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Laboratory incubation of *Toxocara canis* eggs and their development

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Key words: *Toxocara canis*, Incubation, Zygote, Morula, Blastomeres

Abstract

The current study concluded that the laboratory incubation with H2SO4 (0.1N) in 37C° was beneficial for embryonation of *T. canis* eggs during ten days. This incubation showed all the stages of development as follow: the interphase, the early embryo, the Zygote, the blastomeres formation, Morula, Gastrula and early larvae formation inside the egg. All these stages were described and photographed with a digital camera.

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Introduction

Toxocara canis and *T. cati*, roundworms of dogs and cats, are zoonotic parasites, which contribute to visceral and ocular damages in humans especially in children (Overgaauw, 1997 and Sadjjadi *et al.*, 2000). Ingestion of the embryonated eggs of *Toxocara* initiates infection in both definitive and aberrant host (Fisher, 2003).

In man, infection can provoke syndromes like visceral larva migrans (VLM), ocular lava migrans (OLM), covert toxocariasis (CT), and neurotoxocariasis (NT) (Chieffi *et al.* 2009, Fillaux & Magnaval 2013, Macpherson 2013, Overgaauw & van Knapen 2013). All of whose diagnoses mainly depend upon serological techniques (Moreira *et al.* 2014).

The infective stage of this parasite is second larvae inside the egg, which is developed about 7-14 day or more depending on the environmental conditions such as temperature and humidity (Glickman *et al.*, 1986). *T. canis* eggs are oval or spherical shapes, pitted surface , measure about 72 to 85µm (Gillespie, 1988). They are thick-walled, contain four layers, in which the embryonic materials were surrounded by thick membrane of four layers, that are uterine layer, vitelline layer, chitinous layer and lipid layer (Thapa *et al.*, 2013). These eggs are very resistance against various weather and chemicals conditions (Roberts & Janvory, 2000). In Iraq, little previous studies about immunology of *Toxocara canis* like Awad and Al-Azizz, (2007) and Hadi, (2017), but there was no studies about incubation of eggs and their development stages.

The aims of current study are two, first: to refer to the correct way to incubate the eggs of *Toxocara canis* and the development of embryos within them in a short period of time in vitro. Second: describe all the stages of development that appeared in vitro for the first time in Iraq.

Materials and methods

Samples collection

A total of twelve positive fecal samples infected with *Toxocara canis* eggs were collected from the rectum of domestic dogs from different veterinary clinics of Baghdad city.

Laboratory examination

Samples were placed in special cups and transported to the Iraq Natural History Research Center and Museum where the research was conducted. Flotation method with sheather's solution was applied for all samples to obtain the eggs (Tom, 2006).

Laboratory incubation

The eggs were embryonated in 0.1N H_2SO_4 in an Erlenmeyer flask at 37C^0 for two weeks (Borecka *et al.*, 2008) in order to allow embryonic development. The samples were examined daily and the images were taken for embryonic changes with a digital camera.

Results and discussion

Laboratory incubation showed all the stages of development of un embryonated eggs of *T. canis* after incubation that as follow: first and second days not appeared any change (Table 1); the eggs still thick, hard, dark, pitted casing that does not show embryonic tissue (Fig. 1); that stage may be consider as interphase stage which last many hours or many years depend on the metabolic activity this stage is called "resting phase" (Camparoto *et al* 2008). Third day, Cleavage of the cells started in the early embryo (Fig. 2). Then rapid cell cycle divisions with no significant growth to form the Zygote and Bone morphogentic protein pathway (Fig. 3) which was growth factors known as cytokines or metabologins (Reddi and Reddi, 2009).

After many cell cycles a cluster of cells were produced as the same size of the original zygote. On the fourth day the different cells derived from cleavage are called blastomeres that were 8-16 blastomer in eggs of *T. canis* (Fig. 4); Those which differentiate later to neural, muscular and epidermal type of blastomeres (Hirano *et al.*, 1984). After a series of cleavage divisions a compact mass called Morula was distinguished that appeared the apical and pedicle lobes about the fifth and sixth day (Fig. 5). Cleavage end with the formation of the blastula which is a hollow sphere of cells referred to as blastomeres which surrounding an inner fluid- filled cavity called the blastocoele.

That is widened and sealed with tight junctions to create a cavity. The single layer blastula is reorganized into a multilayered structure known as Gastrula on the seventh and eighth day (Forgács and Newman, 2005).

The embryo has begun differentiation to appear distinct cell lineages as: anterior- posterior, dorsal-ventral (Martín-Durán *et al*., 2016), then, specialty one or more cell types including the prospective gut that blastoporal opining (Fig.6). Goldstein and Hird, (1996) revealed to the nematode side development: "the decision as to which end will become the anterior and which the posterior seems to reside with the position of sperm pronucleus. When it enters the oocyte cytoplasm, the centriole associated with the sperm pronucleus initiates cytoplasmic movements that push the male pronucleus to the nearest end of the oblong oocyte.

This end becomes the posterior pole" on the ninth and tenth day an early larva appeared and distinguished the mouth at anterior end and posterior end (Fig. 7).

Table 1. Stages of development of *Toxocara canis* eggs during days of incubation period.

Days	Stages of development
First &Second	Interphase
Third	Early cleavages and Zygote formation
Forth	Blastomeres formation
Fifth & Sixth	Morula formation
Seventh& Eighth	Blastula formation
Ninth &Tenth	Larva formation

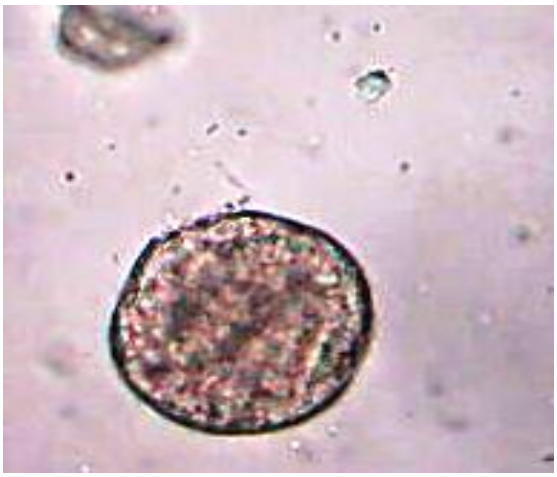

Fig. 1. Un embryonated egg of *T. canis*, thick and pitted casing, 40X.

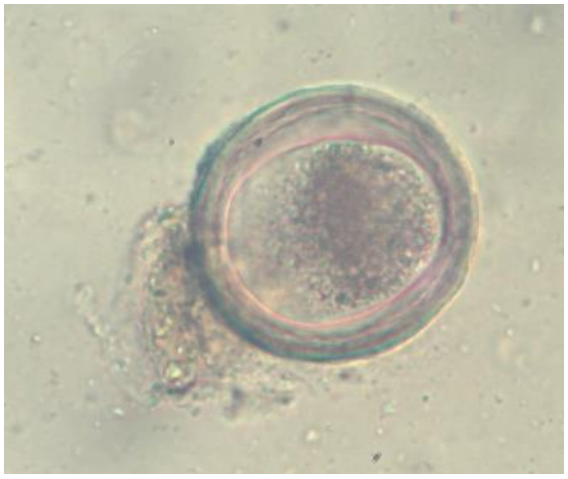

Fig. 2. Cleavage started in the early embryo of *T. canis* egg, 40X.

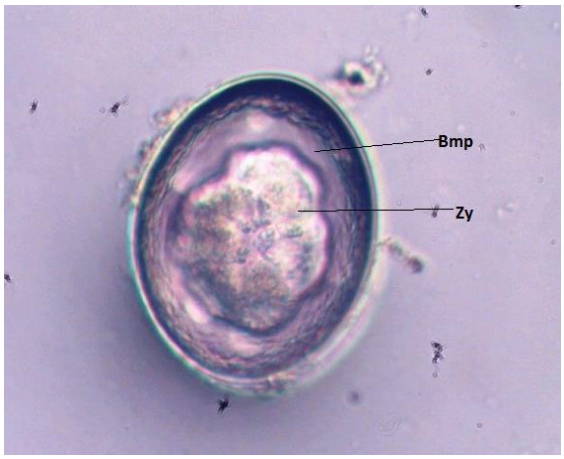

Fig. 3. The Zygote was produced by rapid cell cycle divisions with no significant growth, Zy=zygote, Bmp=Bone morphogentic protein pathway, 40X.

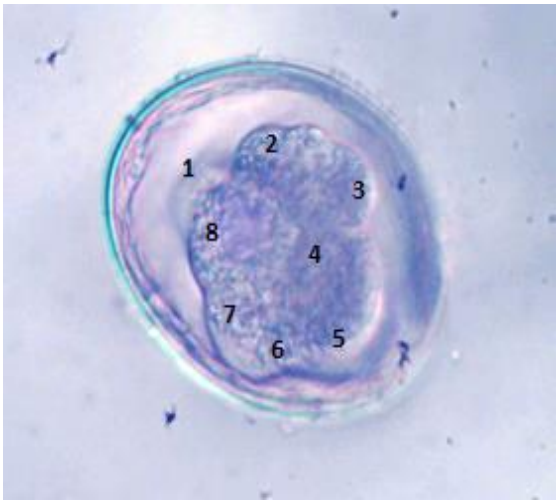

Fig. 4. Blastomers appeared about 8-16 blastomer in eggs of *T. canis*,40X.

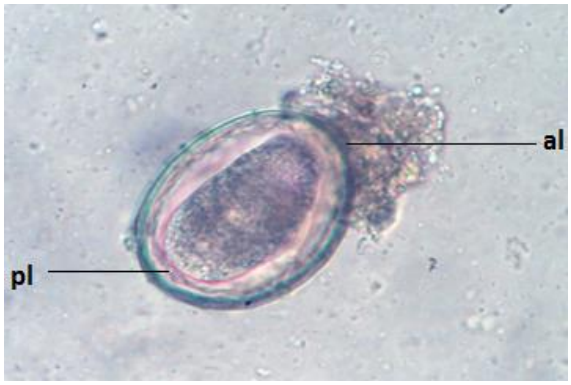

Fig. 5. A compact mass called Morula was distinguished, al=apical lobe and pl=pedicle lobe, 40X.

Fig. 6. Blastula cells differentiation to the basic axes of the body: anterior- posterior, dorsal-ventral, and bp=blastoporal opining, 40X.

Fig. 7. An early larva appeared and distinguished the mouth =mo, posterior end=pe, 40X.

The current laboratory incubation of *T. canis* eggs showed stages of development from unembryonated eggs to larva formation during ten days that means the incubation with Sulfuric acid (H2SO4) 0.1N was useful and can be recommended in future researches which was similar to Borecka *et al.* (2008). But Camparoto *et al.* (2008) described the culturing of *T. canis* eggs *In vitro* and embryonated eggs only within 7 days. However, other studies showed that the development of *T. canis* larvae required at least one month to develop (Rodriguez-Caballero *et al.*, 2007). These differences due to the difference in cultures and temperature of incubation. The current study concluded that the laboratory incubation with H2SO4 (0.1N) in 37C⁰ was beneficial for embryonation of *T. canis* eggs during ten days and appeared all the stages of development.

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