Trichinella britovi newborn larvae obtain in vitro

Obținerea de larve nou-născute de Trichinella britovi in vitro

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ABSTRACT
The aim of the present work was to obtain in vitro newborn larvae of Trichinella britovi, and to determine the viability and the mobility of the larvae in the RPMI media. 5 rats were infested, orally, with 1000 larvae each. After 6 days p.i. the small intestine of the rats was incubated at 37 C for 4 hours. The adults Trichinella britovi were passed on the RPMI media and incubated for 48 hours. When examined on the microscope, we observed female adults with larvae inside and also, newborn larvae with excellent viability.

Key words: trichinella britovi, newborn larvae, in vitro, RPMI (newborn)

Introduction
All stages in the life cycle of trichinella occur in individual mammalian hosts. When skeletal muscle containing the infective larvae is ingested by another mammal, the larvae are released by the action of gastric fluids and pass into the small intestine. There, the parasites invade the small intestine epithelial wall, and molt four times before becoming sexually mature. After copulation, the females begin to expel newborn larvae about six or seven days after infection. This process continues for the life of the female. Although it is generally believed that the adult worms may persist in the intestine for only several weeks, there is evidence that they may survive for much longer periods, especially if the host’s immune system is compromised. Most of the newborn larvae penetrate into the submucosa and are carried in the circulatory system to various organs. However, only the larvae that invade the skeletal muscle survive (Bruschi and Murrell, 2002).

Materials and methods
We used 5 rats who were orally infected with Trichinella britovi larvae (1000 larvae/animal). Infected-stage larvae of Trichinella britovi, were recovered from a naturally infected fox by the standard pepsin digestion method. On day 5 p.i., food was removed from those infected rats, and on the following day (day 6 p.i.), the rats were killed. The entire small intestine was removed from each animal and incubated in 0.9% saline solution at 37ºC with gentle shaking. Adult Trichinella were collected and washed with saline containing 2 % antibiotics (antibiotic-antymicotic mixture) four times at an interval of 0.5 h. The worms were then transferred into culture flasks containing RPMI medium 1640 with fetal bovine sera (5%) and 2% penicillin-streptomycin mixture and incubated for 24 h at 37ºC in 5% CO2 atmosphere.

After 24 h we examined the sample and observed the pregnant Trichinella females, who were very mobile. After another 24 h of incubation, in the sample appeared the newborn larvae.

Results and discussions
There are several studies in culturing Trichinella, different researchers used different mediums for this purpose. For example Gagliardo and col. used cultured intestinal epithelial cells with essential medium (Earle's salts) (MEM) supplemented with sodium pyruvate, L-glutamine, nonessential amino acids and 10% fetal bovine
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sERA OR IN Dulbecco'S MEM WITH THE SAME SUPPLEMENTS (Gagliardo AND col, 2002). WE WERE INSPIRED BY THE KA BIAN PROTOCOL (KA BIAN AND COL., 2004), BUT AFTER 3 UNSUCCESSFUL ATTEMPTS, WE SUPPLEMENTED THE RPMI MEDIUM WITH 5 % FETAL BOVINE SERA AND INCUBATED THE SAMPLES IN 5% CO2 ATMOSPHERE. AFTER 24 H OF INCUBATION WE OBSERVED THE PREGNANT FEMALES AS SHOWN BELOW:

The females presented a lot of larvae inside uterus, they were very mobile. We didn’t capture the exact moment of hatching.

After another 24 h we observed the newborn larvae in the medium:

So, 8 days post-infection Trichinella britovi adult female, hatch. Despite our results, other authors claim that Trichinella spiralis females hatch in natural conditions 4 days p.i. (Harley and Garllichio, 1971). Other authors sustain that Trichinella spiralis females lays living larvae within the small intestine beginning the fifth or sixth day after the infection (Barnes, 1987; Lapage, 1957; Olsen, 1974; Read, 1972; Wassom, 1988).

It is known that newborn larvae are very immunogenic, (Kazura J.V., D.I. Grove, 1978), (Marti and col., 1987 ). that’s why their obtaining in vitro could be only the first step in developing an immunogenic protocol.

Conclusions:
1. For incubation, Trichinella britovi needs special supplements such as fetal bovine sera 5% and special incubating conditions: 5% CO2 atmosphere.
2. 6 days post-infestation Trichinella britovi females are mature.
3. 7 days post-infestation the females present larvae in utero (after 24 h of incubation).
4. The females hatch, in vitro, 8 days post-infestation or after 48 h of incubation when newborn larvae, with excellent viability, were observed in the medium.
REZUMAT
Scopul acestui experiment a fost obţinerea larvelor nou-născute de *Trichinella britovi* şi observarea viabilităţii şi mobilităţii larvelor în mediul RPMI. 5 şobolani au fost infestaţi per os cu 1000 de larve fiecare. La 6 zile p.i., şobolani au fost sacrificaţi, li s-a recoltat intestinul subţire şi s-au izolat adulţii de *Trichinella*. Aceştia au fost păsaţi pe mediul RPMI şi incubaţi în atmosferă de 5% CO2. La 8 zile p.i. şi după 48 de ore de incubare am obţinut larve nou-născute, care prezentau o excelentă mobilitate şi viabilitate.

Cuvinte cheie:

Reference


