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Project title:

STUDY ON GASTROINTESTINAL HELTHMINTH INFECTION AND MONEZIOSIS CAUSED BY *Moniezia* spp IN GOATS IN BAC GIANG PROVINCE, PREVENTION AND TREATMENT OF THE DISEASE

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INTRODUCTION

1. The urgency of the project

Bac Giang is a province of midland and mountainous region, with an area of hills and mountains, wide grazing grounds, very convenient for the development of animal husbandry in general and goat farming in particular. On January 1, 2020, there were 28.694 goats in Bac Giang province (GSO, 2020). Goats are animals that are easily adapted to different living conditions, able to live and grow well in hot and humid climates. However, if goats are raised according to the free grazing method but the hygiene of the environment is not good, the goats may be susceptible to helminth infection.

Gastrointestinal helminth infection in general and tapeworm infection in particular cause great harm to goat farming in developing countries. Helminth infection results in health decline, poor growth , decreased weight gain, decreased milk production, fertility lowering and even death can occurs if disease in goat is severe (Torres - Acosta JFJ et al., 2012).

Parasitic tapeworms cause diarrhea, weight loss, intestinal obstruction, and even death. Tapeworm infection not only affects the development of the goat herds, decreasing economic efficiency, creating favourite conditions for infectious diseases to occur but also has a negative impact on the scale of developing goat production in different. mountainous areas for hunger eradication and poverty, reducion and transforming agricultural structure (Chikweto A. et al., 2018).

Up to now, study on curent status of gastrointestinal helminth infectione and Moniezia tapeworm infection in goat herds of Bac Giang province has not been paid attention to, so there are no effective measures for prevention of the disease.

From the urgent requirements of disease control, ensuring the health of the goat herds, contributing to improvement of the productivity of goat production in Bac Giang province, we carry out the project "study on gastrointestinal helminth infection and , tapeworm infection caused by Moniezia spp. in goats in Bac Giang province, prevention and treatment of the infection"

2. Objective of the project

Identification of some characteristics of gastrointestinal helminth infection in goats in Bac Giang province. Some characteristics of infection caused by *Moniezia* tapeworm spp. in goats in Bac Giang province. Developing prevenvention and treatment measures, to make contribution to limiting the consequences of *Moniezia* tapeworm spp in goats in Bac Giang

3. Scientific and practical significance of the project

3.1. *Scientific significance*

The project has provided scientific information on the situation of gastrointestinal helminth infection in goats in Bac Giang province; epidemiological, pathological and clinical characteristics of tapeworm disease, Thereby setting a scientific basis for developing highly effective prevention and treatment measures of tapeworm infection in goats**.**

3.2**.** *Practical significance*

On the basis of the the project finding, goat producers are recommended to apply the measures for prevention and treatment of helminth infection in general, and tapeworm infection in particular to limit the prevalence of helminth infections in goats and limit damage caused by tapeworms, to make contribution to the development of goat farming in Bac Giang province.

4. New contributions of the project

Parasitic tapeworm species M. expansa has been Identified in goats in Bac Giang province by PCR technique.

- 9 species of soil mites - intermediate host of M. expansa tapeworm are identified; It is determined that the time to complete the life cycle of the M. expansa tapeworm in goats is 47 - 48 days.

- Epidemiological mapping for tapeworm infection in goats in edemic areas in Bac Giang province ihas been developed.

5. The dissertation structure

The disertation consists of 115 pages (not included the list of references): 03 pages of introduction; 24 - pages of document overview; objects, materials, 21 pages of materials, contents and methods ; 64 pages of results and discussion of the study ; 03 pages of conclusion and recommendations. In the dissertation there are 33 tables, 22 charts and graphs, 90 colour pictures showing the results of the project. 148

documents have been consulted (of which 59 were published in the past 5 years).

Chapter 1

DOCUMENT OVERVIEW

1. Scientific basis of the projject

Gastrointestinal, healminths in ruminant animals in general and in goats in particular consist of many types. Skrjabin K. I. and Petrov A. M. 1977, Soulsby E. J. L. 1982, Nguyen Thi Le et al. 1996 showed that helminth parasites were found in goats included: *Fasciola hepatica, Fasciola gigantica, Eurytrema pancreaticum, Paramphistomum liorchis, Calicophoron cauliorchis, Calicophoron calicophorum, Ceylonocotyle scoliocoelium, Gatrothylax crumenifer, Carmyerus crumennifer, Fischoederius cobboldi, Moniezia expansa, Moniezia benedeni, Oesophagostomum columbianum, Trichocephalus axei, T. colubriformis, Cooperia pectinata, Haemonchus contortus.*

1.2. Study on gastrointestinal helminths and *Moniezia* infection in goats in the country and in the world

1.2.1 Studies in the world

The prevalence of helminth infections: in various geographical regions varied in different season: Velusamy R. et al. (2015), Jas R. et al. (2017), Sivajothi S. and Reddy B. S. (2018) all reported that the prevalence of helminth infections was highest in spring, lowest in summer while Bansal D. K. et al. (2015), Pal P. et al. (2017) stated that the prevalence of helminth infections was the highest in the summer, and the lowest in the winter. In addition, other factors such as age as well as goat raising methods also greatly affect the prevalence of gastrointestinal helminth infections in goats [Rabbi K.M.A et al. (2011), Shah H. et al. (2017)].

The identification of tapeworm species is significant in the prevention and treatment of tapeworm infection in goats. Previously, the identification of tapeworm species was mainly based on morphology of

the tapeworm. Nowadays, with the development of technology, the use of PCR technique for identifying tapeworm species has resulted in more accurate finding [Diop G. et al. (2015)].

When the small intestine of goats is parasitized by Moniezia tapeworm spp. in large quantities, goats exhibit stunted, bloated, ruffled hair coat, anemia, and neurological signs. Examination of goats at slaughter showed that the small intestine was distended, the number of parasitic tapeworms was hight, filling the small intestine and obstructing the intestinal lumen (Patil R. J et al., 2016, Maity M. et al., 2017).

To ensure the health of the goat herds, Bagde V. and Jumde P. (2015) used aqueous extracts from *butea frondosa* seed at dosage of 50 ml/goats for 3 days; Neem oil, (*azadicta indica*) at dosage of 1ml/goat/ 3 days exhibited high anthemitic efficaciy in treating tapeworm infection. Cedillo J. et al. (2015) reported that using aqueous extracts from willow tree at dosage of 40 ml/goat/day was efficacious for treatment of *Moniezia* tapeworm infection.

1.2.2. Study in VietNam

The hot and humid tropical climate in our country is very favourable for the existence and development of helminth parasites and helminth infections. Currently, goat husbandry is quite developed, but the problem of prevention of helminth parasites in goats has not been paid enough attention to.

A number of research works have been done and published in the past decades. That was the work of Nguyen Thi Kim Lan (1999) who studied the situation of helminth in Co goat herds of Thai Nguyen, Bac Can, Tuyen Quang and Cao Bang provinces. 12 species were identified and anthemintic drugs were used effectively by the author. In 2011, Nguyen Huu Hung published study results in goats of Tra Vinh province infected with 8 helminth species and their infection prevalence of these species.

Moniezia tapeworm infection in goats has caused significant economic losses to goat producers. However, studies on tapeworm infection in goats in our country and preventive measures for tapeworm in goats are very little applied.

Some authors have studied the pathology and clinical manifestation of goats infected with tapeworm and indicated that tapeworm infection in goats was characterised by emaciation, severe weakness due to nutritional loss, anemia, pale eye mucosa; and many tapeworm proglottids were found in the feces, the tapeworm segments can be found in the perianal region of the goats (Nguyen Thi Kim Lan 1999).

To use anthemintics in treating tapeworm infection in goats, a number of pharmaceutical chemicals were used including niclosamide at dosage of 100 mg/kg B.W, praziquantel at dosage of 15 mg/kg B.W, vermitan at a dosage of 35 mg/kg B.W. In addition to medicinal chemicals to treat diseases**,** scientists have studied the effect of anthemintics in treating tapeworm infection in goats by using herbal medicine in order to limit the state of drug residues in the goat's body, which is harmful to the health of consumers. The herbal medicine contains flavonoids, alkaloids, phenols, tannins, saponins and acids, which are able to kill gastrointestinal helminths in goats (Bui Thi Tho, 2009).

According to Bui Thi Tho et al. (2009), pomegranate consists of chemical composition of alkaloids (Pelletierin, isopelletierin, N-methyl pelletierin; pseudopelletinerin) and tannins. In particular, isopelletierin is an alkaloid with hight effect on tapeworms than other alkaloids

Chapter 2
OBJECTS, MATERIALS, CONTENTS AND METHODS OF STUDY

2.1. Objects, time period and places of study

2.1.1. Objects of study

- Goats reared in Bac Giang province.
- Gastrointestinal parasitic helminth species in goats reared in Bac Giang province.
- Infection caused by *Moniezia* tapeworm spp. in goats

2.1.2. Time period and place of study

***** *Study period***:** from 2017- 2020

***** *Places of Study*

The project was carried out in 5 districts of Bac Giang province including: Yen The, Lang Giang, Luc Nam, Luc Ngan and Son Dong.

Sample testing places: laboratory of Faculty of Animal science and veterinary medicine - Bac Giang University of Agriculture and Forestry, Pathology laboratory and veterinary Hospital - National university of Agriculture, Institute of ecology and biological resources, National Institute of Hygiene and Epidemiology, Center for Biodiversity Resources, Education and Development - Hanoi Pedagogical University 2.

2.2. Study materials:

Experimental animals: goats reared in 5 districts of Bac Giang province.

* Types of study samples: fresh feces samples collected from goats of all ages; samples of helthminths were obtained from infected goats; blood samples of goats with tapeworm infection and healthy goats; samples from small intestine, heart, lung, liver and kidney sections from goats infected with tapeworms.

Instruments, equipment and chemicals: light microscope, olympus microscope CX 221, scanning electron microscope; feces test kits; blood collection devices; Automatic Erma PCE-210, Hematology Analyzer and TC Matrix - Microtom; ABI Prism 3130 Genetic Analyzer; centrifuge, micropipetes, electrophoresis, Transilluminator PCR machine, DNeasy Tissue Kit (qiagen).

Chemicals used to make specimens for microscopic examination; saturated saline solution; Barbagallo solution; solution used to drain from the body of helminths; DNeasy Tissue Kit (qiagen), QIAquick PCR purification kit (QIAGEN Inc. USA), BigDye Terminator Cycle Sequencing Kit (Applied Biosystem). Using 3 anthelmintic regimens for treatment of tapeworm infection in goats: anthelmintic drug praziquantel; Pomegranate bark decoction combined with saline purgative drug $MgSO_4$.

Sequencing Kit (Applied Biosystem) and other laborial chemicals and instruments.

2.3. Contents of study

2.3.1. Study on gastrointestinal helminth infection in goats in Bac Giang province

- Investigatigation of the current situation of the prevention and control of gastrointestinal helminth infection in Bac Giang province

- Prevalence and intensity of gastrointestinal helminth infection in goats through dissection of goats:

+ Prevalence and intensity of gastrointestinal helminth infection of goats in the district

+ Prevalence of single infection and mixed infection of helminths in gastrointestinal tract of goats.

- Prevalence of gastrointestinal helminth infection in goats through fecal test:

+ Prevalence and intensity of gastrointestinal helminth infection in goats through fecal test.

+ Prevalence of single infection and mixed infection of helminths in gastrointestinal tract of goats.

+ Prevalence of gastrointestinal helminth infection according to the age of goats, goat breeds, methods of goat farming and seasons.

- Composition and distribution of gastrointestinal helminth spp in goats in Bac Giang province.

2.3.2. Study on tapeworm infection caused by Moniezia spp in goats in Bac Giang province

**** Identification of tapeworm species as pathogens of goats in Bac Giang province.***

- Dissection and collection of tapeworms from goats in Bac Giang.

- Identification of tapeworm species in goats by using morphological techniques.

- Exalmination of tapeworm species in goats by using molecular biology techniques.

**** Study of tapeworm infection through stool test***

- Prevalence and intensity of tapeworm infection in goats in different districts.

- Prevalence and intensity of tapeworm infection according to goat age, goat breed, method of farming and season.

**** Comparation of the risks of tapeworm infection in goats by different methods of rearing used by goat producers.***

* Studying the intermediate host of *M. expansa* tapeworm

+ Identification of the composition of soil mite species isolated in Bac Giang province.

- Prevalence of tapeworm larvae infection in soil mites in the the field.

- Prevalence of tapeworm larvae infection in experimentally infected soil mites.

* Study on clinical and pathological characteristics of tapeworm infection in experimentally infected goats and naturally infected goats.

* Study and recommendation of measures for prevention and treatment of tapeworm infection in goats

- Trial and determining a hight effective and safe anthelmintic regimen in treating tapeworm infection.

- Recommendation of measures for prevention and treatment of tapeworm infection in goats.

2.4. Method of study

2.4.1. Method of study on gastrointestinal helminth infection in goats in Bac Giang province:

2.4.1.1.Investigation of current situation of prevention of gastrointestinal helminth infection in goats in Bac Giang province: by using method of direct observation, interviewing goat raising households and recording questionnaires.

2.4.1.2. Method of study on infection of gastrointestinal helminth in goats

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

- The minimum sample size was calculated using Win episcope 2.0 epidemiological software.

- Samples were collected according to method of Multi-stage cluster sampling.

- Dissection of goats was performed according to the method of incomplete helminthological dissection of gastrointestinal tract described by Skrjabin K. I. (1928), to collect samples of helminths in the gastrointestinal tract of the goats.

- Performing to fix worm specimens and identification of species according to classification key described by Phan The Viet et al. (1977).

- Detecting helminth eggs, and helminths in goat feces according to the Fulleborn's method and sedimentation technique based on Benedek. The prevalence of infection was determined by the number of goats infected with helminths of the total number of goats with tested feces. The

intensity of helminth infection through dissection was determined by counting the number of helminths in each goat during the dissection and classification process.

2.4.2. Method of study on tapeworm infection in goats

2.4.2.1. Method of identification of tapeworm spp. by using morphological.

Methods based on identification key described by Phan the Viet et all. (1977).

2.4.2.2. Molecular method for identification of tapeworms

- Using PCR technique to examine 5 tapeworm samples collected from goats and 5 tapeworm samples collected from cows that had been identified by routine morphological techniques. Comparing the obtained sequences with those on the gene bank using the MEGA 6 programe (Tamura K. et al., 2013), plotting the phylogenetic tree based on the Maximum Likelihood (ML) method with the most suitable.model.

2.4.2.3. Comparision of the risks of tapeworm infection in goats according to different methods of goat farming applied

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

Applying epidemiological analysis methods according to documents of Nguyen Nhu Thanh (2001).

2.4.2.4. Method of study on soil mites - intermediate hosts of Moniezia tapeworm

** Method of collecting soil mites of the Oribatid mite family*

- Soil samples of the top soil (10 cm thick) were taken from goat keeping households and goat grazing grounds, with the size of each soil sample of 5 x 5 x 10 cm.

- Oribatid mites were extracted by the Berlese -Tullgren modified funnel method" based on the behavior of negative light direction and burrowing, collecting soil mites and preserving them at 70% ethanol or 4% formaldehyde solution.

** Method to identify soil mites of Oribatid family and determining the infection rate of tapeworm larvae in soil mites*

Classification of soil mites based on the documents of Vu Quang Manh (2007). Ultrastructural soilmites was photographed under a scanning electron microscope.

After identification of the soil mites, each soil mite individual was crushed to examine under the microscope to find cysticercoid larvae of the *Moniezia* tapeworm, thereby determining the infection rate of larvae.

* Method of experimental *Moniezia* tapeworm larvae infection in Oribatid mites and determining the infection rate of larvae in soil mites after infection.

Old proglottids were selected then crushed to release eggs The morphology and structure of eggs were examined before experimental infecting. Infective eggs must be old eggs, with 3-sided or 4-sided shapes, with the oncophere (6 - hook embryonated form) inside. Tapeworm eggs were mixed with dried leaf debris and placed in a glass jar containing soil mites.According to Narsapur V. S. et al. (1979), Polec W. et al. (1994): 27 - 29 days after exprimental infecting soil mites, soil mite infective larvae as pathogens were obtained, each soil mite was crushed to find *Moniezia* tapeworm larvae to identify soil mites infected with tapeworm larvae. From these, the prevalence and intensity of larvae infection of soil mites were calculated.

2.4.2.5. The way of experimental tapeworm infection to study on tapewworm infection

2 phases of experimental tapeworm infection were designed in healthy Co goats at the age of 5 months to study tapeworm infection. Phase 1 of infection was to study the main pathological features of the disease; Phase 2 specifically experimental infection for trial of anthelmintic drugs. Phase 1: 5 experimentally infected goats were divided into 2 groups: group 1 included 3 goats, which were allowed to swallow soil mites containing tapeworm larvae capable of causing disease at infectious dosage of 200 larvae per goat, group 2 included 2 non-infected goats. Phase 2 included 12 goats that were experimentally infected with tapeworms being divided into 4 groups each group included 3 goats that were infected. Each goat was allowed to swallow 200 pathogenic larvae.

2.4.2.6. Study on clinical and pathological characteristics of tapeworm in experimentally infected goats and in naturally infected goats in the field

** Study on experimentally infected goats*

- Method of identification of clinical signs: the clinical signs in experimentally infected goats were observed and compared to goats in the control group.

Identification of macroscopic lesions: goats experimentally infected with tapeworms were dissected to observe heart, liver, lungs and GI tract with naked eye and magnifying glass.

Study of microscopic lesions: method of making tissue snears was used according to the impregnation and tisue embedding paraffin and staining with Haematoxilin - Eosin to observe under the microscope to determine the microscopic lesions.

** Methods of studying tapeworm infection in goats in the studied places*

2.4.2.7. Experimental designing anhelmnthics to treat tapeworm infection in goats.

Three experimental anthelminthic treatment regimens were used as follows: regimen1 praziquantel at dosage of 15 mg/kg body weight; regimenII - Pomegranate bark decoction at dosage of 45g/goat/day, regimen III Pomegranate bark decoction at dosage of 45g/goat/day combined with saline purgative (MgSO4) 50g/goat.

Experimental treatment consisted of 3 steps: Step 1: anhelminthic treatment for tapeworm experimentally infected goats (each treatment regimen was used for 3 goats). Step 2: anhelminthic treatment of tapeworm infection on a narrow scale in the field (each treatment regimen used for 20 goats). Step 3: anhelminthic treatment for tapeworm infection of goats on a lare scale (each treatment regimen used for 182 - 185 goats). Goat stool test was performed 15 days after taking the drug to evaluate the efficacy of each regimen. Safety was determined by monitoring the expression of goats before and after taking the drug.

2.4.3. Data processing methods

The data were processed by biological statistical method (according to the document of Do Duc Luc et al., 2017), on Microsoft Excel 2010 software, on minitab 16.0 software.

Chapter 3
RESULTS AND DISCUSSION

3.1. Study on gastrointestinal helminth infection in goats in Bac Giang province

*3.1.1. Investigation of the current status of the prevention and treatment of helminth diseases in Bac Giang***.**

The findings are shown in table 3.1, investigation of 130 goat raising households in Bac Giang province on performing 3 preventive measures of helminth infection in goats, specifically as follows: Anthelmnthics treatment of goats was rarely applied by goat producers: the number of households that used anthelmnthics in treating tapeworms infection 3 times/ year accounted only for 10.77%; number of households that used anthelmnthics in treating tapeworms infection twice a year accounted for 25.38%; The number of households that did not take this prevention measure accounted for 63.85%. The treatment of goat manure was rarely done: in 130 households raising goats, up to 73.08% of which did not treat goat manure; number of households that applied this preventive measure made up only 26.92%. Besides this, up to 43.85% of goat raising households said that they did not apply any preventive measures for the goats during the raising process.

3*.1.2. The prevalence of infection and intensity of gastrointestinal helminths in goats*

3.1.2.1. The prevalence and intensity of infection of gastrointestinal helminths in goats through dissection.

** In the districts:*

Table 3.2. The prevalence and intensity of infection of gastrointestinal helminth species in goats in the districts (*through dissection***)**

Location (district)	**Number of dissectedgoats(goat)**	**Number of infected goats(goat)**	**Percentage (%)**	The infection intensity **(number ofhelminths/goat (min ÷ max)**
Yen The	43	41	95,35[a]	3 - 1631
Lang Giang	32	23	71,88[b]	1 - 942
Luc Nam	36	29	80,56[ab]	2 - 1064
Luc Ngan	47	42	89,36[ab]	3 - 1120
Son Đong	42	36	85,71 [ab]	2 - 1306
Total	**200**	**171**	**85,50**	**1 - 1631**

** Note: Numbers followed by the different letters in the column differ significantly (P< 0,05).*

The results are shown in Table 3.2: out of the total of 200 goats examined, there were 171 goats infected with gastrointestinal helminths, accounting for 85.50%, with overall infection intensity varying from 1 - 1631 helminths / goat. The prevalence of infection and intensity of gastrointestinal helminths among the districts were different: the highest prevalence and intensity of helminth infection in goats raised in Yen The district were (95.35% and 3-1631 helminths / goat respectively); the lowest was in Lang Giang district (71.88% and 1-942 helminths/ goat respectively). The difference in the prevalence of helminth infection in goats in Yen The district compared with Lang Giang district was statistically significant (P <0.05)

Table 3.3. Prevalence of single and mixed infection of gastrointestinal helminths in goats (through dissection)

Number of goats infected with helminths (worm)	Infection status	Number of infected goats (goat)	Percentage (%)
171	Only infected with tapeworms	2	1.17^{d}
	Only infected with trematodes	7	4.09^{cd}
	Only infected with nematodes	94	54.97^{a}
	Infected with trematodes and , tapeworms	10	5.85^{cd}
	Infected with nematodes and, tapeworms	17	9.94^{bc}
	Infected with nematodes and trematodes	27	15.79^{b}
	Infected with trematodes nematodes and tapeworms	14	8.19^{c}

** Note Numbers followed by the different letters in the column differ significantly (P< 0,05).*

The results are shown in Table 3.3: there were differences in the prevalence of single prevalence of goats infected only with nematodes was the highest (54.97%), followed by mixed infection of nematodes and trematodes (15.79%); the mixed infection of nematodes and tapeworms; the mixed infection with tapeworms and nematodes; The mixed infection

of trematodes tapeworms and nematodes was 9.94%, 8.19%, 5.85% respectively. The lowest prevalence was found in goats infected only with tapeworms (1.17%).

3.1.2.2. The prevalence of infection of gastrointestinal helminth species in goats in 5 districts of Bac Giang province through fecal test

** In the districts:*

Table 3.4. Prevalence of of gastrointestinal helminth species in goats in 5 districts of Bac Giang province *(through fecal testing)*

Localitiy (District)	Number of examined goats (goat)	Number of infected goats (goat)	Percentage (%)
Yen The	422	381	90,28[a]
Lang Giang	325	229	70,46[c]
Luc Nam	362	287	79,28[b]
Luc Ngan	456	346	75,88[bc]
Son Dong	412	352	85,44[ab]
total	**1977**	**1595**	**80,68**

** Note Numbers followed by the different letters in the column differ significantly (P< 0,05).*

The results are shown in Table 3.4: there were goats infected with helthminths in all of 5 districts of Bac Giang province; In a total of 1977 examined fecal samples 1595 of which were infected with helminths, accounting for 80.68%, varying from 70.46% - 90.28%. The prevalence of helminthin infection was the highest in goats raised in Yen The district (90.28%); in Son Dong, Luc Nam and Luc Ngan districts the prevalence was 85.44%, 79.28% and 75.88%, respectively; prevalence of infection in goats raised in Lang Giang district was the lowest (70.46%). The differences in the prevalence of gastrointestinal helminth infection, among goats in Yen The district, Lang Giang district and Luc Nam district were statistically significant (P <0.05).

** The prevalence of single and mixed infection of gastro intestinal helthminth spp. through fecal test*

The results are shown in Table 3.5: In total 1595 goats were infected with helthminths, there were 829 infected only with nematodes,

accounting for the highest prevalence (51,97%); followed by 288 goats infected with a mixture of nematodes and trematodes, accounting for 14.29%; 188 goats were infected with a mixture of nematodes and tapeworms, 154 goats were. Infected with a mixture of tapeworms ,trematode and nematodes 11.79%, and 9.66% respectively. Number of goats was infected only with tapeworms accounts for a very low rate (1.57%).

** Prevalence of gastrointestinal helminth infection varied with age through fecal test*

The results are shown in Table 3.6: goats of all ages were infected with gastrointestinal helminths but with different prevalence. The prevalence of infection of goats under 3 months of age was 63.69% The prevalence of infection increased rapidly when goats were > 3 - 6 months of age (89.08%). This difference was statistically significant (P <0.05). Goats at the age of over 6 months, the prevalence of helminths tended to decrease but not apparently. Comparing the prevalence of helminth infection in goats > 6-12 months old and goats over 12 months old the difference was not statistically significant (P> 0.05).

** Breed variation in prevalence of gastrointestinal helminth infection in goats through fecal test*

The results are shown in Table 3.7: there were different prevalence of helminth infection in different goat breeds. The prevalence of helminth infection of three goat breeds varied from 70.97% - 93.94%. of which, the highest prevalene of helminth infection was in indigenous (Co) breed (93.94%), followed by Boer breed (73.52%), the prevalence of helminth infection of Bach Thao goat breed was the lowest (70.97%). The differences in the prevalence of gastrointestinal helminth infection among Co breed Bach Thao and Boer breed were statistically significant (P < 0.05).

** Variation in Prevalence of gastrointestinal helminth infection by goat farrming method through fecal test*

The results are shown in Table 3.8: prevalence of helminth infection in goats reared by traditional methods was 87.78%, higher than that in goats under semi-intensive rearing (prevalence of infection was 73.67%). This difference was statistically significant. Thus, rearing methods had a

apparent influence on the prevalence of gastrointestinal helminth infection in goats.

** Seasonal prevalence of helminth infection in goats through fecal test.*

The results are shown in Table 3.9: the prevalence of gastrointestinal helminth infection in goats varied from 69.10% - 89.74%. The highes prevalence of helminth infection was observed in summer (89.74%), followed by autumn and spring (83.33%; 77.04% respectively). In winter, the helminth infection was the lowest (69.10%). The prevalence of helminth infection in goats was significantly different among spring, summer and winter ($P < 0.05$). Thus, goats were infected with gastrointestinal helminths all year round, but they were more infected in summer and autumn, less infected in spring, and the prevalence of infection was the lowest in winter.

3.1.2.3. Composition and distribution of gastrointestinal helminths in goats in Bac Giang province.

The results are shown in Table 3.10: goats raised in 5 districts of Bac Giang province that were infected with relative abundace of helminth species. During dissection of goats, we detected 8 helminth species in the gastrointestinal tract, including : F. gigantica, P. cervi, E. pancreaticum, M. expansa, Strongyloides papillosus, Haemonchus spp., Oesophagostomum spp., Trichocephalus spp.. Of the 8 species, mentioned above 7 were found commonly in the studied places (occurrence frequency was 100%) only Eurytrema pancreaticum species was found in 3/5 studied districts, its occurrence frequency was 60%.

3.2 Study on *Monieziosis* caused by *Moniezia* tapeworm spp in goats in Bac Giang province

3.2.1. Identification of tapeworm species causing parasite infection in goats

3.2.1.1. Results of dissection and collection of tapeworms from goats in Bac Giang province

Table 3.11. Results of dissection and collection of tapeworms from goats in Bac Giang province

Place (district)	Number of dissected goats(goat)	Number of goats infected with tapeworms (goat)	Percentage (%)	Infection intensity (min – max tapewworms /goat)
Yen The	43	11	25,58[ab]	2 - 7
Lang Giang	32	4	12,50[b]	2 - 6
Luc Nam	36	5	13,89[b]	2 - 9
Luc Ngan	47	8	17,02[ab]	2 - 8
Son Dộng	42	15	35,71[a]	2 - 10
Total	**200**	**43**	**21,50**	**2 - 10**

* *Note Numbers followed by the different letters in the column differ significantly (P< 0,05).*

The results are shown in Table 3.11: out of 200 dissected goats , 43 were infected with tapeworms, accounting for 21.50%, The infection intensity was 2-10 tapeworms/goat. The prevalence and intensity of tapeworm infection varied in different districts.: The prevalence and intensity of tapeworm infection in goats reared in Son Dong district was the highest (35.71% and 2 - 10 tapeworms/goat respectively); the prevalence and intensity of tapeworm infection in goats reared in Lang Giang district was the lowest (12.50% and 2 - 6 tapeworms/goat respectively). The differences in the prevalence of tapeworm infection in goats in Son Dong district compared with Lang Giang and Luc Nam districts was statistically significant ($P < 0.05$).

3.2.1.2. Results of identification of tapeworm species in goats by morphological techniques

The identification results showed that: The examined tapeworm individuals all had interproglottidal *glands*. All tapeworms collected from 50 goats were *M. expansa* species because of having rosette-pattern interproglottidal glands; 50 individuals of tapeworms obtained from bovines were *M. benedeni species* because of their linear pattern interproglottidal glands and located in the middle of the tapeworm. The size of the scolex and suckers of the two species was not markedly different. In contrast, their body length, length and width of mature proglottids and gravid proglottids were markedly different. The

proglottids of *M. expansa* species were narrower and shorter, but thicker than those of *M. benedeni.*

3.2.1.3. Results of identification of tapeworm species in goats using molecular biology techniques

*** Cox1 gene analysis**

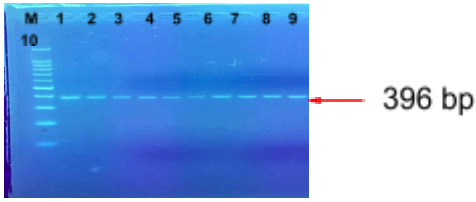

Figure 3.12. Electrophoresis image of PCR products from cox1 gene sequence

Results obtained werre very good sequences. After comparison, primer was excised, cox1 sequences of all samples reached 396 bp long 396 bp.

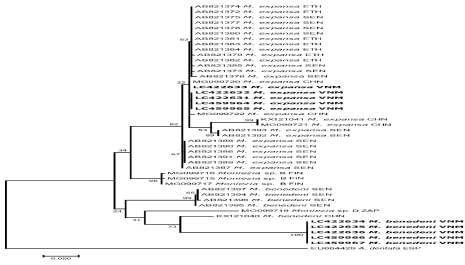

Figure 3.13. Phylogenic tree was constructed based on cox1 sequences using Maximum Likelihood method.

Cox1 sequences of *M. benedeni* tapeworm from cattle were 100% genetically identical, while there was a slight difference (0.0 - 0.4%) between cox1 sequences of *M. expansa* tapeworm from the goat. The phylogenic tree (Figure 3.13) from the cox1 gene sequence dataset showed that *M. expansa* samples from Vietnamese goats were grouped together with samples of this species from Ethiopia and Senegal and two

sequences (MG099720, MG099722) from China. The genetic distance between samples in *M. expansa* species varied from 0.3 - 7.2%.

*** ITS sequence analysis**

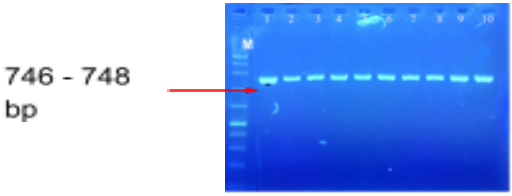

Figure 3.14. Electrophoresis image of PCR products from ITS2 sequences

The results of electrophoresis of ITS2 PCR products from 5 tapeworm samples collected from 5 districts of Bac Giang province are shown in Figure 3.14. Ihe electrophoresis bands corresponded to approximate 746 - 748 bp.

** Note Numbers followed by the different letters in the column differ significantly (P< 0,05).*

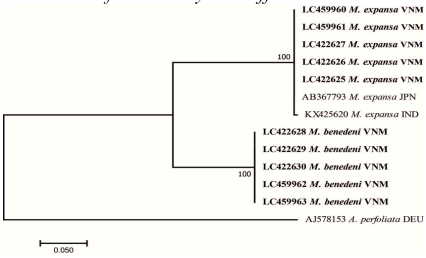

Figure 3.15. The phylogenic tree was constructed from ITS2 sequences using the Maximum Likelihood method

The phylogenic tree was constructed from ITS2 sequence dataset using the Maximum Likelihood (ML) method are shown in Figure 3.15. The results revealed that the sequences in *M. expansa* species from Bac Giang, Vietnam was 99.70% similar to the sequence.number AB367793 of *M. expansa* tapeworm from Japan and KX425620 from India , Our

results of study showed that the parasitic tapeworm in goats in Bac Giang province belongs to *M. expansa* species.

3.2.2. Study on epidemiological characteristics of tapeworm infection in goats in Bac Giang province

3.2.2.1. Prevalence and intensity of tapeworm infection in goats in various districts

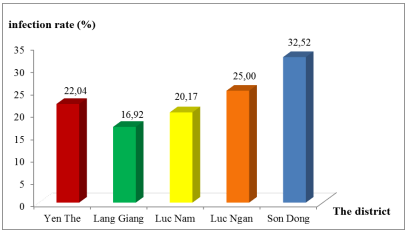

Figure 3.16. Bar chart of tapeworm infection prevalence in goats in investigated districts

The results are shown in, Table 3.13 and Figure 3.16, in all of 5 districts of Bac Giang province, goats were infected with tapeworms. The overall prevalence of infection was 23.72% higher than the infection prevalence through dissection (21.50%). There was different prevalence of infection in goats in different districts. The highest infection prevalence (32.52%) was in goats in Son Dong district, followed by Luc Ngan district (25.00%); Yen The district (22.04%); Luc Nam district (20.17%) and the lowest infection was in Lang Giang district (16.92%). The difference in the prevalence of *M. expansa* tapeworm infection among Son Dong, Luc Ngan, and Lang Giang districts was significant ($P < 0.05$).

3.2.2.2. Variation in prevalence and intensity of tapeworm infection in goats related toage

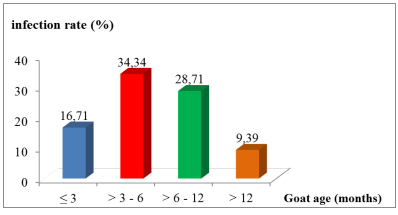

Figure 3.18. Bar chart of age related variation in prevalence of tapeworm infection in goats

The results are shown in Table 3.14 and Figure 3.18, the prevalence of tapeworm infection in different age of goats varied from 9.39 % - 34.34%. The highest prevalence of infection was in Goats at 3 - 6 months of age (34.34%) and the intensity of infection at level 2 and level 3 was hight (30.42% and 18.43%)respectively; followed by goats at 6 - 12 months of age (28.71%); Prevalence of infection in goats under 3 months of age (16.71%); the lowest prevalence of infection was in goats over 12 months of age (9.39%) and goats infected with tapeworms mainly at levels 1 and 2. Thus, the prevalence and intensity of tapeworm infection declined gradually with the increase of the age of goats**.**

3.2.2.3. Breed related variation in prevalence and intensity of tapeworm infection in goats

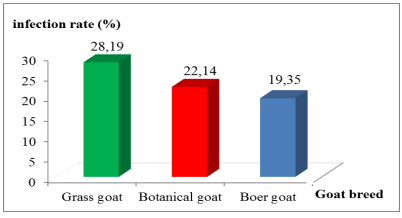

Figure 3.19. Bar chart of prevalence of tapeworm infection varied by goat breed

The results are shown in Table 3.15 and the bar chart in Figure 3.19: there were different prevalences of tapeworm infection in different goat breeds. The highest rate of tapeworm infection was in Co goat (Vietnamese indigenous breed) of goat (28.19%), and the infection at levels 2 and 3 was quite hight (34.11% and 18.69%); followed by goats of Bach Thao breed (22.14%), the lowest prevalence infection was in Boer goat (19.35%). The difference in the prevalence of tapeworm infection among Co breed, Bach Thao and Boer goat breeds was significant (P <

0.05), but there was no difference between Bach Thao and Boer goat breeds (P > 0.05).

3.2.2.4. Goat farrming method related variation in prevalence and intensity of tapeworm infection

The results are shown in Table 3.16 and Figure 3.20: Goats in both farming ways were infected with tapeworms. In particular, the prevalence of tapeworm infection in goats reared by traditional grazing practice was 29.12% significantly higher than that of semi-intensive farming methods (18.39%). In term of Intensity of infection: Goats reared by traditional practice were infected with tapeworms more severely than those reared by semi-intensive farming.

3.2.2.5. Seasonal related variation in prevalence and intensity of tapeworm infection in goats

The results are shown in Table 3.17 and Figure 3.21. Prevalence of tapeworm infection by fecal test from goats in the summer was the highest (29.85%), followed by the autumn (26.06%) and the spring (19. 62%). In winter, goats were infected with tapeworms with the lowest prevalence (17.09%). The differences in the prevalence of tapeworm infection in goats among summer and other seasons of the year was statistically significant (P < 0.05). Intensity of infection: Goats were infected with tapeworms with all of 3 levels of infection intensity. However, in the summer, goats infected with tapeworms, infection intensity at level 3 were the highest and the lowest number was goats tested in the winter.

3.2.2.6. Comparison of the risk of goats infected with tapeworms by farming method

The results are shown in Table 3.18: Goats reared by traditional grazing methods were infected with tapeworms 1.58 times higher than those reared by semi-intensive farming methods (.about 50-60% of time period was free range, 40 - 50% of time period was reared in barns and grass in combination with concentrated feed was fed) The OR index = 1.82 reflected that goats reared by traditional methods were 1.82 times more likely to be infected with tapeworms than goats in insemi-inintensive farming. Free ranged goats in uncultivated hills and grazing grounds were infected with tapeworms 2.31 times higher than those free ranged in cultivated hills and grounds. The OR index = 3.01

reflected that goats were free ranged in uncultivated hills and grazing grounds were 3.01 times more likely to be infected with tapeworms than goats that were free ranged in cultivated hills and grounds**.**

3.2.2.7. Study on the intermediate host of the M. expansa tapeworm

** Identification of composition of soil mite species isolated from Bac Giang province*

The results are shown in Table 3.19: In 16 soil mite species were isolated from Bac Giang province, *Scheloribates mahunkai* was the obatid mite species that occured in all studied places (occurrence frequency wa*s 100%). Javacarus kuehnelti, Arcoppia arcualis, Tectocepheus velatus, Scheloribates africanus, Scheloribates spp., Punctoribates spp., Protoribates paracapucinus* and *Trachyoribates ovulum* occurred with the lowest frequency *(20.00%).* The remaining species were found in 40 - 60% of the studied places.

** Identification of naturally tapeworm larvae infected soil mites*

The results are shown in Table 3.20*,* Among 629 soil mite individuals examinated, there were 47 individuals from 9/16 species containing cysticercoid larvae, accounting for 7.47%, of which *Protoribates paracapucinus* species was the highest prevalence of infection (26.32%); followed by the species*: Scheloribates mahunkai, Pergalumna margaritata, Scheloribates fimbriatus,* which the prevalence of infection varied from 13.19% - 18.75%; *Galumna flabellifera orientalis* species accounted for 10,34%; *Lamellobates ocularis* species (9.09%); *Allozetes pusillus s*pecies *8.54%; Acrogalumna ventralis*, *Scheloribates praeincisus* were infected with the lowest (3.13% - 3.70%) resspectively. The average intensity of larvae infection was 1-3 cysticercoid/mite. In addition to the 9 species mentioned above, in 7 remaining species cysticercoid larvae were not found .

** Determining the soil spiders species of tapeworm infection larval due to cause infection*

The results are shown in Table 3.21, Among 16 experimentally larvae infected soil mite species, there were only individuals from 9 species carrying cysticercoid larvae with different prevalence. These 9 species were all of species that natural larvae infection had been tested and found infected with tapeworm larvae. In this experimental infection, number of individuals in the following species*: Scheloribates fimbriatus,*

*Scheloribates mahunkai, Acrogalumna ventrali*s, and *Galumna flabellifera orientalis* were infected with the highest prevalence of rhe larvae. The remaining species were lower infected (44.44% - 68.75%). In general, the prevalence of larval tapeworm infection was 48.68%, the intensity of infection was 1 - 5 *Cysticercoid* per mite.

3.2.3. Study on pathological and clinical characteristics of tapeworm infection in experimetally infected goats and naturally infected goats in the field

3.2.3.1. Results of laval tapeworm infection in experimental goats

The results are shown in Table 3.23: After infection, progllotids were shed by all of 3 experimentally infected goats in feces . Goat 1 and goats 3 after 47 days experimentally infected with lavae, shed proglottids in feces, and goat 2 after 48 days experimentally infected with lavae shed proglottids in feces. Thus, after infection, goats began to shed proglottids on day 47 - 48 so the timr period to complete the life cycle of tapeworms in goats was 47 - 48 days.

3.2.3.2. Study on clinical signs of goats experimentally infected with tapeworms.

The results are shown in Table 3.24: Goats infected with *M. expansa* tapeworm manifessted the following clinical signs: dry skin, ruffled hair, emaciation, diarrhea, abdominal distension, pale mucous membranes, and neurological symptoms. Very low weight gain of goats were found at 48 and 95 days post infection. While goats in the control group were active, ate normally and had smooth hair. The weight of the control goats at the two time points was higher than that of the experimental group.

3.2.3.3. Study on several hematological indices of experimentally infected goats

** Changes in blood hematology of goats experimentally infected with tapeworms*

The results are shown in Table 3.25: the average red blood cells count (RBC), hemoglobin content and hematocrit (HCT),in experimentally infected goats were sgnificant lower than that of the control (healthy goats) with $P < 0,05$.

** Change in white blood cells count (WBC), and leucocyte formula of goats* experimentally *infected with tapeworms*

The results are shown in in Table 3.26, the white blood cells count in experimentally infected goats had a marked increase compared to control goats (P < 0 .05). In terms of leukocyte formula: the percentage of neutrophils of experimentally infected goats was lower,the percentage of eosinophils of experimentally infected goats was much higher than that of control goats with statistical significance (P < 0,05), (6.89% compared to 2.03%). The differences in basophils, lymphocytes and monocytes of experimentally infected goats and control goats were not statistically significant (P > 0.05).

3.2.3.4. Macroscopic and microscopic lesions in goats experimentally infected with tapeworms

(*) Macroscopic lesions caused by tapeworms in experimentally infected goats

The results are shown in Table 3.27: 95 days after infection, through dissection of 3 experimentally infected goats, 13 - 15 tapeworms/goats were obtained, whereas in the control group, no parasitic tapeworms were found and there were no lesions along the gastrointestinal tract. Macroscopic lesions caused by *M. expansa* tapeworm in goats included:enteritis, ntestinal hemorrhage, intestinal obstruction, intestinal necrosis, intesstinal mucosal sloughing, enlarged liver , pericardial effusion and pneumonia.

(*) Microscopic lesions in goats from experimental tapeworm infection

The resuls are shown in Table 3.28: the microscopic lesions in the small intestine mainly included peeling and degeneration of the villi (there were 77.33% of the specimens with these lesions, eosinophilic infiltration (86.67%) of specimens), intestinal villi were broken (100%), proliferated lesions in intestinal glands (66.67%); The percentage of intestinal specimens containing tapeworms was 100%. Damage to other organs: The toxin from the tapeworms also caused damage to a number of other organs. In the liver: they caused vacuolar degeneration in some hepatocytes and portal vein, congestion in the liver. In the lungs: there was edematous fluid mixed with inflammatory fluid in the bronchial lumen and edematous fluid in the alveolar lumen (33.33% of the specimens had lesions); Lymphocyte proliferation, inflammatory cell infiltrattion in bronchial wall, bronchial epithelial cell degeneration and peeling (26.67%

of specimens had this lesion). In the heart: the percentage of specimens with damaged erythrocytes in the vasculature in the heart accounted for 20%.

3.2.3.5. Symptoms and macropic lesions of goats infected with tapeworms in the field

** Clinical signs*

The results are shown in Table 3.29: 10/25 goats infected with *M. expansa* tapeworm exhibited clinical signs, accounting for 40%. The main manifestations were poor weight gain, anemia, emaciation, ruffled hair; abdominal distention; loose stool ,stool was not well fomed; with many proglottids. There were a lot of mucus in stool; neurological signs were seen in 2 goats with severe tapeworm infection.

** Macropic lesions:*

The results are shown in Table 3.30: Dssection of 25 goats naturally infected with tapeworms, all of which had macropic lesions, accounting for 100%. Lesions concentrated mainly in the small intestines of goats. Number of ptapeworm parasities in the small intestines of goats was 5 - 10 tapeworms per/goat. The macropic lesions were similar to those of goats experimentally infected with tapeworms (tremor and stagger).

In respect of 15 in the group of goats that had no clintcal signs 15/15 goats had macropic lesions but were milder, number of tapeworm parasites in the small intestine was 1-3 tapeworms per /goat, with lesions as follows: petechiae and echymosis at the site of the intestine where the scolex of the tapeworm attached.

3.2.4. Study on infection in goats

The results are shown in Table 3.31: all of 3 anthelmintic treatment regimens were as follows: praziquantel at dosage of 15mg/kg B.w; decoction of pomegranate stem bark 45g/goat/day; Pomegranate bark decoction at dose 45g/goat/day combined with saline pugative $MgSO_4$ at dosage of 50g/goat for oral administration were all effective in deworming tapeworms and were safe for experimental goats.

** Efficacy of tapeworm anthelmintics in a large and narrow scale in the field*

The results of anthelmintics in treating tapeworm infection in a large and narrow scale in the field are shown in Tables 3.32, 3.33 (in the full text dissertation).

The results are presented in tables 3.32, 3.33: when anthelmintics were used in a large and narrow scale in the field. it showwed that goats in narrow and large scale in the field, both regimens 1 and 3 were hight efficacious (90,27% - 94,51%) and safe (100%) in treating tapeworm infection in goats. However, regimen III had the advantage as using herbal medicine, it did not cause harm to goats, and at the same time, there were no drug residues in the goat's body, causing no harm to consumers. Therefore, goat rearing households should use regimen III to treat tapeworms infection in their goat herds

3.2.4.2. Recommendation of integrated prevention and coltrol measures against tapeworm infection in goats

From the study results of the project we recommend prevention and treatment of tapeworm infection in goats as follows: strengthening goat herd management, monitoring tapeworm infection in goats; deworming tapeworm for goats; manure treatment to destroy pathogens; not leaving the hills uncultivated, regularly improvement of the hill farming land; not grazing goats in time of weak light, taking care of goats to raise resistance of their body to infection

CONCLUSION AND RECOMMENDATION

1. Conclusion

1. 1. In the situation of gastrointestinal helminth infection in goats in Bac Giang province:

- The current status of prevention and control measures of gastrointestinal helminth infection in goats in Bac Giang province is not good, up to 43.85% of households did not apply preventive measures against helminth infection in goats.

- Goats in Bac Giang province are infected with a high prevalence of gastrointestinal helminth (85.50% through dissection and 80.68% through stool testing.)

- 8 species of gastrointestinal helminths have been detected including *F. gigantica, P. cervi, E. pancreaticum, M. expansa, S. papillosus, Haemonchus* spp., *Oesophagostomum* spp. and T*richocephalus* spp..

- The prevalence of helminth infection was the highest in goats at the age of 3 - 6 months (89.08%), then graduallydeclines. Prevalence of

tapeworm iinfection in Co goats is 93.94%. The prevalence of tapeworm infection in Boer goats and Bach thao goats is lower. Goats reared by the traditional method are infected with helminths higher than those reared by the semi-intensive method. In Summer and Autumn, goats are infected with gastrointestinal helminths higher than in winter and spring.

1.2. Tapeworm infection in goats in Bac Giang province

** Identification of tapeworm species and epidemiological characteristics of the disease*

- By using morphological techniques and molecular biology, the tapeworm parasites in the gastrointestinal tract of goats in Bac Giang province has been identified as *Moniezia expansa Rudolphi, 1810.*

- The prevalence of tapeworm infection through goat dissection is 21.50%, infection intensity is 2 - 10 tapeworms/goat. The prevalence of tapeworm infection through stool test was 23.72%; 30.70% of goats have 10 - 20 proglottids/faecal shedding, 15.35% of goats have more than 20 proglottids/faecal shedding.

Goats 3 - 6 months old are infected with tapeworms most and most severely, then gradually decrease.

The prevalence of tapeworm infection is 28.19% in Co goat breed, 19.35% in Boer goats and 22.14% in Bach Thao goats.

The prevalence of tapeworm infection in goats was 29.85%, 26.06%, 17.09% and 19.62% in summer, autumn, winter and spring respectively.

The prevalence of tapeworm infection in goats reared by traditional methods was higher than that of goats reared by the semi-intensive imethod (18.39% compared to 29.12%).

- Rearing goats by traditional methods increases the risk of tapeworm infection 1.58 times higher than those reared by semi-intensive methods; Grazing goats in uncultivated hills increases the risk of tapeworm infection 2.31 times compared to grazing goats in regularly cultivated hills.

16 species of soil mites have been identified in Bac Giang, of which 9 species are intermediate hosts of *M. expansa* tapeworm and, 8 have not been published. Epidemiology mapping of the endemic tapeworm infection in goat herds in the studied areas has been developed.

** Pathological and clinical characteristics of tapeworm infection in experimetally infected goats and naturally infected goats in the field*

- Goats experimentally infected with tapeworm are characterized by ruffled hair coat, emaciation, pale mucous membranes, abdominal distention, loose stools with many tapeworms; staggered walk.

- Blood indices in goats infected with tapeworms including red blood cell count, hemoglobin content, and hematocrit are decreased; while white blood cell count is increased, the percentage of eosinophils is increased compared to the control.

Goats infected with tapeworm demonstrate macroscopic lesions: including hemocharrgic enteritis, intestinal obstruction, and necrosis, sloughing of intestinal lining,… Microscopic lesions include: villi degeneration, rupture; proliferative intestinal glands; tapeworms are observed in intestinal specimen; and liver degeneration...

** Treatment of tapeworm infection in goats*

- Regimen I (praziquantel at dosage of 15 mg/kg B.W is uadministered for treatment of tapeworm infection in goats achieves high efficacy (94.51%).

- Regimen III (decoction of pomegranate stem bark at dosage of 45g/goat/day, combined with saline purgative $MgSO_4$ at dosage of 50g/goat) in treating tapeworm infection reaches efficacy of 90.27%;

- Both regimens are safe, do not cause side effects after receiving the drug.

- Intergration of prevention and control measures for tapeworm infection in goats include 6 main contents.

2. Recommendation

- widespread application of intergration of prevention and control measures for tapeworm infection in goats is allowed to be implemented in Bac Giang and other provinces in order to reduce damage caused by the disease, contribute to raising livestock productivity, and promote development of goat production.

- The State management agencies and animal health organization can use the epidemiology map of the endemic tapeworm infection in goats in the studied places to focus on monitoring and have an effective plan to prevent tapeworm infection in goats.

List of studies related to the project

1. **Tran Thi Tam,** Nguyen Thi Kim Lan Phan Thi Hong Phuc (2019), "Study on *Moniezia* tapeworm disease in experimentally infected goats in Bac Giang province ", Journal of Veterinary Science and Technology, Vol. XXVI, No. 7, pages 80 - 86.
2. **Tran Thi Tam,** Nguyen Thi Kim Lan, Pham Ngoc Doanh (2020), "Morphological differences and molecular phylogenetic relationship of two tapeworm species, *Moniezia expansa* and *Moniezia benedeni*, collected from domestic ruminants in northern Vietnam", *Parasitology International*, 74.