

**THAI NGUYEN UNIVERSITY**  
**UNIVERSITY OF AGRICULTURE AND FORESTRY**

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**LE THI KHANH HOA**

**STUDY ON SEVERAL BIOLOGICAL  
CHARACTERISTICS OF Spirocercosis  
CAUSED BY THE NEMATODA *Spirocerca* spp.  
IN DOGS IN THAI NGUYEN AND ITS MEASURES  
OF PREVENTION AND TREATMENT**

**Speciality: Veterinary parasitology and microbiology**  
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**Supervisors: 1. Professor doctor. Nguyen Thi Kim Lan**  
**2. Doctor. Phan Thi Hong Phuc**

**Reviewer 1:.....**

**Reviewer 2: .....**

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at.....on.....in ....

**The dissertation can be found in:**

- National library in Viet Nam**
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## LIST OF STUDIES RELATED TO THE PROJECT

1. Le Thi Khanh Hoa, Hoang Van Hien, Phan Thi Hong Phuc, Nguyen Thi Kim Lan, Pham Ngoc Doanh (2021), “Morphological and molecular characterization of *Spirocerca lupi* (Nematoda: Spirocercidae) from domestic dogs in Vietnam with reference to *Spirocerca vulpis*”, *Parasitology International*, 84, 102381.
2. Le Thi Khanh Hoa, Phan Thi Hong Phuc, Dang Thi Mai Lan (2021), “Situation off the nematoda *Spirocerca lupi* infection in dogs in some localities in Thai Nguyen province”, *The Journal of Agriculture and Rural Development*, 1+2 periodic, No 402+403, pages 95 - 100.

## INTRODUCTION

### 1. Urgency of the project

Currently, dogs are popularly raised in many provinces and cities throughout the country, including Thai Nguyen province. Foreign dog breeds are raised in confinement with good nurturing conditions. While from native dog breeds are usually raised in free-ranging, leading to indiscriminate discharge of faeces into the surrounding environment, increasing the risk of helminth infections, including nematode *Spirocerca lupi* (*S. lupi*).

The parasitic nematode *Spirocerca* produces pea-or apple-shaped nodules in the esophagus and stomach of dogs, infected animals manifest salivary, vomiting, regurgitation, in some cases vomiting with blood, and blood in faeces occur.. If the disease is not diagnosed and treated in time, it will adversely affect the dog's digestive function, making the dog gradually emaciated and die.

According to Rojas A. et al. (2017), *S. lupi* is parasitic nematode that causes Spirocercosis, a severe disease in dogs. The life cycle of *S. lupi* involves intermediate hosts, the dung beetles. Different physicochemical conditions affect the development of nematode *S. lupi*. However, study on biological characteristics and Spirocercosis caused by nematode *S. lupi* in dogs in Vietnam is still very limited.

According to data from the Department of Agriculture and Rural Development of Thai Nguyen province in 2021, there were 231,071 dogs in Thai Nguyen province, of which dogs raised in confinement accounted for nearly 40%, the rest ones were raised largely in free-range or semi confinement, especially in rural and remote places from urban areas. Therefore, the risk of dogs suffering from Spirocercosis caused by *S. lupi* was quite high. However, so far, there have been no studies on Spirocercosis caused by esophageal worm in dogs, so there are no data on the prevalence of the disease and no effective prevention and treatment measures applied.

Starting from the above explanations, we conducted a study on the project “Study on several biological characteristics of Spirocercosis caused by nematode *Spirocerca* spp. in dogs in Thai Nguyen province and prevention measures”.

### 2. Objective of the study

Species identification and determination of some biological characteristics of nematode *Spirocerca* spp.

Determination of some characteristics of nematode *Spirocerca* spp.

Evaluating the effectiveness of some measures for prevention and treatment of Spirocercosis caused by nematode *Spirocerca* spp. in dogs in Thai Nguyen.

### **3. Scientific and practical significance of the project**

#### **3.1. Scientific significance**

The project provides scientific information on some biological, epidemiological, pathological and clinical characteristics and measures of prevention and treatment of the disease caused by nematode *Spirocerca* spp. in dogs in Thai Nguyen.

#### **3.2. Practical significance**

The results of the study are the scientific basis for recommending animal producers to apply preventive measures against nematode *Spirocerca* spp. in order to limit the prevalence of infection in dogs and limit the damage caused by the nematode *Spirocerca* spp. contribute to protecting the health of dogs, thereby contributing to improving the quality of life of the community.

### **4. New contributions of the project**

Parasitic nematode *Spirocerca lupi* in dogs has been identified and the status of *S. lupi* nematode infection in dogs has been identified in Thai Nguyen.

Two species of beetles have been identified - intermediate hosts of parasitic esophageal worm *S. lupi* in dogs in Thai Nguyen including *Catharsius molosus* and *Copris szechouanicus*

The time taken for *S. lupi* larvae to develop into pathogenic larvae in beetles was identified as 16 - 17 days.

The life cycle of esophageal worm *S. lupi* was successfully studied in experimentally infected dogs, the time period to complete the life cycle is 126 - 135 days.

Preventive and control measures of Spirocercosis caused by *S. lupi* in dogs was recommended.

### **5. The structure of the dissertation**

The dissertation consists of 110 pages (not included the list of references and appendices), 2 pages of introduction, 29 pages of the overview of the document 14 pages of the object, materials, content and methods of study ,63 pages of results and discussion , 2 pages of conclusions and recommendations . The dissertation consists of 32 tables, 97 images and charts, 137 references (30 Vietnamese documents, 104 in foreign languages, 3 internet documents, of which 52 documents are from 2017 - 2022).

## CHAPTER 1: OVERVIEW OF DOCUMENTS

Nematode *S. lupi* are red worms with 2 elongated ends with hexagonal shaped buccal cavity, funnel-shaped, buccal capsule slightly swelled anteriorly, 0.140 - 0.168 mm depth, 0.020 - 0.088 mm wide. The nerve ring, the excretory pore are 0.29 - 0.37 mm and 0.48 - 1.25 mm from the top of the head, respectively (Nguyen Thi Quyen, 2017).

Life cycle of nematode *S. lupi* is complex, the life cycle is indirect through intermediate hosts of dung beetles (Ballweber L.R., 2001).

The parasitic nematode *S. lupi* creates hard nodules in the esophageal wall inside which is filled with a bright red pus, in which there are often *S. lupi* worms living and wrapped together in bundles. Symptoms of dogs with Spirocercosis include: vomiting, difficulty swallowing, regurgitation, coughing, salivary, difficulty swallowing.

The prevalence of nematode *S. lupi* infection increases gradually with dog age. In addition, the weather conditions, climate, farming methods also affect the distribution and development of nematode *S. lupi* in dogs.

In order to prevent spirocercosis caused by nematode *S. lupi* in dogs, it is necessary to take good care of the dogs, dogs are not allowed to be free ranging, and the dogs are restricted to contact with the dung beetles - intermediate hosts of the disease. Anthelmintic drugs including ivermectin, doramectin, milbemycin oxime can be used in treating Spirocercosis.

## CHAPTER 2: OBJECTS, MATERIALS, CONTENTS AND METHODS OF STUDY

### 2.1. Objects, time period and places of the study

#### \* Study objects

- Dogs at different age raised in 6 districts and cities of Thai Nguyen province.

- Spirocercosis caused by esophageal worms *Spirocerca* spp.

#### \* Study time period: from 2018 - 2021

#### \* Places of study:

- The study was carried out in 6 districts and cities of Thai Nguyen province, including: Thai Nguyen city, Pho Yen city; Phu Binh, Dinh Hoa, Dong Hy and Dai Tu districts.

- Places of sample testing: Laboratory of Faculty of Animal science and veterinary medicine - Thai Nguyen University of Agriculture and Forestry; Department of Parasitology; Systematic Entomology Laboratory - Department of Systematic Entomology - Institute of Ecology and Biological Resources; National Institute of Hygiene and Epidemiology;

Thai Nguyen National General Hospital, Veterinary Clinic, Vn Pet - Thai Nguyen.

## **2.2. Materials of study**

*\* Experimental animals:*

Dogs of different ages were raised in 6 districts and cities of Thai Nguyen province.

Healthy Dogs at 2 - 3 months of age: 28 dogs (designed for experimental nematode *S. lupi* infection of which 10 dogs were infected and 6 control dogs to study Spirocercosis, 12 dogs experimentally infected were used for experimental efficacy of anthelmintics).

*\* Sampling: Spirocerca* spp. was collected through necropsied dogs in Thai Nguyen province. Fresh stool samples from dogs. Blood samples from dogs with spicercosis caused by nematode *S. lupi* and healthy dogs. Samples were collected from the esophagus, stomach, aorta, and lungs of infected dogs.

*\* Equipment, instruments and chemicals:* Chemicals and instruments essential in studying parasites and molecular biology, gene sequencing,...

## **2.3. Contents of study**

### **2.3.1. Study on some biological characteristics of nematode *Spirocerca* spp. in dogs**

Necropsy and identification of nematode *Spirocerca* spp.

Study on the development of nematode *S. lupi* larvae in the intermediate hosts

### **2.3.2. Study on Spirocercosis caused by *Spirocerca lupi* in experimentally infected dogs**

Study on some epidemiological characteristics of nematode *S. lupi* in dogs

Study on some characteristics of nematode *S. lupi* in experimentally infected dogs

### **2.3.3. Study on measure of prevention and treatment of Spirocercosis caused by nematode *Spirocerca lupi* in dogs**

Study on the efficacy of some anthelmintic drugs against *S. lupi* in dogs

Study on prevention measures against nematode *S. lupi* in dogs in Thai Nguyen province

### **2.3.4. Epidemiological mapping of the prevalence of nematode *Spirocerca lupi* in dogs in Thai Nguyen province**

## **2.4. Methods of study**

### **2.4.1. Methods of study on some biological characteristics of nematode *Spirocerca* spp. in dogs**

#### **2.4.1.1. Method of dissection and identification of nematode *Spirocerca* spp.**



In order to find the parasitic nematode *Spirocerca* spp. In gastrointestinal tract, the dogs were dissected according to the Skrjabin method of incomplete helminthological dissection (1928), collecting all the nematode suspected for *Spirocerca* spp.

Method of species identification by using morphological techniques: Nematodes were treated until the body parts were transparent to clearly observe the internal organs under the microscope. The measurements and images were taken under an optical microscope (ECLIPSE E 100 Nikon) combined with the observation of the microscopic structures of the nematode *S. lupi* under the scanning electron microscope FE-SEM S4800.

Molecular identification method: DNA was extracted from adult worms (3 males and 3 females) using QIAamp DNA MiniKit (Qiagen, Hilden, Germany). PCR products were sequenced by using an ABI 3100 analyzer, an automated capillary electrophoresis system. The obtained sequences were compared to those on GenBank. The homology sequences of *Spirocerca* species on GenBank were used for molecular evolution analysis using MEGA7 software (Kumar S. et al., 2016). Phylogenetic tree was built using Maximum Likelihood method. with the most suitable model.

#### 2.4.1.2. *Methods of study the development of nematode S. lupi larvae in the intermediate hosts*

All beetles present in cattle manure were caught. First, the beetle samples were observed under a stereo microscope and classified at the subfamily level, then the beetle samples in the subfamilies were identified to the lowest taxonomic level by taxonomic key of Choate P.M. (2001). After number of beetles collected was identified, each beetle individual was dissected, their muscles and organs were removed and put in a petri dish containing 0,9% NaCl physiological saline, the sediment was crushed and removed then examined under a stereo microscope to look for *S. lupi* larvae.

The way of collecting *S. lupi* worm eggs to infect beetles: Nematode *S. lupi* was collected from nodules from dog's esophagus and continued raising in physiological saline, nematode *S. lupi* continued to live for 2 - 3 days and did not stop seding eggs. After 3 days, physiological saline was centrifuged to collect worm eggs.

The way of infecting *S. lupi* worm eggs for beetles: The morphology and structure of nematode *S. lupi* eggs obtained before infection was examined. Infected eggs must be old eggs, containing larvae inside. The number of eggs was counted on the Mc. Master chamber then mixed about 500.000 eggs of nematode *S. lupi* with about 1 kg of dog faeces, placed in 2 plastic boxes with about 0,5 kg of faeces/ each. 100 beetles of *Ca. molosus* species were placed in a plastic box and 100 beetles of *Co.*

*zsecouanicus* species in the remaining box. The beetles ingested dog faeces with *S. lupi* eggs in plastic containers and infected with larvae.

Starting from the second day after infection, 3 beetles were collected from each box every day, dissected and found *S. lupi* larvae in the beetles, and at the same time the time period of *S. lupi* larvae to develop to pathogenic larvae in beetles was determined.

#### **2.4.2. Methods of study on Spirocercosis associated with nematode *Spirocercia lupi* in dogs in Thai Nguyen**

##### **2.4.2.1. Methods of study on some epidemiological characteristics of Spirocercosis associated with nematode *Spirocercia lupi* in dogs**

Direct interviews with dog owners were implemented in the study area about the implementation of measures of prevention of parasitic diseases in dogs.

Sampling was performed according to multistage cluster sampling method. Fresh dog stool samples were randomly collected in the morning from dog raising households. Fecal examination was performed by using Fulleborn method.

The intensity of *S. lupi* infection at necropsy was determined by the number of parasitic worms/dog, and by collecting and counting the number of parasitic nematode *S. lupi* in each dog.

The intensity of nematode *S. lupi* infection via stool examination was determined by counting the number of worm eggs per 1 gram of faeces in the Mc. master.

Chamber Based on the origin of the domestic dogs, dogs were divided into 3 groups: native dog breeds, foreign dog breeds and cross dog breeds.

Dogs were at 4 different age were monitored  $\leq 5$  months;  $> 5 - 12$  months;  $> 12 - 24$  months and  $> 24$  months of age.

Methods of raising dogs: dogs raised in free range, dogs kept in confinement, and dogs raised in semi confinement.

Study on *S. lupi* worm infection in dogs in 4 seasons of the year: Spring, Summer, Autumn and Winter.

##### **2.4.2.2. Method of studying some characteristics of Spirocercosis associated with nematode *Spirocercia lupi* in experimentally infected dogs**

*\* Dogs were experimentally infected with nematode *S. lupi**

After *S. lupi* larvae developed to pathogenic larvae in the infected beetles, the beetles were dissected to collect *S. lupi* larvae (after 25 days of infection).

2 phases of infection were designed with 16 healthy native dogs at 2 - 3 months of age, each phase 8 dogs were used (5 infected dogs and 3 control dogs). Experimentally infected dogs were swallowed pathogenic *S.*

*lupi* larvae, at a dose of about 150 larvae/dog. Dogs in control group, larvae were not swallowed.

Dogs in the experimental and control groups were provided with the same food and water (No anthelmintics were administered).

*\* The time period to complete the life cycle of nematode S. lupi in the body was monitored*

After 80 days of infection, every dog's faeces were tested daily by using Fulleborn method to determine the time *S. lupi* worm eggs began to emerge in dog faeces. After *S. lupi* worm eggs began being passed in faeces, stool samples were tested once a week until dogs were necrosied to monitor the eggs shedding from the worms during the experiment. The time to begin emerging nematode *S. lupi* eggs in the dog's faeces was the time the worm to complete the life cycle in the dog - the definite host.

*\* Spirocercosis associated with nematode Spirocerca lupi in experimentally infected dogs:* each infected dog was thoroughly daily observed the clinical manifestations of each dog was recorded at the same time, the clinical signs in the control group of dogs were observed

*\* Blood test method was used to determine hematological indicators of infected dogs and control dogs:* Blood samples were collected after the *S. lupi* worm completed its life cycle, the second time blood was taken before the dogs were necropsied. Some hematological parameters of experimentally infected dogs and control dogs were analyzed on an automatic hematology analyzer Osmetech OPTI – CCA/Blood Gas Analzyen.

*\* Methods of identification of gross and microscopic lesions*

After 5 months of infection, the experimentally infected dogs and control dogs were necropsied to observe the lesions of the dogs and examine the number of parasitic nematode *S. lupi* in each dog.

Applying Skrjabin method of incomplete heminthological dissection (1928) to collect nematode *S. lupi*. The gross lesions were thoroughly observed with the naked eye and magnifying glass. Then samples from oesophagus, aorta, lung, stomach were taken fixed in 10% formalin solution to make microscopic slides.

### **2.4.3. Methods of prevention and treatment of Spirocercosis in dogs**

#### **2.4.3.1. Methods of determining the efficacy of some anthelmintics against nematode *S. lupi* in dogs**

*\* Design of experiments was conducted to evaluate the efficacy of anthelmintics against nematode S. lupi from experimentally infected dogs*

3 anthelmintics were experimentally administered in 12 dogs experimentally infected with nematode *S. lupi*. After infection, when dogs were tested it was found that the number of nematode *S. lupi* eggs in the

dog's faeces reached about 1,000 eggs/gram of faeces, experiment of anthelmintics in treating Spirocercosis caused by nematode *S. lupi* was carried out in dogs. 12 experimentally infected dogs were divided into 4 groups: 3 groups with anthelmintics administered and 1 control group without anthelmintics, 3 dogs were used in each group.

In experimental group 1: ivermectin was administered at dosage of 0,3 mg/kg body weight, injected subcutaneously once a week.

In experimental group 2: doramectin was administered at 0,5 mg/kg body weight, injected subcutaneously once a week.

In experimental group 3: milbemycin oxime, was administered 0,5 mg/kg body weight, given once a week.

All of three experimental groups were expected the anthelmintic drugs administered for 10 weeks. However, this course may be shortened if the drug works faster than expected.

The efficacy of anthelmintics on nematode *S. lupi* in experimental dogs was determined by: after administration of the anthelmintic drugs once a week number of *S. lupi* worm eggs/gram of faeces counted, stool samples were collected from each dog for testing, counting the number of *S. lupi* worm eggs/gram of faeces. In week 13, all dogs in the experimental and control groups were necropsied, the number of parasitic nematode *S. lupi* in each dog was counted. The results of stool test were compared to results of necropsy in experimental dogs and control dogs, thereby evaluating the efficacy of anthelmintic drugs.

The safety of anthelmintics in treating *S. lupi* was evaluated by the results of monitoring the body state, movement, eating and some other reactions of the dog's body before and after administration of the anthelmintic drugs.

*\* Evaluation of the efficacy of anthelmintics against nematode S. lupi in dogs in the field*

After the experimental results of anthelmintics in experimental dogs was obtained, the 3 anthelmintic drugs administered above were continued being administered at the same dosage as administered against *S. lupi* in experimentally infected dogs each anthelmintic was administered, in treating 45 dogs infected with *S. lupi* in the field for 15 dogs.

The efficacy of anthelmintic against *S. lupi* worms was determined by examining the stool: counting the number of eggs/ in faeces before the anthelmintic drugs were administered. After anthelmintic drugs were administered, the dog's faeces were re-tested to find worm eggs to evaluate the effectiveness of anthelmintics as the evaluation method used

in experimentally infected dogs described above. After the trial of the anthelmintics in the field, an anthelmintic drug that was highly effective and safe to prevent *S. lupi* worms in dogs was selected.

2.4.3.2. *Method of prevention of Spirocercosis caused by nematode S. lupi in dog*

\* *Study on the effectiveness of killing eggs of nematode Spirocerca lupi by some antiseptic including CaO; Ca(OH)<sub>2</sub> 5%, 10%; NaOH 2%, 3%, 4%*

Dog faeces infected with nematode *S. lupi* with severe intensity was placed in a petri dish in a layer of 1 cm thick. Antiseptics were used at different concentrations, the surface of the faeces layer, was sprayed to make it wet. For CaO a layer about 1cm thick on the surface of the stool in the petri dish was covered. After that, every day the number of worm eggs was examined and counted on the counting chamber Mc. Master, *S. lupi* eggs were observed to determine whether the eggs were alive or dead. If the eggshell was broken or wrinkled or larvae in the eggs were lysed or the eggs turned black, the eggs were considered dead.

\* *Some experimental preventive measures against nematode S. lupi in dogs*

#### *Experimental design*

The experiment was carried out in 3 communes of Phu Binh district - Thai Nguyen province. The experiment was conducted in 150 dogs from native breeds was divided into 3 equal groups: Experimental group 1; Experimental group 2 and control group. In each group 50 dogs were used. Dogs in control and two experimental groups were relatively equal in terms of factors: age (8 - 12 months old); body weight (10 - 15 kg); sex (equivalent number of male and female dogs); raising methods (free range and confinement).

- For dogs in experimental group 1 and experimental group 2: the following 4 preventive measures were applied:

+ Anthelmintic used for Prophylactic treating dogs experimentally infected with nematode *S. lupi* by using doramectin 0.5 mg/kg B.W in group 1 the drug was administered 3 times on day 1, 14 and 28. Experimental group 2 was administered 3 times on day 1, 14, 28 then administered once a month. The experiment was monitored for 5 months.

+ Their habitat also needs to be cleaned regularly food and water provided for dogs should be cleaned.

+ Powdered lime was used to treat dog faeces

- For dogs in control group: the above prevention measures need not be applied.

*The prevalence and intensity of nematode *S. lupi* infection after 2 months of experiment was determined*

After 2 months of experiment, stool test to determine the prevalence and intensity of nematode *S. lupi* infection in the two experimental and control groups by using Fulleborn method, counting number of eggs in 1 gram of feces using Mc. Master chamber. There by, the effectiveness of the preventive measures applied was preliminary evaluated.

*The prevalence and intensity of nematode *S. lupi* infection was determined after 5 months of experiment*

After 5 months of experiment, the dog's faeces were tested again to evaluate the effectiveness of the applied preventive measures.

*\* Recommendation of measures for prevention of nematode *S. lupi* in dog*

From the results of study on biological characteristics, epidemiological characteristics of the Spirocercosis and the results of experiment of prevention and treatment measures, integrated approach to Spirocercosis caused by nematode *S. lupi* in dog was recommended

#### **2.4.4. Methods of epidemiological mapping**

The epidemiological map of endemic Spirocercosis caused by esophageal worm *S. lupi* in dogs in 6 districts and cities of. Thai Nguyen province was developed using GIS technique, including the following steps: Developing mapping software, editing paper maps, input data and spatial data normalization using Microstation software.

#### **2.4.5. Data processing methods**

The obtained data were processed by biological statistical method of Do Duc Luc et al., 2017, on Minitab 16.0 software.

## **CHAPTER 3: RESULTS AND DISCUSSION**

### **3.1. Study on several biological characteristics of nematode *Spirocerca* spp. in dogs**

#### **3.1.1. Results of necroscopy examination and identification of the the parasitic nematode *Spirocerca* spp. in dogs in Thai Nguyen province**

##### **3.1.1.1. Result of necropsy and examination of the worm**

**Table 3.1. Prevalence and intensity of *Spirocerca* spp. infection in dogs**

| Locality (district, city) | Number of operated and examined dogs (dog) | Number of infected dogs (dog) | Prevalence of infection (%) | intensity of infection(number of worms/dog) |
|---------------------------|--------------------------------------------|-------------------------------|-----------------------------|---------------------------------------------|
| Thai Nguyen city          | 89                                         | 9                             | 10,11 <sup>b</sup>          | 1 - 13                                      |
| Pho Yen city              | 91                                         | 11                            | 12,09 <sup>b</sup>          | 2 - 15                                      |
| Phu Binh district         | 102                                        | 29                            | 28,43 <sup>a</sup>          | 4 - 27                                      |

|                   |            |            |                    |               |
|-------------------|------------|------------|--------------------|---------------|
| Dinh Hoa district | 96         | 16         | 16,67 <sup>b</sup> | 2 - 19        |
| Dong Hy district  | 93         | 25         | 26,88 <sup>a</sup> | 1 - 29        |
| Dai Tu district   | 103        | 15         | 14,56 <sup>b</sup> | 1 - 12        |
| <b>Total</b>      | <b>574</b> | <b>105</b> | <b>18,29</b>       | <b>1 - 29</b> |

(Through necropsy and examination)

The results were shown in table 3.1 and figure 3.1: Out of 574 domestic dogs necropsied and examined, 105 (18,29%) were infected with *Spirocerca* spp. varying from 10,11% to 28,43%. With an intensity ranging from 1 - 29 nematodes per dog. Dogs in Phu binh were infected with the highest prevalence (28,43%), followed by dogs in Dong Hy district (26,88%), the lowest prevalence of infection was in dogs in Thai Nguyen (10,11%).

### 3.1.1.2. The results of species identification by using morphological methods

\* The results of identification of parasitic worms in the dog's esophagus by using morphological method

Table 3.2 showed that all of 240 individuals of parasitic worms in canine esophageal nodules in Thai Nguyen belonged to the *Spirocerca lupi* species (Rudolphi, 1809), genus *Spirocerca* (Railliet et Henry, 1911), family Spirocercidae (Chitwood and Wehr, 1911), suborder Spirurina (Railliet et Henry, 1915), order Rhabditida (Chitwood, 1933), subclass Chromadoria (Pearse, 1942), class Chromadorca (Inglis, 1983), phylum Nematoda (Potts, 1932).

\* Morphological characteristics and size of parasitic nematode *S. lupi* in dogs in Thai Nguyen

Parasitic Esophageal worm in dogs in Thai Nguyen was identified as *S. lupi* species with the main morphological and structural features as follows:

- When was alive, the worm's body was in red colour, twisted, the female worms were larger than the male worms.

- There was absence of teeth structures in the buccal capsule in *S. lupi*. Around the mouth, there were two cephalic papillae and one pair of sensory papillae (Amphids) (Figure 3.3A). The ratio of glandular and muscular esophagus of male and female worms was almost the same (about 10:1) (Figure 3.3B, 3.3C).

- The tail of the male worm had two copulatory spicules with different sizes. The size of the copulatory spicule was 4 times longer than that of the short spine (Figure 3.4A). The papillae that were located near the anus included four pairs of large anal papillae, one broad papillae just before the anus, two pairs of posterior papillae, and four pairs of small papillae near the caudal end (Figure 3.4A, 3.4B).

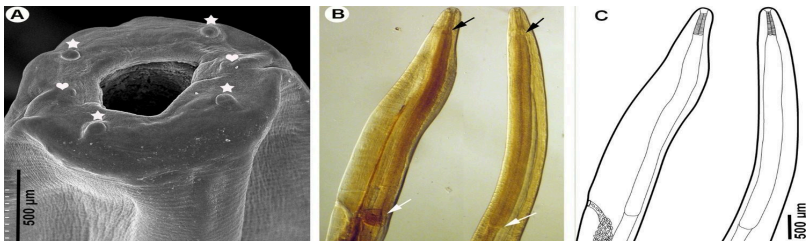
- In female worms, the fat tail was found in the anterior part of the body, usually 0.5 - 2.5 mm behind the esophagus (Figure 3.6A, 3.6B), with the exception of one individual whose vulva was located at the end of the esophagus (Figure 3.6C). In adult females, the vulva was obscured by the uterus when filled with eggs (Figure 3.7A, 3.7B). The uterus extended to the anterior portion of the esophagus, turning round backward to form circles that were easily confused with the vulvar opening (Figure 3.7C). The uterus was filled with eggs. The nematode *S. lupi* was raised in physiological saline, the worm laid a lot of eggs. Eggs were elongated, with thick shells, and fully developed embryos were coiled in eggs (Figure 3.8).



**Figure 3.2A.** The *S. lupi* worm's body was twisted

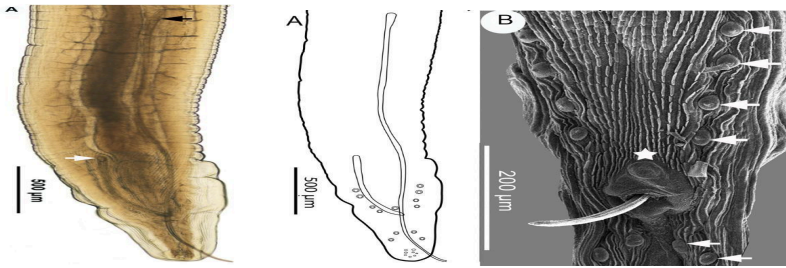


**Figure 3.2B.** Twisted body of nematode *S. lupi* female (upper) larger than male (lower)

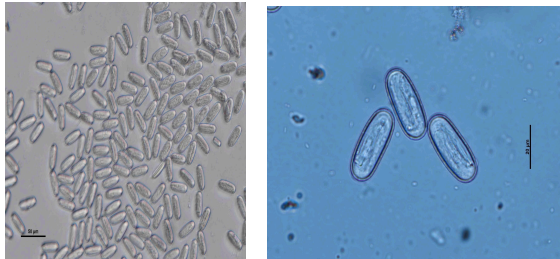


**Figure 3.3.** Anterior body part of the roundworm *S. lupi*





**Figure 3.4. Male worm tail**



**Figure 3.8. Roundworm eggs *S. lupi***

### ***3.1.2. Study on the development of the nematode in larvae of Spirocerca lupi in the intermediate hosts***

#### ***3.1.2.1. Identification of the intermediate host species of the nematode Spirocerca lupi***

*\* Beetle species found in Thai Nguyen*

The results were shown in table 3.4: 2 species of dung beetles of the family Scarabaeidae (*Catharsius molosus* and *Copris szechouanicus*) were identified in Thai Nguyen. Among them, *Catharsius molosus* was the highest (63.23%), followed by *Copris szechouanicus* (32.96%). In addition, there were some beetles of the genus *Onitis*, which were not identified, accounting for 3.81%.

**Table 3.4. Beetle species found in Thai Nguyen**

| Locality (district, city) | Number of Beetles | Species <i>Catharsius molosus</i> |       | Species <i>Copris szechouanicus</i> |       | Onitis spp. |       |
|---------------------------|-------------------|-----------------------------------|-------|-------------------------------------|-------|-------------|-------|
|                           |                   | n                                 | %     | n                                   | %     | n           | %     |
| Thai Nguyen city          | 88                | 51                                | 57,95 | 37                                  | 42,05 | 0           | 0,00  |
| Pho Yen city              | 95                | 47                                | 49,47 | 48                                  | 50,53 | 0           | 0,00  |
| Phu Binh district         | 297               | 195                               | 65,66 | 81                                  | 27,27 | 21          | 7,07  |
| Dinh Hoa district         | 109               | 61                                | 55,96 | 48                                  | 44,04 | 0           | 0,00  |
| Dong Hy district          | 280               | 210                               | 75,00 | 70                                  | 25,00 | 0           | 0,00  |
| Dai Tu district           | 102               | 50                                | 49,02 | 36                                  | 35,29 | 16          | 15,69 |

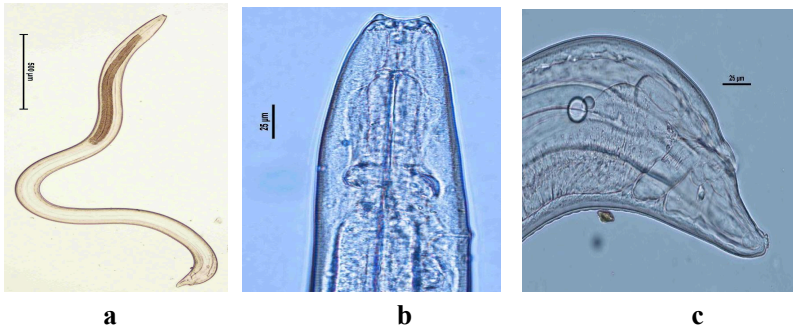
|              |            |            |              |            |              |           |             |
|--------------|------------|------------|--------------|------------|--------------|-----------|-------------|
| <b>Total</b> | <b>971</b> | <b>614</b> | <b>63,23</b> | <b>320</b> | <b>32,96</b> | <b>37</b> | <b>3,81</b> |
|--------------|------------|------------|--------------|------------|--------------|-----------|-------------|

\* *S. lupi* larvae-infected beetles and their position in the animal taxonomy

**Table 3.5. Prevalence and intensity of *Spirocerca lupi* larvae infection in the collected beetles**

|   | Beetle species              | Number of tested beetles (beetle) | Number of <i>S. lupi</i> larvae infected beetles (beetle) | Percentage of infection (%) | Number of larvae / beetle (min - max) |
|---|-----------------------------|-----------------------------------|-----------------------------------------------------------|-----------------------------|---------------------------------------|
| 1 | <i>Catharsius molosus</i>   | 614                               | 39                                                        | 6,35                        | 1 - 22                                |
| 2 | <i>Copris szechouanicus</i> | 320                               | 19                                                        | 5,94                        | 1 - 7                                 |
| 3 | <i>Onitis</i> spp.          | 37                                | 0                                                         | 0,00                        | 0                                     |
|   | <b>total</b>                | <b>971</b>                        | <b>58</b>                                                 | <b>5,97</b>                 | <b>1 - 22</b>                         |

The results in Table 3.5 show that the prevalence of *S. lupi* larvae infection in beetles in the field was relatively low (5.97%), the average intensity of infection was 1 - 22 larvae/beetle. Of the 3 beetle species collected in Thai Nguyen, only 2 species contained *S. lupi* larvae, namely *Catharsius molosus* (percentage of larvae infection was 6.35%) and *Copris szechouanicus* species (percentage of larvae infection was 5.94%).



**Figure 3.12. larvae in the third stage of nematode *S. lupi***

Table 3.6 and figure 3.12 show that larvae in 3rd stagel (L3) of the esophageal worm (*S. lupi*) acquired from beetles had the following main characteristics: elongated body, about 2 mm in size ranging from 1.8 - 2.6 mm) (Figure 3.12a). The anterior part of the body had 2 papillae (Figure 3.12b), the tail was cup-shaped with short tufts of hair (Figure 3.12c). The buccal capsule was short, the esophagus was divided into two parts: the muscular esophagus in the anterior part and the glandular esophagus was in in the posterior. The buccal capsule accounted for about 1/3 the length of the muscular esophagus. Nerve loop at the end of the muscular esophagus, the excretory hole opens to the side of the body at the level of the junction

between the muscular esophagus and the glandular esophagus (Figure 3.12b). The anal opening was located in the posterior.

### 3.1.2.2. Prevalence and intensity of *S. lupi* larvae infection in experimentally infected beetles

**Table 3.7. Prevalence and intensity of experimental *Spirocerca lupi* larvae infection in beetles**

| Beetle species              | Number of experimentally infected beetles (beetle) | Number of <i>S. lupi</i> larvae infected beetles ((beetle) | Percentage (%) | Number of larvae/beetle |           |
|-----------------------------|----------------------------------------------------|------------------------------------------------------------|----------------|-------------------------|-----------|
|                             |                                                    |                                                            |                | $(\bar{x} \pm m_x)$     | min - max |
| <i>Catharsius molosus</i>   | 100                                                | 95                                                         | 95,00          | $110,5 \pm 104,9$       | 1 - 415   |
| <i>Copris szechouanicus</i> | 100                                                | 90                                                         | 90,00          | $9,8 \pm 8,5$           | 1 - 30    |

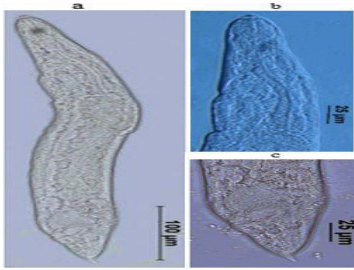
The findings in table 3.7 showed that both dung beetle species containing *S. lupi* eggs were found to have larvae of this worm when tested. The prevalence and intensity of larva infection in *Ca. molosus* was 95% and 1 - 415 larvae/beetle, while the prevalence of *Co. szechouanicus* species was 90% and 1 - 30 larvae/beetle. Thus, the *Ca. molosus* was infected with *S. lupi* larvae with higher rate and intensity than that of *Co. szechouanicus*. In addition, the prevalence and intensity of infection in experimentally infected beetles was much higher than in natural infestations.

### 3.1.2.3. Time spent by *Spirocerca lupi* larvae to develop into pathogenic larvae in beetles

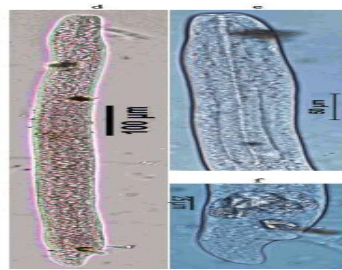
**Table 3.8. Time period for development of *Spirocerca lupi* larvae in beetles**

| Dung beetle species         | Number of experimentally infected beetle individuals (beetle) | Time of appearing larval stages (day) |               |                |                  |
|-----------------------------|---------------------------------------------------------------|---------------------------------------|---------------|----------------|------------------|
|                             |                                                               | L1                                    | L2            | L3             | highly active L3 |
| <i>Catharsius molosus</i>   | 95                                                            | 1 - 11                                | 9 - 15        | 16             | 23               |
| <i>Copris szechouanicus</i> | 90                                                            | 1 - 14                                | 11 - 16       | 17             | 25               |
| <b>Total</b>                | <b>185</b>                                                    | <b>1 - 14</b>                         | <b>9 - 16</b> | <b>16 - 17</b> | <b>23 - 25</b>   |

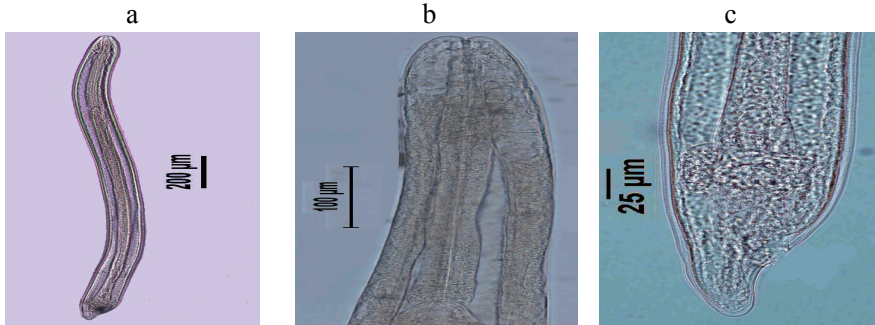
The results in Table 3.8 show that The development time of the larval stage of esophageal worms in the body of the two dung beetle species was similar. At ambient temperature of about 22 - 27°C, the development time period of L1 stage larvae was 1 - 14 days after infection; L2 stage was 9 - 16 days; L3 stage was 16 - 17 days; about 23 - 25 days after infection, L3 larvae were highly active, this was the larva stage capable of infecting the definite host (dog).



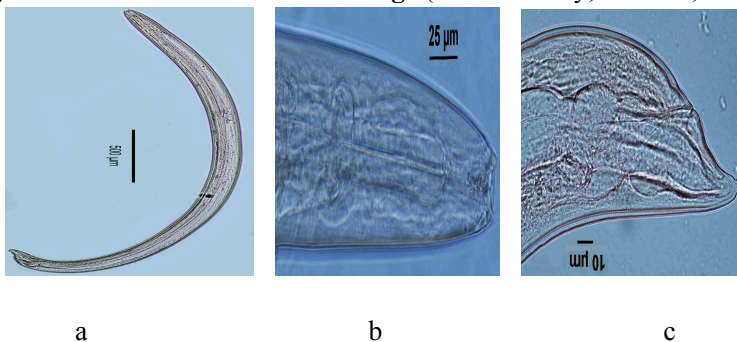
**Figure 3.13. Larvae in L1 stage**  
(a: entire body; b: head; c: tail)



**Figure 3.14. Larvae in young L2 stage**  
(d: entire body; e: head; f: tail)



**Figure 3.15. Larvae in the old L2 stage** (a: entire body; b: head; c: tail)



**Figure 3.16. Larvae in the L3 stage** (a: entire body; b: head; c: tail)

Table 3.8 and figures 3.13 - 3.16 show that after the embryonated egg were ingested by the dung beetle, the embryo was released from the egg and underwent development of the larval stages L1, L2 and L3. The results showed that the size of the larval stages varied greatly. The characteristics of the larval stages were as follows: in L1 larvae (Figure 3.13) there was absence of cephalic horn, there was a pointed tail tip of about 7 - 9  $\mu\text{m}$ , and no tail hair in L2 larvae (Figure 3.14; 3.15) there was absence of cephalic

horn, tail tip and tail hair, the tail was obtuse. In the young L2 larvae (Figure 3.14) buccal capsule was not clearly seen, in the old L2 larva (Figure 3.15) the buccal cavity was clearly seen. In L3 larvae (Figure 3.16) there were 2 cephalic horns, no tail tip and there was a tuft of short hair in the tail.

### 3.2. Study on nematode *Spirocerca lupi* in dogs in Thai Nguyen

#### 3.2.1. Study on some epidemiological characteristics of nematode *Spirocerca lupi* in dogs

##### 3.2.1.1. Current status of parasitic disease prevention for dogs in Thai Nguyen

The above results show that the prevention of parasitic diseases in dogs in Thai Nguyen has not been really interested in by dog owners. Periodic administration of anthelmintics to remove parasitic worms in dogs has not been widely applied in the area.

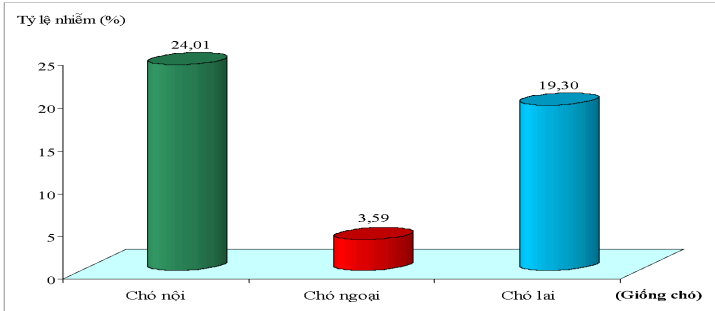
##### 3.2.1.2. Prevalence and intensity of *S. lupi* infection in dogs at localities

**Table 3.11. Prevalence and intensity of *Spirocerca lupi* infection in dogs at localities (via stool test)**

| Locality<br>(district, city) | Number of<br>tested<br>samples | Number of<br>infected<br>samples | Percentage of<br>infection (%) | intensity of infection |              |            |              |           |              |
|------------------------------|--------------------------------|----------------------------------|--------------------------------|------------------------|--------------|------------|--------------|-----------|--------------|
|                              |                                |                                  |                                | +                      |              | ++         |              | +++       |              |
|                              |                                |                                  |                                | n                      | %            | n          | %            | n         | %            |
| Thai Nguyen city             | 474                            | 44                               | 9,28 <sup>c</sup>              | 35                     | 79,55        | 7          | 15,91        | 2         | 4,55         |
| Pho Yen city                 | 481                            | 55                               | 11,43 <sup>bc</sup>            | 42                     | 76,36        | 10         | 18,18        | 3         | 5,46         |
| Phu Binh district            | 492                            | 128                              | 26,02 <sup>a</sup>             | *65                    | 50,78        | 42         | 32,81        | 21        | 16,41        |
| Dinh Hoa district            | 485                            | 76                               | 15,67 <sup>b</sup>             | 52                     | 68,42        | 16         | 21,05        | 8         | 10,53        |
| Dong Hy district             | 478                            | 113                              | 23,64 <sup>a</sup>             | 60                     | 53,10        | 37         | 32,74        | 16        | 14,16        |
| Dai Tu district              | 489                            | 70                               | 14,31 <sup>b</sup>             | 43                     | 61,43        | 21         | 30,00        | 6         | 8,57         |
| <b>Total</b>                 | <b>2899</b>                    | <b>486</b>                       | <b>16,76</b>                   | <b>297</b>             | <b>61,11</b> | <b>133</b> | <b>27,37</b> | <b>56</b> | <b>11,52</b> |

Prevalence and intensity of esophageal worm (*S. lupi*) infection in dogs was varied in various localities and cities, the highest prevalence of infection was in Phu Binh district (26.02%), followed by Dong Hy district (23.64%), Dinh Hoa (15.67%), Dai Tu (14.31%), Pho Yen city (11.43%) and the lowest was in Thai Nguyen City (9.28%). Thus, the prevalence of esophageal worm infection at the localities was significantly different ( $P < 0.05$ ).

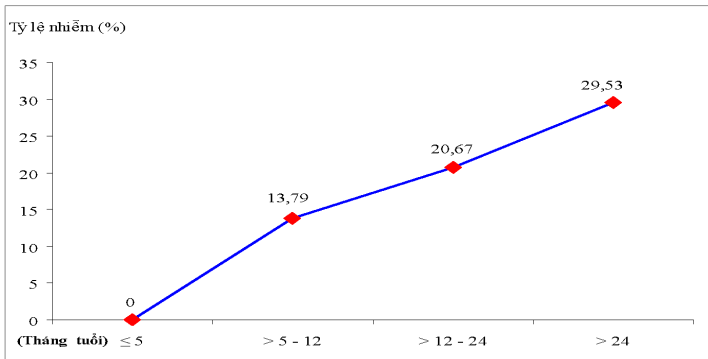
### 3.2.1.3. Prevalence and intensity of nematode (*Spirocerca lupi*) infection dependent on dog breed (via stool test)



**Figure 3.19. A bar chart represent for Prevalence of Nematode (*S. lupi*) infection dependent on dog breed**

The results in Table 3.12 showed that via 2,899 tested samples of dog faeces in all of 3 groups of dogs (native dog breeds, foreign dog breeds, cross dog breeds), the prevalence of nematode *S. lupi* infection in native dog breeds was the highest (24.01%). Followed by cross dog breeds (19.3%) and the lowest was foreign dog breeds (3.59%).

### 3.2.1.4. Prevalence and intensity of *Spirocerca lupi* infection with dog age (via stool test)



**Figure 3.20. Chart represent the prevalence of *S. lupi* infection with dog age**

Table 3.13 shows that The prevalence of *S. lupi* infection increased with dog age. In dogs over 24 months old, the prevalence of infection was the highest (29.53%); the second highest was in dogs > 12 - 24 months old (20.67%) followed by dogs > 5 - 12 months (13.79%). The number of dogs under 5 months of age whose faeces was tested no *S. lupi* eggs were found in the fecal samples.

*3.2.1.5. The prevalence and intensity of the Spirocercca lupi infection in different sex of dogs (via stool tests)*

**Table 3.14. The prevalence and intensity of the *Spirocercca lupi* infection in different sex of Dogs (via stool tests)**

| Sex          | Number of tested samples | Number of infected samples | percentage of infection (%) | Intensity of infection |              |            |              |           |              |
|--------------|--------------------------|----------------------------|-----------------------------|------------------------|--------------|------------|--------------|-----------|--------------|
|              |                          |                            |                             | +                      |              | ++         |              | +++       |              |
|              |                          |                            |                             | n                      | %            | n          | %            | n         | %            |
| Male         | 1464                     | 240                        | 16,39 <sup>a</sup>          | 148                    | 61,67        | 67         | 27,92        | 25        | 10,41        |
| Female       | 1435                     | 246                        | 17,14 <sup>a</sup>          | 149                    | 60,57        | 66         | 26,83        | 31        | 12,60        |
| <b>Total</b> | <b>2899</b>              | <b>486</b>                 | <b>16,76</b>                | <b>297</b>             | <b>61,11</b> | <b>133</b> | <b>27,37</b> | <b>56</b> | <b>11,52</b> |

Regarding the prevalence of infection: out of 1,464 tested fecal samples of male dogs, 240 were infected with esophageal worms, accounting for 16.39%. Out of 1,435 fecal samples of the female dog, 246 were infected accounting for 17.14%. Thus, the prevalence of esophageal worm infection was higher in the male dogs than that of the female dogs, but this difference had no statistical significance ( $P > 0.05$ ).

*3.2.1.6. The prevalence and intensity of Spirocercca lupi infection by raising method (via stool test)*

**Table 3.15. The prevalence and intensity of *Spirocercca lupi* infection by dog raising method (via stool tests)**

| Method of raising             | Number of examined samples | Number of infected samples | Percentage of infection (%) | Intensity of infection |              |            |              |           |              |
|-------------------------------|----------------------------|----------------------------|-----------------------------|------------------------|--------------|------------|--------------|-----------|--------------|
|                               |                            |                            |                             | +                      |              | ++         |              | +++       |              |
|                               |                            |                            |                             | n                      | %            | n          | %            | n         | %            |
| Free range                    | 982                        | 234                        | 23,83 <sup>a</sup>          | 125                    | 53,42        | 75         | 32,05        | 34        | 14,53        |
| Confinement                   | 927                        | 59                         | 6,36 <sup>b</sup>           | 47                     | 79,66        | 10         | 16,95        | 2         | 3,39         |
| When confined when free range | 990                        | 193                        | 19,49 <sup>a</sup>          | 125                    | 64,77        | 48         | 24,87        | 20        | 10,36        |
| <b>Total</b>                  | <b>2899</b>                | <b>486</b>                 | <b>16,76</b>                | <b>297</b>             | <b>61,11</b> | <b>133</b> | <b>27,37</b> | <b>56</b> | <b>11,52</b> |

The results in table 3.15 show that when the dog was raised by different raising methods, the prevalence and intensity of the esophageal worm infection was different. In dogs raised in free range, the prevalence of esophageal worm (*S. lupi*) infection was the highest and the intensity of infection was the most severe of the three raising methods.

*3.2.1.7. The prevalence and intensity of Spirocercca lupi infection in different seasons of the year (via stool tests)*

**Table 3.16. The prevalence and intensity of *Spirocerca lupi* infection in different seasons of the year (via stool tests).**

| season       | Number of tested samples | Number of infected samples | Percentage of infection (%) | Intensity of infection |              |            |              |           |              |
|--------------|--------------------------|----------------------------|-----------------------------|------------------------|--------------|------------|--------------|-----------|--------------|
|              |                          |                            |                             | +                      |              | ++         |              | +++       |              |
|              |                          |                            |                             | n                      | %            | n          | %            | n         | %            |
| Spring       | 727                      | 128                        | 17,61 <sup>ab</sup>         | 71                     | 55,47        | 40         | 31,25        | 17        | 13,28        |
| Summer       | 744                      | 191                        | 25,67 <sup>a</sup>          | 98                     | 51,31        | 61         | 31,94        | 32        | 16,75        |
| Autumn       | 719                      | 102                        | 14,19 <sup>b</sup>          | 76                     | 74,51        | 21         | 20,59        | 5         | 4,90         |
| Winter       | 709                      | 65                         | 9,17 <sup>b</sup>           | 52                     | 80,00        | 11         | 16,92        | 2         | 3,08         |
| <b>Total</b> | <b>2899</b>              | <b>486</b>                 | <b>16,76</b>                | <b>297</b>             | <b>61,11</b> | <b>133</b> | <b>27,37</b> | <b>56</b> | <b>11,52</b> |

The results in table 3.16 show that dogs were infected with esophageal worms *S. lupi* in all of 4 seasons of the year. In particular, the prevalence of infection in dogs tested in the summer was the highest, followed by the one in the spring, the autumn and the lowest was in the winter.

### **3.2.2. Studying some characteristics of experimental *Spirocerca lupi* infection in dogs**

#### **3.2.2.1. The result of experimental *S. lupi* infection in dogs in Thai Nguyen**

2 experimental infections were carried out, in each infection, 5 dogs were used (150 *S. lupi* larvae/ dog) and compared with 3 control dogs that were not infected. The results showed that: 100% of the experimental dogs were infected with esophageal worms *S. lupi*, in the control groups *S. lupi* worms were not detected.

#### **3.2.2.2. Time period to complete the life cycle of *Spirocerca lupi* in dogs**

The results in table 3.18 show that: 100% of the experimentally infected dogs in both infections of Thai Nguyen began passing the *S. lupi* eggs in faeces after 126 - 135 days of infection. The number of eggs was passed out in experimental group 1 varied from 1125.6 to 1372.9 eggs/grams of stool, in experimental group 2 varied from 1025.6 - 1165.9 eggs/grams of stool. Thus, the time period for worm larvae of *S. lupi* developed into adult worms in the infected dog was 126 - 135 days. This is the time to complete the life cycle of the esophageal worms *S. lupi* in dogs.

#### **3.2.2.3. Clinical signs of dogs infected with *Spirocerca lupi***

The results in Table 3.19 show that the dogs infected with the esophageal worms *S. lupi* expressed symptoms such as anorexia, weakness, salivary, vomiting, difficulty swallowing, coughing, difficulty breathing. While the control dogs had no clinical signs, healthy and had good appetite.

Weight gain in experimentally infected dogs was relative less than that of control dogs despite the same care and nurturing conditions. Specifically, in both experiments revealed that before infection, the weight of dogs in the 2 experimental groups was relatively equal and there was no significant difference ( $P>0.05$ ). However, 5 months after infection, the



average weight of dogs in the 2 groups changed significantly ( $P<0.05$ ). In the infected group, the average weight of dogs increased from 3.62kg to 13.06kg (experimental phase 1) and from 3.42kg to 11.94kg (experimental phase 2); while the weight of dogs in the control group increased from 3.63kg to 15.43kg.

#### 3.2.2.4. Gross lesions found in dogs experimentally infected with nematode *Spirocerca lupi*

After 5 months of infection, the results of necropsy showed that parasitic adult *S. lupi* were found in esophageal nodules of 100% of experimentally infected dogs and *S. lupi* worms were found in the aorta of 30% of infected dogs. Number of worms varied from 5 to 14 individuals/dog. All of 10 dogs showed gross lesions, these lesions concentrated mainly in the esophagus, stomach, aorta, and lungs with different degrees. While dogs in the control group, parasitic *S. lupi* worms were not found and no lesions were detected.

The gross lesions in infected dogs that we observed were that 1 - 3 hard esophageal nodules from each dog inside which there was red liquid pus and *S. lupi* worms. There were nodules in the aorta containing immature *S. lupi* worms. The aortic wall had internal, rough pits. hemorrhagic gastritis, pneumonia and Pulmonary hepatisation occurred.

#### 3.2.2.5. Microscopic lesions found in dogs infected with nematode *Spirocerca lupi*

**Table 3.21. Microscopic lesions in dogs experimentally infected with nematode *S. lupi***

| Type of specimens | Number of studied specimens | Number of specimens with lesions | Percentage (%) | Main micropic lesions                                                                                                                                                                                                                                                                                                                                       |
|-------------------|-----------------------------|----------------------------------|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Esophagus         | 10                          | 10                               | 100            | The epithelial layer structure was destroyed, the buffer layer appeared many inflammatory cells (the inflammatory cells were mainly plasma cells and macrophages), the mucous glands were degenerated. In the structure of the muscular layer of the esophagus, there were many slices of worms in different layers, around the developing fibrous tissues. |
| Aorta             | 10                          | 7                                | 70,00          | The endothelium disappeared, the middle layer was fibrous, making the artery lumen narrow, the elastic fibers were degenerated into fibrous fibers resulting in <u>lost of the elasticity of the aorta.</u>                                                                                                                                                 |
| Stomach           | 10                          | 3                                | 30,00          | The epithelial layer structure was destroyed, the buffer layer appeared many inflammatory cells, the gastric mucosa and mucous glands were degenerated                                                                                                                                                                                                      |

|              |           |           |              |                                                                                               |
|--------------|-----------|-----------|--------------|-----------------------------------------------------------------------------------------------|
| Lungs        | 10        | 5         | 50,00        | Bronchial mucosa degenerated, peeled inflammatory cells infiltrated densely and proliferated. |
| <b>Total</b> | <b>40</b> | <b>25</b> | <b>62,50</b> | <b>The lesions mentioned above</b>                                                            |

### 3.2.2.6. *Changes in some hematological parameters of dogs experimentally infected with nematode Spirocerca lupi*

When dogs suffered from Spirocercosis caused by esophageal worm *S. lupi*, the red blood cell count was only  $4.57 \pm 0.10$  million/mm<sup>3</sup>, significantly lower than the control dogs ( $6.42 \pm 0.16$  million/mm<sup>3</sup>) ( $P < 0.05$ ). Platelet count of dogs infected with *S. lupi* ( $514.67 \pm 24.25$  thousand/mm<sup>3</sup>) increased significantly compared to control dogs ( $395.66 \pm 10.23$  thousand/mm<sup>3</sup>) ( $P < 0.05$ ). The average white blood cell count of healthy dogs was  $12.15 \pm 0.98$  thousand/mm<sup>3</sup>. In dogs with Spirocercosis, the white blood cell count increased to  $23.71 \pm 0.69$  thousand/mm<sup>3</sup>, a marked increase compared to healthy dogs,  $11.56$  thousand/mm<sup>3</sup> ( $P < 0.001$ ), especially in neutrophils and eosinophils.

## 3.3. Study on prevention and treatment of roundworm *S. lupi* for dogs

### 3.3.1. Study on the efficacy of some anthelmintics for treatment of Spirocercosis caused by *S. lupi* in dogs

#### \* Trial treatment for Experimentally infected dogs

All of 3 anthelmintics used in dogs were effective, but the activity of doramectin (0.5mg/kg B.W) was to remove *S. lupi* eggs faster than milbemycin oxime (0.3mg/kg B.W) and ivermectin (0.3mg/kg B.W). All of the three drugs showed no side effects in dogs.

#### \* Trial treatment of dogs infected with *S. lupi* in the field

From the above results, all of three drugs were safe for dogs, but the anthelmintic doramectin was higher effective than milbemycin oxime and ivermectin. Besides, doramectin is quite popular on the market today. Therefore, dog owners should use doramectin in treating Spirocercosis caused by *S. lupi* in dogs.

### 3.3.2. Study on prevention measures of Spirocercosis caused by *S. lupi* in dogs

#### 3.3.2.1. Study on the effect of killing eggs of nematode *Spirocerca lupi* by using some antiseptics

From the results in Tables 3.27 and 3.28, we have the following reviews: Nematode *S. lupi* eggs have high resistance to common antiseptics, little affected by Ca(OH)<sub>2</sub> solutions (traditionally called slaked lime) with concentrations of 5% and 10%, NaOH solution concentrations of 2%, 3% and 4%. Only powdered lime (CaO) is effective in destroying the eggs of this nematode. Therefore, in practice it is possible to collect dog faeces and treat with lime powder to kill eggs of *S. lupi* worms.

### 3.3.2.2. Trial of prevention measures of Spirocercosis caused by *S. lupi* in dogs

#### \* Prevalence and intensity of nematode *S. lupi* infection in dogs before the experiment

The results in Table 3.29 show that in experimental group 1, the pre-experiment prevalence of *S. lupi* infection was 10%, the prevalence in experimental group 2 was 16% and that in control group was 12%. Regarding the intensity of infection, dogs in the experimental and control groups were mainly infected with *S. lupi* with mild and moderate infection intensity, only 1 dog in experimental group 2 was infected with severe intensity. The difference in the prevalence of *S. lupi* infection among experiment 1, experiment 2 and control groups was not significant ( $P>0.05$ ).

#### \* Prevalence and intensity of nematode *S. lupi* infection in dogs after 2 months of experiment

The results after 2 months of experiment showed that there was different in the prevalence and intensity of esophageal worm infection in the experimental and control groups. In 2 experimental groups doramectin was administered, the prevalence and intensity of infection were decreased compared to before experiment. The application of preventive measures against Spirocercosis in dogs was initially effective, but not so obvious after 2 months of application.

#### \* Prevalence and intensity of nematode *S. lupi* infection in dogs after 5 months of experiment

After 5 months of applying preventive measures against Spirocercosis in dogs, there was a clear effect. In experimental group 1 when the drug was administered only 3 times in the first month, the preventive effectiveness after 5 months was not clear. However, the prevalence of infection did not increase further. In experimental group 1, after only 3 times of anthelmintic drug administered in the first month. The effectiveness was not apparent. However the prevalence of infection was not increased. In experimental group 2 after 3 times of anthelmintic drug administered in the first month. From the second month, anthelmintic drug administered once a month, after 5 months, 100% of affected dogs were recovered and there were no further infected cases. In the control group that no measures were applied, the prevalence and intensity of nematode *S. lupi* infection was the higher and more severe than those in experimental group 1 and group 2.

### 3.3.2.3. Recommendation of preventive measures against nematode *S. lupi* in dogs

Periodic deworming should be done

Cleaning cages, kennels, rearing areas. Treating faeces to kill pathogens.

Nurturing care

### **3.4. Developing an epidemiological map of the prevalence of nematode *Spirocerca lupi* in dogs in Thai Nguyen province**

The results in table 3.32 and the epidemiological map in figure 3.35 show that dogs in 30 communes, wards and towns in 6 districts and cities of Thai Nguyen province were infected with esophageal worm *S. lupi*, but the prevalence of infection was different in each locality. Epidemiological maps are important documents to help managers, specialists and animal producers enhance the care, rearing and management of dogs in localities within the scope of the study. In particular and other regions in general. Thereby, effective control and prevention measures can be carried out.

## **CONCLUSIONS AND RECOMMENDATIONS**

### **1. Conclusion**

#### **1.1. Some biological characteristics of nematode *Spirocerca lupi* in dogs**

##### **1.1.1. Results of necropsy and species identification**

The prevalence of esophageal worm *S. lupi* infection through necropsy of dogs in Thai Nguyen province was 18.29%, the intensity of infection was from 1 to 29 worms/dog.

Identification of species by using morphological methods and identification of species by molecular techniques, esophageal worms causing Spirocerosis in dogs in Thai Nguyen was identified as *Spirocerca lupi* (Rudolphi, 1809)

##### **1.1.2. The development of nematode *S. lupi* larvae in the intermediate hosts**

There were 2 species of dung beetles of Scarabaeidae family found in Thai Nguyen and infected with *S. lupi* larvae, namely belonged to *Ca. molosus* species (6.35%) and *Co. szechouanicus* (5.94%).

The prevalence of *S. lupi* larvae infection of *Ca. molosus* species infection after the experimental infection was 95% with 1 - 415 larvae/beetle, The prevalence of *S. lupi* larvae infection of *Co. szechouanicus* was 90% with 1 - 30 larvae/beetle. The development time period of L1 stage of *S. lupi* was 1 - 14 days; L2 stage was 9 - 16 days; L3 stage was 16 - 17 days; 23 - 25 days after experimental infection, L3 larvae were highly active, maybe this was the time when the larvae have best capacity to infect the definite host.

#### **1.2. Spirocerosis caused by nematode *Spirocerca lupi* in dogs**

### *1.2.1. Some epidemiological features of nematode Spirocerca lupi in dogs*

The prevalence of nematode *S. lupi* infection in dogs in 6 districts and cities of Thai Nguyen province through stool testing was 16.76%. The prevalence of infection in native dog breeds was 24.01%, in cross dog breeds was 19.3% and in foreign dogs was 3.59%. The prevalence of *S. lupi* infection increased gradually with dog age and did not depend on sex.

The percentage of dogs raised by free-range method infected with *S. lupi* worms was 23.83%, dogs raised in confinement was 6.36% and dogs raised in semi confinement was 19.49%. The prevalence of esophageal worm *S. lupi* infection in dogs in the summer was 25.67%, higher than that in other seasons of the year.

### *1.2.2. Some characteristics of Spirocercosis caused by nematode S. lupi in dogs*

The time period to complete the life cycle of *S. lupi* worms in dogs was 126 - 135 days. Dogs infected with *S. lupi* worms manifested with anorexia, weakness, salivary, vomiting, difficulty swallowing, coughing, difficulty breathing, fever, diarrhea, and weight loss. The gross lesions caused by *S. lupi* was concentrated mainly in the esophagus, stomach, aorta, lungs with different extents.

Microscopic lesions such as: esophageal epithelial layer structure was destroyed, many macrophages, plasma cells, and fibrous tissues appeared. Aortic fibrosis, loss of elasticity, ...

Dogs infected with esophageal worms have a markedly decreased red blood cell count; increased white blood cell counts, platelets; especially increased neutrophils and eosinophils compared to controls.

### ***1.3. Prevention and treatment of Spirocercosis caused by nematode Spirocerca lupi in dogs***

Doramectin 0.5 mg/kg B.W was effective against nematode *S. lupi* in dogs and was safer than ivermectin and milbemycin oxime. This anthelmintic drug should be used to remove *S. lupi* in dogs in the locality.

Lime powder to kill esophageal worm eggs effectively; solution of  $\text{Ca(OH)}_2$  5, 10%, NaOH 2 - 4% was almost not effective on killing esophageal worm eggs.

## **2. Recommendation**

Widely applied measures to prevent Spirocercosis in dogs in Thai Nguyen province and neighbouring provinces: in order to minimise the risk of disease, and contribute to improving the health of dogs.

Doramectin should be administered in treating Spirocercosis in dogs, and powdered lime should be used to treat dog feces.

The Sub-Department of Animal Husbandry and veterinary, and fishery should use the map of endemic *pirocercosis* caused by nematode *Spirocerca*

*lupi* in dogs in localities in the surveillance and prevention of Spirocerosis in dogs.