ISTANBUL UNIVERSITY
CERRAHPASA

Effect of Temperature on the Survival Rates of the Embryonic States of Development of *Trichuris skrjabini* Nematodes Parasitizing Sheep

Valentyna Oleksandrivna YEVSTAFIEVA[1], Maksym Oleksandrovich PETRENKO[2], Vitaliy Vasyliovych MELNYCHUK[1], Yuliia Valentynivna VAKULENKO[3], Tetiana Ivanivna BAKHUR-KAVALIAUSKENE[4], Olena Viktorivna TITARENKO[2], Bohdan Serhiyovych SHAFERIVSKYI[5], Marina Anatolievna PISHCHALENKO[6], Sergii Vasyliovych FILONENKO[7], Serhii Volodymyrovych SHEIKO[8]

[1]Department of Parasitology and Veterinary-Sanitary Examination, Poltava State Agrarian University, Faculty of Veterinary Medicine, Poltava, Ukraine
[2]Department of Infectious Pathology, Hygiene, Sanitation and Biosafety, Poltava State Agrarian University, Faculty of Veterinary Medicine, Poltava, Ukraine
[3]Department of Information Systems and Technologies, Educational and Scientific Institute of Economics, Management, Law and Information Technologies Poltava State Agrarian University, Poltava, Ukraine
[4]Department of Parasitology and Pharmacology, Bila Tserkva National Agrarian University, Faculty of Veterinary Medicine, Bila Tserkva, Kyiv region, Ukraine
[5]Department of Animal Productivity Biology named after Academician O.V. Kvasnytskyi, Poltava State Agrarian University, Faculty of Technology of Production and Processing of Animal Husbandry Products, Poltava, Ukraine
[6]Department of Plant Protection, Educational and Scientific Institute of Agrotechnology, Breeding and Ecology, Poltava State Agrarian University, Poltava, Ukraine
[7]Department of Plant Growing, Educational and Scientific Institute of Agrotechnology, Breeding and Ecology, Poltava State Agrarian University, Poltava, Ukraine
[8]Department of Humanities and Social Sciences, Poltava State Agrarian University, Faculty of Accounting and Finance, Poltava, Ukraine

Cite this article as: Yevstafieva, V.O., Petrenko, M.O., Melnychuk, V.V., Vakulenko, Y.V., Bakhur-Kavaliauskene, T.I., Titarenko, O.V., Shaferivskyi, B.S., Pishchalenko, M.A., Filonenko, S.V., & Sheiko, S.V. (2023). Effect of temperature on the survival rates of the embryonic states of development of *Trichuris skrjabini* nematodes parasitizing sheep. *Acta Veterinaria Eurasia*, 49(2), 105-112.

ORCID IDs of the authors: V.O.Y. 0000-0003-4809-2584; M.O.P. 0000-0002-5275-9401; V.V.M. 0000-0003-1927-1065; Y.V.V. 0000-0002-6315-0116; T.I.B.-K. 0000-0001-8271-8267; O.V.T. 0000-0002-7370-8523; B.S.S. 0000-0001-5742-5016; M.A.P. 0000-0001-8954-8256; S.V.F. 0000-0001-8360-8852; S.V.S. 0000-0002-4635-4643

Abstract

The prevention of the trichurosis of sheep requires understanding the terms of development of pathogens and their survival in the environment under the influence of abiotic factors. This study was conducted to determine the tolerance to a temperature factor of *Trichuris skrjabini* nematode eggs isolated from sheep. *Trichuris* eggs were obtained from the gonads of female nematodes in laboratory conditions and cultivated in a thermostat until the appearance of motile larvae in the eggs at the temperatures of 20°C, 25°C, and 30°C. It was determined that the time of formation of infectious eggs decreases with increasing temperature. The temperature of 25°C was found to be the most favorable for the development of *T. skrjabini* eggs in laboratory conditions, and 80.3% of eggs with motile larvae formed on the 54th day. Temperatures of 20°C and 30°C were less advantageous to the process of embryogenesis. The motile larvae develop in eggs in 63 days at 20°C and in 45 days at 30°C, and their respective survival rates are 77.0% and 75.3%. The obtained data increase the effectiveness of planning measures to prevent the trichurosis of sheep, taking into account the period of development of the pathogen depending on the temperature of the environment.

Keywords: Exogenous development, nematodes' eggs, optimal environmental conditions, trichurosis

Introduction

The gastrointestinal nematodes of sheep are recognized among the most common pathogens of sheep farms in many parts of the world. This group of parasites is commonly represented in sheep by the helminths of the genus *Trichuris*, usually of the species *Trichuris skrjabini* (Baskakov, 1924; Nematoda, Trichuridae), *T. ovis* (Abildgaard, 1795; Nematoda, Trichuridae), or *T. globulosa* (Linstow, 1901; Nematoda, Trichuridae) (Asmare et al., 2016; Bhattacharjee et al., 2021; Jadidoleslami et al., 2022; Melnychuk et al., 2020; Yevstafieva et al., 2018).

The environment plays a significant role in the spread of gastrointestinal nematodes, including trichurosis. This is due to the fact that the causative agents of nematodosis complete a certain stage of their biological cycle in the environment, and their corresponding adaptations are characterized by the emergence of tolerance to the influence of various environmental factors. Such resistance of parasites to the influence of environmental factors is extremely necessary for exogenous stages because they persist in the environment for quite a long time (Aleuy & Kutz, 2020; Moskvina et al., 2016; Natalini et al., 2021).

Corresponding author: Tetiana Ivanivna BAKHUR-KAVALIAUSKENE • **E-mail:** fly_13@ukr.net

Received: December 07, 2022 • **Accepted:** March 23, 2023 • **Publication Date:** June 7, 2023 • DOI: 10.5152/actavet.2023.22119
Available online at actavet.org

The infected animal hosts are the main source of the parasite eggs in the environment. Moreover, the amount of invasive material released into the environment depends on the type of infection, fecundity, number, and life span of parasites. In particular, it is established that the helminths of the suborder Trichinellida and the genus *Trichuris* are the most fertile and tolerant during the process of embryogenesis and long-term persistence in the environment (Lindgren et al., 2020; Mkandawire et al., 2022; Saldanha et al., 2022). These parasites are geohelminths. According to their cycles of development, the eggs are released with the feces of definitive hosts and mature to the infectious stage outside the host's body under the influence of various environmental conditions. Such development is possible in the presence of oxygen and favorable levels of temperature and moisture. Moreover, the abovementioned abiotic factors directly affect the duration of the biological cycle and viability of parasites (Cable et al., 2017; Melnychuk & Berezovsky, 2018). The temperature plays a decisive role in the survival of eggs of the geohelminth nematodes. The pathogens die in the early stages of embryonic development under unfavorable temperature regimes (Molnár et al., 2013; Parija, 2022). In particular, eggs of helminths of the genera *Trichuris* do not develop well in the environment under the influence of high and low temperatures, and their viability decreases (Vejzagić et al., 2016).

It is extremely important to study the biological properties of the causative agents of parasitosis, especially the exogenous stages of parasites, as well as the influence of the external environment on them and the possibilities of their adaptation and survival. Therefore, the aim of the work was to determine the resistance of *T. skrjabini* nematode eggs isolated from sheep to the influence of different temperature regimes during their embryonic development.

Method

Animals and Research Model

Experimental research was conducted in the laboratory of parasitology of the Poltava State Agrarian University (Poltava, Ukraine) in 2022. The nematodes were collected by complete helminthological dissection of the large intestines of sheep slaughtered at specialized slaughterhouses of the Poltava region. *T. skrjabini* were microscopically recognized by the standard taxonomic keys (Skrjabin et al., 1957; Taylor et al., 2016).

In total, 43 sheep with age ranging from 6 months to 5 years, of the Romanov and Askanian fine-wool breeds, were studied.

Parasitological Studies

Trichuris nematodes were removed from the intestines of sheep in laboratory conditions and washed in a 0.9% NaCl solution. Live, mature *Trichuris* females were selected for the experiment. *Trichuris* eggs were obtained from their gonads by dissecting the area of the distal part of the vagina. The resulting culture of *T. skrjabini* eggs was transferred to separate Petri dishes with a 0.9% NaCl solution. Then, the eggs were placed in each Petri dish (100 eggs per dish) and cultivated in a thermostat at different temperatures (20°C, 25°C, and 30°C) until the formation of the motile larvae. *Trichuris* egg cultures were examined under a microscope every 3 days and aerated daily. If necessary, 0.9% NaCl solution was added to egg cultures. The degree and stage of egg development were determined by the morphological structure. The term of formation of invasive eggs was determined. Each experiment was performed in triplicates.

Microphotography was performed using a digital camera SIGETA M3CMOS 14000 14.0 MP (TM SIGETA, Kyiv, Ukraine) with the microscope Sigeta MICROmed XS 5520 (Ningbo Shengheng Optics & Electronics Co., Ltd, Ningbo, China).

Statistical Processing of Results

All datasets were expressed as mean (*M*) $\pm$ standard deviation of the mean. Statistical processing of the experimental results was carried out using Microsoft Excel software.

Results

Under all temperature regimes, the exogenous development of *T. skrjabini* eggs occurred in six stages that differed in the morphological structure of the embryo: zygote, formation of blastomeres, formation of a bean-like and tadpole-like embryos, formation of a larva, and last a mobile larva (Figure 1).

With increasing temperature, the time for the formation of mobile larvae in *T. skrjabini* eggs decreased, and the percentage of survival ranged from 75.3% to 77.0%. In particular, at a temperature of 20°C, the maturation period of eggs to the invasive stage happened in 63 days, and their survival rate was 77.0 $\pm$ 3.6% (Table 1).

The zygote was found until the 27th day of cultivation, at which point the amount of eggs at that stage gradually decreased from 100.0% to 4.0 $\pm$ 2.0%. The stage of blastomere formation lasted from 3 to 33 days. The amount of eggs at that stage gradually

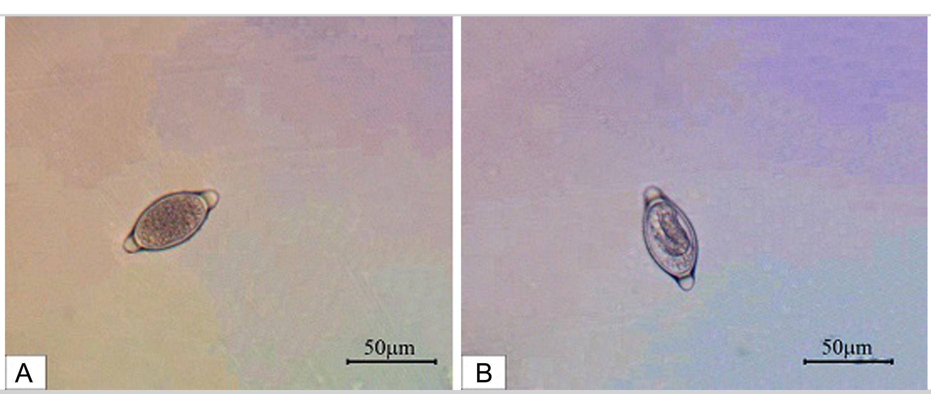

Figure 1.
Eggs of Trichuris skrjabini in the process of their embryogenesis at the stage: (A) zygote and (B) formation of a mobile larva.

Table 1.

Survival Rates of Trichuris skrjabini Nematode Eggs In Vitro at a Temperature of 20°C

	Stage of Development, %					
Day	**Zygote**	**Cleavage**	**Bean-Like embryo**	**Tadpole-Like Embryo**	**Larva**	**Motile Larva**
1	100.0	–	–	–	–	–
3	93.3 ± 3.5	6.6 ± 3.5	–	–	–	–
6	86.6 ± 5.5	13.3 ± 5.5	–	–	–	–
9	78.6 ± 5.7	21.3 ± 5.7	–	–	–	–
12	50.3 ± 4.0	26.3 ± 4.0	8.3 ± 5.0	–	–	–
15	35.0 ± 5.2	30.6 ± 0.5	19.0 ± 3.0	–	–	–
18	20.6 ± 2.3	34.6 ± 1.5	26.3 ± 2.1	2.3 ± 1.5	–	–
21	12.6 ± 4.7	27.6 ± 3.5	34.3 ± 1.5	8.6 ± 2.5	–	–
24	8.0 ± 2.6	18.3 ± 3.5	41.0 ± 1.0	16.0 ± 6.2	–	–
27	4.0 ± 2.0	14.6 ± 4.0	45.6 ± 3.1	18.6 ± 7.5	–	–
30	–	7.0 ± 3.4	48.0 ± 3.6	24.3 ± 2.8	2.0 ± 1.0	–
33	–	2.0 ± 1.0	34.6 ± 5.7	36.6 ± 7.2	7.6 ± 0.5	–
36	–	–	20.0 ± 1.7	48.0 ± 2.6	12.6 ± 1.5	–
39	–	–	12.3 ± 3.1	43.0 ± 4.3	25.0 ± 1.0	–
42	–	–	4.0 ± 1.7	32.3 ± 5.1	39.3 ± 1.5	3.3 ± 1.5
45	–	–	–	19.3 ± 0.5	49.0 ± 2.6	10.3 ± 1.2
48	–	–	–	11.0 ± 2.6	40.6 ± 1.5	26.0 ± 3.6
51	–	–	–	5.6 ± 3.2	32.0 ± 3.6	39.3 ± 1.5
54	–	–	–	–	25.0 ± 4.5	52.0 ± 2.0
57	–	–	–	–	13.3 ± 5.1	63.6 ± 6.1
60	–	–	–	–	6.3 ± 3.5	70.6 ± 4.5
63	–	–	–	–	–	77.0 ± 3.6

Note: – no eggs at the stage of development.

increased until 18 days (34.6 ± 1.5%) and then decreased until 33 days (2.0 ± 1.0%). The stages of bean-like and tadpole-like embryo formation in eggs took place during 12–42 days and 18–51 days, respectively, and the numbers of such eggs varied during this period from 4.0 ± 1.7% to 48.0 ± 3.6% and from 2.3 ± 1.5% to 48.0 ± 2.6%, respectively. The maximum numbers of such eggs were detected on the 30th and 36th day of cultivation for the bean-like and the tadpole-like embryos, respectively. The two final stages in eggs lasted 30–60 days for the formation of larva (the number of such eggs varied from 2.0 ± 1.0% to 49.0 ± 2.6%) and 42–63 days for the formation of motile larva (the amount of infectious eggs ranged from 3.3 ± 1.5% to 77.0 ± 3.6%).

At a temperature of 25°C, the maturation period of eggs to the invasive stage lasted 54 days, and their survival rate was 80.3 ± 2.1% (Table 2).

At this temperature, the zygote stage lasted until the 24th day of cultivation, and the number of eggs also gradually decreased from 100.0% to 3.0 ± 2.0%. The stage of blastomere formation lasted from 3 to 30 days. The number of such eggs ranged from 2.0 ± 1.0% to 38.6 ± 2.1%. *Trichuris* eggs at the stages of bean-like and tadpole-like embryo formation were detected in the culture at 9–39 days and 21–45 days. The maximum number of eggs at these stages of development was observed on the 27th day (52.0 ± 1.7%) and the 33rd day (39.6 ± 4.6%), respectively, and the minimum at the 9th day (5.3 ± 3.2%) and the 45th day (3.3 ± 1.2%). Eggs of *T. skrjabini* in the stages of larva formation and motile larva were detected in the culture during 27–51 days and 36–54 days. The maximum number of eggs at these stages of development was noted on the 39th day (44.0 ± 2.0%) and the 54th day (80.3 ± 2.1%), respectively, and the minimum at the 27th day (4.3 ± 1.5%) and the 36th day (4.0 ± 3.4%).

At a temperature of 30°C, the egg matured to the invasive stage in 45 days, and its survival rate was 75.3 ± 2.5% (Table 3).

In that case, the zygote stage was found up to the 18th day of cultivation. Moreover, the number of eggs at that stage gradually decreased from 100.0% to 5.3 ± 2.5%. The next stage of blastomere formation lasted from the 3rd to the 24th days, and their numbers increased until the 9th day (from 30.3 ± 2.5% to 41.0 ± 1.0%) and then decreased until the 24th day (to 6.0 ± 3.6%). The stages of bean-like and tadpole-like embryo formation in *T. skrjabini* eggs took place over 6th–33rd days and 15th–36th days, respectively. The number of eggs at these stages increased up to the 21st day (from 4.6 ± 2.3% to 46.6 ± 3.1%) and up to the 30th day (from 4.6 ± 3.1% to 34.3 ± 2.1%) and then decreased until the 33rd and 36th days (to 8.3 ± 5.0% and 8.3 ± 3.1%), respectively. The stages of formation of larvae and mobile larvae in eggs occurred at

Table 2.
Survival Rates of Trichuris skrjabini Nematode Eggs In Vitro at a Temperature of 25°C

	Stage of Development, %					
Day	**Zygote**	**Cleavage**	**Bean-Like Embryo**	**Tadpole-Like Embryo**	**Larva**	**Motile Larva**
1	100.0	–	–	–	–	–
3	88.3 ± 1.5	11.6 ± 1.5	–	–	–	–
6	78.6 ± 1.5	21.3 ± 1.5	–	–	–	–
9	66.3 ± 3.7	28.3 ± 1.1	5.3 ± 3.2	–	–	–
12	48.6 ± 1.5	31.3 ± 1.5	5.6 ± 1.2	–	–	–
15	30.0 ± 1.0	38.6 ± 2.1	17.0 ± 1.0	–	–	–
18	21.0 ± 1.7	33.3 ± 1.5	31.0 ± 1.0	–	–	–
21	11.0 ± 2.0	24.0 ± 2.0	45.6 ± 2.5	4.6 ± 2.1	–	–
24	3.0 ± 2.0	19.3 ± 1.2	50.0 ± 1.7	11.0 ± 3.6	–	–
27	–	7.0 ± 3.6	52.0 ± 1.7	20.0 ± 1.7	4.3 ± 1.5	–
30	–	2.0 ± 1.0	39.6 ± 2.1	34.6 ± 3.2	6.6 ± 2.1	–
33	–	–	28.6 ± 3.2	39.6 ± 4.6	14.6 ± 1.2	–
36	–	–	15.6 ± 3.5	32.6 ± 4.0	30.3 ± 1.2	4.0 ± 3.4
39	–	–	7.0 ± 4.5	20.6 ± 6.6	44.0 ± 2.0	10.0 ± 3.4
42	–	–	–	12.0 ± 6.2	41.3 ± 1.2	27.0 ± 3.4
45	–	–	–	3.3 ± 1.2	23.6 ± 4.7	53.3 ± 3.7
48	–	–	–	–	11.6 ± 2.1	68.6 ± 2.1
51	–	–	–	–	6.6 ± 2.1	73.6 ± 2.1
54	–	–	–	–	–	80.3 ± 2.1

Note: – no eggs at the stage of development.

Table 3.
Survival Rates of Trichuris skrjabini Nematode Eggs In Vitro at a Temperature of 30°C

	Stage of Development, %					
Day	**Zygote**	**Cleavage**	**Bean-Like Embryo**	**Tadpole-Like Embryo**	**Larva**	**Motile Larva**
1	100.0	–	–	–	–	–
3	69.6 ± 2.5	30.3 ± 2.5	–	–	–	–
6	56.3 ± 3.2	39.0 ± 1.0	4.6 ± 2.3	–	–	–
9	30.3 ± 2.5	41.0 ± 1.0	9.3 ± 4.5	–	–	–
12	22.3 ± 1.5	39.0 ± 1.0	18.0 ± 3.6	–	–	–
15	12.3 ± 2.3	32.6 ± 2.5	28.6 ± 2.5	4.6 ± 3.1	–	–
18	5.3 ± 2.5	28.3 ± 1.5	35.6 ± 3.7	8.6 ± 2.5	–	–
21	–	19.6 ± 1.5	46.6 ± 3.1	11.6 ± 3.1	–	–
24	–	6.0 ± 3.6	41.6 ± 2.1	22.6 ± 1.1	6.6 ± 3.1	–
27	–	–	34.0 ± 3.0	28.6 ± 3.2	9.6 ± 3.1	4.6 ± 2.5
30	–	–	20.0 ± 2.0	34.3 ± 2.1	15.0 ± 3.6	7.6 ± 2.5
33	–	–	8.3 ± 5.0	27.3 ± 1.5	27.6 ± 2.1	12.0 ± 1.0
36	–	–	–	8.3 ± 3.1	32.6 ± 4.7	34.3 ± 10.0
39	–	–	–	–	18.3 ± 3.5	57.0 ± 6.0
42	–	–	–	–	6.3 ± 3.7	69.0 ± 4.5
45	–	–	–	–	–	75.3 ± 2.5

Note: – no eggs at the stage of development.

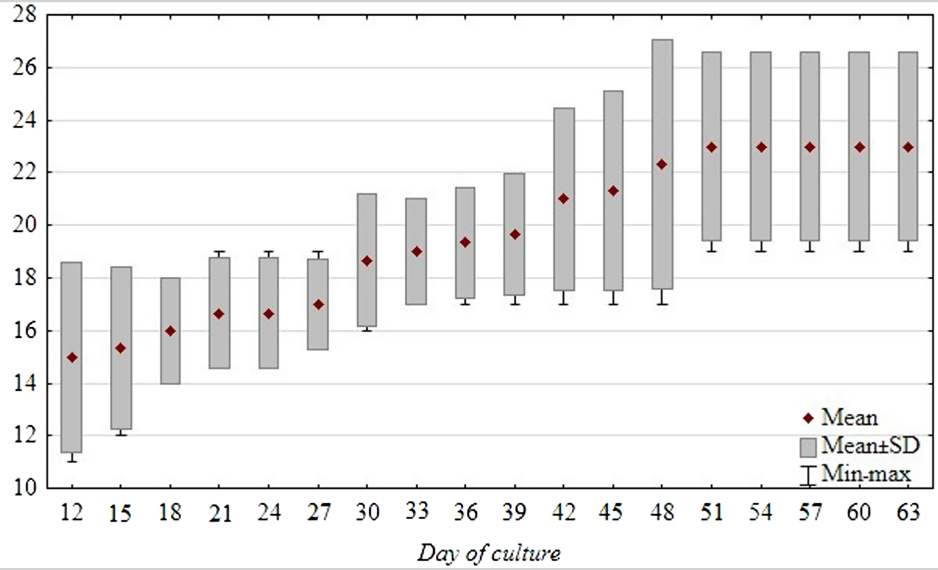

Figure 2.
Lethality of Trichuris skrjabini eggs during embryogenesis at a temperature 20°C.

the 24th–42th days and 27th–45th days, respectively. The number of eggs at the stage of larval formation increased until the 36th day (from 6.6 ± 3.1% to 32.6 ± 4.7%) and then decreased until the 42nd day (to 6.3 ± 3.7%). The number of eggs at the stage of a mobile larva formation during cultivation increased from 4.6 ± 2.5% to 75.3 ± 2.5%.

The number of dead eggs during cultivation varied according to the temperature regime. At a temperature of 20°C, 15.0 ± 3.6% (on the 12th day) to 23.0 ± 3.6% (on 51st–63rd days) of eggs died during cultivation (Figure 2). As the temperature increased, the number of dead eggs decreased. At a temperature of 25°C, 14.3 ± 1.2% (on the 12th day) to 19.6 ± 2.1% (on 42nd–54th days) of eggs died during cultivation (Figure 3). In contrast, at a temperature of 30°C, the number of non-viable eggs increased to 24.6 ± 2.5% (within the 33rd–45th days). Thus, 19.3 ± 2.5% of eggs died on the 9th day, 20.6 ± 3.2% on the 12th day, 21.6 ± 4.0% on the 15th day, and 22.0 ± 4.3% on the 18th–21st day (Figure 4).

Discussion and Conclusion and Recommendations

The relevance of studying the influence of abiotic factors on the specifics of survival of geohelminths in the process of their embryonic development is evidenced by the works of many scientists, as this will allow effective planning of measures for the control and prevention of animal and human parasitosis (Blaxter & Koutsovoulos, 2015; Charlier et al., 2018; Oyewole & Simon-Oke, 2022; Vejzagić

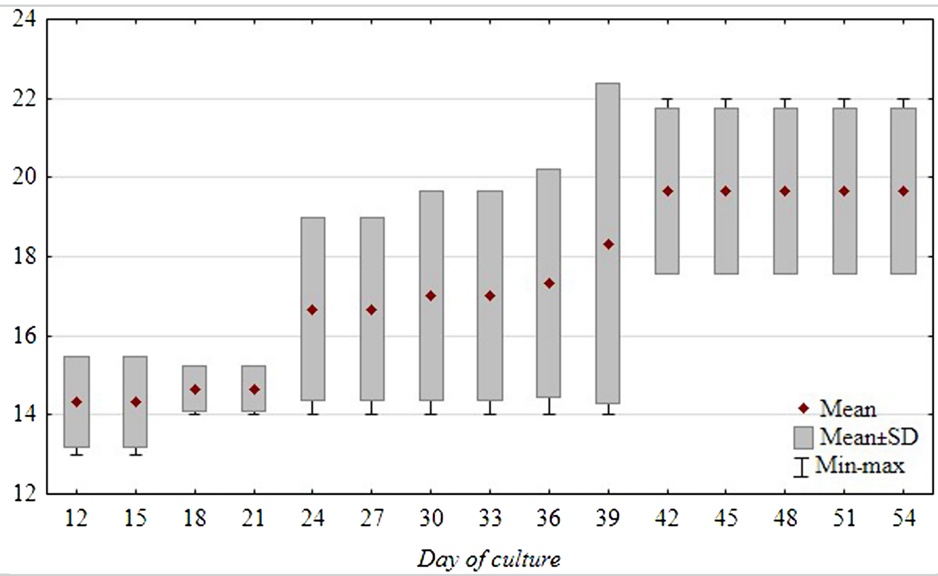

Figure 3.
Lethality of Trichuris skrjabini eggs during embryogenesis at a temperature 25°C.

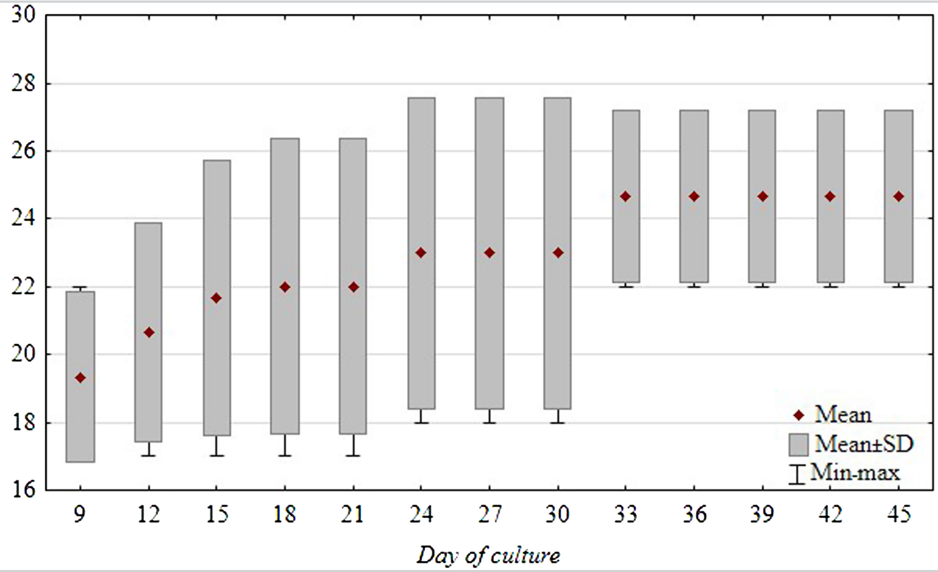

Figure 4.
Lethality of Trichuris skrjabini eggs during embryogenesis at a temperature 30°C.

et al., 2016). Therefore, we determined the tolerance of *T. skrjabini* nematode eggs, isolated from sheep, to the temperature factor. The chosen direction of research is due to the fact that the trichurosis of sheep is a widespread nematode infection at farms in many countries of the world. One of the frequently recorded species is *T. skrjabini* (de Souza et al., 2013; Melnychuk et al., 2020; Zhou et al., 2021).

It has been proved that the causative agents of trichurosis are sufficiently resistant in the embryonic stages of their development to the influence of abiotic factors of the external environment (Mkandawire et al., 2022; Saldanha et al., 2022). In this study, the survival rates of *T. skrjabini* eggs during their embryogenesis ranged from 75.3% to 80.3%. Our earlier studies also established a high survival rate of *Trichuris* eggs in the process of their maturation. In particular, the formation of invasive *T. suis* (Schrank, 1788; Nematoda, Trichuridae) eggs in laboratory conditions at a temperature of 27°C occurred in 40 days, and their viability ranged from 89.3% to 96.6% (Yevstafieva et al., 2016). At the same temperature, the development of *T. vulpis* eggs to the invasive stage occurred in 18 days, and their survival rate was 76.6% (Yevstafieva et al., 2019).

However, it has also been noted that the viability of *Trichuris* eggs depends on the temperature of environment. Moreover, the development period of these pathogens in the external environment gradually decreases with increasing temperature, and the limit of their survival is 37–38°C (Forman et al., 2021; Manz et al., 2017).

Our results also indicate a significant effect of temperature on the tolerance and survival of *T. skrjabini*, a parasite of sheep, in the embryonic stages of development. In particular, with an increase in temperature from 20°C to 25°C, the period of formation of motile larvae in eggs decreased from 63 to 54 days. Their survival rates and the formation of invasive eggs increased and ranged from 77.0 ± 3.6% to 80.3 ± 2.1%, while the number of dead eggs, on the contrary, decreased from 23.0 ± 3.6% to 19.6 ± 2.1%.

At a temperature of 30°C, the period of formation of invasive *T. skrjabini* eggs was the shortest, i.e., 45 days. However, this temperature turned out to be more unfavorable for the development of *T. skrjabini*. The survival rate of eggs was the lowest, 75.3 ± 2.5%, and the number of dead eggs was the highest, 24.6 ± 2.5%.

It was also found that regardless of the temperature regime, the embryogenesis of nematode eggs of this species occurred in six stages. At the same time, the duration of each stage depended on the temperature index.

The obtained data support the findings of other authors, which show that the viability of parasitic organisms is determined by the influence of biotic and abiotic factors (Botero-Cañola et al., 2019; Bommarito et al., 2022). Moreover, the temperature of the environment is the most significant abiotic factor of the external environment. This is due to the fact that the life processes of living organisms occur through various chemical reactions that proceed according to the law of thermodynamics. The temperature affects both the speed of these reactions and a certain structural rearrangement of chemical and organic compounds, as well as the structure of water. It has been proven that for all living organisms, including parasitic ones, there are temperature limits that ensure their viability. A decrease in temperature below a certain limit negatively affects the processes of assimilation and dissimilation if water crystallization occurs. An increase in temperature to the upper limit leads to coagulation of proteins. Also, scientists have determined that parasite eggs are more resistant to low temperatures than to high ones. This feature developed in the process of evolution and adaptation of parasites, as low temperatures slow down the metabolic processes of their embryonic stages of development (Darimani et al., 2016; Kines et al., 2021; Senecal et al., 2020).

Therefore, it can be concluded that the exogenous development of *T. skrjabini* nematodes of sheep lasts from 45 to 63 days in laboratory conditions, depending on the different temperature regimes. The duration of each developmental stage, the rate of egg survival, and the number of invasive egg formation depend on the temperature. The temperature of 25°C was optimal for the survival rate of *T. skrjabini* eggs (80.3%). Under temperature regimes of 20°C and 30°C, the survival rates of eggs in the process of embryogenesis did not exceed 77.0% and 75.3%. The development periods of *T. skrjabini* eggs gradually decreased with increasing temperature. At a temperature of 20°C, *T. skrjabini* embryogenesis lasts 63 days, at 25°C it occurs in 54 days, and at 30°C it happens in 45 days. The obtained data make it possible to more effectively plan measures to combat sheep trichurosis, taking into account the period of development of the pathogen depending on the temperature of the environment.

Ethics Committee Approval: The research protocol of the current study was approved by the Ethic Committee of the Poltava State Agrarian University (Approval number: 2022/03).

Informed Consent: Verbal/written informed consent was not obtained from the animals because the nematodes as a source of eggs were collected by complete helminthological dissection of the large intestines of sheep slaughtered at specialized slaughterhouses.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.O.P., V.V.M.; Design – T.I.B.-K., Y.V.V.; Supervision – M.O.P., B.S.S.; Resources – V.O.Y., V.V.M.; Materials – V.O.Y., V.V.M., M.O.P., M.A.P., S.V.F.; Data Collection and/or Processing – Y.V.V., T.I.B.-K., O.V.T.; Analysis and/or Interpretation – V.O.Y., V.V.M., M.O.P.; Literature Search – S.V.S., B.S.S., Y.V.V.; Writing Manuscript – V.O.Y., M.O.P., T.I.B.-K.; Critical Review – S.V.S., T.I.B.-K.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The research was carried out within the framework of the initiative topic of scientific work "Monitoring, implementation of improved methods of diagnosis, treatment and prevention of invasive animal diseases" (state registration no. 0121U100644).

References

Aleuy, O. A., & Kutz, S. (2020). Adaptations, life-history traits and ecological mechanisms of parasites to survive extremes and environmental unpredictability in the face of climate change. *International Journal for Parasitology. Parasites and Wildlife*, *12*, 308–317. [CrossRef]

Asmare, K., Sheferaw, D., Aragaw, K., Abera, M., Sibhat, B., Haile, A., Kiara, H., Szonyi, B., Skjerve, E., & Wieland, B. (2016). Gastrointestinal nematode infection in small ruminants in Ethiopia: A systematic review and meta-analysis. *Acta Tropica*, *160*, 68–77. [CrossRef]

Bhattacharjee, K. B., Deka, D. K., & Deka, D. K. (2021). Prevalence of gastrointestinal parasites in sheep of Assam, India. *International Journal of Current Microbiology and Applied Sciences*, *10*(2), 1805–1812. [CrossRef]

Blaxter, M., & Koutsovoulos, G. (2015). The evolution of parasitism in Nematoda. *Parasitology*, *142*(1), S26–S39. [CrossRef]

Bommarito, C., Wahl, M., Thieltges, D. W., Pansch, C., Zucchetta, M., & Pranovi, F. (2022). Biotic and abiotic drivers affect parasite richness, prevalence and abundance in Mytilus galloprovincialis along the northern Adriatic Sea. *Parasitology*, *149*(1), 15–23. [CrossRef]

Botero-Cañola, S., Dursahinhan, A. T., Rácz, S. E., Lowe, P. V., Ubelaker, J. E., & Gardner, S. L. (2019). The ecological niche of Echinococcus multilocularis in North America: Understanding biotic and abiotic determinants of parasite distribution with new records in New Mexico and Maryland, United States. *Therya*, *10*(2), 91–102. [CrossRef]

Cable, J., Barber, I., Boag, B., Ellison, A. R., Morgan, E. R., Murray, K., Pascoe, E. L., Sait, S. M., Wilson, A. J., & Booth, M. (2017). Global change, parasite transmission and disease control: Lessons from ecology. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *372*(1719), 20160088. [CrossRef]

Charlier, J., Thamsborg, S. M., Bartley, D. J., Skuce, P. J., Kenyon, F., Geurden, T., Hoste, H., Williams, A. R., Sotiraki, S., Höglund, J., Chartier, C., Geldhof, P., van Dijk, J., Rinaldi, L., Morgan, E. R., von Samson-Himmelstjerna, G., Vercruysse, J., & Claerebout, E. (2018). Mind the gaps in research on the control of gastrointestinal nematodes of farmed ruminants and pigs. *Transboundary and Emerging Diseases*, *65*(1), 217–234. [CrossRef]

Darimani, H. S., Ito, R., Maiga, Y., Sou, M., Funamizu, N., & Maiga, A. H. (2016). Effect of post-treatment conditions on the inactivation of helminth eggs (*Ascaris suum*) after the composting process. *Environmental Technology*, *37*(8), 920–928. [CrossRef]

de Souza, M., Pimentel-Neto, M., de Pinho, A. L., da Silva, R. M., Farias, A. C., & Guimarães, M. P. (2013). Seasonal distribution of gastrointestinal nematode infections in sheep in a semiarid region, northeastern Brazil. *Brazilian Journal of Veterinary Parasitology*, *22*(3), 351–359. [CrossRef]

Forman, R., Partridge, F. A., Sattelle, D. B., & Else, K. J. (2021). Un-'egg'-plored: Characterisation of embryonation in the whipworm model organism *Trichuris muris*. *Frontiers in Tropical Diseases*, *2*, 790311. [CrossRef]

Jadidoleslami, A., Siyadatpanah, A., Borji, H., Zarean, M., Jarahi, L., Moghaddas, E., & Budke, C. M. (2022). Prevalence and seasonality of adult and arrested larvae of gastrointestinal nematodes of sheep from Mashhad City, Northeastern Iran. *Iranian Journal of Parasitology*, *17*(2), 214–222. [CrossRef]

Kines, K. J., Fox, M., Ndubuisi, M., Verocai, G. G., Cama, V., & Bradbury, R. S. (2021). Inactivating effects of common laboratory disinfectants, fixatives, and temperatures on the eggs of soil transmitted helminths. *Microbiology Spectrum*, *9*(3), e0182821. [CrossRef]

Lindgren, K., Gunnarsson, S., Höglund, J., Lindahl, C., & Roepstorff, A. (2020). Nematode parasite eggs in pasture soils and pigs on organic farms in Sweden. *Organic Agriculture*, *10*(3), 289–300. [CrossRef]

Manz, K. M., Clowes, P., Kroidl, I., Kowuor, D. O., Geldmacher, C., Ntinginya, N. E., Maboko, L., Hoelscher, M., & Saathoff, E. (2017). *Trichuris trichiura* infection and its relation to environmental factors in Mbeya region, Tanzania: A cross-sectional, population-based study. *PLOS ONE*, *12*(4), e0175137. [CrossRef]

Melnychuk, V. V., & Berezovsky, A. V. (2018). Comparative embryonic development of nematodes of the genus *Trichuris* (Nematoda, Trichuridae) obtained from sheep (*Ovis aries*). *Biosystems Diversity*, *26*(4), 257–262. [CrossRef]

Melnychuk, V., Yevstafieva, V., Bakhur, T., Antipov, A., & Feshchenko, D. (2020). The prevalence of gastrointestinal nematodes in sheep (*Ovis aries*) in the central and south-eastern regions of Ukraine. *Turkish Journal of Veterinary and Animal Sciences*, *44*(5), 985–993. [CrossRef]

Mkandawire, T. T., Grencis, R. K., Berriman, M., & Duque-Correa, M. A. (2022). Hatching of parasitic nematode eggs: A crucial step determining infection. *Trends in Parasitology*, *38*(2), 174–187. [CrossRef]

Molnár, P. K., Dobson, A. P., & Kutz, S. J. (2013). Gimme shelter--The relative sensitivity of parasitic nematodes with direct and indirect life cycles to climate change. *Global Change Biology*, *19*(11), 3291–3305. [CrossRef]

Moskvina, T. V., Bartkova, A. D., & Ermolenko, A. V. (2016). Geohelminths eggs contamination of sandpits in Vladivostok, Russia. *Asian Pacific Journal of Tropical Medicine*, *9*(12), 1215–1217. [CrossRef]

Natalini, M. B., Cuervo, P. F., Gennuso, M. S., Romero, V. L., Jouliá, R. B., Beldomenico, P. M., & Kowalewski, M. M. (2021). Influence of extraordinary floods on wildlife parasites: The case of gastrointestinal helminths and protozoa of wild canids from the Iberá ecoregion, Argentina. *Parasitology Research*, *120*(11), 3827–3835. [CrossRef]

Oyewole, O. E., & Simon-Oke, I. A. (2022). Ecological risk factors of soil-transmitted helminths infections in Ifedore district, Southwest Nigeria. *Bulletin of the National Research Centre*, *46*(1), 13. [CrossRef]

Parija, S. C. (2022). Climate adaptation impacting parasitic infection. *Tropical Parasitology*, *12*(1), 3–7. [CrossRef]

Saldanha, B., Pucu, E., Chame, M., & Leles, D. (2022). Back to basics: Could simple experiments resolve important parasitology enigmas? The rarity of Ascaris eggs compared with Trichuris eggs in archeology and other contexts. *Acta Tropica*, *228*, 106229. [CrossRef]

Senecal, J., Nordin, A., & Vinnerås, B. (2020). Fate of Ascaris at various pH, temperature and moisture levels. *Journal of Water and Health*, *18*(3), 375–382. [CrossRef]

Skrjabin, K. I., Shikhobalova, N. P., & Orlov, I. V. (1957). *Trichocephalids and capillariids of animals and man and the diseases caused by them. The essentials of nematodology*. Russian Academy of Sciences.

Taylor, M. A., Coop, R. L., & Wall, R. L. (2016). *Veterinary parasitology. Chichester*. Willey-Blackwell.

Vejzagić, N., Kringel, H., Bruun, J. M., Roepstorff, A., Thamsborg, S. M., Grossi, A. B., & Kapel, C. M. (2016). Temperature dependent embryonic development of *Trichuris suis* eggs in a medicinal raw material. *Veterinary Parasitology*, *215*, 48–57. [CrossRef]

Yevstafieva, V. A., Kravchenko, S. O., Gutyj, B. V., Melnychuk, V. V., Kovalenko, P. N., & Volovyk, L. B. (2019). Morphobiological analysis of *Trichuris vulpis* (Nematoda, Trichuridae), obtained from domestic dogs. *Regulatory Mechanisms in Biosystems*, *10*(2), 165–171. [CrossRef]

Yevstafieva, V. A., Yuskiv, I. D., & Melnychuk, V. V. (2016). An investigation of embryo and eggshell development in *Trichuris suis* (Nematoda, Trichuridae) under laboratory conditions. *Vestnik Zoologii*, *50*(2), 173–178. [CrossRef]

Yevstafieva, V. A., Yuskiv, I. D., Melnychuk, V. V., Yasnolob, I. O., Kovalenko, V. A., & Horb, K. O. (2018). Nematodes of the genus *Trichuris* (Nematoda, Trichuridae) parasitizing sheep in central and South-Eastern regions of Ukraine. *Vestnik Zoologii*, *52*(3), 553–556. [CrossRef]

Zhou, M., Shen, D., Wang, J., Lu, Y., Su, Y., Peng, Z., Teng, L., Liu, Z., & Hou, Z. (2021). First isolation of Trichuris from wild blue sheep (Pseudois nayaur) in the Helan Mountains, China. *Parasitology Research*, *120*(7), 2665–2670. [CrossRef]