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In vitro STUDY ON TARGETING DEVELOPMENTAL EMBRYONATION PATTERN OF EGGS OF ASCARID SPECIES OF WILD ANIMALS

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ABSTRACT

The present study was aimed to investigate the time intervals required by the eggs of two ascarid species - *Baylisascaris transfuga* retrieved from *Melursus ursinus* (Sloth bear), and *Toxascaris leonina,* retrieved from *Panthera leo persica* (Asiatic/Indian lion) to reach infective stages at controlled temperature (27±1°C) in 0.4% formalin solution as well as in faecal samples as such. The incubated material revealed that the eggs advanced to two-cell stage at day 2 and 3 post-incubation in case of *T. leonina* and *B. transfuga,* respectively, in formalin solution as well as in faecal samples. The final infective stages having 2nd stage larvae inside the eggs were observed at day 12 and 9 post incubation in 0.4% formalin solution and at day 14 and 9 post-incubation in faecal samples in case of *B. transfuga* and *T. leonina,* respectively. To check the reoccurrence of infection of these parasites having direct life cycle, better management practices involving anthelmintic therapy of the infected individuals with complete disinfection of the habitat area should be carried out.

Key words: Ascarids, *Baylisascaris transfuga*, Embryonation, *Toxascaris leonina*

INTRODUCTION

The ascarid species of wild and domestic animals are geohelminths of veterinary and medical concern due to their significant zoonotic potential (Kumar *et al.*, 2005; Chhabra and Singla, 2009). These parasites do not prove detrimental to their definitive hosts, until heavy infections are encountered. But, accidental intrusion of ascarid parasites in accidental hosts including human beings (O'Lorcain and Holland, 2000), mammals (Sato *et al.*, 2005) and birds (Kazacos, 1986) with their infective stages have generated utmost concern amongst health workers. The accidental consumption of larvated eggs through contaminated food and water are the prime causes of zoonotic infections, primarily *neural* (Sato *et al.*, 2004), *visceral* (Beaver *et al.*, 1969) and *ocular larva migrans* (Kazacos, 1997). These zoonotic parasites may not only prove detrimental to the accidental hosts (especially human beings) but also play significant role in the reduction of vulnerable wild animal species (Kristen, 2013). As the majority of emerging parasitic diseases (~72%) originate in wildlife populations (Jones *et al.*, 2008), it is important to determine the contribution of ecological variables (temperature and relative humidity) in disease transmission among wildlife. The present study was aimed to evaluate the developmental stages and compare the time interval required to acquire final infective stages by the eggs of two ascarid species of wild animals, so that they become capable to infect the definitive hosts or accidental hosts, including human beings.

MATERIALS AND METHODS

The eggs of targeted ascarid species *i.e. Baylisascaris transfuga* (Baylis and Daubney, 1922) and *Toxascaris leonina* (Leiper, 1907) were retrieved from the faecal samples of *Melursus ursinus* (Sloth bear) and *Panthera leo persica* (Asiatic/ Indian lion), respectively, kept at Mahendra Mohan Choudhury Zoological Park, Chhatbir, Punjab (India). The samples were collected from the respective cages of the host animals in early morning or at the instance of faecal voiding. The samples were immediately kept in plastic bags at 4°C and then transferred to Department of Veterinary Parasitology, COVS, GADVASU, Ludhiana (India) for further processing. The egg per gram (epg) values for the eggs of *B. transfuga* and *T. leonina* were 2800 and 3900, respectively.

The faecal material of *Melursus ursinus* and *Panthera leo persica* was divided into two subsets to evaluate the embryonation pattern and interval in faecal sample as such and in 0.4% formalin solution in distilled water at 27±1°C (O'Lorcain, 1995). For incubation in 0.4% formalin solution, approximately 500 eggs were harvested by floatation concentration technique using Sheather's sugar solution (Soulsby, 1982) and then suspended in a solution, kept in petri-dishes (63.5 mm dia.). On the other hand, the faecal samples of both the animals were kept as such separately in petri-plates (with 50-70% relative humidity) and sprinkled with 1 mL 2% formalin. The suspension material was stirred daily to maintain the oxygenation of eggs. A small subsample (by counting the number of eggs in one drop of suspension material and by floatation concentration technique from faecal samples) of the material from each petri-dish containing approximately 100 eggs were examined thrice through light microscopy (10X) daily to study developmental stages. Thus, the sequences and intervals of embryonation were studied. The criteria adopted for evaluation of developmental stages (Caceres *et al*., 1987) is presented in Table 1.

Table 1: Criteria to identify viability and stage of development of ascarid eggs by microscopy

Non-viable egg	Viable egg
i. Poorly defined structures ii. Contraction, rupture, and loss of membrane continuity iii. No larval movement observed by microscope light stimulation iv. Vacuolization of cytoplasm and cellular condensation in unicellular stage with granulated and vacuolated cytoplasm	*One to four cells*: 1, 2, 3, or 4 cells within the egg *Early-morula*: 5 to 10 cells within the developing embryo *Late-morula*: 11 or more cells within the developing embryo *Blastula*: a spherical layer of cells surrounding a fluid-filled cavity *Gastrula*: a layer of cells surrounding the embryo plus a kidney shape invagination in one side of the embryo *First-stage larva*: a larval structure within the developing embryo without moult and with motility in response to light stimulation *Second-stage larva*: a larval structure within the developing embryo with moult and motility in response to light stimulation

After 14 days of initiation of embryonation study, 100 eggs were examined microscopically (10X) to assess their viability as per Cruz *et al.* (2012). The larval movement observed by microscope light stimulation was used to evaluate the viability of larval stages, infective for definitive as well as accidental and paratenic hosts. The data was statistically analyzed using SPSS 16 software to calculate mean and standard deviation values of the developmental stages of the ova of parasites.

RESULTS AND DISCUSSION

Detailed developmental studies associated with embryonation pattern of *Baylisascaris transfuga* or *Toxascaris leonina* had not been carried out earlier. However, the descriptive developmental studies of ascarids of domestic animals mainly dogs (*Toxocara canis*), cattle (*T. vitulorum*) and pigs

(*Ascaris suum*) have been carried out by O'Lorcain (1995), Devi *et al*. (2001) and Cruz *et al.* (2012), respectively, at different temperature conditions and observed maximum embryonation at 27±1°C. Thus, the present study was planned to evaluate the developmental pattern of ascarid eggs at reported optimum temperature *i.e.* 27±1°C. The complete description of embryonic development of eggs of *B. transfuga* and *T. leonina* in faecal sample as well as in 0.4% formalin has been depicted in Fig. 1. In case of *B. transfuga,* the eggs remained in one cell stage for first two days and further development to two-cell stage was noticed at day 3 post-incubation in both the subsets (faecal sample and 4% formalin). In case of eggs of *T. leonina*, development started on the very 1st day and hence, next stage was observed at day 1 post-incubation in both the subsets (Fig. 1). At the end of 1st week, the majority of eggs (~48%) was in 1st larval stage in *T. leonina* and at two- and four

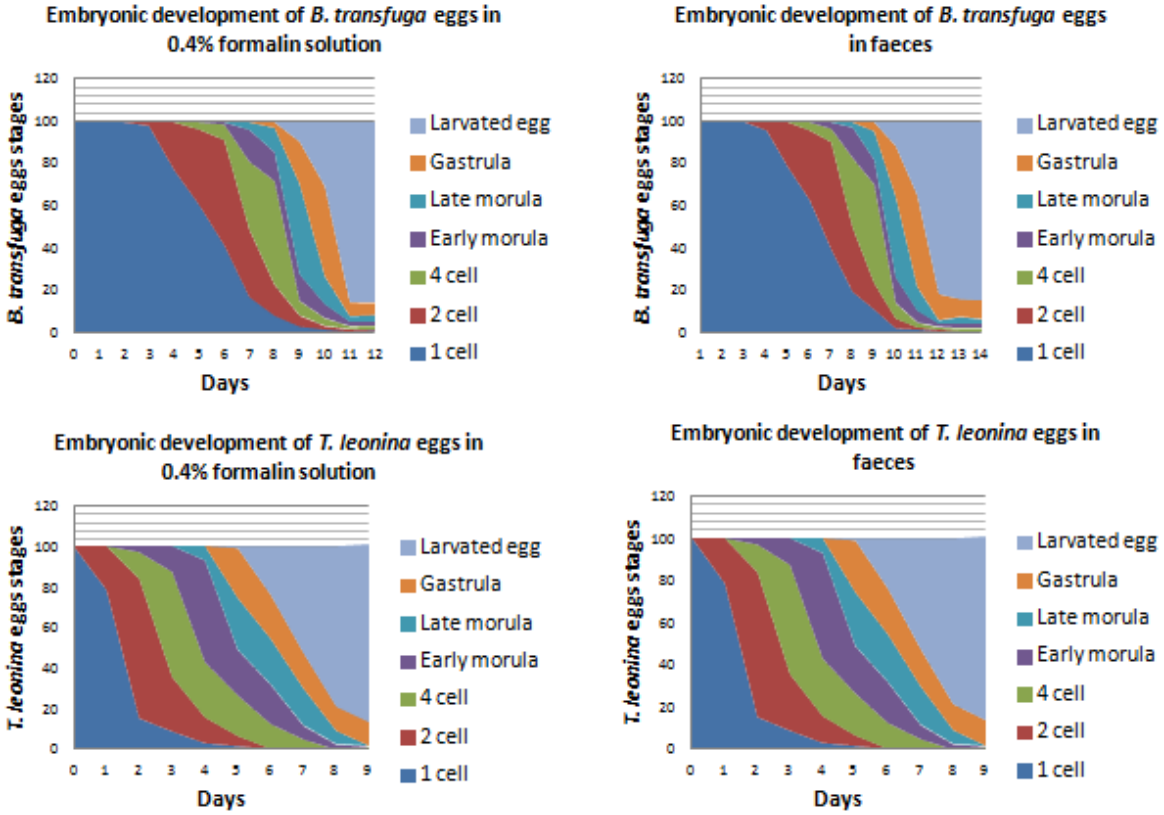

Fig. 1: Embryonic developmental stages of *Baylisascaris transfuga* and *Toxascaris leonina* in 0.4% formalin solution and faecal sample at 27±1°C temperature

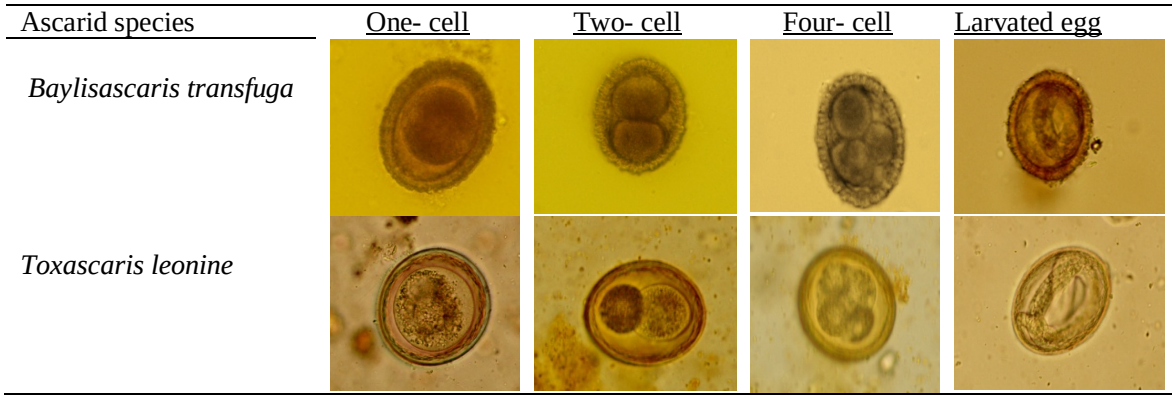

Fig. 2: Various developmental stages of ascarids

and four-cell stage in *B. transfuga* (approx. 32% each) in case of formalin solution. Similar observations were observed in case of faecal samples. On the other hand, Cruz *et al.* (2012) reported most of the embryos (72.5%) of *A. suum* eggs in late morula stage at the end of the 1st week at 28°C.

The larvated egg stages containing 1st stage larva in *B. transfuga* and *T. leonina* were observed on days 9 and 5 post-incubation in both formalin solution and faecal samples, respectively. Kim *et al.* (2012) reported the presence of 1st stage larvae in the eggs of *A. suum* at day 19 post-incubation but at a controlled temperature of 25°C in an environmental chamber. The observation revealed that a little variation in temperature affects embryonation process of ascarid eggs. The final infective stages (*i.e.* all the larvated eggs of subsamples were at 2nd larval stages) along with other developmental stages, was observed on days 12 and 9 post-incubation in 0.4% formalin solution and on days 14 and 9 post-incubation in faecal samples in *B. transfuga* and *T. leonina*, respectively. The extended time interval required by the eggs of *B. transfuga* to acquire final infective stage could be attributed to its thicker cell wall as compared to *T. leonina* (Bauer, 2013). The purpose of carrying out study in 0.4% formalin solution and in faecal samples simultaneously was to evaluate the variation in time interval to reach the infective stage, which did not significantly differ. Various developmental stages of ascarids have been presented in Fig. 2.

The viability of eggs was assessed by evaluating the movements of larvae inside the eggs after light stimulation and approximately 86 and 84% of *B. transfuga* eggs were found viable in 0.4% formalin solution and faecal sample, respectively. About 88 and 87% *T. leonina* eggs were found viable in 0.4% formalin solution and faecal sample, respectively. Cruz *et al.* (2012) observed 87.5% viability of *A. suum* eggs at 21 days of incubation, which is in consonance of present study.

There are extensive studies targeting pathology (visceral larva migrans, ocular larva migrans and neural larva migrans) caused by larval stages of ascarids of domestic and wild animals. But the information about the embryonic development upto infective stage (responsible for causing pathologic implications) outside the hosts is limited, especially in case of ascarids of wild fauna. The present study highlighted the comparative time interval required by different ascarids of *Melursus ursinus* and *Panthera leo persica* to reach final infective stage. The interval required by the eggs of *B. transfuga* and *T. leonina* was 12 and 9 days, respectively, under *in vitro* conditions of 27±1°C temperature. The study may prove valuable to zoo keepers and veterinarians to adopt management practices at appropriate time to control the infections caused by ascarids.

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