MINISTRY OF EDUCATION AND TRAINING
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STUDY ON BLACKHEAD DISEASE CAUSED BY *HISTOMONAS MELEAGRIDIS* PROTOZOAN IN CHICKENS IN THAI NGUYEN, BAC GIANG AND PREVENTION - TREATMENT MEASURES

Speciality: Veterinary parasitology and microbiology

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INTRODUCTION

1. Urgency of the dissertation

Blackhead disease (*Histomonosis)* in a dangerous parasite/ protozoan in poultries, especially chickens and turkeys. This disease is caused by anaerobic protozoan parasite which its science name is *Histomonas meleagridis*. Diseased poultries are depression, reduced appetite, Sulphur-yellow diarrhea, skin of head becomes pale or cyanotic, ceca and liver is swollen, caseous cores with white and liver appears gangrene sports as "Chrysanthemum". Diseased chickens were died, if they are not treated immediately, the mortality may be 85%- 95%.

Histomonosis is detected in backyard chickens at some of provinces of the North from March, 2010 (Lê Văn Năm, 2010). Recently, this disease occurs in provinces, cities over the country. The disease is out breaking in Thai Nguyen and Bac Giang provinces, cause huge damages about economy for farmers. Hence, in Viet Nam there are not yet dissertation discovering about blackhead disease in chickens, there are not effective treatment and prevention process.

In order to make contribution to controlling disease, improve productivity of raising chickens; we implement the dissertation ***"Study on blackhead disease characteristics caused by Histomonas meleagridis protozoan in raising chickens in Thai Nguyen, Bac Giang provinces and recommendation for prevention and treatment measures"***.

2. Objective of the dissertation

Evaluation on epidemiologic and pathological characteristics and measures of disease prevention caused by ***Histomonas meleagridis*** in raising chickens in two provinces Thai Nguyen and Bac Giang, contribute to improving the chickens husbandry productivity in areas.

3. Scientific and practical significance of the dissertation

3.1. Scientific significance

The results of the dissertation are the scientific information of epidemiological and pathological features and preventive process of blackhead disease in chickens in Thai Nguyen and Bac Giang provinces and others Northern mountain provinces.

3.2. Practical significance

The results of the study are scientific basis to recommend animal producers in applying preventive and control measures of chicken's blackhead disease contribute to improving the productivity in animal husbandry.

3.3. New contribution of the dissertation

- It in the first work in studying systematically about the disease, epidemiological and pathological characteristics and prevention and treatment measures of blackhead disease in chickens.

- Building prevention and treatment process of blackhead disease caused by ***Histomonas meleagridis*** protozoan in chickens effectively, disseminating and applying widely them to various farmer households and raising chicken farms.

4. Structure of dissertation

Dissertation includes 128 pages (primary content) divided into chapter: Introduction: 2 pages, chapter 1: Overview of document (39 pages), chapter 2: Materials, contents and methodology (24pages), Chapter 3: Study results and discussion (66 pages). Conclusion and recommendation (2 pages).

References (25 pages); Pictures of dissertation (17 pages); Appendix (24 pages).

The dissertation has 33 tables, 14 graphs, 68 pictures showing results of dissertation, 148 references (13 Vietnamese documents, 135 foreign language documents, including documents from 2010 – 2015 are 35%).

Chapter 1

OVERVIEW OF DOCUMENT

Basing on results of analyzing 18s rRNA genetic order of 18S rRNA of *H. meleagridis* Cepicka I. et al (2010) showed, *H. Meleagridis's* position: Protozoan genders , *Parabasalia* phylum, *Tritrichomonadea* class, *Tritrichomonadea* order, *Dientamoebidae* family , *Histomonas* genus , *H. meleagridis* species.

Lund E. E. and chute A. M. (1974) said: *H. meleagridis* protozoan exist in two forms, amoeboid and flagellated. Within the tissue, it is present as an amoeboid protozoan, while in the lumen or free in the contents of cecum, it lives as an elongated flagellated form.

H. meleagridis protozoan has weak resistance. After it follows feces to go out environment, the most life time is no more than 24 hours. Nevertheless, *H. meleagridis* may exit annual in egg of pinworms (Le Van Nam, 2011).

Dwyer D. M. (1970) researched and made successfully H.me rearing environment including 85 - 95%, M199, 5 - 10% serous horse, 5% 5% chick embryo extract and 1% rice powder.

Infecting *H. meleagridis* protozoan in chickens and turkeys may be occurred individually or simultaneously by some ways. Firstly, chickens eat fresh feces, internal organs of diseased chickens or anus connect with H. meleagridis protozoan. Secondly, chickens swallow *Heterakis gallinarum* - egg of pinworms which have germ and contain *H. meleagridis*. Thirdly, chickens eat earthworms containing eggs pinworm's egg with *H. meleagridis*. When they are in chicken's body, *H. meleagridis* reproduces by binary fission to rapidly increase.

Chickens which have blockhead disease have typical symptoms: Sulphur – yellow diarrhea, skin of head pale or cyanotic. With diseased chickens, lesions concentrate mostly on liver and ceca, , caseous cores with white, liver was swollen twice – three times, inflamed ceca in swollen, gangrene spots as chrysanthemum (Mc Douglad L. R., 2005).

Preventing blackhead disease from chickens by combining measures: hygiene in taking are, using paromonycin drug, nitarsone (Histostat M)... mix into food for chickens, or using *H. meleagridis* for poultry.

Hess M. et al (2015), nitromidazdes and nitrofuraus drugs are two preventive and treatable medicine groups effectively. However, on 1990 years, many other countries in the word banned using two products because these existed within products for a long time and caused cancer for human. Because couldn't find pharmaceutical chemistries which replaced to treat blackhead disease has out broken in countries and posed on damage heavily in economy.

Chapter 2
MATERIALS, CONTENTS AND METHODOLOGY

2.1 Object, time and place of study

2.1.1. Object of study

Blackhead disease in chickens in Thai Nguyen and Bac Giang province

2.1.2. Place and time of study

** Place of study*

- The dissertation was carried out at farm households, farms with various sizes in two provinces Thai Nguyen and Bac Giang.

- Places where samples were tested: Laboratory of Veterinary medicine and Animal science faculty – Thai Nguyen university of Agriculture and Forestry: Laboratory Agriculture and Forestry technology and economy – Thai Nguyen university Surgery – Pathology genre Viet Nam national university of Agriculture.

* *Time of study*: 2012- 2015

2.2. Materials of study

2.2.1. Animals and various types of study samples

* *Animals of study*: Raising chickens in Thai Nguyen and Bac Giang healthy 2 month–old chickens are good health and chickens were vaccinated (to design blackhead disease infection experiments).

* *The samples of study include*: Internal organs of diseased chickens and healthy chickens, *H. meleagridis* protozoan, samples of pinworms collecting through chickens necropsy, sample of blood, samples of manure and samples of farming areas of chickens.

2.2.2. Instruments and chemicals

Instruments and chemicals include light microscopes, blood gas analyzer, *H. meleagridis* protozoan culture medium, anthelmintic

drugs and drugs for blackhead disease of chickens and other instruments and chemicals.

2.3. Contents of study

2.3.1. Nomenclature of parasitic protozoan (H. meleagridis) in raising chickens in Thai Nguyen and Bac Giang by using PCR method

2.3.2. Investigation of characteristic of blackhead disease in chickens

2.3.2.1. Investigation of present status of prevention and control of parasitic diseases and blackhead disease in chickens in Thai Nguyen and Bac Giang.

2.3.2.2. Study on H. meleagridis infection in chickens through necropsy.

2.3.2.3. Study on relation between blackhead disease and pinworm disease in chickens.

2.3.3. Study on blackhead disease by H. meleagridis in chickens.

2.3.3.1. Study on blackhead disease in experimentally infected chickens.

2.3.3.2. Study on blackhead disease in chickens in Thai Nguyen and Bac Giang.

2.3.4. Study on prevention and treatment measures for chickens caused by blackhead disease.

2.3.4.1. Study on measures killing immediate hosts to prevent blackhead disease in chickens.

2.3.4.2. Determining effect of (killing/destroying) H. meleagridis protozoan by using benkoacl destroying antiseptic drugs, providine 10%, Qm-supercide in condition of laboratory.

2.3.4.3. Determining the efficacy and safety level on two blackhead disease treatment regimens for chickens.

2.3.4.4. Recommendation of preventive and treatment measures of this disease.

2.4. Methods of study

2.4.1. Nomenclature of the protozoan Histomonas spp. caused black disease in chickens in Thai Nguyen and Bac Giang by molecular biology measure

2.4.2. Methods of studying on epidemiological characters of blackhead disease in chickens in Thai Nguyen and Bac Giang

2.4.2.1. Methods of field investigation of present status of prevention and control parasitic disease in chickens.

Establishing evaluation criteria, direct observation of present status of chickens raising in the places studied, interviewing and giving investigation from on a number of criteria designed.

2.4.2.2. Method of studying on epidemiological characteristics of blackhead disease in chickens: using method of studying on describe epidemiology and epidemiological analysis.

* *Determining capacity of samples collecting in areas:* collecting samples by using stratified cluster sampling capacity of samples were calculated by Win Episcope 2.0 software.

* *Determining infection proportion of H. meleagridis in chickens:* The proportion of the infection of *H. meleagridis* protozoan in chickens were determined by combination between methods: observing) clinical symptoms, dissection and check of lesions. Making specimen of liver and ceca to dye Giemsa of dye Hematoxilin- Eosin and observe them under light microscope.

* *The internal organs were dissected incomprehensively*, found parasitic pinworm to determine infection intensity of pinworms.

* *Method of detecting eggs of pinworm in around the area of chicken pen, pig pen floors and garden where raises chickens:* collecting samples and using Gefter measure to detect eggs of pinworm.

2.4.4. Method of studying on blackhead disease caused by H. meleagridis protozoan in chickens experimentally

2.4.4.1. Study on blackhead disease in infected chickens

a) *Method of culturing H. meleagridis protozoan in artificial environment*

* *Prepare of culture environment*

Dwyer medium includes: M199 with salt of hanks (85%), 5% chicken embryo extract 8 – 10 days old, serous horse (10%), rice powder 1mg/ 1ml, pH= 7,4. Modified Dwyer medium includes: M199 with salt of hanks (90%), serous horse (10%), rice powder 10mg/ 1ml, pH= 7,4.

* *Method of culture:* spots of liver cassation and all agents contained in ceca were separated into an aseptic glass and the were covered with Dwyer environment of advanced Dwyer environment (the proportion between medical waste and culture medium 1 : 9), they were kept in fastidious environment at 40˚C in 48h. 1ml environment containing the protozoan is moved into aseptic test-tube containing 9 ml culture solution on 3 days. Replication of *H. meleagridis* is evaluated annual by counting quantity of *H. meleagridis* into 1 ml environment in Neubauer clamber, determining dose admin steered in experimental chickens.

- Infecting *H. meleagridis* for chickens: using aseptic cylinder suck environment containing *H. meleagridis* with detailed ml member, pumps into chickens' mouth and anus. Chicken are abstained from eating and drinking in 5h before and after an infection, stimulate chickens to.

** Study on pathology of blackhead disease in infected chickens through the gross injury level in the liver, ceca and other internal organs:* after infecting *H. meleagridis* for chickens through mouth and anus, every day a chicken in dissected to follow the injury level in experimental infected chickens. Body temperature of chickens were checked daily at 8 - 9 am; clinical signs of them were observed and taken notes simultaneously. The earliest and latest and death time of diseased chickens also is determined.

** Testing blood of experimental and control chickens.*

** Checking gross and minor injuries and determining change of weight and volume in internal organs of experimental infected chickens by necropsy examination in chickens* which were died and alive in sixteenth day after being experimentally infected. Their internal organs are observed by naked eyes and magnifier, taking picture of areas that manifested typical injuries. Experimentally infected and control are weighed weight and the internal organs. Chickens liver and ceca were made based on Histology Technique of cutting tissues, the tissues can be mounted on a microscope slide stained with Hematoxilin – Eosin and examined under light microscope to observe microscopic changes.

2.4.5. Method of studying on preventive and treatment measures in blackhead disease in chickens

2.4.5.1. *Prevention of blackhead disease in chickens by using anthelmintic drugs for deworming pinworms.*

Using mebendazole 10%, levamisole and fenbenclazole drugs denormes for chickens in small areas and after in large areas.

2.4.5.2. Determining effect of killing H. meleagridis by antiseptic drugs: absorbing 5ml of advanced Dwyer's culture medium that contains *H. meleagridis* into each petri spreading/making thin and then sparing benzoic, povidine 10% and QM – Supercide on its surface, observing ability of killing *H. meleagridis* of antiseptic drugs.

2.4.5.3. Determining the efficacy and the safely level of blackhead disease: Establishment for two blackhead disease treatment regimens for chickens, experimental treatment for chickens which have blackhead disease from experimental infection, then experimental treatment for chickens in places. First regimen consists of: sulfamonomethoxine, doycyclin, paracetanol, detoxication drug of liver, spleen and kidney, unilyte Vit-C. Second regimen consists of: Cloroquin phosphat, Holarrhena antidyesenterica, detoxication drug of liver, spleen and kidney, unilyte Vit-C.

2.5 Method of treatment of data

Data collected in treated by methods of biostatistics (Nguyen Van Thien, 2008), on Excel software 2007 and Minitab software 14.0.

Chapter 3

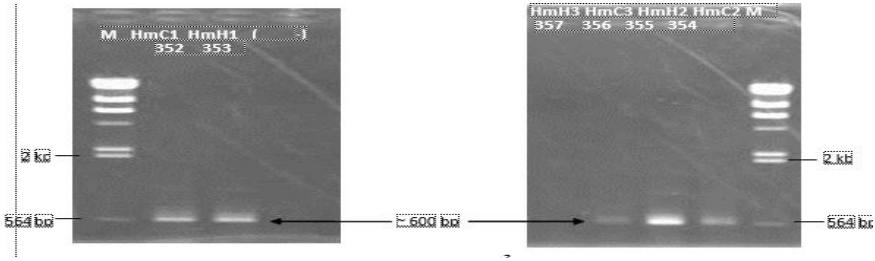
REULTS AND DISCUSSION

3.1. Results of nomenclature of parasitic protozoan (*Histomonas* spp) by using molecular bidogy method

3.1.1. Implementation of PCR technique for receiving 18S ribosomal gene

Implementation of PCR technique has received 18S gene which has about 600bp length the results are presented in picture 3.1

Picture 3.1 shows that the samples Hm-C1-TN-VN, Hm-H1-TN-VN; Hm-C2-BG-VN, Hm-H2-BG-VN and the ceca samples Hm-C3-TN-VN have PCR product. Two couples samples Hm-C1-TN-VN, Hm-H1-TN-VN and Hm-C2-BG-VN, Hm-H2-BG-VN are selected to analyse gene sequence directly.



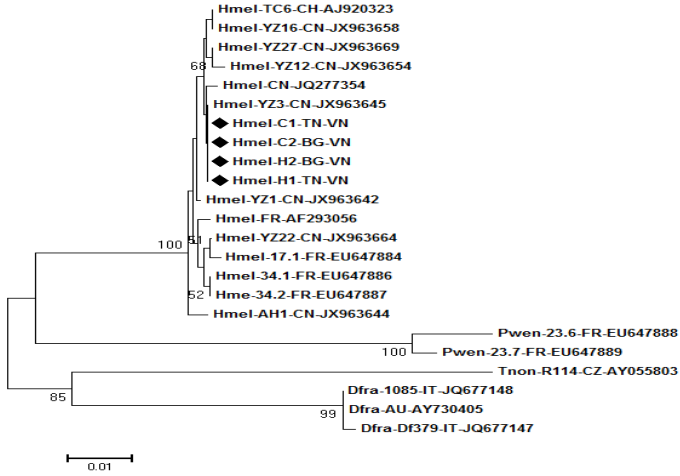
Picture 3.1: Pictures of electrophoresis in PCR product of 18S gene in *Histomonas* spp checked in agerose 1%.

3.1.2. The results of identificating gene sequence 18S ribosomal and accessing Gen bank of Histomonas spp.

The results of retrieving gene sequence, comparing nucleotide sequence of 18S gene ribosomal and accessing Gen bank of 4 *Histomonas* spp. samples are presented in table.3.1 and Picture 3.1 (appendix of dissertation).

The results Picture 3.1 and table 3.1 show that: When comparing and collating nucleotide sequence of gene 18S ribosomal of 4 *Histomonas* spp samples isolated with the samples of the world, the samples of Vietnam have nucleotide sequence which are similar 86 – 100 % with the samples in the world.

3.1.4. Analyzing genealogy relation



Picture 3.2. Genealogy tree showing relation about species based on amino acid sequence of gene

The results in Picture 3.2 show that: 4 *Histomonas* spp samples of Vietnam have similar relation with *H. meleagridis* sample signed H.mel –YZ3- CN-5X963645 and locates into the same group with sample signed H. mel – CN- 5Q277354 of China.

3.2. Epidemiological characteristics of blackhead disease caused by *Histomonas meleagridis* in chickens in Thai Nguyen and Bac Giang

3.2.2. H. meleagridis infection in chickens in various places

3.2.2.1. Infection rates of H.me in chickens in various places

The results of table 3.3 show that: there were 244 chickens' infected *H. meleagridis* of total 1276 chickens dissected. The highest proportion of infected chickens was in Yen The district (34,83%), the second was in Phu Binh district (29,43%), Tan Yen district (16,74%), Pho Yen district (8,52%), Hiep Hoa district (8,24%) and the lowest rate was in Vo Nhai district (4,60%).

Table 3.3. Infection rates of *H. meleagridis* in chickens in various places

Place (province)		Number of chickens tested (chicken)	Number of chickens Infected (chicken)	Infection rate (%)
Thai Nguyen	Phu Binh	265	78	29,43
	Vo Nhai	174	8	4,60
	Pho Yen	176	15	8,52
	Sum	**615**	**101**	**16,42[a]**
Bac Giang	Tan Yen	215	36	16,74
	Yen The	264	92	34,85
	Hiep Hoa	182	15	8,24
	Sum	**661**	**143**	**21,63[b]**
Total		**1276**	**244**	**19,12**

Notes: In vertical line, the figures carrying different letters are in statistically significant difference.

In Yen The, Phu Binh and Tan Yen, the number of families, have raised chickens with the large amount, long term, they have not any time for, exposing surface of hen- house to kill germs. Chickens in these places raised in blackhead disease essentially they contacted with many germs; hence the infection rates chickens in these places were very high.

3.2.2.2. *Infection rates of H. meleagridis from chickens ages*

H. meleagridis infection proportion with aging in chickens was illustrated table 3.4 (Primary dissertation).

The results of table 3.4 show that: chickens at different ages also infection *H. meleagridis*, but chickens at different ages had different infection rates. Infection rates of *H. meleagridis* in chickens aged 1-3 months (32,53%).

3.2.2.3. Infection rates of H. meleagridis in chickens by crop

The proportion of *H. meleagridis* infection in chickens with crop was described in table 3.5 (In primary dissertation).

The results of Table 3.5 show that: the highest infection rate of *H. meleagridis* (26,98%) was in chicken raised in Summer, next was spring (20,56%), autumn (16,57%) and the lowest rate was in chickens raised winter (11,74%).

The weather of spring and summer was warm, humid, rainy which creates advantaged conditions for development of immediate hosts and vector hosts and infected chickens with blackhead disease, hence the infection rate was very high. In contrast, the weather of autumn and winter was dry and cold, this weather was disadvantaged conditions for development of immediate hosts and vector hosts so this rate was low.

3.2.3. Study on relation between blackhead disease and pinworm disease in chickens

3.2.3.1. The infection rate and intensity if pinworm is chickens dissected

Table 3.9. The infection rates and intensity if pinworm is chickens dissected

Place (province/ district)		Number of chickens dissected	Number of chickens infected	Infection rate (%)	Rate Infected intensity (number of pinworms/chickens)					
					< 150		150 – 300		> 300	
					n	%	n	%	n	%
Total		**615**	**272**	**44,23**	**74**	**27,21**	**126**	**46,32**	**72**	**26,47**
Thai nguyen	Phu Binh	265	159	60,00	42	26,42	69	43,40	48	30,19
	Vo Nhai	174	38	21,84	12	31,58	20	52,63	6	15,79
	Pho Yen	176	75	42,61	20	26,67	37	49,33	18	24,00
Total		**661**	**345**	**52,19**	**87**	**25,22**	**161**	**46,67**	**97**	**28,12**
Bac Giang	Tan Yen	215	106	49,30	25	23,58	53	50,00	28	26,42
	Yen The	264	177	67,05	43	24,29	78	44,07	56	31,64
	Hiep Hoa	182	62	34,07	19	30,65	30	48,39	13	20,97
Total		**1276**	**617**	**48,35**	**161**	**26,09**	**287**	**46,52**	**169**	**27,39**

The results of table 3.9 show that: In Thai Nguyen province, the infection rate of pinworms was 44,45 % of total 615 chickens

tested, the highest infection rate of pinworms in chickens was in is Phu Binh district, the lowest rate was in Viet Nam district (21,84 %). In Bac Giang province, this rate was 52,19 % of total 611 chickens tested, this rate was the highest Yen The district (67,05%) and the lowest in Hiep Hoa (30,07 %). The infection rate of blackhead disease had a relation with the infection rate of pinworms in chickens because, chickens in places infected pinworms also infected blackhead disease more than others places and vice versa.

3.2.3.2 Determining correlation coefficient between the infection rate of pinworm (x) and the infection rate of H.meleagridis (y) in chickens

Correlation between the infection rate of pinworm (x) and the infection rate of *H.meleagridis* (y) was illustrated in table 3.12 and picture 3.12.

The result of table 3.12 show that: regression equation between the infection rate of pinworm and *H.meleagridis* in chickens was y = 15,2 + 0,708x. Correlation coefficient was R = 0,947, show that this correlation was advantaged and close.

3.3 Study on blackhead disease in experimentally infected chickens and in the field research

3.3.1 Study on blackhead disease in experimentally infected chickens.

3.3.1.1. Culture of H.meleagridis

Table 3.14. Result of culturing *H. meleagridis* in Dwyer environment

Time of culture	*Number of H. meleagridis / ml culture medium* (x 10^3)						
	Start	After					
		24 h	48 h	72 h	96 h	120 h	144h
1	4,16	20,87	145,44	1154,32	1062,4	489,72	50,86
2	3,84	18,24	121,25	803,65	740,38	371,46	56,23
3	5,86	28,79	225,61	2118,49	1692,78	751,35	71,92
4	1,3	6,34	38,54	264,58	212,64	112,34	5,26
Average	**3,79**	**18,56**	**132,71**	**1080,76**	**927,05**	**431,22**	**46,07**

The result of table 3.14 and 3.15 were showed:

H. meleagridis protozoans were isolated from ceca and liver of disease chickens kept 48h, when they were cultured and moved to

Dwyers and modified Dwyers environment they also developed well, the number of protozoan infection quickly and they decreased gradually. However, *H. meleagridis* developed better in modified environt.

Table 3.15. Result of culturing *H. meleagridis* in modified Dwyer environment

Time of culture	*Number of H. meleagridis/ ml culture medium* (x 10^3)						
	Start	After					
		24 h	48 h	72 h	96 h	120 h	144h
1	2,64	13,37	95,86	732,94	583,15	412,95	78,32
2	4,58	25,12	190,87	1556,8	1245,36	648,74	125,37
3	1,86	8,96	53,94	371,25	297,34	217,38	48,62
4	7,28	45,19	368,45	3597,38	2870,28	1379,4	237,85
Average	**4,09**	**23,16**	**177,28**	**1564,59**	**1249,03**	**664,62**	**122,54**

3.3.1.3. Study on the infection rate in chickens through infection ways

The infection rate by infected ways was described on table 3.16 (primary dissertation). Table 3.16 shows that: number of diseased chickens by infecting experimentally through chicken's vent were100%, while this rate was low when it used through mouth way.

3.3.1.4. Appearance time and clinical signs in chickens infected

* Appearance time and clinical signs in chickens infected were illustrated on table 3.17. (primary dissertation).

Table 3.17 shows that: appearance time of symptoms in chickens experimentally infected through chicken's vent was earlier than chickens experimentally infected by month way (9.58 ± 0.17 compared with 13.33 ± 0.88 days).

With experimentally infecting *H. meleagridis* by month way, the distance of protozoans moved to suitable parasitic location was long. Beside, In movement to parasitic, protozoan faced nevertheless obstacles such as: acid environment in gastric juice of chickens proventriculus and gizzard, digestive juice like gall juice, hence, the infection rate was low and appearance time of symptoms was long.

By contrast, when chickens were experimentally infected through chicken's vent *H. meleagridis* penetrated quickly into ceca with mo influence of any agents, simultaneously the distance of

movement was short, so the infection rate was high and appearance time of symptoms was earlier.

* Clinical signs of diseased chickens caused by infecting experimentally

Table 3.18. The rates and signs of chickens had blackhead disease

(Watching chickens being experimentally infected through chicken's vent infected)

Number of experi-mentally	Number of chickens showing signs	Rate (%)	The result of monitoring: Primary clinical signs	Number of chickens showing clinical	Rate (%)	Rate time appearance of clinical signs after being experimentally min ÷ max (day)
40	40	100	Standing together depression rough house coat	40	100	7 ÷ 11
			Dinking a lot water, reducing or quitting appetite	40	100	8 ÷ 15
			High fever 43- 44˚ C	40	100	8 ÷ 15
			Chickens don't exercise, stand with closing eyes tightly and hide head under wings	40	100	10 ÷ 18
			cockscomb was pale or cyanotic	40	100	11 ÷ 21
			Sulphur-yellow diarrhea	40	100	11 ÷ 22
			Died	35	87,50	14 ÷ 27

Table 3.18 show that: diseased chickens by being experimentally infected had signs such as: depression; rough hair coat, reduced appetite of quitted appetite chickens often hide their head under wings, high fever 43- 44˚C, comb and wattle were pale or cyanotic, Sulphur – yellow diarrhea. Time of death was 14-27 days after being infected.

3.3.1.6 Changes of some hematology indices experimentally infected chickens

The result of table 3.20, 3.21, 3.22 show that: chickens infected blackhead disease with number of erythrocytes and hemoglobin content were decreased; number of leucocytes erythrocytes and volume of erythrocytes were increased more than healthy chickens, rate of neutrophil was decreased, rat of eosinophil was increased, lymphocytes and monocytes were increased, basophils had unclear changes (P>0,05); total protein content was decreased, various enzymes GDT, GPT, LHD were increased compared with that in control chickens.

Table 3.20. Changes of some blood cell indices of chickens after being experimentally infected

Group	Control Group $\overline{X} \pm m_{\overline{X}}$	Infected Group $\overline{X} \pm m_{\overline{X}}$
Number or blood samples	**20**	**20**
Number or erythrocytes (million/mm^3)	$3,01 \pm 0,05^a$	$2,49 \pm 0,06^b$
Number or leucocytes (thousand/mm^3)	$30,51 \pm 0,28^a$	$39,59 \pm 0,28^b$
Number or thrombocytes (thousand/mm^3)	$312,42 \pm 4,14^a$	$318,77 \pm 4,45^a$
Hemoglobin (g/%)	$12,64 \pm 0,11^a$	$8,52 \pm 0,14^b$
Average volume of erythrocytes (μm^3)	$122,29 \pm 0,29^a$	$124,85 \pm 0,31^b$

Notes: In horizontal line, the figures carrying different letters are in statistically significant different (P< 0,05).

Table 3.21. Changes of leucocytes equation of experimentally infected

Group	Control Group $\overline{X} \pm m_{\overline{X}}$	Infected Group $\overline{X} \pm m_{\overline{X}}$
Number of blood samples	**20**	**20**
Neutrophils (%)	27, 33 ± 0,14[a]	22,85 ± 0,3[b]
Eosinophils (%)	4,06 ± 0,03[a]	5,52 ± 0,13[b]
Basophils (%)	3,94 ± 0,05[a]	4,01 ± 0,04[a]
Lymphocytes (%)	58,63 ± 0,19[a]	60,28 ± 0,29[b]
Monocytes (%)	6,03 ± 0,05[a]	6,47 ± 0,09[b]

Notes: In horizontal line, the figures carrying different letters are in statistically Significant different (P< 0,05).

Table 3.22. Changes of some of serum biochemistry indices of diseased chickens caused by infection

Group	Control Group $\overline{X} \pm m_{\overline{X}}$	Infected Group $\overline{X} \pm m_{\overline{X}}$
Number of blood sample	**20**	**20**
Total protein (g/ dl)	4,01 ± 0,06[a]	1,95 ± 0,04[b]
Albumin (g/ dl)	2,02 ± 0,04[a]	0,71 ± 0,02[b]
Globulin (g/ dl)	1,98 ± 0,03[a]	1,24 ± 0,04[b]
Ratio A/G	1,02[a]	0,57[b]
GOT (U/L)	106,45 ± 2,78[a]	187,92 ± 4,07[b]
GPT (U/L)	19,49 ± 0,45[a]	24,19 ± 0,48[b]
LDH (U/L)	186,67 ± 5,31[a]	276,39 ± 7,24[b]

Note: In horizontal line, the figures carrying different letters are in statistically significant different (P< 0,05).

3.3.1.7 Study on lesions of diseased chickens caused by infection

The result of table 3.23 show that: diseased chickens caused by infection has big swollen ceca, content in ceca lumen, white, liver was swollen twice - three times, surface of liver appeared gangrene spots as "chrysanthemum" spleen and gallbladder were swollen, some of chickens had peritonitis.

Table 3.23. Gross lesions of chickens infected with blackhead disease after being experimentally infected

Dissected chickens	Number of chickens showing lesions	Rate (%)	Rate of primary lesions		
			Primary gross lesions	Number of chickens	Rate (%)
16	**16**	**100**	*** Lesions in ceca**		
			- Ceca was swollen; the mucous membrane was bled gangrened	16	100
			- Liquid contained in ceca lumen was brown-yellow and thick	6	37,5
			- Liquid contained in ceca lumen was white	10	62,5
			- Ceca was ulcerated and holed	9	56,25
			*** Lesions in liver**		
			Liver was swollen, had many gangrene spots as "chrysanthemum"	16	100
			*** Lesions in other internal organs**	9	56,25
			- Peritonitis		
			- Spleen was swollen	16	100
			- Swollen gall bladder	16	100

3.3.2. Study on blackhead disease in chickens naturally infected in Thai Nguyen and Bac Giang

3.3.2.1. Symptoms and lesions of diseased chickens in Thai Nguyen and Bac Giang

Symptoms and lesions of diseased chickens naturally infected were described on table 3.27 and 3.28 (primary dissertation).

The results of two tables show that: symptoms and lesions of chickens naturally infected were similar with symptoms and lesions of infected chickens caused by infecting experimentally. These symptoms lesions would be scientific foundation for diagnosing chickens infected in various places.

3.4. Study on prevention and control measures of blackhead disease in chickens

3.4.1. Preventing blackhead disease in chickens by worming pinworms in huge areas

3.4.1.2. Efficacy of anthelmintic drugs for norming pinworms in huge areas

Table 3.30. Efficacy of anthelmintic drugs for norming pinworms in huge areas

Name and close of the drugs	Number of chickens dewormed	15 days before and after deworming			Deworming efficacy (%)	
		Number of faeces samples before and after deworming	Number of infected faeces samples before and after deworming	Infection intensity (eggs/gram of faeces) ($\overline{X} \pm m_{\overline{x}}$)	Number of samples were cleared of Pinworms egg	Deworming efficacy (%)
Fenbendazole 16 mg/kg TT	102	121	121	2274,46 ± 65,44	112	92,56
			9	221,78 ± 23,18		
Levamisole 20 mg/kg TT	100	114	114	2076,47 ± 63,72	103	90,35
			11	241,27 ± 18,31		
Mebendazole 10 % (20 mg/kg TT)	118	134	134	2386,82 ± 78,41	126	94,03
			8	280,50 ± 16,50		

The results of table 3.30 show that: all of 3 anthelmintic drugs fenbendazde levamisde and mebendazole 10% used for deworming pinworm in chickens were highly effective, absolute efficacy was 90 - 94%. Hence, with any veterinary anthelmintic drugs for deworming pinworms to prevent blackhead disease in chickens.

3.4.3. Determining effective treatment regimen of blackhead disease in chickens

3.4.3.1. Testing treatment regimen for infected chickens after being infected

Table 3.32 shows that: the result for treating blackhead disease in chickens of number 2 regimen was better than number 1 (63,33 % compared with 26,67 %).

Table 3.32. Effect of treatment regimen of blackhead disease in experimentally infected chickens

Regimen	Treatment drug	Dose	Number of chicken treated (chicken)	Number of chicken recovered (chicken)	Proportion (%)
1	Sulfamonomethoxine	0,5g/ liter of water/ day	30	8	26,67
	Doxycyclin	0,25g/ liter of water/ day			
	Paracetamol	2 g/ liter of water/ day			
	Unilyte Vit – C	3 g/ liter of water/ day			
	detoxication drug of liver, spleen and kidney	1g/ liter of water/ day			
2	Cloroquin phosphat	0,25g/ liter of water/ day	30	19	63,33
	holamrhrena antyday senteria	1g/ liter of water/ day			
	Sulfamonomethoxine	0,5g/ liter of water/ day			
	Paracetamol	2 g/ liter of water/ day			
	Unilyte Vit – C	3 g/ liter of water/ day			
	detoxication drug of liver, spleen and kidney	1g/ liter of water/ day			
Control	10 chicken did not use drugs, died in 14th - 25th day after being infected				

3.4.3.2. Determining efficacy of two treatment regimen for blackhead disease in chickens on large extent

The result of table 3.3 shows that: number 1,2 regimen – every regimen used to treat for 160 infected chickens, the rate of recovering was 51.25% and 83.75% respectively. In conclusion , the result for treating blackhead disease in chickens of number 2 regimen was better than number 1.

Table 3.33. Efficacy of treatment regimen for blackhead disease in chicken in the field

Regimen	Treatment drug	Dose	Number of chicken treated (chicken)	Number of chicken recovered (chicken)	Proportion (%)
1	Sulfamonomethoxine	0,5g/ liter of water/ day	160	22	51,25
	Doxycyclin	0,25g/ liter of water/ day			
	Paracetamol	2 g/ liter of water/ day			
	Unilyte Vit – C	3 g/ liter of water/ day			
	detoxication drug of liver, spleen and kidney	1g/ liter of water/ day			
2	Cloroquin phosphat	0,25g/ liter of water/ day	160	134	83,75
	holamrhrena antyday senteria	1g/ liter of water/ day			
	Sulfamonomethoxine	0,5g/ liter of water/ day			
	Paracetamol	2 g/ liter of water/ day			
	Unilyte Vit – C	3 g/ liter of water/ day			
	detoxication drug of liver, spleen and kidney	1g/ liter of water/ day			
Control	10 chicken did not use drugs, died in 14th - 25th day after being infected				

Two regimens used to treat for diseased chickens is the field gave higher results than treatment in chickens diseased by infection way this result was explained: In experimentally infected chickens, we treated for diseased chickens in 16th day after being experimentally infected. At that time, chickens infected, the member of protozoans parasitized in liver, the rate of curing chickens of

blackhead disease was low in chickens which their liver were gangrened and destroyed seriously by contrast, in the field we treads for diseased chickens in many different period, because the number of diseased chickens were low, the effect of treatment was higher.

From the experimental results of two regimens treated blackhead disease for chickens on large and small extents, we recommend farmers should treat diseased chicken by using two regimens to achieve higher treatment efficacy.

3.4.4. Recommending procedure of prevention and control of blackhead disease in chickens

(1). Killing H. meleagridis in chicken's body

When chickens appearance sign and lesions of blackhead disease, the second regimen should be used to treat for all chickens absolutely.

(2). Killing the intermediate host infected

- Deworming pinworms in chickens: basing on the conditions of places, they can use some of drugs: fenbendazole 16ml/kg BW, mebendazole 20mg/kg BW or levamisol 20mg/kg BW for deworming pinworms in chickens.

- Tackling chicken's feces to kill eggs of pinworms collecting from feces, litter and around coop and garden raising chickens are collected for composting to kill eggs and larval of pinworms to avoid spreading of disease germs into the environment around them.

(3). Cleaning coop and garden raising chickens

Which chickens raise in intensive system or kept in a semi-intensive system coop must be ventilated cool in summer and warm in winter, always dry and clean with suitable density. With chickens raised in free-range or extensive system framer should be made for sleeping chickens. Bricks are pared under framer to clean and collect feces favorably. If are husbandry is large, this region should be divided into 2-3 part to raise chicken alternately. Coops, play grounds, gardens are sterilized 2 time/ month by using benkocid, povidine 10%, QM - Supercide to kill *H. meleagridis*. Grass have to be cut, sewers are cleaned one time/ month to environment of chicken husbandry because dean and dry. After selling chickens: Floor, ceiling, wall, cribs, various tools used in raising chickens are scoured, dried and them spray antiseptic drugs all coops and tools after cleaning.

(4). *Strengthening care and management of chickens*

Chicken need to be cared and managed, especially chickens which are under 3 months to improve resistance of chickens to infection, including pinworm disease and *H. meleagridis* protozoan disease. If chickens are raise by keeping in a semi intensive system, they should be put into coop on rainy days to avoid eating pinworms - host of *H. meleagridis* protozoan.

CONCLUSION AND RECOMMENDATION

1. Conclusion

***(1). Nomenclature of parasitic protozoan Histomonas* spp**

Histomonas meleagridis genus has been identified as protozoan causing blackhead disease in chickens in VietNam.

(2). Regarding the epidemiological characteristics

- The proportion of infection *H. meleagridis* in chicken in Thai Nguyen is 16,42 % and Bac Giang in 21,63 %. The proportion of infection *H. meleagridis* in 1 - 3 month chickens is highest (32.53%) and them less than. The highest infection rate of *H. meleagridis* is chickens is in summer. The infection rate *H. meleagridis* is chickens raising by intensive semi intensiv and free-range or extensive system are 8,6 %; 36,47 %; 25,10 % respectively with chickens raised in coops with land floor, the infection rate of *H. meleagridis* is higher than chickens raise is cement floor brick (24,63 % compared with 13.75%).

The infection rate of *H. meleagridis* was 5,78 %, 16,02 %, 32,46 % respectively in chickens raised in condition of good, medium, low veternary hygiene.

- The proportion of infection pinworm in chicken in Thai Nguyen and Bac Giang are from 30,95 % to 69,52 %. Blackhead disease and pinworm disease have advantaged correlation with regression equation (y = - 15,4 + 0,708x). The correlation coefficient R = 0,947. Pinworm infection-prone chicken blackhead disease than non-infected chickens pinworm.

(3). Blackhead disease causing in chickens from H. meleagridis

- Were cultured and successfully infect single-cell experiments *H. meleagridis* to chicken

- Blackhead diseased chickens cause by experimentally and naturally and infected also has typical symptoms such as: high fever 43˚C – 44˚C, comb and wattle are pale or cyanotic, diarrhea, Sulphur-yellow feces. Time of death is 14 – 27 days after being experimentally infected.

- Blackhead disease diseased chickens have number of erythrocytes, the content of hemoglobin and average volume of erythrocytes decrease, number of leucocytes, thrombocytes, the rate of neutrophils, lymphocytes and monocytes also increase, total protein and albumin decrease, the content of globulin, enzyme glutamate oxaloacetate transaminase, glue and lac increase.

- The lesion of diseased chickens caused by infection concentrates on liver and ceca. After 7 days, injures appearance in ceca, in live it is after 9 day.

- Chickens had blackhead disease by infecting and naturally infected, lesions are: cecum is swollen, bled and gangrened, the ceca lumen is fifed with a dense liver in swollen, and the surface of liver has a lot of experimentally infected or naturally infected.

(4). The prevention and treatment measures of blackhead disease

- **Fenbendazole, mebendazole** used to deworm pinworms for chicken also hare high efficacy

- Antiseptic drugs such as Benkocid, povidine 10 % and QM - Supercide can destroy *H. meleagridis*.

- Regimen includes: cloroquin phosphat, hollarhrena antydy seteria, sulfamono-methoxin, paracetamol, detoxication drug of liver, spleen and kidney, unilyte vit – C have high efficacy in treating blackhead disease for chickens, this regimen can apply in places.

2. Recommendation

Procedure for prevention and treatment of blackhead disease in chickens should be applied in Thai Nguyen and Bac Giang provinces.

LIST OF PAPERS PUBLISHED
RELATING TO DISSERTATION

1. **Truong Thi Tinh**, Nguyen Thi Kim Lan, Le Van Nam, Do Thi Van Giang, Nguyen Thi Bich Nga (2015), "Prevalence of black head disease of chickens in Thai Nguyen and Bac Giang provinces", *Journal of veterinary science and technical*, vol XII, No. 3, pp. 53-59..

2. **Truong Thi Tinh**, Nguyen Thi Kim Lan, Le Van Nam, Do Thi Van Giang, Nguyen Thi Bich Nga (2015), "The correlation between the prevalence of *Heterakis gallinarum* and blackhead disease of chickens", *Journal of Science and Technology Thai Nguyen University*, vol 142 (12), pp. 17-22.

3. **Truong Thi Tinh**, Nguyen Thi Kim Lan, Le Van Nam, Do Thi Van Giang, Nguyen Thi Bich Nga (2015), "The results of *Histomonas meleagridis* in dwyers's culture and artificial infection on chickens in Thai Nguyen province", *Journal of Agriculture and Rural development*, No. 269, pp. 193-198.