647 703 Experimental Parasitology: Maintenance and cultivation of Nematodes: In vivo - in vitro

Demonstration and Practice : *Trichinella spiralis*January 3rd, 2023

Objectives:

- 1. Know the objectives of in vivo & in vitro cultivation of nematode
- 2. Know the life cycle of *Trichinella spiralis* in mouse model
- 3. Know how to culture *T. spiralis* in mice for preparation of adult stage in small intestine, muscle larvae and new born larvae (lab practice)

Learning process:

- 1. Lecture and brief lab according the given handout and by power point presentation, VDO in the procedure of *T. spiralis* cultivation in mice (MD3504 room at 10.00-11.00)
- 2. Demonstration and practice (Lab 8 at 13.00-17.00)
 - 2.1 Prepare muscle larvae of *T. spiralis* from *T. spiralis* infected mice by Barnette and Justus
 - 2.2 Prepare *T. spiralis* adult stage from small intestine of *T. spiralis* infected mice
 - 2.3 Collect and differential count female and male of T. spiralis from small intestine of infected mouse
 - 2.4 Observe the newborn larvae release from *T. spiralis* female adult
 - 2.5 Count and infects each mouse with 30 muscle larvae of *T. spiralis* by gastric intubation (one mouse / person)

Evaluation:

- 1. Do the assignment report
- 2. Attention /attitude during laboratory work and from pretest
- 3. Marks from examination

Objective for in vitro and in vivo cultivation of nematodes

- 1. For definite diagnosis or to improve the sensitivity of routine method eg. agar plate culture
- 2. To prepare a large amount of the required nematodes use.....
 - for preparation of protein / antigen / DNA / RNA in research works
 - in immunology, molecular biology, immunopathology diagnosis
 - in biology and physiology studies
 - for studying the gross morphology: (macroscopic, microscopic), size (morphometry),
 - for studying the structures of nematodes (scanning electron microscopy, transmission electron microscopy)
 - for studying the pathology and pathogenesis in nematodes infection
 - for study in drug tests eg. drug susceptibility or drug resistant for new drug development and pharmacogenetic field
- 3. To prepare the fresh nematode parasites use for teaching at that time has no infected patient
- 4. To feed the susceptible animals for preparation of each stage of nematode
- 5. To maintain their life cycle
- 6. To prepare the reference strain of nematodes

Lab practice:

I. Collection of encysted muscle larvae of *T. spiralis* from infected mice

T. spiralis infected mice (after 1-2 month post infection)



Skinless, remove visceral organs, extremities; and then chop

Blending with artificial juice (1%pepsin, 1% HCl, 0.85% NaCl solution)

Incubate at 37°C, for 1-1.5 hrs

Pour suspension in Erlenmeyer flask, seal with 2 layers gauze

Upside down on beaker filled with 10-15 ml of NSS

Collect the suspension in the centrifuge tube

Examine the muscle larvae (L1) under stereoscope every 20-30 min

Count the larvae and infect to new mouse by gastric intubation (30-50 larvae/mouse)

II. Collection of adult stage of *T. spiralis* from small intestine of infected mice *T.*

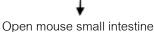
spiralis infected mice (6-7 days post infection)



Euthanasia



Open abdominal cavity and collect small intestine in petri dish containing NSS



Collect adult stage (female, male) and new born larvae of T. spiralis