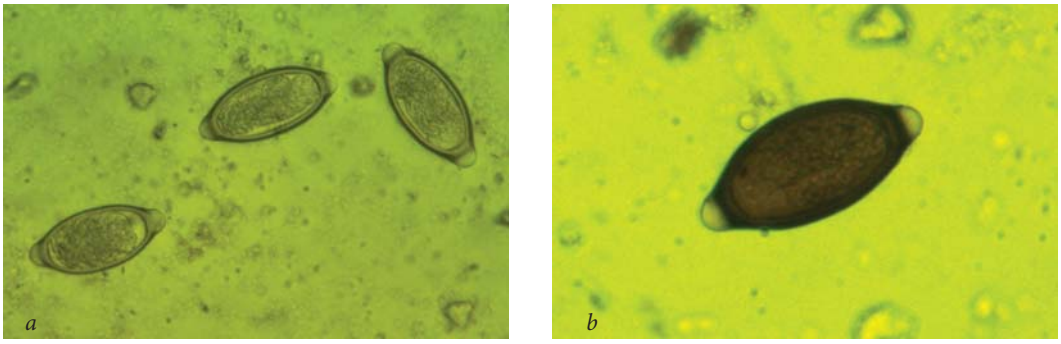

Fig. 4. Eggs of *Trichuris suis* at the stage of developing larvae: *a* — sampled from nematode gonads, 30th day of cultivation (×300); *b* — sampled from faeces of infected pigs, 20th day of cultivation (×450).

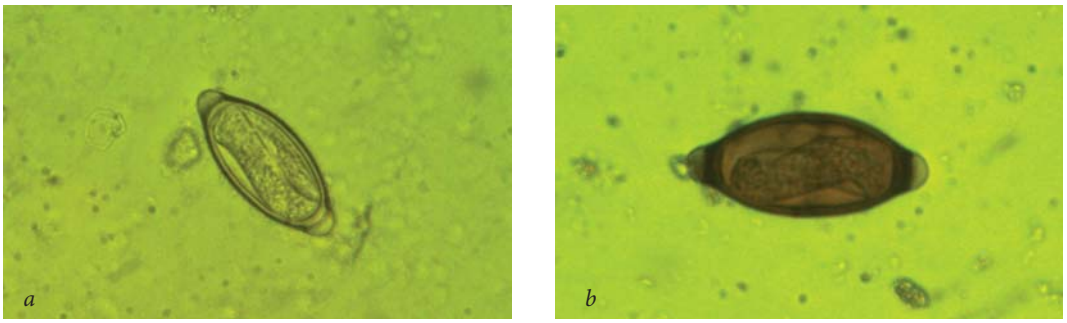

Fig. 5. Infectious *Trichuris suis* egg: *a* — sampled from nematode gonads, 40th day of cultivation (×350); *b* — sampled from faeces of infected pigs, 30th day of cultivation (×450).

Analysis of morphometric characters of developing *T. suis* eggs from faeces of infected pigs showed significant biometric changes only after 30th day of cultivation (table 3).

For example, Ef egg length increased insignificantly by 1.54 % (71.2 ± 0.40 μm, $p < 0.05$ relative to that before cultivation, 70.1 ± 0.25 μm). On the 40th day, egg length and width and egg plug width increased by 2.23 % (71.7 ± 0.31 μm, $p < 0.001$), 2.39 % (33.4 ± 0.24 μm, $p < 0.01$), 4.27 % (11.7 ± 0.21 μm, $p < 0.05$), respectively, relative to same characters before cultivation. On 45th day of cultivation, such morphometric characters still significantly increased, plug width by 5.08 % (11.8 ± 0.20 μm, $p < 0.05$). Eggshell significantly thinned by 6.89 % (2.7 ± 0.09 μm, $p < 0.05$).

In total, the morphometric studies of experimentally cultured *T. suis* eggs collected from faeces of infected pigs revealed the egg biometric changes that characterized 45 days of their growth and development. At that, egg length increased on 30–45th day, egg width and plug width increased on 40–45th day. Only on the 45th day the eggshell thinning was registered.

Our data confirm previous results (Olekhnovich, Yatusevich, 1994, 2001) according to which eggs of *T. suis* either in laboratory conditions or in environment undergo certain stages of development: protoplast, blastomere formation, blastomere cleavage, bean-like embryo, tadpole embryo, larva formation (end of development). We also established the duration of development of infectious *T. suis* eggs obtained from various biological

Table 2. Morphometric characters of developing *Trichuris suis* eggs, sampled from nematode gonads (Eg) (n = 50)

Day of cultivation	Characters, M ± m (min–max), μm				
	length	width	length	width	Eggshell thickness
	egg		egg plug		
Before cultivation	65.1 ± 0.39 (59.6–69.9)	30.9 ± 0.22 (25.9–34.7)	7.8 ± 0.18 (4.9–10.4)	10.7 ± 0.20 (8.2–15.7)	3.4 ± 0.10 (1.6–4.9)
10th	66.1 ± 0.39 (61.9–76.7)	30.9 ± 0.18 (28.7–35.6)	7.9 ± 0.21 (5.4–12.0)	10.7 ± 0.18 (8.5–13.3)	3.0 ± 0.11** (1.6–5.5)
20th	68.9 ± 0.41*** (62.3–76.8)	31.3 ± 0.23 (28.9–35.7)	8.4 ± 0.25* (5.7–13.6)	10.8 ± 0.20 (8.5–15.6)	2.9 ± 0.10*** (1.5–4.0)
30th	69.6 ± 0.56*** (62.3–77.2)	32.9 ± 0.30*** (28.9–37.0)	9.9 ± 0.35*** (5.9–14.7)	11.0 ± 0.21 (8.7–15.9)	2.9 ± 0.10*** (1.2–4.0)
40th	69.7 ± 0.46*** (62.7–77.1)	32.9 ± 0.28*** (29.0–37.0)	9.9 ± 0.35*** (6.4–14.7)	11.0 ± 0.19 (8.7–15.8)	2.9 ± 0.10*** (1.2–3.9)
45th	69.7 ± 0.52*** (62.7–77.2)	32.9 ± 0.26*** (30.1–37.0)	9.9 ± 0.34*** (5.9–14.7)	11.0 ± 0.19 (8.7–15.9)	2.9 ± 0.10*** (1.5–3.9)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ — relative to characters before cultivation.

Table 3. Morphometric characters of developing *Trichuris suis* eggs from faeces of sick pigs (*Sus scrofa domesticus*) (Ef) (n = 50)

Day of cultivation	Characters, M ± m (min–max), μm				
	length	width	length	width	Eggshell thickness
	egg		egg plug		
Before cultivation	70.1 ± 0.25 (65.9–74.4)	32.6 ± 0.19 (30.3–35.3)	10.3 ± 0.27 (7.0–13.9)	11.2 ± 0.17 (8.7–13.6)	2.9 ± 0.08 (2.0–4.7)
10th	70.3 ± 0.43 (62.1–75.7)	32.9 ± 0.22 (30.7–36.2)	10.4 ± 0.23 (7.0–13.8)	11.2 ± 0.22 (7.0–14.1)	2.9 ± 0.10 (2.0–4.7)
20th	70.5 ± 0.46 (62.1–76.9)	32.9 ± 0.22 (30.1–36.2)	10.5 ± 0.22 (7.0–13.8)	11.3 ± 0.19 (8.4–14.1)	2.9 ± 0.08 (2.0–4.0)
30th	71.2 ± 0.40* (61.9–75.6)	33.0 ± 0.16 (31.1–36.4)	10.7 ± 0.26 (7.1–13.7)	11.4 ± 0.17 (8.7–13.6)	2.8 ± 0.09 (1.5–3.9)
40th	71.7 ± 0.31*** (65.9–76.2)	33.4 ± 0.24** (30.9–37.0)	10.8 ± 0.19 (8.1–13.7)	11.7 ± 0.21* (8.9–14.1)	2.7 ± 0.09 (1.1–3.8)
45th	71.7 ± 0.35*** (65.8–76.9)	33.4 ± 0.21** (31.0–37.9)	10.9 ± 0.21 (8.1–13.7)	11.8 ± 0.20* (9.1–14.9)	2.7 ± 0.09* (1.0–3.8)

* $p < 0.05$, ** — $p < 0.01$, *** $p < 0.001$ — relative to characters before cultivation.

substrates and cultured *in vitro* at the optimum temperature (27 °C) and that the mobile larvae develop in 40 days. According to Nasonova (1975), development of infectious larva in *T. suis* eggs sampled from porcine faeces continued 48–57 days. We assume that this inconstancy in duration of *T. suis* egg development indicates the variability and adaptability of parasites to different climate conditions of their hosts' environment. We also for the first time determined morphometric features of pig whipworm egg development, reliably confirmed by characters of egg length and width and egg plug width, and eggshell thickness. We found no similar studies in available literature.

Conclusion

It should be noted that *T. suis* egg maturation *in vitro* at 27 °C (the eggs sampled from nematode gonads and from faeces of infected pigs) and development of mobile larva last 40 days. Morphologically, seven specific stages of embryogenesis were identified. Egg development of *T. suis*, sampled from porcine faeces, is faster and their survivability is higher (96.6 ± 0.33 %), than of *T. suis* eggs obtained from the nematode gonads (89.3 ± 0.33 %). Variations of embryological transformation of *T. suis* eggs collected from different organic substrates are confirmed biometrically. For example, *T. suis* eggs collected from nematode gonads, exhibit such peculiarities: from 10th to 20th days of culture, eggshell thinned (by 14.71 %, $p < 0.001$); from 20th to 30th days, plug length increased (by 21.21 %, $p < 0.001$); on the 30th day, egg width increased (by 6.08 %, $p < 0.001$); from 20th to 40th days, egg length grew (by 6.59 %, $p < 0.001$). Development of *T. suis* eggs sampled from porcine faeces was characterized by insignificant changes, as follows: from 30th to 45th days of culture, egg length increased (by 2.23 %, $p < 0.001$); on 40th day egg width grew (by 2.39 %, $p < 0.01$); from 40th to 45th day, plug width increased (by 5.08 %, $p < 0.05$), on 45th day the eggshell thinned (by 6.89 %, $p < 0.05$).

Authors are thankful to V. V. Kornyushin and V. F. Galat (Kyiv, Ukraine) for consultations.

References

Beer, R. J. S. 1973 a. Studies on the biology of the life-cycle of *Trichuris suis* Schrank, 1788. *Parasitology*, **67**, 253–262.

Beer, R. J. S. 1973 b. Morphological descriptions of the egg and larval stages of *Trichuris suis* Schrank, 1788. *Parasitology*, **67,** 263–278.

Yevstafieva, V. A. 2011. Epizooty of associated porcine infections in forest-steppe and steppe conditions. *Visnyk Poltavskoyi Derzhavnoyi Agrarnoyi Akademii*, 98–100 [In Ukrainian].

Kotelnikov, G. A. 1984. *Helminthological studies of animals and environment*. Kolos, Moscow, 40–47 [In Russian].

Lapach, S. N., Chubenko, A. V., Babich, P. N. 2001. *Statistical methods in medical and biological research using Excel*. Morion, Kyiv, 11–35 [In Russian].

Nasonova, M. V. 1974. Resistance of porcine whipworm eggs to various environmental factors. *Nauch. Trudy Voronezh. Selskohos. Instituta*, 60, 55–60 [In Russian].

Nasonova, M. V. 1975. Duration of development and survivability of porcine trichocephalosis eggs in Voronezh oblast conditions. *Nauch. Trudy Voronezh. Selskohos. Instituta*, 70, 72–77 [In Russian].

Nejsum, P., Thamsborg, S. M., Petersen, H. H., Kringel, H., Fredholm, M., Roepstorff, A. 2009. Population dynamics of *Trichuris suis* in trickle-infected pigs. *Parasitology,* **136**, 691–697.

Olekhnovich, N. I., Yatusevich, A. I. 1994. Pig trichocephalosis. *Uchenye zapiski Vitebskoi gosudarstvennoi akademii veterinarnoi mediciny*, 31, 127–130 [In Russian].

Olekhnovich, N. I., Yatusevich, A. I. 2001. *Pig trichocephalosis*. VGVAM, Vitebsk, 1–11 [In Russian].

Safiullin, R. T. 1991. Economic losses caused by ascariasis, trichocephalosis and oesophagostomosis. *Bull. VIGIS*, 1–121 [In Russian].

Stybel, V. V. 2004. Peculiarities of intestinal porcine nematodoses in western Ukraine. *Visnyk Sumskogo Nasionalnogo Agrarnogo Universitetu. Sumy*, **7** (12), 144–148 [In Ukrainian].

Toluzarova, T. A. 1982. Duration of development and survivability of Trichocephalus eggs in environment in North-Western zone of Russia. *Bull. Vsesouznogo Instituta Gelmintologii*, **33**, 83–84 [In Russian].

Received 22 February 2016
Accepted 23 February 2016