

Short Communication

A Method for Accelerating the Maturation of *Toxocara cati* Eggs

*Zibaei M¹, Sadjjadi SM¹, Jahadi Hosseini SH², Sarkari B³

¹ Dept. of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Iran

² Dept. of Ophthalmology, Shiraz University of Medical Sciences, Iran

³ Dept. of Microbiology and Immunology, School of Medicine, Yasuj University of Medical Sciences, Iran

(Received 27 Sep 2006; accepted 24 Feb 2007)

Abstract

Background: The effect of temperature and humidity on the maturation of *Toxocara cati* eggs in an in vitro system was investigated.

Methods: Suspensions of *Toxocara cati* eggs, with 5% formalin/saline or 2.5% formalin/ringer were prepared and maintained at 37 °C under 40% humidity or at 25 °C under 98% humidity for 3 weeks for egg development.

Results: The suspension sample mixed by 2.5% formalin/ringer and maintained at 25 °C and 98% humidity could fully embryonate the eggs of *Toxocara cati* in 3 weeks.

Conclusion: The main advantage of this method is the increase of recovery and also reducing of the eggs maturation time.

Keywords: *Toxocariasis*, *Toxocara cati*, Eggs maturation

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 (1, 2). Ingestion of the embryonated eggs of *Toxocara* initiates infection in both definitive and aberrant host (3). *Toxocara cati* is the common roundworm of cats. It is related to *T. canis*, the roundworm of dogs and, more distantly, to *T. leoninae* which occurs in cats and dogs (3).

Toxocara eggs are un-embryonated and non-infectious when passed in the feces of dogs and cats into the environment. The eggs of *Toxocara* are extremely resistant

to chemical agents and it is assumed that they may survive in an appropriate environment for more than a year like the related nematodes, but they are quite sensitive to desiccation and temperatures above 37 °C (1). Within a period of 3 to 6 wk to several months, depending on soil type and climatic conditions such as temperature and humidity, *Toxocara* eggs develop to an infectious stage (1, 4). In many biological or diagnostic studies embryonated eggs and large number of second stage larvae of *Toxocara* species are needed.

Different substances, including formalin and sulfuric acid with different condition of temperature and humidity have been used to find an optimal system for *in vitro*

development of *Toxocara* eggs (5-8). These systems need long time incubation of the samples and yield relatively low number of embryonated eggs.

In this study, considering the simultaneous effect of temperature and humidity, a novel method was used in order to accelerate and improve the maturation of *T. cati* eggs.

Materials and Methods

Eggs were extracted from the uteri of female *T. cati* worms. Two suspensions of *T. cati* eggs, having the same number of eggs, were prepared and maintained under different temperature and humidity conditions. The first suspension with 5% formalin/ saline was maintained at 37 °C under 40% humidity and the second with 2.5% formalin/ringer was kept at 25 °C under 9.8% humidity. Samples were monitored and oxygenated, using vacuum pump,

every day for a total of 3 wk for development of eggs. At the end of 3 wk the numbers of larvae in two samples were carefully counted.

Results

In the first group including three samples with 5% formalin/saline at 37 °C under 40% humidity, only 46% of eggs were embryonated (Fig. 1), whereas in the second group including three samples with 2.5% formalin/ringer at 25 °C under 98% humidity, 93.3% of eggs were embryonated (Fig. 2).

Our findings demonstrated that an increase in humidity and decrease in temperature produced a rise in the number of developed eggs. The results indicated that suspension sample mixed by 2.5% formalin/ ringer and maintained at 25 °C and 98% humidity could fully embryonate the eggs of *T. cati* within 3 wk.

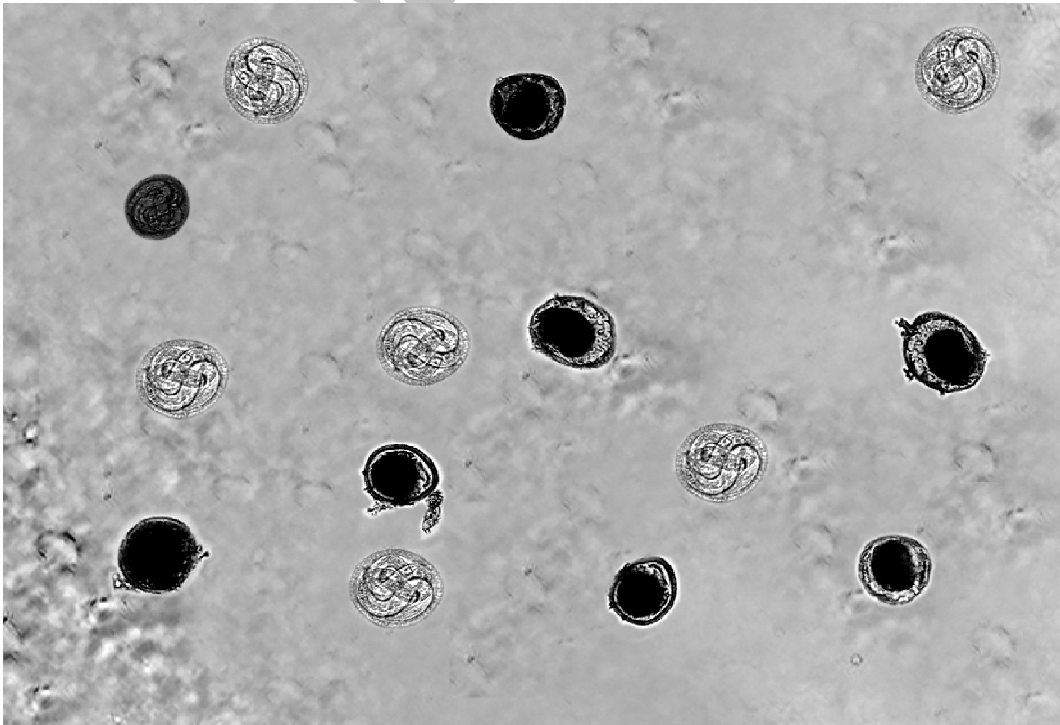


Fig. 1: Embryonated and unembryonated *Toxocara cati* eggs in 5% formalin/saline at 37°C under 40% humidity at the end of 3 weeks

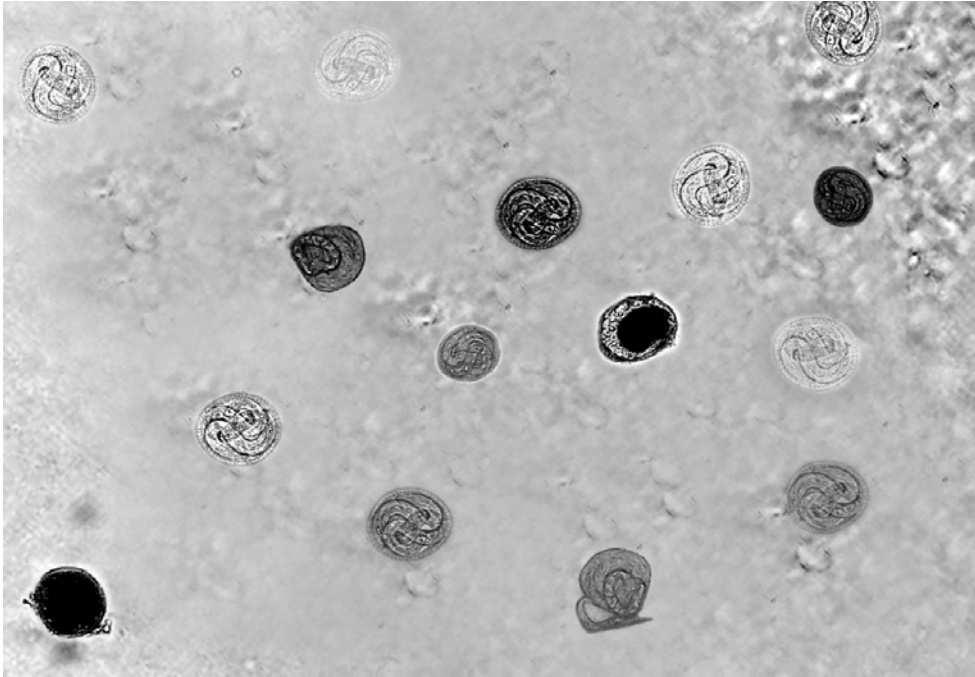


Fig. 2: Embryonated and unembryonated *Toxocara cati* eggs in 2.5% formalin/ringer at 25°C under 98% humidity at the end of 3 wk

Conclusion

The main advantage of the mentioned method is the increase of recovery and also reducing of the eggs maturation time. Using this novel system a large number of infective eggs and live larvae of *Toxocara cati* could be harvested for production of *Toxocara cati* excretory/ secretory antigens or larval antigens for different purposes.

Acknowledgments

The authors thank Mrs Kazemian and Mrs Habibi for their cooperation and also Mr Farhangmehr and Mr Hakkani for their technical assistance. This study was financially supported by grant No. 85-2893 from Office of Vice Chancellor for Research of Shiraz University of Medical Sciences, Iran.

References

1. Overgaauw PAM. General introduction, Aspects of *Toxocara* epidemiology, toxocariasis in dogs and cats. *Critical Review in Microbiology*. 1997; 23: 233-51.
2. Sadjjadi SM, Khosravi M, Oryan, A, Mehrabani, D. Sero-prevalence of toxocariasis in school children of Shiraz, Iran. *Journal of Tropical Pediatrics*. 2000; 46: 327-30.
3. Fisher M. *Toxocara cati*: an underestimated zoonotic agent. *Trends in Parasitology*. 2003; 19(4): 167-70.
4. Despommier D. Toxocariasis: clinical aspect, epidemiology, medical ecology, and molecular aspects. *Clinical Microbiology Reviews*. 2003; 16(2): 265-72.
5. De Savigny DH. In vitro maintenance of *Toxocara canis* larvae and a simple method for the production

- of *Toxocara* ES antigen for use in serodiagnostic tests for visceral larvae migrans. *Journal of Parasitology*. 1975; 61(4): 781-82.
6. Villanueva EJ. A method for the recovery of infective larvae from *Toxocara canis* eggs for collection of larval secretory/ excretory products. *Annals of the New York Academy of Sciences*. 1992; 653: 211-16.
 7. Smith HV. A rapid method for hatching infective eggs of *Toxocara canis*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1989; 83: 215.
 8. Crowcraft NS, Gillespie SH. Hatching of second-stage larvae of *Toxocara canis*: a rapid method for processing large numbers of worms. *Journal of Helminthology*. 1991; 65: 311-12.