In Vitro Development of *Toxocara canis* Nematode Eggs

Sardorbek No’Monjon o’g’li Turgunov* and Erkinjon Berdikulovich Shakarboev

**ABSTRACT**

This study aims to describe the morphological changes of the embryo inside the developing egg during incubation in vitro at 30 °C, as well as to study the differences in the viability of the eggs during the incubation period. *T. canis* eggs were placed in a thermostat for incubation at 30 °C in an isotonic solution of 0.9% sodium chloride for eleven days. Development, morphological changes and viability of 100 eggs were observed. No significant differences were found in recorded viability from day first to day eleventh of incubation.

**Keywords:** Larva, stage, *Toxocara canis*, viability.

1. **INTRODUCTION**

*Toxocara canis* and *Toxocara cati* are gastrointestinal helminths commonly found in dogs and cats. It is known from the literature that female nematodes of the genus *Toxocara* lay up to 200,000 eggs per day, polluting the environment and posing a potential threat to humans [1]. *T. canis* is distributed worldwide and is the main zoonotic helminth causing human toxocariasis. Dogs excrete faeces corresponding to 3% of their body weight per day, which is approximately 270 gr per animal [2].

Human infection usually occurs through accidental consumption of eggs and ingestion of reservoir hosts including chickens, cattle, lambs, pigs, and worms [3], [4]. Insect faeces also play a role in the spread of invasive eggs, and once the insects ingest the eggs, the eggs do not lose their invasive properties [5].

The purpose of this research work is to study the structural features of the stages of development of the nematode *T. canis* in eggs in vitro, as well as the differentiation of viable and non-viable eggs. In addition, to compare the viability of eggs in the early stages of development and after reaching the invasive stage. The presence of embryonated eggs developing in these favorable conditions helps to assess the risk to public health.

2. **MATERIALS AND METHODS**

Adult nematodes of *T. canis* were collected from the intestines of a naturally infected dog from the Andijan region, Andijan district by the method of complete helminthological dissection of Skryabin [6] (Fig. 1).

Fig. 1. Nematodes of *Toxocara canis*.

Fig. 2. Eggs isolated from the female nematode of *Toxocara canis*. 
Helminth eggs were isolated from female *T. canis* nematodes and placed in a 0.9% isotonic solution of sodium chloride (Fig. 2).

The level of the solution was measured in a Petri dish and 0.9% isotonic sodium chloride solution was added when evaporation was observed to maintain the desired concentration. Cultivation of eggs was carried out under constant control in a thermostat at 30 °C for 11 days. At this time, the eggs were found to have reached their highest invasive stage.

A total of 100 eggs were placed in a Petri dish and examined using a light microscope. During the examination, attention was paid to the mobility of the larvae inside the eggs under the light of a microscope. The number of each developmental stage within the egg and the number of eggs with embryos that lost viability out of 100 eggs examined were recorded. Daily inspection was repeated several times.

Egg viability was assessed at the early embryonic stage of development, day 1 of incubation, and compared with viability determined on day 11. In the early stages of development, viable eggs contain dividing embryos with well-defined structures. Non-viable eggs are eggs that do not divide or stop developing at any stage of development. Eggs with abnormal morphology, unclear internal structure, damaged shell and contents are also not considered viable. The viability of eggs with larvae was determined by observing the structure and motility of the larvae inside the eggs under the light of a microscope [7].

### 3. Results and Discussion

During 11 days of incubation and microscopic examination, twelve developmental stages were identified: one-cell, two-cell, three-cell, four-cell, early morula, late morula, blastula, gastrula, pre-larva 1, pre-larva 2, first-stage larva (L1) and second stage larva (L2) (Fig. 3). During the first 1 day of incubation, no cell division was observed inside the egg. At the beginning of the second day, the first cleavage occurred in 90% of them. After six hours, 4% of the developing embryos were at the 2-cell stage, 16% at the 3-cell stage, 69% at the 4-cell stage, and only 2% at the 1-cell stage. Development of embryos from the 2-cell stage to the 4-cell stage occurred rapidly, and the 3-cell stage was observed very rarely (Fig. 3).

At the end of the second day, 46% of the embryos were at the early morula stage, 39% at the 4-cell stage, and the rest at the 2-cell stage. Early morula stage embryos reached their maximum development on the third day (87%), while the late morula stage started at the end of the third day and reached a maximum on the fourth day (87%). At the beginning of the fifth day, most of the developing embryos were in the late morula stage (75%), while others were in the blastula stage (12%). At the beginning of the third quarter of the day, the blastula stage reached its maximum level (77%), and at the end of the day, gastrula-stage embryos appeared (15%). At the beginning of the sixth day, the gastrula stage peaked (72%) and several blastula stages (13%) were observed. At the beginning of the second quarter of the day, movements were observed...
In Vitro Development of *Toxocara canis* Nematode Eggs

Turgunov and Shakarboev

Fig. 5. Timeline of in vitro development of the nematode *T. canis* at 30 °C.

![Timeline of in vitro development of *T. canis* at 30 °C.](image)

Fig. 6. *T. canis* embryonic stages inside the egg: a) one-cell stage, b) two-cell stage, c) three-cell stage, d) four-cell stage, e) early morula, f) late morula, g) blastula, h) gastrula, i) pre-larva 1, j) pre-larva 2, k) first stage larva, l) second stage larva.

![Embryonic stages of *T. canis*](image)

in the developing embryos using a light microscope, and the pre-larva 1 stage (18%) was recorded. The pre-larva 2 stage was observed at the end of the day (13%). Both stages were observed for at least 2 consecutive days. On the seventh day, pre-larvae 2-stage eggs (73%) reached a maximum and first-stage larvae (9%) were observed for the first time. All developing embryos were in the first larval stage on day eight (83%). Eggs with second-stage larvae were not observed on day nine, but most embryos were in second-stage larvae (62%) on day ten (Fig. 4).

Based on these obtained results, a timeline was prepared to describe the developmental stages observed and the time when the changes occurred (Fig. 5). Photographic documentation of the observed stages is included in Fig. 6. Each stage was typically observed for approximately 2 hours to 3 days, and each stage took varying amounts of time to peak development.

Viable eggs are eggs that contain dividing embryos with a well-defined structure. Eggs that lost their viability were also observed during the research. Some eggs did not divide and remained in the one-cell stage. Some other eggs had damaged outer shell (Fig. 7a) or stunted embryo development (Fig. 7b). After larval development, non-viable eggs were found to contain malformed larvae (Fig. 7c). First-stage larvae in eggs showed movement immediately after exposure to light when examined under a light microscope. The second-stage larvae responded to light exposure a little later.

The viability of *T. canis* eggs was 83% on the 11th day of incubation. As shown in Fig. 8, there was no significant difference between the proportion of viable eggs from day 1 to day 11 of incubation.

According to the results of the study, no significant differences in the viability of developing embryos from the 1st to the 11th day of incubation of *T. canis* nematode eggs
were revealed. In addition, no significant difference was noted with the development stages of the parasite outside the host reported in the literature. Twelve developmental stages were determined in an incubator at 30 °C in vitro for 11 days. These stages are one-cell, two-cell, three-cell, four-cell, early morula, late morula, blastula, gastrula, pre-larva 1, pre-larva 2, first-stage larva and second-stage larva. Natural hatching of larvae was not observed in our study. However, in other research studies, the nematodes Toxocara canis [8] and Ascaris suum [7] observed occasional larvae emerging from the thin part of the shell.

In our research, a U-shaped embryo grew inside the egg, formed a ring, and began to form a new ring. These stages, called pre-larva 1 and pre-larva 2, were first noted in the embryogenesis of T. canis in the studies of Abou-El-Naga [8]. These stages preceded the first larval stage and were observed consecutively for at least 1 day before progressing to the next stage of development, and a distinctive structure was identified that distinguished them from each other and from other stages. The structure of the embryo at these stages was previously studied by Cruz et al., [7] in the nematode A. suum. Both stages were recorded on the same day (day 6). In our study, the first-stage larva was observed on the 7th day of incubation.

The differences between T. canis developmental stages in different studies are mainly due to the temperature factor of incubation. T. canis eggs were found to fail to develop fully at temperatures above 37 °C and below 11.8 °C. The highest percentage of eggs with viable larvae was observed after incubation at 28 °C [9]. In this study, the two-cell stage or more was reached after 24 hours, Johnson et al. [10] found that the nematode Ascaris suum reached the two or more-cell stage after 48 hours. Abou-El-Naga [8] incubated T. canis nematodes at 28 °C and cell division occurred after 24 hours, similar to our results.

Pre-larva-1 and pre-larva-2 stage larvae inside eggs were found to move very slowly when viewed under a light microscope. And the first-stage larvae responded to light exposure with immediate movement.

It is important to study the viability of T. canis nematode eggs, to determine the level of environmental pollution and to study the effects of various disinfectants. In our study, no significant difference was found when comparing the viability of eggs from the first day to the eleventh day. In another study on T. canis, Abou-El-Naga [8] also found no significant difference in egg viability.

During our study, we encountered problems in identifying pre-larva-1 and pre-larva-2 stages. One-cell, two-cell, three-cell, four-cell, early morula, and late morula stages became much easier to identify. These stages did not require a lot of time and magnification. Based on our experiments, it was concluded that the most convenient time to determine the viability of developing embryos is the first days of incubation.

4. Conclusion

In this study, it was reported that viable and non-viable eggs of the nematode T. canis can be clearly distinguished, as well as the exact initial rate of development can be determined. During the study, 12 stages of the developing embryo of T. canis nematode in vitro were described. The results of the study indicate that the presence of early-developing embryos of the nematode T. canis in soil, wastewater and sewage is a potential risk to public health.

Acknowledgment

The work was carried out within the framework of the program, “Ways of the Development of Helminth Fauna in Vertebrates. Taxonomy and Improvement of Control Measures”, implemented by the Academy of Sciences of the Republic of Uzbekistan.
In Vitro Development of Toxocara canis Nematode Eggs

Turgunov and Shakarboev

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

REFERENCES


