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## LIST OF PAPERS PUBLISHED RELATING TO DISSERTATION

1. **Tuyen N. V.**, Lan N. T. K., Doanh P. N (2019), "Morphological and molecular characteristics of adult worms of *Gnathostoma* Owen, 1836 (Nematoda) collected from domestic pigs in Dien Bien Province, northern Vietnam", *Folia Parasitologica*, 66 : 010.
2. **Nguyen Van Tuyen**, Nguyen Thi Kim Lan (2019), "Situation of *F. buski* infection in indigenous pigs in Dien Bien provine", *Journal of Veterinary Science and Technology*, 26 (7) : 74 - 79.
3. Nguyen Thi Kim Lan, **Nguyen Van Tuyen**, Nguyen Thi Ngan (2020), "Some epidemiological characteristics of lungworm disease in indigenous pigs in Dien Bien Dong and Muong Ang districts, Dien Bien province", *Journal of Veterinary Science and Technology*, 27 (5) : 66 - 73.
4. **Nguyen Van Tuyen**, Nguyen Thi Kim Lan, Nguyen Thi Ngan (2020), "Study on clinical and pathological characteristics of lungworm disease in indigenous pigs in Dien Bien province", *Journal of Veterinary Science and Technology*, 27 (7) : 59 - 66
5. **Tuyen N. V.,** Lan N. T. K., Doanh P. N (2021), Molecular phylogenetic relationships of *Metastrongylus* nematodes with emphasis on specimens from domestic pigs in Vietnam, *Journal of Helminthotogy*, 95, e52, 1 - 6.
6. **Nguyen Van Tuyen**, Nguyen Thi Kim Lan, Nguyen Thi Ngan, Pham Dieu Thuy (2021), "Prevalence of *Macracanthorhynchus hirudinaceus* infection in indigenous pigs at Dien Bien province", *Journal of Veterinary Science and Technology*, 28 (6) : 67 - 74.

# INTRODUCTION

## 1. The urgency of the dissertation

Pig farming occupies a very important position in the livestock industry in countries in the world as well as in Vietnam. Currently, the development of pig farming in our country is facing many difficulties in disease, in addition to infectious diseases, pigs are also susceptible to parasitic diseases. Statistics show that more than 50 species of parasites that cause disease in pigs have been detected (Pham Sy Lang et al., 2006). Parasitic diseases have affected the growth of pigs, resulting in increased feed consumption, reduced weight gain, and costs such as drugs for treatment, and nursing care compared to healthy pigs. Not only that, parasitic helminths cause damage in pigs but also open the way for many types of bacteria and viruses to invade and cause disease. Among them, there are parasitic lungworm species that cause diseases in the pigs.

Lungworm disease is caused by some nematode species of the genus *Metastrongylus*. Lungworms cause damage to the respiratory tract, bronchitis, pneumonia, reducing weight gain compared to healthy pigs and are easy to die if not treated in time (Nguyen Thi Kim Lan, 2012; Heinz Mehlhorn, 2016).

Dien Bien is a mountainous province in the Northwest region of Vietnam. According to statistics, currently there are over 290,000 pigs in the province (GSO, 2021), of which indigenous pigs are commonly raised with a few pigs in households. Indigenous pigs can stand poor farming conditions, getting few diseases, and their meat is delicious. However, due to the difficult economic conditions, knowledge of people is low, pig raising in the province is still in extensive farming, making use of the free-range method, and the application of advanced technology in livestock production is still limited. Therefore, pigs raised in Dien Bien province are susceptible to helminths, especially pìg lungworms.

Stemming from the urgent need of husbandry practice and disease prevention for indigenous pigs in Dien Bien province, we carried out the project: “Study on gastrointestinal and respiratory helminth infections in indigenous pigs in Dien Bien province, characteristics of lungworm infection caused by *Metastrongylus* spp. measures of prevention and treatmentof the disease”.

## 2. Objectives of the project

Identification of species composition, distribution, situation of helminth infections in gastrointestinal and respiratory tracts, characteristics of lungworm infection caused by *Metatrongylus* spp in indigenous pigs in Dien Bien province. Identification of risk factors of lungworm infection in indigenous pigs according to pig farming methods in Dien Bien province. From this to recommend effective measures for prevention and treatment of helminthic infection in general and lungworm infection in particular in indigenous pigs in Dien Bien province.

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

**3.1. Scientific significance**

The project is a systematic study on the status of helminth infection in the gastrointestinal and respiratory tracts of indigenous pigs in Dien Bien province; epidemiological characteristics, biological life cycle, pathological and clinical characteristics of lungworm infection; From this, there is a scientific basis to develop a highly effective procedure of prevention and treament of lungworm infection in pigs.

**3.2. Practical significance**

The results of the dissertation are the scientific basis for recommending pig producers in Dien Bien province to apply effective measures in order to minimise harmful effects caused by helminths in pigs in general and limit the rate of lungworm infection fin pigs in particular, contributing to the development of pig farming in a sustainable way.

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

- Species composition and infection status of helminth in gastrointestinal and respiratory tracts in indigenous pigs were identified.
- A species of nematode belonging to the genus Gnathostoma was detected completely new in genetic characteristics compared to those published on the international gene bank was determined.
- Species *M. elongatus* and *M. pudendotectus* causing lungworm infection in indigenous pigs was identified by using molecular biology techniques.
- Identification of two earthworm species - the intermediate host of pig lungworm was *Pontoscolex corethrurus* and *Pheretima aspergillum*, the time period to complete the life cycle of lungworm in experimentally infected pigs was determined as 31 - 36 days.

- Effective measures of prevention and treatment of lungworm (*Metastrongylus* spp.) infection have been, recommended and widely applied in pig farming households in Dien Bien province and other provinces.

**5. The dissertation structure**

The dissertation consists of 115 pages (not included appendices and list of references), 3 pages of introduction, 28 pages of document overview, 17 pages of study objects, materials, pages of contents and methods of study, 65 pages of results and discussion, 02 pages of conclusions and recommendations, The dissertation includes 32 tables, 107 colour pictures and charts and 134 references, of which 35 in vietnamese, 98 in foreign languages, 01 from internet. (52 references are from 2016 - 2021).

## Chapter 1
## OVERVIEW OF DOCUMENT

Nematode class has the richest and most diverse number of species (Pham Sy Lang et al., 2006). Up to now, 5 species of pig lungworm have been discovered, of which *M. elongatus, M. pudendotectus* and *M. salmi* are wider distributed than 2 species of *M. asymetricus* and *M. confusus*. *M. asymetricus* species is restricted in Europe and East Asia, while *M. confusus* species is found only in Africa and Europe. Pham Van Khue (1982), Phan Dich Lan et al. (2005), Truong Vinh Yen (2008) reported that, in Vietnam, there are 3 species of nematodes belonging to the genus *Metastrongylus* parasitizing in pig trachea, including: *M. elongatus*, *M. pudendotectus* and *M. salmi* of which *M. salmi* species is capable of causing disease in humans (Calvopina et al., 2016).

At different ages, the rate of lungworm infection is different. (Giovanni et al., 2016; Nguyen Huu Hung, 1997) all said that lungworm infection in pigs was highest at 3 - 6 months of age, then gradually decreased. However, Phan Dich Lan et al. (2005) stated that: the prevalence of lungworm infection increases with pig age, the older the pig, the higher the prevalence of lungworm infection. In addition, weather, climate, soil, pig farming practices have great influence on the distribution and development of lungworm species in pigs.

The life cycle of the lungworm (*Metastrongylus* spp) involved. with intermediate hosts includingsome earthworm species. When pigs with mild lungworm infection, there are no obvious symptoms, the animals

still eat normally but gradually lose weight. When severe infected, animals are characterized by poor appetite, emaciation malnutrition, coughing, difficulty breath, especially in the early morning and evening. Without timely treatment, good management and care, the mortality rate is about 15 - 30% (Nguyen Thi Kim Lan, 2012).

Histologically, the lung tissue becomes hard, solid and gray or white in colour, emphysema in the lung, pulmonary edema and hemorrhage; eosinophilia, fibrosis, and enlarged lymph nodes (Patra et al., 2013; Lechner et al., 2016).

In order to prevent lungworm disease in pigs, it is necessary to avoid contact with earthworms - the intermediate host of the disease (Schwartz, 2004; Stewart and Hoyt, 2006) and doramectin, moxidectin and albendazol are used tin treating the disease (Calvopina et al., 2016).

# Chapter 2
# OBJECTS, MATERIALS, CONTENTS AND METHODS OF STUDY

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

### *2.1.1.Objects of study*

- Indigenous pigs raised in 5 districts of Dien Bien province.
- Parasitic helminths in the gastrointestinal and respiratory tracts of indigenous pigs.
- Lungworm infection caused by *Metastrongylus* spp. in indigenous pigs.

### *2.1.2. Time and places of study*

* Study time period: 2017 - 2021.

* Places of study:

- Indigenous pig raising households in 5 districts of Dien Bien province.
- Sample testing places: laboratory of Dien Bien Technical Economic College; Institute of Ecology and Biological Resources; National Institute of Hygiene and Epidemiology; Thai Nguyen Central general Hospital; Dien Bien Provincial General Hospital; Thanh Hai General Clinic, Dien Bien province; Can Tho University.

## 2.2. Study Materials

### *2.2.1. Animals and types of study samples*

* Animals for study: pigs raised in 5 districts of Dien Bien province

* Study samples: stool samples from pigs of every age; helminth samples collected through surgical examination, earthworm samples; blood samples from pigs experimentally infected with lungworms and healthy pigs; trachea, bronchi, lung, heart, liver, and intestinal samples of pigs infected with lungworm and coresponding samples from healthy pigs were collected

***2.2.2. Instruments, equipment and chemicals*** needed for study parasites, molecular biology, gene sequencing were used.

**2.3. Study contents**

***2.3.1. Study helminth composition and characteristics of helminth infections in gastrointestinal and respiratory tracts in indigenous pigs in Dien Bien province***

- Identification of parasitic helminth composition in the gastrointestinal and respiratory tracts of indigenous pigs in Dien Bien province.

- Identification of the characteristics of helminth infections, in gastrointestinal and respiratory tract in indigenous pigs in Dien Bien province.

***2.3.2. Study on lungworm (Metastrongylus* spp.*) and disease caused by lungworm in indigenous pigs***

*2.3.2.1. Identification of lungworm collected from pigs*

- Identification of pig lungworm species by morphological technique.

- Evaluation of pig lungworm species by molecular biology technique

*2.3.2.2.Epidemiological characteristics of lungworm (Metastrongylus* spp.*) in indigenous pigs*

** Through surgical examination:*

- Prevalence and intensity of lungworm infection in indigenous pigs in Dien Bien province.

- Prevalence and intensity of lungworm single infection and mixed infection

** By stool test:*

- Prevalence and intensity of lungworm infection in indigenous pigs in Dien Bien province.

- Prevalence and intensity of lungworm infection associated with pig age, rearing practices, topographical region and season of the year.

*2.3.2.3. Study on risk of lungworm infection in pigs in various pig farming methods practices.*

*2.3.2.4. Study on earthworm species - the intermediate host of lungworms*

- Identification of earthworm species collected from Dien Bien province.

- Identifying earthworm species as intermediate host of lungworms in pigs by determining the prevalence and intensity of lungworm larvae infection in earthworms.

*2.3.2.5. Study on pathological and clinical characteristics of lungworm infection in experimentally infected indigenous pigs and naturally infected pigs in the field.*

*2.3.2.6. Study on measures of prevention and treatment of lungworm infection in indigenous pigs in Dien Bien province*

*2.3.2.7. Recommendation of measures of prevention and control of lungworm infection in indigenous pigs in Dien Bien province*

**2.4. Methods of study**

***2.4.1. Methods of studying helminth composition and characteristics of helminth infections in gastrointestinal and respiratory tracts in indigenous pigs in Dien Bien province***

- Using cross-sectional descriptive epidemiology (Nguyen Nhu Thanh et al., 2001).

- Collecting samples by using multi-stage cluster sampling method.

- Using the method of complete helminthological dissection, by Skrjabin (1928), fixation speciments and identifying helminhts according to the key of Nguyen Thi Le et al. (1996); Nguyen Van Duc et al. (2017). For lungworms and suspected species such as the stomach worm (*Gnathostoma* sp.) was assessed by using molecular analysis, using the ITS2 insertion sequence of the nuclear gene and part of the cox1 gene of the mitochondrial genome for analysis. Identification of infection was performed by counting the number of parasitic helminths in pigs

- Testing feces for worm eggs was performed by using cherbovick method and Benedek sedimentation method (1943). Intensity of helminth infection was determined by counting the number of eggs in 1 gram of feces using the Mc Master counting chamber.

***2.4.2. Methods of studying lungworm (Metastrongylus* spp.*) and lungworm disease in indigenous pigs***

*2.4.2.1. Identification of parasitic lungworm species in indigenous pigs in Dien Bien province*

** Method of identifying pig lungworm species by morphology*

According to the identification key of Nguyen Thi Le et al. (1996), Nguyen Van Duc et al. (2017), based on the morphological characteristics, size and structure of adult worms combined with observing the microscopic structure of lungworms under the scanning electron microscope FE-SEM S4800.

* Method of identifying lungworm species in pigs by using PCR and sequencing techniques

Using molecular biology techniques to confirm 6 lungworm samples collected from indigenous pigs that were identified by morphological techniques. of which, *M. elongatus* species (3 samples) and *M. pudendotectus* (3 samples). The above worm samples were preserved in 96% Ethanol untilthe species was identified by molecular analysis to species. Using the ITS2 insert (second internal transcribed spacer) of the nuclear gene and a part of the *cox*1 gene (Cytochrome c oxidase subunit 1) of the mitochondrial genome to analyze the 6 sample mentioned above.

***DNA extraction.***

***Performing PCR reaction***

ITS2 and *cox*1 sequences were cloned by using standard PCR with primers 3S, A28 for ITS2 sequences and primers JB3, JB4 for cloning the *cox*1 gene fragment (Bowles et al., 1993).

**Table 2.1. Primer sequence corresponding to the gene fragment**

| Gene fragment | primer | Primer sequence (5' - 3') |
|---|---|---|
| ITS2 | 3S | 5'-AGC GGT GGA TCA CTC GGC TCG TG- 3' |
| | A28 | 5' GGG ATC CTG GTT AGT TT CTT TTC CGC-3' |
| CO1 | JB3 | 5'-TTT TTT GGG CAT CCT GAG GTT TAT-3' |
| | JB4 | 5'-TAA AGA AAG AAC ATA ATG AAA ATG-3' |

Analyzing, comparing, drawing phylogenetic tree by using Maximum Likeklihood method on MEGA 7.0 software (Tamura et al., 2013).

*2.4.2.2. Method to determine epidemiological characteristics of lungworm (Metastrongylus* spp.*) in indigenous pigs through stool testing*

The method of testing to determine the rate and intensity of parasitic lungworm infection in indigenous pigs was carried out according to the method described in section 2.4.1.

*2.4.2.3. Methods of studying the risk of lungworm infection in*

*ndigenous pigs according to pig farming methods*

Using relative risk (RR) and Odds ratio (OR) to compare the risks mentioned above.

*2.4.2.4. Methods of studying earthworms - intermediate hosts of pig lungworms*

- Method of identification and classification of earthworms was based on documents of Thai Tran Bai (1983), Do Van Nhuong (1995) and Nguyen Thanh Tung (2013). On the basis of identification, the earthworm species was identified as an intermediate host used for infection of pigs.

- Method of determining the prevalence of lungworm larvae in earthworms each individual earthworm obtained:was dissected,and their internal organs were pressed onto a glass slide and observed under the microscope at 100-400x magnification. Identifcation and distinguishing of lungworm larvae from some of other larvae in earthworms was based on description and identification through characteristics such as parasitic sites and parasite shape which werw described by Nguyen Duc Tan (1996).

*2.4.2.5. Study on pathological and clinical characteristics of lungworm infection in indigenous pigs*

*a. Study on experimentally infected pigs*

** Earthworms were collected for infecting pigs*

Earthworms were collected from places where no pigs were raised and the earthworm species was tested and accurately identified as the intermediate host of lungworms in pigs. Several individual worms were examined to ensure that the collected worms were not contaminated with lungworm larvae and eggs. Then 320 earthworm inviduals were reared in boxes, each box was 60 x 30 x 20 cm in size, soil and humus were put into the box.

** Cultivation of lungworm larvae*

Lungworms were collected from nsurgical examination of pigs ` at households and slaughterhouses. All survival worms were placed in a glass containing a 0.9% saline solution. Under laboratory conditions, with normal temperature, lungworms can survive in physiological saline for about 3-5 days. During this time, the female lungworm continued to sed a large number of eggs into the worm culture environment. Large numbers of eggs was collected from the sediment. The obtained lungworm eggs were spread evenly on the soil surface in

the earthworm rearing box. Moisture was monitored daily, not let the soil get too dry or too wet.

The lungworm larvae were examined in earthworms every 3 days under the microscope. Each time 2 earthworms were taken, washed, cut along the body from the head of the earthworms,their internal organs were pressed onto a slide and were observed under the microscope. The pathogenic larvae were used to infect pigs. Pathogenic lungworm larvae were crescent, of which some locations were thick like buttons, the top of the tail was blunt.

** Collecting pathogenic larvae from earthworms .*

After eggs and lungworm larvae were used to infect earthworms, if the lungworm larvae in the earthworms examined to be seen as crescent shape, of which some locations were thick like buttons, the top of the tail was blunt, the earthworms were collected. All infected earthworms were crushed and put in a glass containing physiological saline solution with a ratio of 1:10 (1 gram of earthworms: 10ml of 0.9% NaCl solution), some marbles were put into the glass and then placed in a shaker for about 30 minutes to dissolve the earthworms, and filtered through a filter with a diameter of 0.5mm to remove raw residues. Filtered water was centrifuged at 2500 rpm for 2 minutes, the part of water above was decanted and the sediment was collected. The sediment was put into a test tube containing Ringer's solution, and continued to centrifuge for 2 times to collect sediment, the number of larvae present in the sediment was counted and then the larvae were concentrated in a glass containing 50 ml of physiological saline solution, ensuring that there were about 100 pathogenic lungworm larvae in 1 ml, from which the dose of pathogenic larvae to infect pigs was calculated.

** Method of monitoring the time period to complete the life cycle of lungworms in experimentally infected pigs*

After infection, each pig was raised separately in a cage, completely isolated, eating feed without anthelmintics for prevention and treatment of helminths. The control group (5 animals) was also reared under similar conditions.

From day 21 post-infection onward, pig feces was examined daily until lungworm eggs were detected in feces. The clinical manifestations of pigs was monitored. The time to start appearing lungworm eggs in

the infected pig's feces was the time to complete the life cycle of the worms in the pig's body.

** Study on pathological and clinical maniffestation of experimentally infected pigs*

*- Clinical signs of infected pigs were observed.*

Monitoring the growth rate of pigs was performed by weighing pigs in both groups of pigs before and 63 days after the experiment. After appeareing lungworm eggs in the feces of the experimentally infected pigs and the pig's body temperature, physical condition, movenment, eating condition, hair coat were observed, and neurological status were monitored. The same was observed in pigs in the control group.

*- Blood test to determine the change in white blood cell count of infected pigs and control pigs.*

*Sampling method:* After testing, if lungworm eggs in the feces of experimentally infected groups of pigs at the same time of rearing.

*Test method:* Leukocyte parameters such as white blood cell count, leukocyte formula (ratio of granulocytes, lymphocytes and large monocytes) were performed on the BK-6310 hematology analyzer.

** Methods of determining the gross lesions*

Surgical examination was carried out in infected and control pigs at 64 and 65 days post-infection by using non-complete dissection method. Along the respiratory tract was thorouthly observed with the naked eye and magnifying glass, lungworms were searched in the bronchi. Areas with typical gross lesions were photograph taken. Lung, liver, heart, trachea and intestinal specimens of infected pigs were collected and soaked in 10% formol solution to make microscopic specimens.

** Methods of identifying microscopic lesions*

Determination of microscopic lesions by using the method of making tissue specimen according to the paraffin impregnation, Hematoxilin - Eosin staining was performed,the results under an optical microscope with magnification 200 - 400 times were read. Typical microscopic changes on the slide was photograph taken.

*b. Study on naturally infected pigs in the field*

Clinical manifestations were monitored and gross lesions were identified like in those in experimentally infected pigs

*2.4.2.6. Study on meassures of prevention and treatment of lungworm infection in indigenous pigs raised in Dien Bien*

Evaluation of the efficacy and safety of 3 types of anthelmintics including tetramisol (15 mg/kg B.W), ivermectin (0.3 mg/kg B.W), fenbendazol (5 mg/kg B.W) on infected pigs, in narrow and large scale in the study localities.

*2.4.2.7. Recommendation of meassures of prevention and control of lungworm infection in indigenous pigs in Dien Bien province.*

Based on the results of the study on the epidemiological characteristics and the results of the anthelmintic trials mentioned above to recommend integrated prevention measures for lungworm infection in indigenous pigs in Dien Bien province.

***2.4.3. Data processing methods***

The obtained data were processed by the biological statistical method of Nguyen Dinh Hien and Do Duc Luc (2017), using Excel 2007, Epicalc 2000 and Minitab 16.0 software.

## Chapter 3
## RESULTS AND DISSCUSSION

### 3.1. Species composition and characteristics of gastrointestinal and respiratory helminth infections,

***3.1.1. Composition of parasitic helminth species in indigenous pigs***

**Table 3.1. Species composition and distribution of gastrointestinal and respiratory helminths in indigenous pigs in Dien Bien province**

| List of helminth species | Parasitic site | Study places (district) | | | | | Frequence of occurrence (%) |
|---|---|---|---|---|---|---|---|
| | | Dien Bien | Dien Bien Dong | Muong Cha | Muong Ang | Muong Nhe | |
| *Fasciolopsis buski* | Small intestine | + | + | + | + | + | 100 |
| *Ascaris suum* | Small intestine | + | + | + | + | + | 100 |
| *Trichocephalus suis* | Caeca | + | + | + | + | + | 100 |
| *Ascarops strongylina* | Stomach | - | + | + | - | + | 60,0 |
| *Gnathostoma* sp. | Stomach | + | + | + | - | + | 80,0 |
| *Metastrongylus elongatus* | Trachea | + | + | + | + | + | 100 |
| *Metastrongylus pudendotectus* | Trachea | + | - | + | - | + | 60,0 |
| *Macracanthorhynchus hirudinaceus* | Small intestine | + | + | + | + | + | 100 |
| **Total secies detected** | | **7** | **7** | **8** | **5** | **8** | |

8 parasitic helminth species were detected, in the gastrointestinal tract and respiratory tract of pigs in Dien Bien including: *F. buski, A. suum, T. suis, A. strongylina, Gnathostoma* sp., *M. elongatus, M. pudendotectus, M. hirudinaceus* of which *F. buski, A. suum, T. suis, M. elongatus, M. hirudinaceus* all appeared in all of 5 districts with 100% occurrence frequency.

Of eight helminth species detected, morphologically, *Gnathostoma* sp. was similar to *G. doloresi*, with a slight difference in the genital spines of the male worms. However, in terms of molecular biology, they were genetically distinct from *G. doloresi* and other *Gnathostoma* species in both ITS2 and *cox*1 sequences. The ITS2 sequence was 578 bp in length and the cox1 sequence was 393 bp in length. Morphological and biological data confirmed that *Gnathostoma* sp. in this study is a new species derived from pigs of Vietnam (the results of description in detail of identification by morphology and molecular biology of *Gnathostoma* spp. are presented in the main dissertation).

### *3.1.2. Characteristics of gastrointestinal and respiratory helminth infection in indigenous pigs*

*3.1.2.1. The prevalence of helminth infection in indigenous pigs in localities in Dien Bien province*

**Table 3.2. The prevalence of helminth infection in indigenous pigs in Dien Bien province**

(through dissection)

| Locality (district) | Number Number of pigs at examination surgery (pig) | Trematode | | Nematode | | Thorny-headed worms | |
|---|---|---|---|---|---|---|---|
| | | Number of infected pigs (pig) | Percentage (%) | Number of infected pigs (pig) | Percentage (%) | Number of infected pigs (pig) | Percentage (%) |
| Dien Bien | 273 | 12 | 4,40[a] | 163 | 59,71[c] | 10 | 3,66[d] |
| Dien Bien Dong | 198 | 4 | 2,02[ab] | 172 | 86,87[a] | 19 | 9,60[b] |
| Muong Ang | 226 | 9 | 3,98[a] | 152 | 67,26[b] | 8 | 3,54[d] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Muong Cha | 251 | 5 | 1,99[ab] | 212 | 84,46[a] | 17 | 6,77[c] |
| Muong Nhe | 215 | 2 | 0,93[b] | 195 | 90,70[a] | 26 | 12,09[a] |
| **Total** | **1.163** | **32** | **2,75** | **894** | **76,87** | **80** | **6,88** |

** Notes: in the vertical row, the prevalence of infection with different letters, the difference is statistically significant (P < 0.05)*

The results are shown in table 3.2: indigenous pigs raised in Dien Bien province were infected with 3 classes of helminths, of which pigs infected with nematode with the highest prevalence (76.87%),the second highest was the prevalence of thorny-headed worms (6.88%) and the lowest was the prevalence of trematode infection (2.75%).

**Table 3.3. Prevalence of helminth infection in indigenous pigs in Dien Bien province**

(through examination of faeces)

| **Locality** (district | **Number of pigs tested** (pig) | **Trematodes** | | **Nematodes** | | **Thorny headed worms** | |
|---|---|---|---|---|---|---|---|
| | | **Number of infected pigs (pig)** | **Percentage (%)** | **Number of infected pigs (pig)** | **Percentage (%)** | **Number of infected pigs (pig)** | **Percentage (%)** |
| Muong Cha | 372 | 8 | 2,15[ab] | 288 | 77,42[b] | 24 | 6,45[bc] |
| Dien Bien | 437 | 18 | 4,12[a] | 285 | 65,22[c] | 12 | 2,75[d] |
| Dien Bien Dong | 381 | 5 | 1,31[b] | 322 | 84,51[a] | 31 | 8,11[b] |
| Muong Ang | 394 | 14 | 3,55[ab] | 275 | 69,80[c] | 21 | 5,33[c] |
| Muong Nhe | 288 | 3 | 1,04[b] | 251 | 87,15[a] | 36 | 12,50[a] |
| **Total** | **1872** | **48** | **2,56** | **1421** | **75,91** | **124** | **6,62** |

** Notes: in the vertical row, the prevalence of infection with different letters, the difference is statistically significant (P < 0.05)*

The results are shown in table 3.3: the prevalence of infection of thorny headed worms and trematodes was lower than that of nematodes. The prevalence of trematodes infection was the highest in Dien Bien district and the lowest was in Muong Nhe district (4.12% and 1.04% respectively). Pigs raised in Muong Nhe district were

infected with the highest prevalence of nematodes, the lowest prevalence of infection was in Dien Bien district with (87.15%,and 65.22% respectively. thorny headed worms were found the highest prevalence in Muong Nhe district, the lowest was in Dien Bien district ( 12.5% and 2.75% respectively).

*3.1.2.2. The prevalence of helminth infections in indigenous pigs was age dependent*

The results are shown in table 3.4: the prevalence of trematode infection increased with age of pigs, pigs under 3 months to over 12 months of age were infected with 0.42%; 1.65%; 3.31% and 5.72% respectively. Pigs started being infected with spiny headed worms from 3 months of age or more, pigs over 6 months of age to 12 months of age were infected with 9,32% and pigs over 12 months of age were infected with the highest prevalence (9.32% and 17.17% respectively). Pigs under 3 months of age examined showed that they were not infected.

The prevalence of nematode infection tended to decrease gradually with pig age, the older the pig, the lower the infection. Particularly, the prevalence of nematode infection was the highest in pigs at the age of 3 - 6 months,the second highest was in pigs at the age of 6 - 12 months and the lowest was in pigs at the age of over 12 months (89.71%, 82.61% and 74.39% respectively).

*3.1.2.3. Prevalence of helminth infections in indigenous pigs related to pig farming practice*

The results are shown in table 3.5: the prevalence of helminth infections in the group of free range pigs was higher than that in the group of pigs raised by semi-confinement and confinement. Free-range pigs were infected with trematodes, nematodes and spiny headed worms at prevalence of 3.66%; 92.91% and 10.18% respectively which were much higher than those in confined pigs (the prevalence of infection was 0.55%, 43.09% and 0.83%, respectively). The difference was significant with $P < 0.05$.

*3.1.2.4. Prevalence of helminth infection in indigenous pigs related to the season of the year*

The results are shown in table 3.6: pigs raised in all of four seasons were infected with helminths, the prevalence of infection varied from 1.28 - 3.53%, 52.04 - 95.91% and 3.06 - 9.48% in trematodes nematodes and spiny headed worms respectively Prevalence of

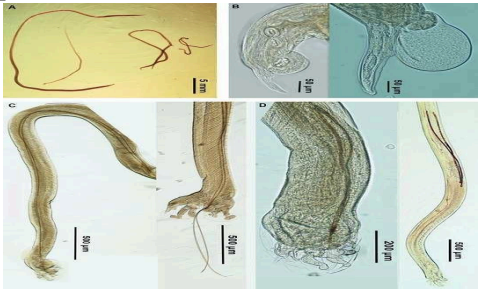
helminth infection in pigs in summer and autumn were higher than that in spring and winter.

### 3.2. Lungworm (*Metastrongylus* spp.) and lungworm disease caused in pigs by this species

#### *3.2.1. Identification of lungworm species collected from indigenous pigs*

*3.2.1.1. Identification of lungworm species by using morphological techniques.*

In Dien Bien province, two species of nematodes of genus parasitic *Metastrongylus* in the trachea of pigs were detected including *M. elongatus* (Gmelin, 1790) and *M. pudendotectus* (Vostokov, 1905) accounting for 98.86% and 1.14% respectively, no nematodes belonging to the species *M. salmi* were detected. (description in detail and photograph taken under the scanning electron microscope of these two worms are presented in detail in the main dissertation).



**Photograph of *Metastrongylus elongatus* and *Metastrongylus pudendotectus* taken from domestic pigs in VietNam.**

A. The whole female and male bodies of *M. elongatus* (left) and *M. pudendotectus* (right)
B. Female tails of *M. elongatus* (left) and *M. pudendotectus* (right)
C. The tail of the male *M. elongatus* contains two long genital spines (left) and protrudes outside the body (right).
D. The tail of the male M. *pudendotectus* contains two genital spines (left) and is retracted into the body (right).

*3.2.1.2. Identification of lungworm species by using molecular biology techniques*

Electrophoresis results of the PCR products, cloning of ITS2 and *cox*1 gene sequences of lungworm samples collected from pig in 5 districts of Dien Bien province showed that the ITS2 sequences of *M. elongatus* species were 100% resemble each other with. length of 564 bp, the ITS2 sequence of *M. pudendotectus* was not ablbe to be obtained because the sequence was messed up due to fungal infection.

The comparison results showed that the sequences of *M. elongatus* from Vietnam differed from the same species in Estonia and Uzbekistan and also from *M. apri* in Denmark by 2.9% (Table 3.10).

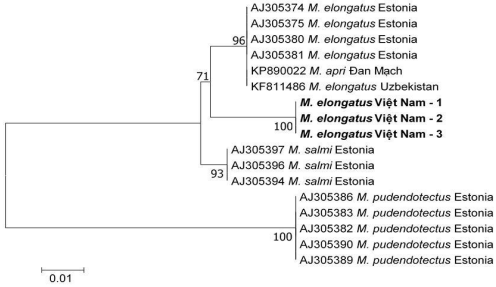



**Figure 3.11. Phylogenetic tree of *Metastrongylus* species was developed from ITS2 sequence by Maximum Likekliwood method**

On the phylogenetic tree constructed by the Maximum Likekliwood method (Figure 3.11)*, M. elongatus* samples from Vietnam formed a separate group, which was grouped together with *M. elongatus* from Estonia, Uzbekistan and *M. apri* from Denmark into a common branch with a bootstrap value of 71%. The sequences of the Estonian species (*M. salmi* and *M. pudendotectus)* also form ed independent clade. *M. elongatus* species is closely related to *M. salmi* species with a genetic distance of 2.9%, but relatively distant (13.3%) from *M. pudendotectus* (table 3.10).

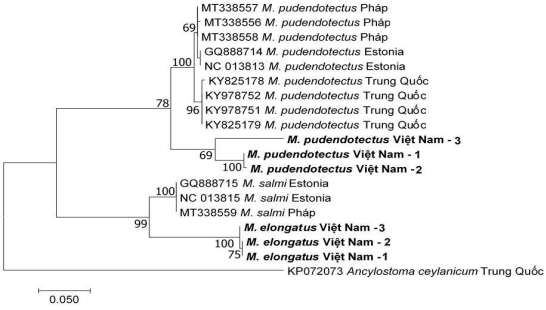



**Figure 3.11. Phylogenetic tree of *Metastrongylus* species was developed from *cox*1 sequence by Maximum Likekliwood method**

Regarding the *cox*1 gene sequence, both species are 393 bp in length. In GeneBank there are only sequences of 2 species: *M. pudendotectus* from Estonia, France, China and *M. salmi* from Estonia, France. The analysis results showed that all sequences of *M.*

*pudendotectus* from Vietnam, China, France and Estonia formed a common clade with a boostrap value of 78% (Figure 3.12). However, Vietnam sequences were by 4.0 - 5.8% different from those of the same species from China, France and Estonia, forming a separate group, while Chinese sequences are only 0.6 - 0.9% different from French and Estonian sequences. The sequences from three countries form a group with a 100% boostrap value (Table 3.11).

The sequences of *M. salmi* from Estonia and France form an independent clade. The Vietnam *M. elongatus* species sequences also form a separate clade, close to *M. salmi* species. The analysis results showed that *M. pudendotectus* of Vietnam has its own molecular characteristics. So far, there is no *cox*1 sequence data of *M. elongatus* species in Gene bank for comparison. These are the first *cox*1 sequences of *M. elongatus.*

### *3.2.2. Epidemiological characteristics of lungworm infection caused by Metastrongylus* spp. *in indigenous pigs*

*3.2.2.1. Prevalence and intensity of lungworm infection in indigenous pigs in Dien Bien province*

**Table 3.12. Prevalence and intensity of lungworm infection in indigenous pigs in Dien Bien province**

(through dissection)

| Locallity (district) | Number of pigs at examination surgery (pig) | Number of infected pigs (pig) | Percentage of infection (%) | Number of pigs infected only with lungworms (pig) | Intensity of worm infection/pig (min - max) |
|---|---|---|---|---|---|
| Dien Bien | 273 | 51 | 18,75[b] | 22 | 7 - 82 |
| Dien Bien Dong | 198 | 46 | 23,23[ab] | 17 | 7 - 83 |
| Muong Ang | 226 | 32 | 14,16[c] | 10 | 6 - 52 |
| Muong Cha | 251 | 67 | 26,67[a] | 27 | 13 - 109 |
| Muong Nhe | 215 | 60 | 27,91[a] | 22 | 10 - 113 |
| **Total** | **1.163** | **256** | **22,14** | **98** | **6 - 113** |

** Note: in the vertical row, the prevalence of infections with different letters, the difference is statistically significant (P < 0.05)*

The results are shown in table 3.12: there were 256/1163 pigs infected with lungworms, the average prevalence of infection was 22.14%, varying from 14.16 - 27.91%; In which, 98 pigs were examined and found only infected with lungworms. The differences in prevalence of infection in Muong Nhe and Muong Cha districts

compared to Dien Bien and Muong Ang districts was statistically significant (P<0.05). Pigs infected with lungworms with an intensity of 6 - 113 worms/pig. In which, pigs raised in Muong Nhe district were infected with the highest intensity (varying from 10 - 113 worms/pig) and the lowest was in Muong Ang district (6 - 52 worms/pig)

**Table 3.13. Prevalence and intensity of lungworm infection in indigenous pigs in Dien Bien province**

(through stool test)

| Place (district) | Number of tested pigs (pig) | Number of infected pigs (pig) | Percentage of infection (%) | Intensity of infection (egg/gram of faeces) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | < 500 | | 500 - 1000 | | > 1000 | |
| | | | | n | % | n | % | n | % |
| Dien Bien | 437 | 78 | 17,85[c] | 51 | 65,38 | 21 | 26,93 | 6 | 7,69 |
| Dien Bien Dong | 381 | 81 | 21,26[b] | 46 | 56,79 | 25 | 30,86 | 10 | 12,35 |
| Muong Ang | 394 | 47 | 11,93[d] | 31 | 65,96 | 14 | 29,79 | 2 | 4,26 |
| Muong Cha | 372 | 95 | 25,54[ab] | 54 | 56,84 | 27 | 28,42 | 14 | 14,74 |
| Muong Nhe | 288 | 86 | 29,86[a] | 42 | 48,84 | 30 | 34,88 | 14 | 16,28 |
| **Total** | **1.872** | **387** | **20,67** | **224** | **57,88** | **117** | **30,23** | **46** | **11,89** |

*Notes: in the vertical row, the prevalence of infections with different letters, the difference is statistically significant (P < 0.05)*

The results are shown in table 3.13: Pigs raised in 5 districts of Dien Bien province were all infected with lungworm, the overall infection rate was 20.67% (varying from 11.93% - 29.86%). Pigs infected with lungworms were the highest in Muong Nhe district (29.86%), followed by Muong Cha, Dien Bien Dong and Dien Bien districts with the infection prevalence of 25.54%; 21.26%; 17.85% respectively. The lowest was in pigs in Muong Ang district (11.93%). Pigs infected with mild intensity was the lowest (11.89%).

*3.2.2.2. Prevalence and intensity of single and mixed lungworm infections in indigenous pigs*

The results are shown in table 3.14: pigs infected with only *M. elongatus* species (239 pigs) or co-infected with both *M. elongatus* and

*M. pudendotectus* species (17 pigs), pigs infected with only *M. pudendotectus* species were not found. Pigs were only infected with *M. elongatus* species with an intensity varying from 7 - 113 (average 30.13 ± 20.03) worms per pig, co-infected with *M. elongatus* and *M. pudendotectus* species with an intensity from 2 - 82 (average 20.79 ± 19.88) worms/pig.

These results were consistent with previous studies in that: *M. elongatus* is the dominant species and often co-infects with *M. pudendotectus*, although the composition of *Metastrongylus* spp. and infection rates vary between sites (Nosal et al., 2010). In Florida, USA, a single case of *M. elongatus* infection and co-infection with *M. elongatus* and *M. pudendotectus* or *M. elongatus* and *M. salmi* was found (Forrester et al., 1982). This may suggest that *M. pudendotectus* infection may depend on the previous presence of *M. elongatus* species. Therefore, further investigations of the nematode (*Metastrongylus*) are needed to conduct in order to confirm this ecological feature.

*3.2.2.3. Prevalence and intensity of lungworm infection in in indigenous pigs was related to age.*

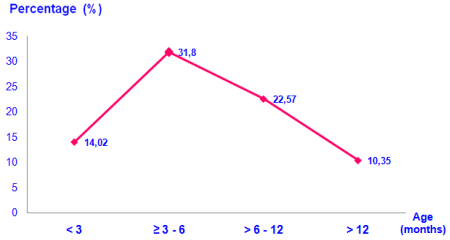



**Figure 3.16. Graph chart of prevalence of lungworm infection in indigenous pigs in different age**

The results are shown in table 3.15 and chart 3.16: Pigs of every age were infected. The prevalence of infection varied from 10.35% - 31.80%, in which, pigs at the age of 3 - 6 months infected with lungworm were the highest (31.80%), and this was also the age group with the highest intensity of severe infection (15.03%). Through statistical analysis, the differences among pig ages was significant ($P < 0.001$).

*3.2.2.4. The influence of seasons on prevalence and intensity of lungworm infection in indigenous pigs*

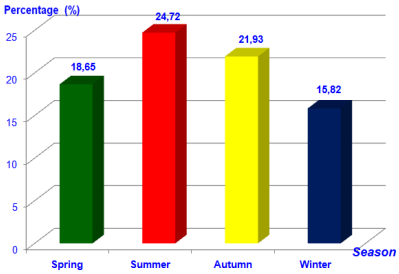



**Figure 3.17**. **Column chart of prevalence of lungworm infectione in indigenous pigs in different season**

The results are shown in table 3.16 and figure 3.17: the prevalence of lungworm infection varied among the seasons, in which, pigs infected with lungworms were the highest in summer (24.72%), followed by autumn (21.93%) and spring (18.65%), the lowest was in winter (15.82%).The intensity of infection varied from mild to severe, in which the intensity of severe infection was more concentrated in summer and autumn. Statistical analysis results showed that the difference between the seasons was cignificant ($P < 0.05$).

*3.2.2.5. Prevalence and intensity of lungworm infection in indigenous pigs in different geographic area*

The results are shown in table 3.17 and figure 3.18:

In terms of prevalence of infection: indigenous pigs raised in mountainous areas infected with the highest prevalence of lungworms (27.89%); The prevalence of lungworm infection in Pigs in semi-mountainous region were lower (19.05%), the lowest was in the flat area (8.29%). Statistical data showed that the difference was very significant ($P<0.001$).

In term of intensity of infection: in the high mountainous area, pigs infected with lungworms with number of more than 1000 eggs/gram of feces was the most common (14.54%).

*3.2.2.6. Prevalence and intensity of lungworm infection in indigenous pigs in different farming practice of pigs*

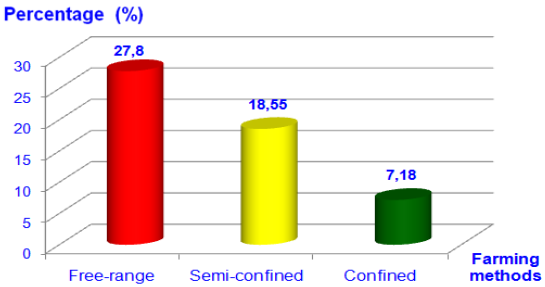



**Figure 3.19. Column chart of prevalence and intensity of lungworm infection in indigenous pigs in different farming practice of pigs**

The resuls are shown in table 3.18 and figure 3.19: the different farming practice of pigs influenced on prevalence of lungworm infection. The highest prevalence of lungworm infection (27.80%) was found in, free range pigs; followed by semi-confined pigs (18.55%) and the lowest was confined pigs (7.18%). There was a very significant difference in the prevalence of lungworm infection among the 3 farming practices ($P < 0.01$).

In term of intensity of infection: the percentage of pigs infected with the number of eggs per 1000 eggs/gram of feces was the highest in the free-range group of pigs (14.40%), followed by semi-confined group of pigs (8.47%) and the lowest was in the confined group of pigs (3.85%).

### *3.2.3. Risk of infection in different farming practices*

The results are shown in table 3.19: in free-range and semi-confined pigs, the risk of lungworms infection in pigs increased from 1.50 - 3.87 times compared to confined pigs. According to the OR index, pigs raised under these methods are 1.69 - 4.98 times more likely to be infected than noninffected.

### *3.2.4.* Intermediate host of lungworms

*3.2.4.1. Composition and distribution of earthworm species in Dien Bien province.*

**Table 3.20. Composition and distribution of earthworm species in Dien Bien province.**

| TT | *Earthworm species* | Locality (district) | | | | | Frequence of occurence (%) |
|---|---|---|---|---|---|---|---|
| | | Dien Bien | Dien Bien Dong | Muong Ang | Muong Cha | Muong Nhe | |
| **Family Rhinodrilidae Benham, 1890** | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Genus *Pontoscolex* Schmarda, 1861** | | | | | | |
| 1 | *Pontoscolex corethrurus* Müller, 1856 | + | + | + | + | + | 100 |
| **Family Megascolecidae family part Rosa, 1891** | | | | | | | |
| | **Genus *Amynthas genus* Kinberg, 1867** | | | | | | |
| 2 | *Pheretima aspergillum* Perrier, 1872 | + | + | - | + | + | 80,0 |
| 3 | *Amynthas robustus* Perrier, 1872 | + | + | + | - | + | 80,0 |
| 4 | *Amynthas morrisi* Beddard, 1892 | - | + | + | + | - | 60,0 |
| 5 | *Amynthas corticis* Kinberg, 1867 | + | + | - | + | - | 60,0 |
| 6 | *Amynthas plantoporophoratus* Thai, 1984 | - | - | + | + | - | 40,0 |
| 7 | *Amynthas pauxillulus* Gates, 1936 | + | - | - | - | - | 20,0 |
| | **Number of *earthworm species detected*** | **5** | **5** | **4** | **5** | **3** | |

*Notes: (+): detected; (-): undetected;*

The results are shown in table 3.20: 7 earthworm species of 2 genera belonging to 2 families were identified. 5/7 specie were found in Dien Bien, Dien Bien Dong and Muong Cha districts, 4/7 species were found in Muong Ang district and only 3/7 species were found in Muong Nhe district. These earthworms belong to the Rhinodrilidae and Megascolecidae families. The Amynthas genus with abundence in quantity including 6 species: *Amynthas plantoporophoratus, Pheretima aspergillum (Amynthas aspergillum), Amynthas morrisi, Amynthas robustus***,** *Amynthas corticis, Amynthas pauxillulus* belongs to the family Rhinodrilidae. Only one species of Pontoscolex genus belonging to the Rhinodrilidae family, *Pontoscolex corethrurus* was found.

*3.2.4.2. Prevalence of earthworm species in Dien Bien province*

The results are shown in table 3.21 and figure 3.27, out of 204 individuals of earthworms collected from 5 districts including Muong Ang, Muong Nhe, Dien Bien, Muong Cha and Dien Bien Dong, the species with the highest prevalence was *Pontoscolex corethrurus* (72.06%), followed by *Amynthas robustus* (13.73%). The species with a lower prevalence were *Amynthas morrisi* (4.90%), *Pheretima aspergillum* (3.43%), *Amynthas corticis* (3.43%), *Amynthas plantoporophoratus* (1.47%) and *Amynthas pauxillulus* (0.98%).

*3.2.4.3. Prevalence and intensity of lungworm larvae infection in earthworms*

Among the individuals of 7 examined earthworm species, of which 2 species whose lungworm larvae were found, namely *Pontoscolex corethrurus* and *Pheretima aspergillum*.

In *Pontoscolex corethrurus* species: there were 48/147 individuals carrying lungworm larvae, accounting for 32.65%, the average intensity of infection was 11.23 ± 1.31 larvae/individual, varying from 1 - 39 larvae.

In *Pheretima aspergillum* species there were 2/7 individuals carrying lungworm larvae, accounting for 28.57%, the average intensity of infection was 3.50 ± 2.50 larvae/individual.

In the remaining individuals including 5 species *Amynthas plantoporophoratus, Amynthas morrisi, Amynthas robustus, Amynthas corticis* and *Amynthas pauxillulus* whose lungworm larvae were not found.

Among the earthworm species collected in the study areas, one species was first detected as the intermediate host of pig lungworm in Vietnam (that was *Pheretima aspergillum* species).

### *3.2.5. Pathological and clinical manifestation of lungworm infection in indigenous pigs*

*3.2.5.1. Pathological and clinical characteristics of lungworm infection in experimentally infected pigs*

** Time period to complete the life cycle of lungworms experimentally infected pigs*

After infection, pigs began to shed eggs from 31 - 36 days it means, that, the time period to complete the life cycle of the lungworm (*Metastrongylus* spp.) in the body of indigenous pigs and was 31 - 36 days and did not depend on number of infective lungworm larvae more or less.

** Clinical development of pigs experimentally infected with lungworms*

Clinically, infected pigs expresed emaciation, dry skin, ruffled hair coats, nasal discharge, dry cough and prolonged cough; weight gain decreased by 30% compared to healthy pigs.

** Change in white blood cell count and while cell formula of pigs infected with lungworms*

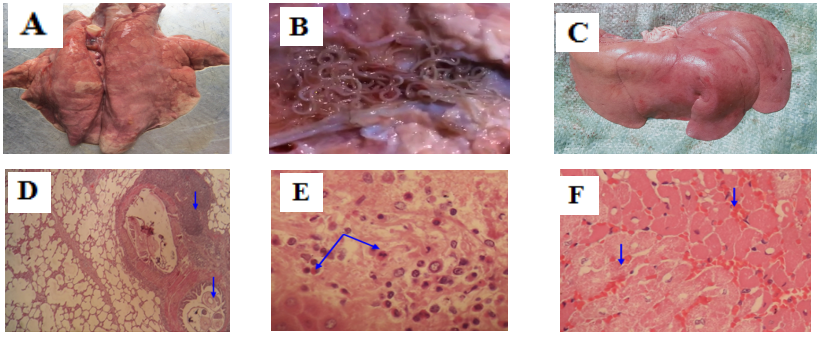
The results are shown in table 3.25: the average white blood cell count of pigs infected with lungworms (21.78 x $10^9$/l) was higher than that of control pigs (15.85 x $10^9$/l). This difference was statistically significant ($P<0.05$). The while cell formula of the group of pigs

experimentally infected with the lungworms (*Metastrongylus* spp.) also changed compared with the control pigs, the most obvious was the change in granulocytocytes, the percentage of granulocytes increased from 57.30% in the healthy pigs to 66.70% in infected pigs; The white blood cell count depended on the physiological state of the body, white blood cell counts increased when the body was inflamed and decreased when bone marrow failure occurred. eosinophils belong to the group of granulocytes, when eosinophils increases, the number of granulocytes also increases. Therefore, determining the white blood cell count is of great significance in the diagnosis of diseases in livestock.

* *Macroscopic and microscopic lesions of pigs experimentally infected with lungworm*

Macroscopic lesions of pigs infected with lungworm (*Metastrongylus* spp.) mainly occurred in the lungs with many white spots on the surface, the lung surface was not smooth, especially the edge and lower lobes of the lung; The lungs were hepatized, congestive and hemorrhagic; The lymph nodes of the the lungs are swollen, the trachea was filled with mucus, the liver was yellow, swollen and solid.

On histopathological specimens, there were inflammatory cell infiltration, congested lung tissues, dilated bronchi, many torn lung alveoli along with proliferation of lymphocytes, macrophages, and macrophages, especially eosinophils. There were large granuloma in the surface of the the liver lobes, the livers were solid and yellow. The hearts were enlarged and hemorrchagic.




*Notes:*

A.Petechiae in lung surface

D. Worms in alveolar lumen were, surrounded by chronic inflammatory cell infiltration.

| | |
|---|---|
| B. Parasitc lungworms in primary bronchi | E. Liver tissues were infiltrated with Inflammatory cells containing, many eosinophils |
| C. The surface of the liver was enlarrged, solid, and yellow. In colour. | F. Myocardial layers degenerated with heavy hemorrhage |

*3.2.5.2. Pathological and clinical characteristics of lungworm disease in naturally infected pigs in the field*

- In 126 pigs infected with lungworm, 31 of which showed symptoms, accounting for 24.60%. The clinical signs of pigs infected with lungworms in the field were similar to those experimentally infected with lungworms.

- The gross lesions in pigs infected with lungworm infection in the districts of Dien Bien province were similar to those in experimentally infected pigs. However, the extent of lesions in experimentally infected pigs was more clearly observed than in field infected pigs.

***3.2.6. Study on of prevention and cotrol measures of lungworm disease in nindigenous pigs.***

*3.2.6.1. Determination of efficacy and safety of anthelmintics in treating infected pigs*

3 anthelmintics were used including ivermectin (0.3 mg/kg B.W), tetramisol (15 mg/kg B.W) and fenbedazol (5mg/kg B.W) for experimental treatment of 9 pigs infectied with lungworms. The results showed that, all of 3 anthelmintics were high effective against lungworms, their efficacy was 100% and safe for experimental pigs.

*3.2.6.2. Determination of efficacy of anthelmintics administed in the field at narrow scale*

The results are showwn in table 3.31, all three anthemintics including ivermectin, tetramisol and fenbendazol were high effectve against lungworms of which tetramisol at dosage of 15mg/kg B.W was the highest effective (100 %), followed by ivermectin at 0.3 mg/kg B.W (96.0%) then fenbendazol at 5 mg/kg B.W (92.0%).

*3.2.6.3. Efficacious anthelmintics were used for treatment of lungworm infection in pigs in localities of Dien Bien province*

The results are shown in table 3.32: when using tetramisol at dosage of 15 mg/kg B.W (mixed with feed) to expel lungworms in 193 pigs, the eradication efficiency reached 95.34%. Teuscher E. et al. (1968), Pham Van Chuc et al (1986) considered, that Tetramisol administered at dosage of 15 mg/kg B.W was effective aganst lungworms in pigs.

Tetramisol could be mixed with feed or S.C. injected to expel adult worms.

***3.2.7. Recommendation of measures for prevention and treatment of lungworm infection inr pigs***

Anthelmintics against lungworms in pigs: ivermectin at a dosage of 0.3 mg/kg B.W, tetramisol at a dosage of 15 mg/kg B.W., fenbendazol at dosage of 5mg/kg B.W. to expel lungworms in pigs; manure was administered to destroy pathogens; construction and cleaning of pigsties, pig management and strengthening pig care and rearing were carried out.

## CONCLUSIONS AND RECOMMENDATIONS

### 1. Conclusions

***1.1. Composition of helminth species and characteristics of helminth infections in indigenous pigs in Dien Bien province***

- There were 8 species of parasitic helminths in the gastrointestinal tract and respiratory tract of pigs in Dien Bien including *F. buski, A. suum, T. suis, A. strongylina, Gnathostoma* sp., *M. elongatus, M. pudendotectus, M. hirudinaceus*. In which, there was 1 species of nematodes belonging to the genus *Gnathostoma* which was completely new in terms of genetic characteristics compared to those published in international gene bank.

- Indigenous pigs infected with trematodes was 2.75%, infected with rnematodes was 76.87% and infected with spiny headed worms was 6.88% (through examination surgery); Through examination of stool speciments: infection of trematodes was 2.56%, nematodes was 75.91% and spiny headed worms was 6.62%;

- Pigs infected with trematodes and spiny headed worms gradually increased with age, rnematode infections tended to decrease gradually. There was much higher prevalence of infection of trematodes, nematodes andspiny headed worms in free-range pigs than that of pigs in semi-outdoor; In summer and autumn, pigs infected with helminths were higher than that in winter and spring. In the high mountainous areas, pigs were infected with helminths higher than that of other ecological regions.

***1.2. Metastrongylus spp. and lungworm disease in indigenous pigs***

** Results of identification of parasitic nematode species in pig lungs*

By using morphological techniques and molecular biology, parasitic nematodes in the lungs of indigenous pigs in Dien Bien province were identified as *M. elongatus* and *M. pudendotectus*.

* *Epidemiological features of lungworm infection in idigenous pigs*

- The prevalence of lungworm infection in indigenous pigs in Dien Bien province through examination surgery was 22.14%, the intensity of infection varied from 6 - 113 worms/pig; the prevalence through stool test was 20.67%, infection intensity was mainly at less than 500 eggs/gram of feces.

- The prevalence of endemic *M. elongatus* species was 20.55%, mixed of *M. elongatus* and *M. pudendotectus* was 1.46%. No single infection with *M. pudendotectus* species was detected.

- Pigs at 3 - 6 months of age were infected with the highest prevalence and intensity of lungworms, then gradually decreased; Pigs raised in summer and autumn, the prevalence of lungworm infection was 24.72% and 21.93%, higher than those in winter (15.82%) and spring (18.65%); the highest prevalence of lungworm infection was found in free-range pigs (27.80%) and the lowest was found in confined pigs (7.18%); The lower incidence of lungworm infection in flat terrain was found lower than that in the high mountainous area.

- 7 species of earthworms in Dien Bien were Identified of which, 2 species (*Pontoscolex corethrurus* and *Pheretima aspergillum*) were intermediate hosts of lungworms in pigs..The prevalence of earthworms carrying lungworm larvae was 24.51%, the average intensity of lungworm infection was 10.92 larvae/earthworm.

Free range and semi confinement of pig farming increased the risk of lungworm infection from 1.50 to 3.87 times compared to confinement of pig farming.

.* *Pathological and clinical characteristics of lungworm infection in indigenous pigs*

- The time period to complete the life cycle of lungworms in infected pigs was 31 - 36 days.

- Pigs infected with lungworms were characterized by emaciation, dry skin, dry cough and prolonged cough; weight gain decreased compared to healthy pigs.

- Gross lesions of pigs infected with lungworms (*Metastrongylus* spp.) mainly occurred in the lungs, with, solid tissues and gray or white in colour; emphysema, the alveolar lumen increased with fluid secrete caused by the process of inflammatory cell infiltration, congested lung

tissue, dilated bronchi, many torn lung alveoli along with proliferation of lymphocytes, macrophages, especially eosinophils, hypertrophy and hemorrhage of myocardia

- In pigs infected with lungworms there were increased white blood cell count, especially granulocytes, compared to that of control pigs.

** Prevention and control measures of of lungworm infection*

Ivermectin at dosage of 0.3 mg/kg B.W., tetramisol at dosage of 15 mg/kg B.W. and fenbendazol at dosage of 5 mg/kg B.W. all showed high anthelmintic efficacy and safety. Among them, tetramisol had the highest anthelmintic efficacy.

Integrated prevention and control measures of lungworm disease for indigenous pigs include 5 main measures could be applied.

**2. Recommendation**

Allow application of integrated measures for prevention and control lungworm infection in pigs in Dien Bien province and other mountainous provinces.