**MD647 703 Experimental Parasitology:** Maintenance and cultivation of Nematodes: *In vivo - in vitro*

**Demonstration and Practice: *Trichinella spiralis***

January 7th, 2025

**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 newborn larvae (lab practice)

**Learning process:**

1. Lecture and brief lab according to the given handout and by power point presentation, VDO in the procedure of *T. spiralis* cultivation in mice (Meeting room 2 on 5th floor, 13.00-13.30 p.m.)
2. Demonstration and practice (Lab F8 at 13.30-17.00)

   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 e.g. 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 e.g. 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)

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Euthanasia

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Skinless, remove visceral organs, extremities; and then chop

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

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Incubate at 37°C, for 1-1.5 hrs.

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Pour suspension in Erlenmeyer flask, seal with 2 layers gauze

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

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Collect the suspension in the centrifuge tube

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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

↓

Open mouse small intestine

Collect adult stage (female, male) and newborn larvae of *T. spiralis*