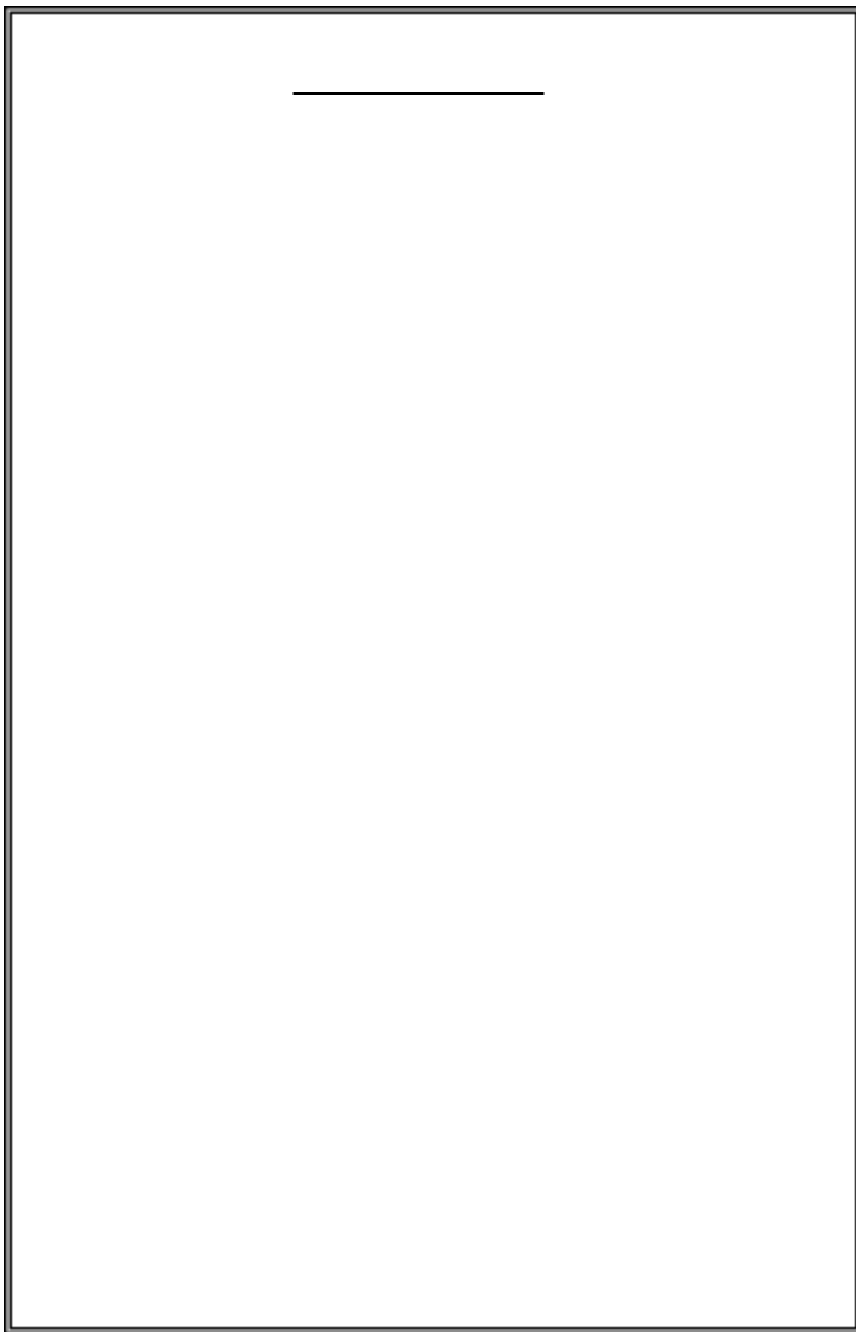




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LIST OF SCIENTIFIC ARTICLES RELATED TO THE DISSERTATION

1. **Tran Nhat Thang**, Pham Dieu Thuy, Nguyen Thi Kim Lan, Pham Ngoc Doanh, Duong Thi Hong Duyen, Madoka Ichikawa Seki (2023), Morphological and molecular characterization of *Eurytrema* spp. Looss, 1907 detected in domestic water buffaloes and cattle in northern Vietnam, Journal of Veterinary Medical Science, 85(9), pp. 929 - 936.
2. **Tran Nhat Thang**, Nguyen Thi Kim Lan, Pham Dieu Thuy, Pham Ngoc Doanh, Duong Thi Hong Duyen (2023). the prevalence of *Eurytrema* spp. in buffaloes and cattle herd in Tuyen Quang province and its medicine testing. TNU Journal of Science and Technology, 228(13), pp. 125 - 131.

INTRODUCTION

1. Urgency of the dissertation

Currently, buffalo and cattle raising in our country primarily adhere to traditional methods of extensive farming and utilization. Therefore, outbreaks still frequently occur in buffalo and cattle herds. In addition to infectious diseases, buffaloes and cattle also suffer from parasitic diseases such as fasciolosis, dicrocoeliosis, paramphistomosis, and Eurytrematosis. These diseases have been causing significant harm, reducing the ability of buffaloes and cattle to grow and reproduce, reducing resistance, thereby making them susceptible to other secondary diseases, or exacerbating existing diseases in the bodies of buffaloes and cattle.

According to Takeuchi-Storm et al. (2018) and Mas-Coma et al. (2020), in addition to causing disease in buffaloes and cattle, fasciolosis also transmits and causes disease in humans. Liver flukes reduce the ability to digest food, cause buffaloes and cattle to lose weight, become weak, reduce the quantity and quality of milk, inhibit estrus, and reduce fertility. Referring to paramphistomosis, Singh et al. (2017) reported that the disease is distributed worldwide, including in tropical and subtropical regions, killing buffaloes and cattle, and reducing livestock productivity. According to De Sousa et al. (2021), Eurytrematosis caused by *Eurytrema* spp. mainly affects ruminant animals such as buffaloes, cattle, goats, and sheep in the Americas and Asia. Eurytrematosis causes buffaloes and cattle to lose weight, become emaciated, have reduced work capacity, and die if the infection is severe (Graydon et al., 1992).

Tuyen Quang is a northern mountainous province with favorable natural conditions for developing buffalo and cattle husbandry. However, buffalo and cattle raising in many localities in the province still mainly follows natural grazing methods, making them susceptible and prone to parasitic diseases in general and Eurytrematosis in particular.

From the urgent needs of the reality of buffalo and cattle husbandry in Tuyen Quang province, we carried out the dissertation: ***"Study on gastrointestinal trematode infection and the disease caused by Eurytrema spp. in buffalo, cattle herds in Tuyen Quang province."***

2. Objectives of the dissertation

Determine the prevalence of gastrointestinal trematode infection in buffaloes and cattle in Tuyen Quang province, identify some epidemiological, pathological, and clinical characteristics of

Eurytrematosis in buffaloes and cattle, and study and propose preventive and treatment measures for Eurytrematosis in Tuyen Quang province.

3. Scientific and practical significance of the dissertation

3.1. Scientific significance

The results of the dissertation are a scientific reference on the current situation of gastrointestinal trematode infection in buffaloes and cattle; species of pancreatic trematodes and the epidemiological, pathological, and clinical characteristics of diseases caused by pancreatic trematodes in buffaloes and cattle in Tuyen Quang province. These results form a scientific basis to develop highly effective integrated prevention and treatment measures.

3.2. Practical significance

The results of the dissertation form the basis for recommending that buffalo and cattle farmers apply measures to prevent and treat gastrointestinal trematode disease and Eurytrematosis. Applying probiotics to treat buffalo and cattle manure reduces the prevalence of gastrointestinal trematode infection, contributing to improving the productivity of buffalo and cattle husbandry in Tuyen Quang province.

4. New contributions of the dissertation

The prevalence of gastrointestinal trematode infection in buffalo and cattle herds in Tuyen Quang province has been determined according to localities, age, season, husbandry method, and topographical region.

Two species of pancreatic trematodes have been identified. The prevalence and infection intensity of Eurytrematosis in buffalo and cattle herds were determined according to localities, age, season, husbandry method, and topographical region.

For the first time, the ITS2 gene of *Eurytrema coelomaticum* was published on GenBank.

One species of land snail, *Bradybaena similaris* - the first intermediate host, and two species of grasshoppers, *Conocephalus maculatus* and *Conocephalus chinensis* - the second intermediate host of pancreatic flukes, have been identified in Tuyen Quang province.

5. Structure of the dissertation

The dissertation consists of 118 pages (not including the list of references and appendices), including 3 pages of the introduction part; 30 pages of bibliography; 18 pages of research content and methods; 65 pages of the research results and discussion, 2 pages of conclusions and recommendations. The dissertation includes 33 tables, 13 charts and graphs, 62 color photos showing the results, and 177 references (29

Vietnamese, 134 English, and 14 Portuguese references, including 47 documents from the year 2018 up to now).

Chapter 1 BIBLIOGRAPHY

There are many research documents around the world on parasitic trematodes in the digestive tract of buffaloes and cattle, focusing on common trematodes in most countries such as *Fasciola* spp., *Dicrocoelium* spp., *Paramphistomum* spp. and *Eurytrema* spp. flukes. In each region, the geographical location, climatic conditions, customs, animal husbandry methods and people's awareness of preventing parasitic diseases in buffaloes and cattle are different, thereby affecting the prevalence and composition of gastrointestinal trematodes in buffaloes and cattle (Huang et al., 2012; Nzalawahe et al., 2014; Rinca et al., 2019; Nurhidayah et al., 2020; Brahmabhatt et al., 2021).

Eurytrematosis is caused by trematode species of the genus *Eurytrema* in ruminants, including three main species: *Eurytrema coelomaticum*, *Eurytrema pancreaticum* and *Eurytrema cladorchis* of the Dicrocoeliidae family. According to some authors, the age of buffaloes and cattle affects the prevalence of Eurytrematosis, the prevalence is often high in elder buffaloes and cattle (Manga - González et al., 2007).

Roy and Tandon (1992) reported that the prevalence of Eurytrematosis in cattle in winter is higher than in other seasons of the year, due to the high presence of second intermediate hosts (grasshoppers) during the summer and autumn, thus, cattle are infected in summer and fall, and then the disease manifests in winter.

In many parts of the world, including Southeast Asia, the land snail *Bradybaena similaris* belonging to the family Bradybaenidae, class Gastropoda, is considered the first intermediate host of *Eurytrema* spp. According to many authors, the second intermediate host of *Eurytrema* spp. flukes is grasshoppers. Research documents around the world showed that Metacercaria of *Eurytrema* spp. flukes develop in the hemocoel cavity of grasshopper species such as *Conocephalus maculatus*, *C. chinensis*, *C. gladiatus*, *C. dorsalis*, *C. discolor*, *C. percaudatus*, *Oecanthus longicaudus* and *Epocromia* spp. in Japan, China, Korea, Russian Federation and Brazil.

Eurytrema spp. flukes parasitize in the pancreas and pancreatic ducts, therefore lesions of infected cattle are mainly concentrated in the pancreas (Schwertz et al., 2015; Grosskopf et al., 2016).

To date, there is no vaccine to prevent Eurytrematosis in buffalo and cattle. The disease prevention still focuses mainly on the use of anthelmintic drugs (Jiraungkoorskul et al., 2005).

Chapter 2

RESEARCH CONTENT AND METHODS

2.1. Subjects, time and location of study

2.1.1. Study subjects

- Buffaloes and cattle raised in Tuyen Quang province.
- Gastrointestinal trematodes in buffaloes and cattle.
- Eurytrematosis in buffaloes and cattle.

2.1.2. Study time and location

* Study period: 2020 - 2023.

* Study location:

- Buffaloes and cattle owners and slaughterhouses in 5 districts and cities of Tuyen Quang province (Chiem Hoa, Ham Yen, Yen Son, Son Duong district and Tuyen Quang city).

- Sample testing location: Faculty of Animal Science and Veterinary Medicine - Thai Nguyen University of Agriculture and Forestry; Institute of Ecology and Biological Resources; National Institute of Hygiene and Epidemiology; Thai Nguyen National Hospital, Vietnam National Museum of Nature.

2.2. Study materials

2.2.1. Study animals and samples

* Study animals: Buffaloes and cattle of all ages in 5 districts and cities of Tuyen Quang province (collecting and testing fecal samples to determine gastrointestinal trematode infection); buffaloes and cattle infected with pancreatic trematodes (dissecting, collecting of pancreatic trematodes and examining lesions caused by *Eurytrema* spp.).

* Study samples: Fresh fecal samples of buffaloes and cattle of all ages; Pancreatic trematode samples collected from buffaloes and cattle at necropsy; lesional pancreas; Blood samples from buffaloes and cattle infected with pancreatic trematode disease and healthy buffaloes and cattle; Land snail and grasshopper samples collected on grazing lands and around buffalo and cattle barns; Buffalo and cattle feces samples before and after bleach testing; before and after incubation with biological products.

2.2.2. Tools, equipment and chemicals

All necessary chemicals and tools in parasitological study and molecular biology, gene sequencing were provided.

2.3. Study contents

2.3.1. Characteristics of gastrointestinal trematode infection in buffaloes and cattle in Tuyen Quang province through fecal testing

- The prevalence of gastrointestinal trematode infection according to localities.

- The prevalence of gastrointestinal trematode infection according to age.
- The prevalence of gastrointestinal trematode infection according to seasons.
- The prevalence of gastrointestinal trematode infection according to husbandry method.
- The prevalence of gastrointestinal trematode infection according to topographical regions.

2.3.2. Study on diseases caused by *Eurytrema* spp. flukes in buffaloes and cattle in Tuyen Quang province

2.3.2.1. Identification of pancreatic trematode species parasitized in buffaloes and cattle

- Necropsy and collection of *Eurytrema* spp. flukes in buffaloes and cattle in Tuyen Quang province.
- Identification of *Eurytrema* species through morphological characteristics.
- Evaluation of *Eurytrema* species in buffaloes and cattle using molecular techniques.

2.3.2.2. Study on some epidemiological characteristics of Eurytrematosis in buffalo and cattle herds in Tuyen Quang province through fecal testing

- The prevalence and infection intensity of Eurytrematosis in some localities.
- The prevalence and infection intensity of Eurytrematosis according to age.
- The prevalence and infection intensity of Eurytrematosis according to season.
- The prevalence and infection intensity of Eurytrematosis according to husbandry method.
- The prevalence and infection intensity of Eurytrematosis according to topographical region.

2.3.2.3. Study on intermediate hosts of *Eurytrema* spp. flukes in Tuyen Quang province.

- Identify land snail species - the first intermediate host of *Eurytrema* spp. in Tuyen Quang province.
- Identify grasshopper species - the second intermediate host of *Eurytrema* spp. in Tuyen Quang province.

2.3.2.4. Study on pathological and clinical characteristics of Eurytrematosis in buffaloes and cattle in Tuyen Quang province

- Clinical symptoms of buffaloes and cattle infected with pancreatic flukes.
- Gross lesions of buffaloes and cattle infected with pancreatic flukes.

- Microscopic lesions in buffaloes and cattle infected with pancreatic flukes.

- Study on changes in some physiological and biochemical blood hematological parameters of buffaloes and cattle infected with pancreatic flukes.

2.3.3. Study and propose preventive, treatment measures of Eurytrematosis for buffaloes and cattle in Tuyen Quang province

2.3.3.1. Approving medicines to deworm pancreatic flukes for buffaloes and cattle

- Select effective medicines to deworm pancreatic flukes for buffaloes and cattle.

- Determine the effective dose of eligible medicine to deworm pancreatic flukes for buffaloes and cattle.

2.3.3.2. Study on preventive measures of Eurytrematosis in buffaloes and cattle

- Determine the effect of composting buffalo and cattle manure with probiotics to eliminate pancreatic fluke eggs.

- Trial preventive medicine of Eurytrematosis for buffaloes and cattle in Tuyen Quang province.

- Propose integrated prevention and control measures of Eurytrematosis for buffaloes and cattle.

2.4. Study Methods

2.4.1. Method to study characteristics of gastrointestinal trematode infection in buffaloes and cattle in Tuyen Quang province

- Using cross-sectional descriptive epidemiological method (according to Nguyen Nhu Thanh et al., 2001).

- Collect fecal samples using the multi-step cluster sampling method.

Sampling size: 1.750 samples (350 samples per district or city).

- The total number of necropsied buffaloes and cattle in 5 districts and cities was 150 buffaloes and 150 cattle.

- Buffalo and cattle feces were determined by using the sedimentation method (Benedek, 1943) to detect trematode eggs in each fecal sample.

2.4.2. Methods to study Eurytrematosis in buffaloes and cattle

2.4.2.1. Identification of pancreatic fluke species using morphological techniques

- Identification of pancreatic trematode species according to the identification key of Nguyen Thi Le et al. (1996), Mohanta et al. (2015), Leite et al. (2020).

* *Evaluation of pancreatic trematode species using molecular technique*

6 pancreatic flukes were used to evaluate by using molecular technique (3 samples for each species). The DNA fragment of 18S rRNA was amplified using polymerase chain reaction (PCR) using SSU18A and SSU26R primers (Jaleta et al., 2017), whereas the primer set 3S and A28 was used for ITS2 (Bowles et al., 1995). After performing the PCR method, select 1 representative sample for each species for gene sequencing.

Table 2.1. The primer sequences correspond to the target genes

Target gene	Primer set	Primer sequences (5'- 3')
18S rRNA	SSU18A	5'-AAAGATTAAGCCATGCATG-3'
	SSU26R	5'-CATTCTTGGCAAATGCTTTTCG-3'
ITS2	3S	5'-AGC GGT GGA TCA CTC GGC TCG TG-3'
	A28	5'-GGG ATC CTG GTT AGT TTC TTT TCC GC-3'

A phylogenetic tree was built for each target gene by using the Maximum Likelihood (ML) method on MEGA X software (Kumar et al., 2018) with an appropriate model.

2.4.2.2. Method to determine the prevalence and infection intensity of *Eurytrematosis* in buffaloes and cattle

The prevalence of *Eurytrematosis* in buffaloes and cattle was determined by using the Benedek method (1943).

The infection intensity of *Eurytrematosis* was determined by Mc. Master egg counting method, based on the number of pancreatic fluke eggs per gram of feces and clinical manifestations of infected buffaloes and cattle. Three levels of infection intensity were prescribed as follows:

Level (+): Buffaloes and cattle have less than 300 eggs/g of feces, no clinical symptoms. Level (++) : Buffaloes and cattle have 300 - 600 eggs/g of feces, with clinical symptoms but not obvious. Level (+++) : Buffaloes and cattle have more than 600 eggs/g of feces, with clear clinical symptoms.

2.4.2.3. Method to identify land snails and grasshoppers - intermediate hosts of *Eurytrema* spp. in Tuyen Quang province

- The land snail species in Tuyen Quang were identified at the Centre for Life Sciences - Vietnam National Museum of Nature, according to the classification key of Schileyko (2011), Dang Ngoc Thanh and Ho Thanh Hai (2019). Identified land snails are referred to other authors who identified as the first intermediate host of *Eurytrema* spp.

- The grasshopper species were identified according to the classification key of Luu Tham Muu and Dang Duc Khuong (2000), Cigliano (2020). Identified grasshopper species were referred to other authors who

identified as second intermediate hosts of *Eurytrema* spp.

2.4.2.4. Methods to study some pathological and clinical characteristics of Eurytrematosis in buffaloes and cattle

**** Method to determine clinical symptoms of Eurytrematosis***

Clinical symptoms were observed in buffaloes and cattle infected only with pancreatic flukes and not infected with other types of worms.

**** Method to determine gross lessions***

Lesions were observed visually and with the aid of a magnifying glass on the pancreas and pancreatic ducts, and take pictures of areas with typical lesions caused by pancreatic flukes.

**** Method to determine microscopic lesions***

The method of making microscopic specimens is performed by cutting the tissue and staining with Haematoxylin - Eosin (HE).

**** Method to test some physiological and biochemical hematological parameters of buffalo and cattle blood.***

Blood physiological parameters of infected and healthy buffaloes, cattle were analyzed on an ABX Micros 60 machine (Horiba Medical, France). Blood biochemical parameters were analyzed on a Chemray 240 machine (Rayto, China).

2.4.3. Methods to study and propose preventive, treatment measures of Eurytrematosis for buffaloes and cattle

2.4.3.1. Method to study pancreatic fluke medication for buffaloes and cattle

**** Method to determine the medicine effective in deworming pancreatic flukes for buffaloes and cattle***

Approving three medicines that have the effect of deworming trematodes (according to Vo Thi Tra An, 2010) on buffaloes and cattle infected with pancreatic flukes to choose a medicine with high deworming effect.

Table 2.2. Trial diagram of approving three types of pancreatic fluke medication for buffaloes and cattle

No.	Medicine (dose)	Routes of Administration	Number of medicated	Time for fecal testing after
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			buffaloes and cattle	medicating (days)
1	Albendazole (12,5 mg/kg B.W)	Oral	10	15
2	Praziquantel (15 mg/kg B.W)	Subcutaneous	10	15
3	Nitroxynil (1ml/ 25 kg B.W)	Subcutaneous	10	15

** Method to determine the highly effective dose of praziquantel to deworm pancreatic flukes for buffaloes and cattle*

Step 1: Deworm buffaloes and cattle infected with Eurytrematosis using praziquantel medicine at 3 different dosage levels (table 2.6).

Table 2.6. Deworming pancreatic flukes using 3 praziquantel dosage levels

Group	Number of medicated buffaloes and cattle	Used praziquantel dosage	Routes of Administration	Time for fecal testing after medicating (days)
I	10	15 mg/kg B.W	Subcutaneous	15
II	10	18 mg/kg B.W	Subcutaneous	15
III	10	20 mg/kg B.W	Subcutaneous	15
Control	10	Not used	-	15

After achieving the results, choose 2 higher effective dosage levels to proceed to the next step.

Step 2: Deworming for 60 infected buffaloes and cattle using 2 selected doses of praziquantel. After 15 days of using the medicine, re-test the buffalo and cattle feces to determine the effectiveness of each dose of medicine used. From there, choose the dose of praziquantel with the highest effectiveness in deworming pancreatic trematodes for buffaloes and cattle.

Step 3: Use the highest effective dose of praziquantel to deworm pancreatic flukes for remaining buffaloes and cattle infected with pancreatic flukes in the localities.

* The weight of buffaloes and cattle was calculated to determine the highest dose according to Nguyen Xuan Trach et al. (2021).

2.4.3.2. Methods to study and proposed Eurytrematosis preventive measures in buffaloes and cattle

a) Method to determine the effect of composting with probiotics

Step 1: Select 20 households (those with buffaloes and cattle infected with Eurytrematosis and a large amount of residual feces) for probiotic composting.

Step 2: Collect fecal samples before composting in 20 households to test for pancreatic fluke eggs in feces.

Step 3: Compost buffalo and cattle manure with probiotics as follows: 7 households were composted with EMUNIV product; 6 households incubated with EMIC product and 7 households incubated with EMZEO product.

Step 4: Collect fecal samples after 20 - 25 days of composting to test.

** Method of collecting and testing fecal samples before and after composting*

- At each household, fecal samples were collected before and after composting with probiotics, from 10 manure locations with different depths, each location takes about 15 - 20 grams of feces, mixed well to get 1 test sample. Tested fecal samples by sedimentation method to detect pancreatic fluke eggs.

b) Testing Eurytrematosis preventive medicine for buffaloes and cattle

- Arrange buffaloes and cattle to use disease prevention medicine

A total of 150 buffaloes and cattle are divided into 3 groups as follows:

Experimental group 1: included 50 buffaloes and cattle that were prophylactically dewormed for pancreatic flukes with the praziquantel medicine at a highly effective medicine dose (from the results of testing the medicine's effectiveness on buffaloes and cattle) at the beginning of the experiment. Followed up the experiment for 4 months.

Experimental group 2: including 50 buffaloes and cattle that were dewormed twice (first time at the beginning of the experiment, second time after 2 months of the experiment). Followed up the experiment for 4 months.

Control group: including 50 buffaloes and cattle, not provided the medicine. Followed up the experiment for 4 months.

Testing fecal samples were collected at three time points:

+ 1st time: Testing fecal samples before arranging the experiment to determine the prevalence and infection intensity of Eurytrematosis. From there, 3 experimental groups were arranged to ensure relative uniformity in the prevalence and infection intensity of Eurytrematosis.

+ 2nd time: Testing fecal sample test after 1 month of experiment.

+ 3rd time: Testing fecal sample test after 4 month of experiment.

c) *Propose integrated prevention and treatment measures of Eurytrematosis in buffaloes and cattle in Tuyen Quang province*

Integrated prevention and treatment measures of Eurytrematosis for buffaloes and cattle are proposed based on: study results on the prevalence of Eurytrematosis in buffaloes and cattle; results of approving medicines to treat Eurytrematosis in buffaloes and cattle; study results on manure treatment measures and the use of Eurytrematosis preventive medicine for buffaloes and cattle.

2.4.3. Data processing methods

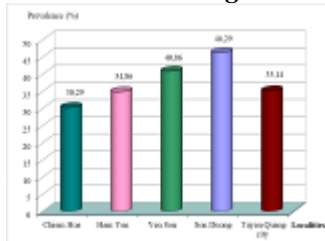
Data were collected and processed using biostatistics methods (according to by Do Duc Luc et al., 2017), on Microsoft Excel 2010 and Minitab 16.0 software.

Chapter 3

RESULTS AND DISCUSSION

3.1. Characteristics of gastrointestinal trematode infection in buffaloes and cattle through fecal testing

3.1.1. The prevalence of gastrointestinal trematode infection in buffaloes and cattle according to localities



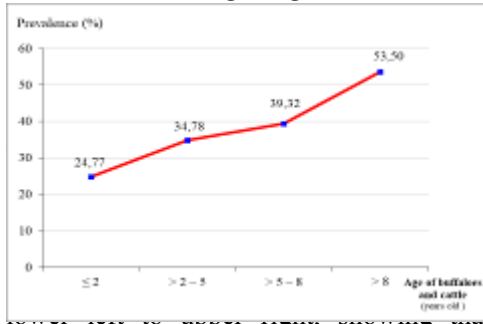
The prevalence of gastrointestinal trematode infection in buffaloes and cattle according to localities

There were buffaloes and cattle infected with trematode infection in general at 5 districts and cities, of which buffaloes were infected, accounting for 37,49%, and cattle were infected, accounting for 46,29%. Observing the morphology of trematode eggs under a microscope showed that they were *Fasciola* spp., *Paramphistomum* spp. and *Eurytrema* spp. eggs.

Kaewnoi et al. (2020) have tested buffalo feces in Southern Thailand and found that the prevalence of gastrointestinal trematode infection was 97,17%. According to Nguyen Kien Cuong and Le Xuan Hien (2022), the prevalence of gastrointestinal trematode infection in cattle in farming households in Hoc Mon and Cu Chi district, Ho Chi Minh City was over 31%.

Therefore, the prevalence of gastrointestinal trematode infection in buffaloes and cattle in Tuyen Quang was lower than the research results of Kaewnoi et al. (2020); higher than the research results of Nguyen Kien Cuong and Le Xuan Hien (2022).

3.1.2. The prevalence of gastrointestinal trematode infection in buffaloes and cattle according to age.



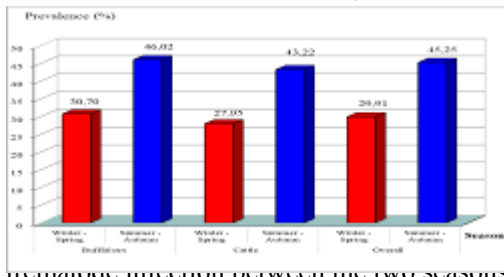
f gastrointestinal trematode infection according to age

The prevalence of gastrointestinal trematode infection in buffaloes and cattle aged under 2 years old was 24.77% and cattle aged over 8 years old was 53.50%. The prevalence of gastrointestinal trematode infection according to age representing the prevalence of gastrointestinal trematode infection in buffaloes and cattle went up from 24.77% to 53.50%, showing that the prevalence of gastrointestinal trematode infection increased in accordance with the age of buffaloes and cattle.

Nurhidayah et al. (2020) mentioned that the prevalence of gastrointestinal trematode infection increases with age in buffaloes raised in Java island, Indonesia. Therein, adult buffaloes (more than 3 years old of age) have the highest trematode infection (66,72%) and the lowest prevalence was in buffaloes age under 1 year old (9.66%).

Thus, fluctuations in gastrointestinal trematode infection according to age of buffaloes and cattle in our study were consistent with the results of Nurhidayah et al. (2020).

3.1.3. The prevalence of gastrointestinal trematode infection in buffaloes and cattle according to seasons.



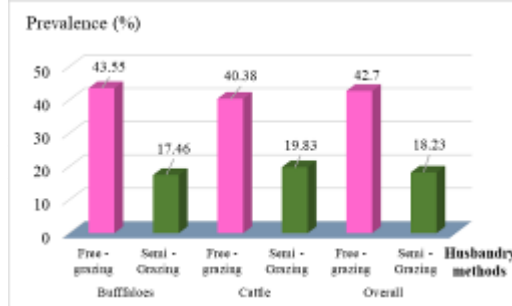
gastrointestinal trematode infection according to seasons

The prevalence of buffaloes and cattle infected in the Winter - Spring was 29.92% and the prevalence of buffaloes and cattle infected in the Summer - Autumn was 44.36%. The prevalence of gastrointestinal trematode infection between the two seasons was significant ($P < 0.05$).

According to Ola - Fadunsin et al. (2020), the prevalence of gastrointestinal trematode infection in cattle in Nigeria occurs mainly in the rainy season, the prevalence is high in the months of April, May, June (83,33% - 85,71%), twice as high as 2 times compared to the dry season.

Therefore, fluctuations of gastrointestinal trematode infection in buffaloes and cattle raised in Tuyen Quang province according to the season of the year were consistent with the above author's research results.

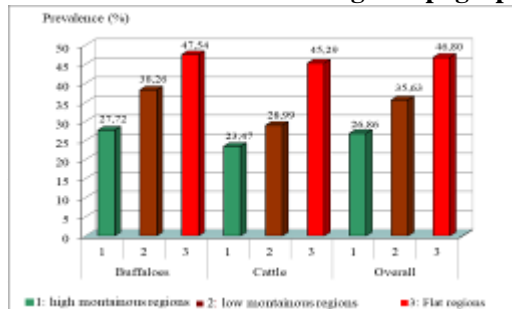
3.1.4. The prevalence of gastrointestinal trematode infection in buffaloes and cattle according to husbandry methods



Turwangsari et al. (2022) studied and reported that: beef cattle raised in free grazing method have the rate of gastrointestinal trematode infection was 67,5%, significantly higher than those raised in confinement method (37,5%).

Thus, fluctuations of gastrointestinal trematode infection according to the husbandry method in buffaloes and cattle in Tuyen Quang province were consistent with the research results of the above author.

3.1.5. The prevalence of gastrointestinal trematode infection in buffaloes and cattle according to topographical regions.



The prevalence of gastrointestinal trematode infection between high mountain and low mountainous region was not significant ($P > 0.05$), but the prevalence in the two mentioned regions compared to flat terrain region was significant ($P < 0.05$).

Pfukenyi et al. (2005) collected and tested 16,264 fecal samples of cattle in two geographical areas, the highlands and lowlands of Zimbabwe. The author reported that the proportion of trematode infection in the highland areas is higher than in the lowland areas (22% compared to 13.9%). According to Nguyen et al. (2017), in Thua Thien Hue province, Vietnam, the prevalence of gastrointestinal trematode infection in cattle varies according to terrain, the rate of trematode infection in the coastal plain is 31,0%, in the low mountainous region is 16,2%.

Our study results were different from those of Pfukenyi et al. (2005), but is relatively consistent with the results of Nguyen et al. (2017).

3.2. Study on the disease caused by *Eurytrema* spp. pancreatic flukes in buffaloes and cattle in Tuyen Quang province

3.2.1. Identification of pancreatic trematode species parasitizing in buffaloes and cattle in Tuyen Quang province

3.2.1.1. Results of necropsy and collection of pancreatic flukes in buffaloes and cattle in Tuyen Quang

Necropsy was conducted on 300 slaughtered buffaloes and cattle in 5 districts and cities of the province, the result showed that the prevalence of Eurytrematosis was 23,67%, with a total number of 1,315 flukes collected. All 1,315 individuals of pancreatic flukes were parasitized in the pancreas of buffaloes and cattle and were not found in other digestive organs.

According to Vu Thi Hen (2014), the proportion of pancreatic trematode infection through cattle necropsy in Nho Quan district, Ninh Binh province was 38,57%. Okajima et al. (2016) studied the circulation of *Eurytrema* spp. pancreatic flukes on beef cattle in some provinces of Japan. The author reported that Eurytrematosis was circulating in 9 provinces in Japan, with the highest infection rate in Okinawa province (5,08%).

Therefore, the prevalence of Eurytrematosis through necropsy in buffaloes and cattle in Tuyen Quang province was higher than the studied results of Okajima et al. (2016), but lower than the results of Vu Thi Hen (2014).

3.2.1.2. Identification of pancreatic fluke species through morphological characterization

**** Morphological characterization of pancreatic flukes parasitized in buffaloes and cattle in Tuyen Quang***

Observing the morphological and morphometric characterization of 1,315 fluke individuals collected from fresh flukes and carmin-stained specimens, we found that the flukes belonged to two different species.

The first species had bodies that were flattened, oval in shape, tapered at both extremities, and lacked distinct caudal appendages. The body dimension were 5,5–8,5 mm long and 2,9–3,6 mm wide, with a red-brown in color. The oral sucker was subterminal and smaller than the ventral sucker. The testes were quite big, with many branches and symmetrical on both sides, situated posterolateral to ventral sucker.

The second species had a leaf-shaped body, bright red in color. The end of the body protruded like a tongue. The body length was 5,2–7,1 mm, and the width was 3,2–4,9 mm. The esophagus was short. Two caeca branches

followed the esophagus. Two globular posterolateral testes were quite small, located between the intestinal bifurcation and the ventral sucker.

** Morphometric measurements of two pancreatic fluke species*

We measured some morphometric dimensions of 15 individual flukes of each species (see the table 3.7 in the main dissertation), and compared them with the dimensions of flukes published by other domestic and foreign authors. The results showed that:

The first species had dimensions consistent with the identification key of Mohanta et al. (2015), which was *Eurytrema cladorchis*.

The second species had dimensions that coincided with the identification key of Leite et al. (2020), which was *Eurytrema coelomaticum*.

** Results of the identification of pancreatic flukes collected from buffaloes and cattle in Tuyen Quang*

The results of identification using morphological techniques showed that out of a total of 1,315 fluke individuals identified, there were 735 individuals of the *Eurytrema cladorchis* specie (accounting for 55,89%), 580 individuals of the *Eurytrema coelomaticum* specie (accounting for 44,11%). Besides the two mentioned species, there were no individual flukes belonging to any other species.

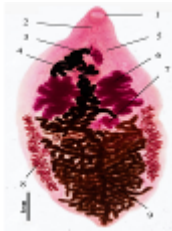


Figure 3.6.
Eurytrema
cladorchis
specie
parasitized in
buffaloes and
cattle in Tuyen
Quang
province.

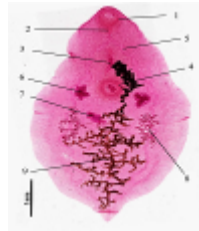


Figure 3.7.
Eurytrema
coelomaticum
specie
parasitized in
buffaloes and
cattle in Tuyen
Quang
province.

3.2.1.3. Evaluation of pancreatic fluke species based on molecular techniques

** Sequence analysis of 18S rRNA gene fragment*

The results of electrophoresis of the PCR product amplified the 18S rRNA gene fragment showed that the electrophoretic bands were very bright and clear, with a length of 756 bp (Figure 3.8). The sequence of the 18S rRNA gene fragment of two pancreatic fluke species has a length of 756 bp. We have conducted to register in the Genbank with the access numbers of *Eurytrema cladorchis* and *E. coelomaticum* as LC760841 and LC760842, respectively.

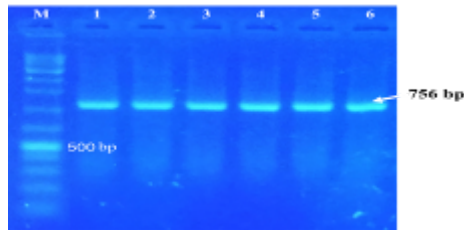


Figure 3.8. Electrophoresis of the PCR product detecting 18S rRNA gene fragment of 6 pancreatic fluke samples

The phylogenetic tree of two pancreatic fluke species collected from buffaloes and cattle in Tuyen Quang province was constructed from the 18S rRNA gene sequences and is presented in Figure 3.9.

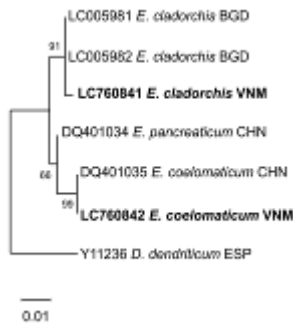


Figure 3.9. A maximum likelihood phylogram was inferred from 18S rRNA sequences.

Figure 3.9 showed that the 18S rRNA gene sequence of *E. cladorchis* from Tuyen Quang, Vietnam showed 99.6% similarity compared to that from Bangladesh (LC005981), whereas 100% similarity was observed between the *E. coelomaticum* from Vietnam and *E. coelomaticum* from China (DQ401035). The Maximum likelihood tree constructed for the 18S rRNA sequences showed that *E. cladorchis* from Vietnam formed a monophyletic clade with *E. cladorchis* from Bangladesh. Similarly, *E. coelomaticum* from Vietnam and China formed a clade distinct from *E. pancreaticum* from China (DQ401034).

***Sequence analysis of ITS2 gene fragment**

Electrophoresis results of the PCR product amplified the ITS2 gene fragment showed that the electrophoretic bands were 407 bp in length (Figure 3.10), clear, and none of the bands were blurred.

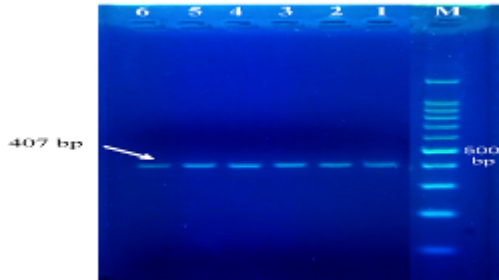


Figure 3.10. Electrophoresis of the PCR product detecting ITS2 gene fragment of 6 pancreatic fluke samples

Sequencing results showed that after removing the primers and trimming, the ITS2 sequences of *E. cladorchis* and *E. coelomaticum* had a length of 229 bp to match other reference sequences, which were then analyzed. These sequences were registered on the Genbank with access numbers LC760843 and LC760844, respectively.

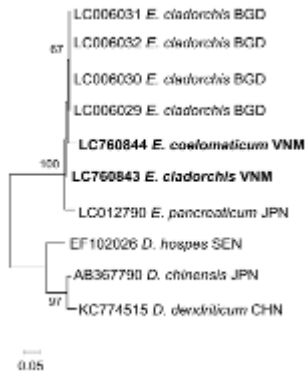
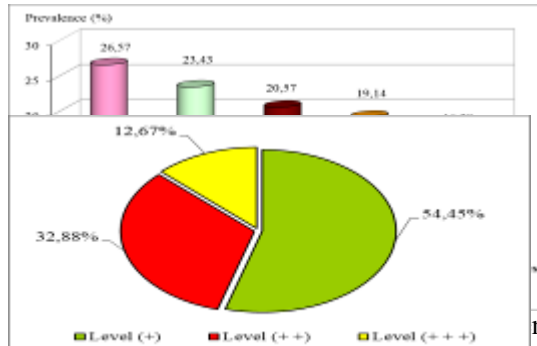


Figure 3.11. A maximum likelihood phylogram was inferred from ITS2 sequences.

Figure 3.11 showed that *E. cladorchis* collected from buffaloes and cattle in Tuyen Quang province, Vietnam showed 99.1% similarity with *E. cladorchis* from Bangladesh (LC006029), whereas *E. coelomaticum* from Vietnam could not be compared to any sample because of the lack of ITS2 reference sequences. Nonetheless, in our study, the nucleotide sequence of *E. coelomaticum* in the present study could be differentiated from *E. cladorchis* from Bangladesh (LC006029) and *E. pancreaticum* from Japan (LC012790) by 3.9% and 5.7% of nucleotide substitutions, respectively.

3.2.2. Study on some epidemiological characteristics of Eurytrematosis in buffaloes and cattle in Tuyen Quang province

3.2.2.1. The prevalence and infection intensity of *Eurytrematosis* according to localities



of *Eurytrematosis* in
s and cities

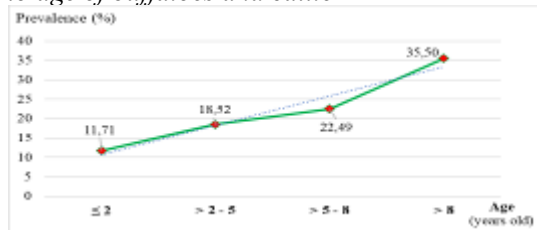
sity of *Eurytrematosis* in

oes and cattle raised in 5
ince were infected with
erences in the prevalence of
n district and Tuyen Quang
city was statistically significant ($P < 0.05$). Buffaloes and cattle raised in
districts and cities of Tuyen Quang province were most infected with a
infection intensity of less than 300 eggs/g of feces (54.45%), followed by
the infection intensity of 300 - 600 eggs/g of feces (32.88%) and the lowest
infection intensity was of over 600 eggs/g of feces (12.67%).

Nguyen Thi Hong Chien et al. (2011) detected through fecal testing, the
prevalence of *Eurytrematosis* in buffaloes and cattle in Vinh Phuc, Ha Nam
and Hanoi was nearly 18%. Tung et al. (2012) tested the fecal samples of
70 yellow cattle in Taiwan, the prevalence of *Eurytrema pancreaticum*
infection was 11.4%.

Therefore, the prevalence of *Eurytrematosis* in buffaloes and cattle in
Tuyen Quang province was higher than the research results of Nguyen Thi
Hong Chien et al. (2011), Tung et al. (2012).

3.2.2.2. The prevalence and infection intensity of *Eurytrematosis* according to age of buffaloes and cattle



ytrematosis according to
cattle

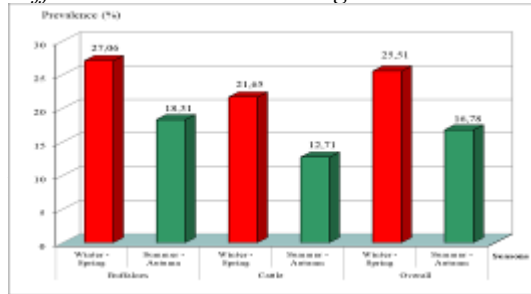
Eurytrematosis increased in
,71%; 18,52%; 22,49% and
g buffaloes and cattle of all
e were 19,61% of buffaloes
and 15% of cattle infected with an infection intensity of over 600 eggs/g of
feces.

Bassani et al. (2006) studied the epidemiological characteristics of
Eurytrematosis in 1,828 cattle in Parana state, Brazil. The author reported
that the proportion of pancreatic trematode infection increased gradually

with the age of the cattle. Cattle aged over 6 years old have the highest infection rate (62.4%).

Fluctuations of Eurytrematosis according to age of buffaloes and cattle in our study were relatively consistent with the results of the mentioned author's research.

3.2.2.3. The prevalence and infection intensity of Eurytrematosis in buffaloes and cattle according to seasons



Eurytrematosis according to

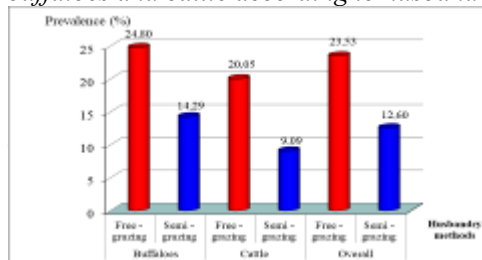
and cattle, the prevalence of was 25,51%, significantly (%). Regarding the infection *Eurytrema* spp. in the Winter - the Summer-Autumn season and cattle infected with an

infection intensity of over 600 eggs/g of feces).

Azevedo et al. (2004) mentioned that in the northern of Parana state, Brazil, the prevalence of pancreatic trematode infection in cattle from January to March was from 13,5% to 14,8%, then gradually decreased; the prevalence from May to August was from 9,9% to 11,9%. According to Sousa and Castro (2022), Eurytrematosis caused by *Eurytrema* spp. in livestock is more common during the rainy season and higher in areas with high rainfall, the prevalence decreases during the cold and dry season, in both definitive and intermediate hosts.

Thus, buffaloes and cattle raised in Tuyen Quang were infected with Eurytrematosis according to seasons at different prevalence and consistent with the results of Azevedo et al. (2004), but different from the comments of Sousa and Castro (2022).

3.2.2.4. The prevalence and infection intensity of Eurytrematosis in buffaloes and cattle according to husbandry methods



Eurytrematosis according to

ence of Eurytrematosis in both etely free-grazing method was semi-grazing method (23,53% difference in the prevalence of

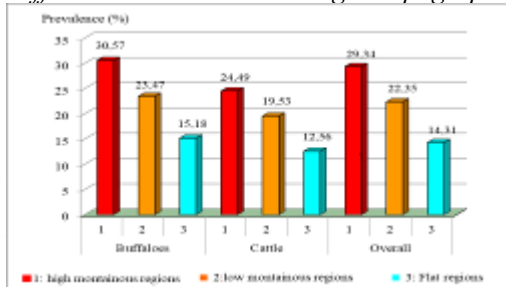
was statistically significant ($P < 0.05$). Regarding the infection intensity:

13,58% of buffaloes and cattle raised in free grazing method were infected with over 600 eggs/g of feces, while only 6,38% of buffaloes and cattle raised on semi-grazing were infected with *Eurytrematosis* at this infection intensity.

According to Ma et al. (2014), goats that are completely grazed in nature have more conditions to contact with intermediate hosts carrying pancreatic trematode larvae, thus the prevalence and the infection intensity of this disease is higher and heavier than those those raise in semi-razing goats.

Our results were consistent with the above author's comments.

3.2.2.5. The prevalence and infection intensity of *Eurytrematosis* in buffaloes and cattle according to topographical regions



Eurytrematosis according to topographical regions

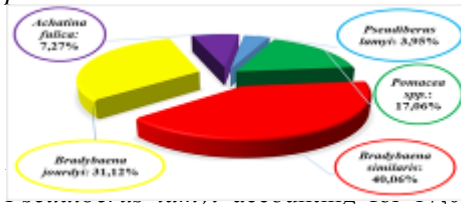
Buffaloes and cattle raised at high mountainous regions were infected with *Eurytrema* spp. at this infection intensity was 16,20%. While buffaloes and cattle in low mountainous regions were infected with *Eurytrema* spp. at this infection intensity was 12,03% and buffaloes and cattle in flat regions were only infected with 8,33% at this infection intensity.

Buffaloes and cattle were infected with *Eurytrema* spp. with an infection intensity of over 600 eggs/gram of feces being the highest (16,20%). While buffaloes and cattle in low mountainous regions were infected with *Eurytrema* spp. at this infection intensity was 12,03% and buffaloes and cattle in flat regions were only infected with 8,33% at this infection intensity.

3.2.2.6. Study on intermediate hosts of *Eurytrema* spp. pancreatic flukes in Tuyen Quang

a. Identify land snail species - the first intermediate host of pancreatic trematodes *Eurytrema* spp. in Tuyen Quang

* Identification of land snail species collected at localities in Tuyen Quang province



Land snails collected in localities

A total of 633 land snails. Figure 3.18 shows the appearance frequency of land snails belonging to *Pomacea* spp., *Pseudobornia jourdyi*, *Achatina fulica* and *Pseudobornia jourdyi*, respectively.

3,95%, respectively.

* Appearance frequency of land snails in localities

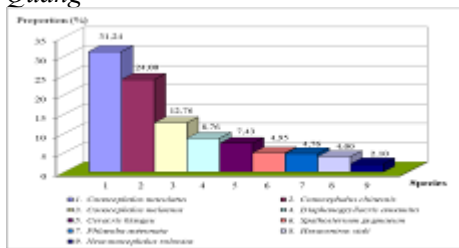
The *Bradybaena similaris* specie appeared in all studied communes (appearance frequency was 100%), followed by *Bradybaena jourdyi*, *Pomacea* spp., *Achatina fulica* with an appearance frequency of 92%; 88% and 56%, respectively; the lowest was of *Pseudiberus lamyi* (36%).

* *Determination of the identified land snail species as the first intermediate host of Eurytrema spp. in Tuyen Quang*

Our results were compared to the research findings of Tang (1950), Basch (1965), Ragusa and Campos (1976), Sakamoto et al. (1980), Chinone et al. (1984), Kozutsumi and Itagaki (1989) showed that among the 5 land snail species identified in Tuyen Quang province, only *Bradybaena similaris* specie has been proven by a number of studies to be the first intermediate host of *Eurytrema* spp.

b. *Identify grasshopper species - the second intermediate host of Eurytrema spp. pancreatic flukes in Tuyen Quang*

* *Identification of grasshopper species collected in localities of Tuyen Quang*



sshopper species collected in

es

For individuals collected in Tuyen Quang province, 9 grasshopper species have been identified. Among them, *Conocephalus maculatus*, *Conocephalus chinensis*, *Ceracris kiangsu*, *Diaphanogryllacris annamita*, *Ceracris kiangsu*, *Spathosternum pygmaeum*, *Phlaeoba antennata*, *Hexacentrus stali* and *Neoconocephalus robustus*. Of which, *Conocephalus maculatus* specie accounted for 31,24% and *Conocephalus chinensis* species reached 24,00%.

* *Appearance frequency of grasshoppers in localities*

Among the 9 identified grasshopper species, *Conocephalus maculatus* was the species that appeared in all studied communes (appearance frequency was 100%), followed by *Conocephalus chinensis* (96%), *Conocephalus melaenus* (56%), *Ceracris kiangsu* (56%), *Diaphanogryllacris annamita* (52%), *Spathosternum pygmaeum* (44%). The three species *Hexacentrus stali*, *Neoconocephalus robustus*, and *Phlaeoba antennata* have the lowest appearance frequency (28% - 40%).

* *Determination of the identified grasshopper species as the second intermediate host of Eurytrema spp. in Tuyen Quang*

Out of the 9 identified grasshopper species, there were only 2 species, *Conocephalus maculatus* and *Conocephalus chinensis*, have been proven by a number of studies to be the second intermediate host of *Eurytrema*

spp. pancreatic flukes. In addition to these two species, the remaining 7 species have not been proven by any author to act as intermediate hosts of pancreatic flukes.

Sakamoto et al. (1980) reported that the rate of *Metacercaria* of *Eurytrema* spp. found in the hemocoel of *Conocephalus maculatus* grasshopper was 18,3% in July and 42% in December.

3.2.3. Study on pathological and clinical characteristics of Eurytrematosis in buffaloes and cattle in Tuyen Quang province

3.2.3.1. Clinical symptoms of buffaloes and cattle infected with Eurytrematosis

Observing 136 buffaloes and cattle only infected with Eurytrematosis, there were 35 animals showing clinical symptoms, accounting for 25,74%. Clinical manifestations were mainly as follows: emaciation; diarrhea, mucosa in feces; emphysema in the neck and chest; milky white cast in eyes; anemia, pale mucous membranes. The rate of buffaloes and cattle manifested these symptoms fluctuating from 14.29 to 42.86% of total buffaloes and cattle have clinical symptoms (Table 3.20).

Rachid et al. (2011) observed clinical symptoms in 3 Nelore cattle infected with Eurytrematosis in Minas Gerais state, Brazil. The author reported that infected cattle showed signs of weight loss, poor appetite, emaciation and lying down.

3.2.3.2. Gross lesions of buffaloes and cattle infected with Eurytrematosis

Necropsy was conducted to examine 71 buffaloes and cattle infected with Eurytrematosis and found that 23 buffaloes and cattle had gross lesions, accounting for 32,39%. Gross lesions were concentrated mainly in the pancreas of buffaloes and cattle, the lesion were as the following: the hard, pale, pancreas increased in size and fibrotic (47,83%), and the intestines were inflamed and hemorrhagic (30,43%). Other lesions such as swollen mesenteric lymph nodes; swollen, pale yellow liver accounted for a lower rate (21,74% and 17,39%, respectively) (Table 3.21).

According to Pantiu et al. (2021), the pancreas, with many *Eurytrema* spp. flukes parasitizing, becomes enlarged, the surface of the pancreas is pale white, has mucus and is wrinkled; part of the pancreatic duct is thickened and pale; some pancreatic lobes were seriously damaged.

The studied results on buffaloes and cattle infected with Eurytrematosis in Tuyen Quang province showed that gross lesions in the digestive tract of infected buffaloes and cattle are relatively consistent with the mentioned author's description.

3.2.3.3. Microscopic lesions in buffaloes and cattle infected with Eurytrematosis

The studied results showed that microscopic lesions caused by *Eurytrema* spp. focusing on the pancreas. The lesion were as follows: *Eurytrema* flukes and eggs caused dilated pancreatic duct, cysts were formed in the pancreatic tissue, when the fluke suckers adhered to the pancreatic tissue, causing an inflammatory reaction, atrophic, degenerated and fibrotic pancreatic tissues. These microscopic lesions lead to pancreatic dysfunction.

According to Grosskopf et al. (2016), *Eurytrema* spp. flukes parasitize in the pancreas and pancreatic ducts, thus the lesions of infected cattle are mainly concentrated in the pancreas: fibrotic, dilated ducts. Additionally, the pancreatic duct may become inflamed. When cattle have a large number of parasitic flukes, the pancreatic duct is obstructed and pancreatic tissues are damaged. In infected, all the histiocytic cells of the pancreatic duct were replaced by fibrotic tissue, and the pancreatic duct is gradually destroyed due to the proliferation of connective tissue.

Microscopic lesions in buffaloes and cattle infected with Eurytrematosis in Tuyen Quang that we observed were relatively consistent with the results of mentioned author.

3.2.3.4. *Study on changes in some physiological and biochemical parameters of blood in buffaloes and cattle infected with Eurytrematosis*

** Changes in some blood physiological parameters of buffaloes and cattle infected with Eurytrematosis*

The total number of red blood cells and white blood cells of buffaloes and cattle infected with Eurytrematosis were compared to those of healthy buffaloes and cattle, the results showed that the number of red blood cells, hemoglobin contents and hematocrits in infected buffaloes and cattle decreased in comparison with those in healthy buffaloes and cattle. Meanwhile, there was a change in the ratio of different types of white blood cells such as: the ratio of neutrophils of infected animals increased significantly in comparison with that in healthy buffaloes and cattle. The ratio of basophils and eosinophils in infected buffaloes and cattle increased in comparison with those in healthy buffaloes and cattle. The ratio of lymphocytes and monocytes in infected animals decreased in comparison with those in healthy buffaloes and cattle (Tables 3.22 and 3.23).

** Changes in some blood biochemical parameters of buffaloes and cattle infected with Eurytrematosis*

Comparison of blood biochemical parameters of buffaloes and cattle infected with Eurytrematosis and healthy buffaloes and cattle, the results showed that the number of protein, albumin and globulin content in infected buffaloes and cattle decreased slightly in comparison with those in

healthy buffaloes and cattle. Gamma glutamyl transferase, aspartate aminotransferase and cholesterol indexes in diseased buffaloes and cattle increased slightly in comparison with those in healthy buffaloes and cattle. Meanwhile, the amylase and glucose indexes of infected buffaloes and cattle increased significantly in comparison with those in healthy buffaloes and cattle (Tables 3.24 and 3.25).

3.2.4. Study and propose preventive and treatment measures off *Eurytrematosis* in buffaloes and cattle

3.2.4.1. Study on highly effective medicines to deworm pancreatic flukes for buffaloes and cattle

** Approving to select a effective medicine in deworming pancreatic flukes for buffaloes and cattle*

The results in the table 3.26 showed that although all three medicines were approved in deworming pancreatic flukes for buffaloes and cattle were safe, but the deworming effectiveness of each medicine was different. Praziquantel medicine at a dose of 15 mg/kg B.W. has the highest deworming effectiveness (80%); Albendazole medicine was almost ineffective (20%), nitroxynil has very low effectiveness (60% in buffalo and 40% in cattle).

** Determining the dose of praziquantel with high effectiveness in deworming pancreatic flukes for buffaloes and cattle*

The results in table 3.27 showed that praziquantel medicine at a dose of 15 mg/kg B.W has an effectiveness of 80% in deworming pancreatic flukes, both doses of 18 mg/kg B.W and 20 mg/kg B.W have an high effectiveness of 100%, respectively.

** Determine the effectiveness of praziquantel medicine at 2 dosage levels (18 mg/kg B.W and 20 mg/kg B.W)*

The results in table 3.28 showed that praziquantel medicine at doses of 18 mg/kg B.W and 20 mg/kg B.W were highly effective in deworming pancreatic flukes (100%), and did not cause side effects in buffaloes and cattle.

** Use praziquantel medicine (at a dose of 18 mg/kg B.W) to deworm pancreatic flukes for buffaloes and cattle in studied localities*

Table 3.29 showed that using the praziquantel medicine at a dose of 18 mg/kg B.W dewormed pancreatic flukes for buffaloes and cattle in Chiem Hoa, Ham Yen, Yen Son, Son Duong district and Tuyen Quang city, the average effectiveness was 98,10%.

Therefor, the praziquantel medicine at a dose of 18 mg/kg B.W widely used for buffaloes and cattle in localities was highly effective and safe in deworming pancreatic flukes.

3.2.4.2. Study on preventive measures of Eurytrematosis in buffaloes and cattle

a) The effect of composting buffalo and cattle manure with probiotics to eliminate pancreatic fluke eggs

The results in table 3.30 showed that composting buffalo and cattle manure with 3 types of probiotics has the effect of reducing or completely eliminating pancreatic fluke eggs in feces. However, EMUNIV and EMZEO probiotics used for composting have a better effect on eliminating pancreatic fluke eggs than that of EMIC probiotic.

b) Testing Eurytrematosis preventive medicine for buffaloes and cattle in Tuyen Quang province

** Determine the prevalence and infection intensity of Eurytrematosis in buffaloes and cattle before conducting the experiments*

Before conducting the experiment, 20% of buffaloes and cattle in the control group were infected with Eurytrematosis; the prevalence of Eurytrematosis of buffaloes and cattle in experimental group 1 and 2 was 22% and 20%, respectively. The infection intensity of buffaloes and cattle in 3 experimental groups was mainly at level (+). Thus, buffaloes and cattle in all three experimental groups had similar prevalence and infection intensity of Eurytrematosis (Table 3.31).

** The prevalence and infection intensity of Eurytrematosis in buffaloes and cattle after 1 month of experiment*

After 1 month of experiment, buffaloes and cattle in experimental group 1 and 2 were provided preventive medicine, the result showed no animals were infected with Eurytrematosis. The difference in the prevalence between buffaloes and cattle in the experimental group 1 & 2 and the control group was significant ($P < 0.05$) (Table 3.32).

** The prevalence and infection intensity of Eurytrematosis in buffaloes and cattle after 4 months of experiment*

After 4 months of experiment, the prevalence of Eurytrematosis in group 1 increased from 0% after 1 month of experiment to 4%, while buffaloes and cattle in group 2 still had no animals infected with Eurytrematosis. Buffaloes and cattle in the control group, the prevalence after 1 month of the experiment was 20%, after 4 months of the experiment increased to 24%, the infection intensity of severe infection increased from 10% to 16,67%. The difference in the prevalence between the 3 experimental groups was significant ($P < 0.05$) (Table 3.33).

c) Propose integrated prevention and treatment measures of Eurytrematosis in buffaloes and cattle

We have proposed comprehensive prevention and treatment measures of Eurytrematosis for buffaloes and cattle, including: using praziquantel medicine at a dose of 18 mg/kg B.W to deworm pancreatic flukes for buffaloes and cattle; composting buffalo and cattle manure with EMUNIV or EMZEO probiotics; ensuring veterinary hygiene during the process of raising buffaloes and cattle; eliminating the intermediate hosts of pancreatic flukes, which are land snails and grasshoppers; Strengthening care and nurturing of buffaloes and cattle to improve disease resistance.

CONCLUSION AND RECOMMENDATIONS

1. Conclusion

1.1. Regarding the characteristics of gastrointestinal trematode infection in buffaloes and cattle in Tuyen Quang province through fecal testing

Buffaloes and cattle raised in Tuyen Quang province infected with gastrointestinal trematode infection at a prevalence of 37,49% (ranging between 30,29% and 46,29%). The prevalence of gastrointestinal trematode infection increased in accordance with age; in the Summer-Autumn season, buffaloes and cattle infected with gastrointestinal trematode infection at a higher prevalence than that in the Winter-Spring season; buffaloes and cattle raised in completely free-grazing method have a higher prevalence than those raised semi-grazing method; buffaloes and cattle raised in flat regions were more infected with gastrointestinal trematode infection than those raised in other regions.

1.2. Regarding the disease caused by Eurytrema spp. pancreatic flukes in buffaloes and cattle in Tuyen Quang province

- By using morphological and molecular techniques, the pancreatic flukes parasitized in buffaloes and cattle in Tuyen Quang province were identified as *Eurytrema cladorchis* and *Eurytrema coelomaticum*.

- All buffaloes and cattle raised in 5 districts and cities in Tuyen Quang province infected with Eurytrematosis at a prevalence of 22,70% in buffaloes and 17,35% in cattle. The prevalence increased gradually in accordance with age (from 14,04% to 36,96% in buffaloes; from 3,92% to 32,26% in cattle). The prevalence of Eurytrematosis in buffaloes and cattle raising in Winter – Spring season was 25.51%, in the Summer - Fall season was 16.78%; in completely free- grazing method was 25,53% and in semi-free grazing was 12,60%. Out of 29,34% of buffaloes and cattle were infected with Eurytrematosis in high mountainous regions, was 22,35% in low mountainous regions, and was 14,31% in flat regions. Buffaloes and cattle infected with Eurytrematosis at the infection intensity of level (+++) accounted for 12,67% of the total of infected buffaloes and cattle.

- The *Bradybaena similaris* land snail was the first intermediate host, the two grasshopper species as *Conocephalus maculatus* and *Conocephalus chinensis* were the second intermediate hosts of *Eurytrema* spp. pancreatic flukes in Tuyen Quang province.

- *Regarding pathological and clinical characteristics of Eurytrematosis in buffaloes and cattle in Tuyen Quang province:*

+ Buffaloes and cattle infected with Eurytrematosis were emaciated, slow; diarrheal, containing mucus in feces; emphysema in the neck and chest; milky white cast in eyes; anemia, pale mucous membranes.

+ Gross lesions included hard, pale pancreas, the pancreas increased in size and fibrotic, swollen mesenteric lymph nodes; swollen liver and pale yellow in color, inflamed and hemorrhagic intestines.

+ Microscopic lesions included: necrosis of large excretory ducts, peeling, the lumen contains adult flukes and eggs, stromal reaction around flukes has degenerated, calcified; the exocrine pancreas was atrophied and fibrotic.

+ Infected buffaloes and cattle have a reduced number of red blood cells, hemoglobin content and hematocrits; white blood cells, neutrophils, basophils and eosinophils increased, while lymphocytes and monocytes decreased significantly in comparison with those in healthy buffaloes and cattle.

+ Infected buffaloes and cattle have protein, albumin and globulin content decreased, gamma glutamyl transferase, aspartate aminotransferase and cholesterol indexes slightly increased, and amylase and glucose indexes clearly increased in comparison with those in healthy buffaloes and cattle.

1.3. Regarding preventive and treatment measures of Eurytrematosis in buffaloes and cattle

- Praziquantel medicine at a dose of 18 mg/kg B.W was safe and highly effective in deworming pancreatic flukes.

- EMUNIV and EMZEO probiotics used to compost buffalo and cattle manure have the effect of eliminating pancreatic fluke eggs in the husbandry environment.

- Use of preventive medicine for buffaloes and cattle twice times per year, each time repeated twice 2 months apart.

- Proposed comprehensive prevention and treatment measures of Eurytrematosis for buffaloes and cattle in Tuyen Quang province including six main measures.

2. Recommendation

Widely apply integrated prevention and treatment measures of Eurytrematosis for buffaloes and cattle in Tuyen Quang province and neighboring provinces.