

MINISTRY OF EDUCATION AND TRAINING
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**STUDY ON *LEUCOCYTOZOOONOSIS* OF CHICKENS IN THAI
NGUYEN, BAC GIANG PROVINCE AND ITS PREVENTIVE,
TREATMENT MEASURES**

Speciality: Veterinary parasitology and microbiology

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SUMMARY OF PhD. DISSERTATION IN VETERINARY

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The dissertation can be found at:

- National Library;**
- Learning Resource Center – Thai Nguyen University ;**
- Library of College of agriculture and forestry – TNU.**

INTRODUCTION

1. Urgency of the dissertation

Leucocytozoon protozoa belong to eukaryotic organism, sporadic blood order (Heamosporidia) parasitize in blood and abdominal organs of livestock, chickens are the most susceptible specie, especially backyard chickens.

Leucocytozoon protozoa parasite in red blood cells causing haemorrhagia, broken red blood cells, resulting of anemia and diarrhoea and green feces, chickens die at high rate from 30 to 50%.

In recent years, poultry husbandry in Thai Nguyen and Bac Giang has strongly developed. These are northern mountainous provinces which have favorable conditions for the development of poultry husbandry, particularly backyard poultry husbandry. However, until this moment there have no study or research about its causes, pathology, preventive and treatment measures in chickens in Thai Nguyen and Bac Giang province. Actually, from real requirements of poultry production, we have begun to realize the thesis: “**Study on *Leucocytozoonosis* of chickens in Thai Nguyen, Bac Giang province and its preventive, treatment measures**”.

2. Objectives of the dissertation

Identify *Leucocytozoon* specie, epidemiological and pathological characteristics caused by *Leucocytozoon* protozoa in backyard chickens in Thai Nguyen, Bac Giang province.

Select a treating regimen and establish preventive measures, avoiding damages caused by *Leucocytozoon* protozoa in chickens in Thai Nguyen and Bac Giang province.

3. Scientific and practical significance of the dissertation

3.1. Scientific significance

The results of dissertation supplied and completed scientific information on the latest epidemiological characteristics, clinical pathology and disease, the effectiveness of treatment regimens for *Leucocytozoonosis* caused in chickens, also was scientific basis for contributing of

Leucocytozoon prevention and treatment measures highly effective in chickens in Thai Nguyen, Bac Giang province and in Viet nam.

3.2. Practical significance

The results of dissertation were basic in recommending for farmers who can apply *Leucocytozoon* preventive, treatment measures to avoid high prevalence and damages caused by *Leucocytozoon* protozoa, contributing to improve poultry productivity, promoting to develop poultry husbandry in particular and livestock husbandry in general.

3.3. New contribution of the dissertation

- The dissertation was the first research in Vietnam which has systematically studied about epidemiological, pathological, clinical and preventive and treatment measures for *Leucocytozoonosis* in chickens in Thai Nguyen, Bac Giang province.

- There was established in preventive, treatment measure of *Leucocytozoonosis* effectively, recommended and widely applied to poultry farms in Thai Nguyen and Bac Giang.

4. Structure of dissertation

Dissertation includes 170 pages which are divided into chapters and sections: Introductory: 3 pages, chapter 1: Overview of document (36 pages), chapter 2: Materials, contents and methodology (20 pages), chapter 3: Study results and discussion (57 pages). Conclusion and recommendation (2 pages).

References 13 pages; Pictures of dissertation (20 pages); Appendix (20 pages).

The dissertation has 30 tables, 11 graphs, 125 references (40 Vietnamese documents, 85 foreign languages documents) and 40 color images (be structured from 110 photos) showing results of dissertation.

Chapter 1

OVERVIEW OF DOCUMENT

Zhao W. et al. (2014) reported that *Leucocytozoon* spp. protozoa infect to many species of birds and cause severe economic impacts to the poultry husbandry of industrial form.

According to Levine N. D. (1985), Pham Sy Lang et al (2011), *Leucocytozoon* protozoa infect chickens being located in classification system as follows: Apicomplexa Phylum (Levine, 1970), Aconoidasida class (Mehlhorn, 1980), Haemosporoda order (Jacques Euzeby, 1988), Leucocytozoidae family (Doflein, 1916), *Leucocytozoon* genus (Sambon, 1908).

According to Nguyen Thi Kim Lan et al. (1999), *Leucocytozoon* protozoa compose by cellular membrane, protoplasm, nucleus. When they parasite in red blood cells, white blood cells, abdominal organs in chickens and birds, *Leucocytozoon* can have two forms: corpuscular form in drumstick or rhombus, sharp in two sides, with size from 15 to 20 μm ; sporadic form in oval with size from 20 to 25 μm .

Le Duc Quyet et al. (2009) considered that the *Leucocytozoon* prevalence depended on many factors such as age of poultry, genus, topography, ecological region, husbandry methods ...

Mark Pattison (2008) informed that the clinical symptoms of *Leucocytozoonosis* in chickens include: anorexia, emaciated, anemic and may be conducted to death.

Lee D. H. et al. (2014) showed: *Leucocytozoon* infected chickens have typical lesions are hemorrhagic subcutaneous at wings, legs, breast and thigh muscle, pancreas and kidneys.

To prevent *Leucocytozoonosis* for chickens and other poultries highly effective, Nguyen Thi Kim Lan et al. (2014) has suggested measures to destroying *Leucocytozoon* protozoa in reservoirs, blackfly - intermediate vector and drug prevention.

According to Le Van Nam (2011), medicines that contain ingredients such as sulfamonomethoxin, sulfadimethoxin and clopidol have beneficial effects in preventing *Leucocytozoonosis*. The author also mentioned that currently parasitic disease cause by blood protozoa often combine with multiple infections, so it is necessary to coordinate new treatment regimen to reach high treatment efficiency.

Chapter 2

MATERIALS, CONTENTS AND METHODOLOGY

2.1. Object, time and place of study

2.1.1. Objects of study

- Semi-backyard chickens in Thai Nguyen and Bac Giang province.
- *Leucocytozoonosis* in chickens.

2.1.2. Place and time of study

2.1.2.1. *Place of study*: Thai Nguyen and Bac Giang province.

2.1.2.2. *Place where samples were tested*:

- Laboratory at faculty of Animal Science and veterinary medicine
- Thai Nguyen University of agriculture and forestry.
- Faculty of Hematology and Anatomy - Thai Nguyen Central Hospital.

2.1.2.3. *Time of study*: 2012 - 2015

2.2. Materials of study

* **Studied samples**: Blood samples of chickens: healthy, dead and sick chickens infected with *Leucocytozoon* protozoa, abdominal organs which have been taken from typical lesions of *Leucocytozoonosis*, blackflies...

* **Equipment and tools**: microtom histological machine, laser hematological analyzer, optical microscope, magnifying glass, small ruminant necropsy toolkits, blood needles, tubes with EDTA solution 1 % anticoagulant, cylinders, needles, glass slides and cover slips...

* **Chemicals and drugs**: hematoxilin eosin staining solution, giemsa staining solution, *Leucocytozoon* drugs for chickens, blackflies insecticides, 10% formalin solution...

2.3. Contents of study

2.3.1. Epidemiological characteristics of *Leucocytozoonosis* in chickens in Thai Nguyen and Bac Giang province.

* *Current situation of parasitic prevention at localities for chickens in Thai Nguyen and Bac Giang*

* *The prevalence of *Leucocytozoon* in chickens in Thai Nguyen and Bac Giang*

- The prevalence and intensity of infection of *Leucocytozoonosis* in chickens at localities.

- The prevalence and intensity of infection of *Leucocytozoonosis* in chickens according to topographical terrain.

- The prevalence and intensity of infection of *Leucocytozoonosis* in chickens according to seasons.

- The prevalence and intensity of infection of *Leucocytozoonosis* in chickens according to age of chickens.

- The prevalence and intensity of infection of *Leucocytozoonosis* in chickens according to poultry husbandry methods.

- The prevalence and intensity of infection of *Leucocytozoonosis* in chickens according to veterinary hygienic conditions in poultry husbandry.

- The prevalence and intensity of infection of *Leucocytozoonosis* in chickens according to sex.

- The prevalence and intensity of infection of *Leucocytozoonosis* in chickens according to poultry concentration.

* *Study on some characteristics of blackfly activities - intermediate vector transmitted *Leucocytozoonosis* to chickens.*

- Specie components of blackflies - intermediate vector transmitted *Leucocytozoonosis* to chickens at localities.

- The percentage of blood biting blackflies containing *Leucocytozoon* protozoa.

- Vacillation of blackflies number in Bac Giang and Thai Nguyen

- Blackfly activities according to year and days

2.3.2. Study on *Leucocytozoonosis* in chickens in Thai Nguyen and Bac Giang

- *Leucocytozoon* protozoal specie composition infected chickens in Thai Nguyen and Bac Giang

- Symptoms of *Leucocytozoon* infected chickens

- Some hematological indicators of *Leucocytozoon* infected chickens in comparison with healthy chickens

- Macroscopical lesions caused by *Leucocytozoon* protozoan in chickens
- Microscopical lesions caused by *Leucocytozoon* protozoan in chickens

2.3.3. Study on *Leucocytozoonosis* preventive, treatment measures

2.3.3.1. Study on treatment regimens of *Leucocytozoonosis* for chickens

Determine on the efficacy and safety of three *Leucocytozoon* treatment regimens in chickens at small scale and large scale.

2.3.3.2. Approve and establish integral preventive and treatment measures for chickens.

* *Approved Leucocytozoon treatment measures for chickens in Thai Nguyen province.*

- The prevalence and intensity of infection of *Leucocytozoonosis* in experimental group and the control group after 1 month trial

- The prevalence and intensity of infection of *Leucocytozoonosis* in experimental group and the control group after 2 month trial

- The prevalence and intensity of infection of *Leucocytozoonosis* in experimental group and the control group after 3 month trial

- The corporal weight of chickens in control and experimental groups after 1, 2 and 3 - month trial

* *Established to recommend Leucocytozoon preventive and treatment measures in chickens.*

2.4. Studied methods

2.4.1. Determine on the status of applied measures in preventing parasitic disease in chickens in studied regions according to interviewed and directly observed method

2.4.2. Methods in determining the prevalence, intensity of infection of *Leucocytozoonosis* in chickens

- Calculate the minimum sample size by WinEpiscope 2.0 software.

- Detect *Lecocytozoon* protozoa in blood samples of chickens by blood templating, giemsa staining and observing under an optical microscope.

2.4.3. Blackfly sample collection and studied method of activity characteristics - intermediate vector transmitted *Leucocytozoon* in Thai Nguyen, Bac Giang

- Collect blood biting blackflies by racket.

- Classify blackflies according to classification key of Eldridge B. F. and Edman J. D. (2004).

- Determine on the percentage of blood biting blackflies containing *Leucocytozoon* protozoa by giemsa staining method, testing staining templates under optical microscope.

- Determine on activity rule of blackflies according to year and days by observing method.

2.4.4. Studied method of *Leucocytozoonosis* in chickens

*2.4.4.1. Identify *Leucocytozoon* spp. protozoa parasite in chickens in Thai Nguyen and Bac Giang province*

The protozoa parasite in red blood cells, on giemsa blood staining templates in which were classified the morphology, size according to classification key of Levine N. D. (1985).

*2.4.4.2. Observing method of clinical symptoms in *Leucocytozoon* infected chickens*

Directly observed changes about the color of crest, corporal statement, color and state of feces, diet, movement ... in chickens, combining with informations of poultry farmers.

*2.4.4.3. Testing method of some hematological indicators of healthy and *Leucocytozoon* infected chickens*

All hematologic indicators of chickens were determined on Cellta - Mek - 6420k - Nihon Kohden machine (Japan).

*2.4.4.4. Determining method of macroscopic and microscopic lesions in abdominal organs, muscles in chickens caused by *Leucocytozoon* protozoan*

- Necropsying chickens under the guidance of Ministry of Agriculture and Rural Development (2005) to determine macroscopic lesions in abdominal organs, muscles caused by *Leucocytozoon* protozoa.

- Studying microscopic lesions by paraffin histological method, hematoxilin – eosin staining, obtaining results under optical microscope (Jones T. C. and Gleiser C. A. (1954)).

2.4.5. Study on preventive and treatment measures of *Leucocytozoonosis* for chickens

Evaluating the efficacy and safety of 03 treatment regimens and approving preventive measures of *Leucocytozoonosis* for chickens according to comparative subdivision method.

2.5. Data processing methods

The obtained data were processed by bio-statistical method (referred by Nguyen Van Thien, 2008), on Excel 2007 and Minitab 14.0 software.

Chapter 3

RESULTS AND DISCUSSION

3.1. Epidemiological characteristics of *Leucocytozoonosis* in chickens in Thai Nguyen and Bac Giang province

3.1.2. Current situation of *Leucocytozoonosis* in chickens in Thai Nguyen and Bac Giang province

3.1.2.1. The prevalence and intensity of infection of *Leucocytozoonosis* in chickens at localities

Table 3.2: The prevalence and intensity of infection of *Leucocytozoonosis* in chickens at localities

Localities (district, city, town)	Tested chickens	Infected chickens	Prevalence (%)	Intensity of infection (% RBC containing protozoa)					
				≤ 5		> 5 - 10		> 10	
				n	%	n	%	n	%
Thai Nguyen	2000	411	20.55	199	48.42	127	30.90	85	20.68
Song Cong	330	51	15.45	31	60.78	13	25.49	7	13.73
Pho Yen	330	59	17.88	31	52.54	19	32.20	9	15.26
Đông Hy	330	68	20.61	32	47.06	22	32.35	14	20.59
Phu Binh	340	71	20.88	34	47.89	22	30.99	15	21.13
Vo Nhai	330	82	24.85	35	42.68	26	31.71	21	25.61
Đinh Hoa	340	80	23.53	36	45.00	25	31.25	19	23.75
Bac Giang	1000	208	20.80	111	53.37	66	31.73	31	14.90
Yen The	250	51	20.40	28	54.90	17	33.33	6	11.76
Tan Yen	250	37	14.80	25	67.57	10	27.03	2	5.41
Son Đông	250	61	24.40	29	47.54	20	32.79	12	19.67
Luc Ngan	250	59	23.60	29	49.15	19	32.20	11	18.64
Total	3000	619	20.63	310	50.08	193	31.18	116	18.74
$\chi^2_{\text{Thai Nguyen \& Bac Giang}} = 0.025$				P= 0.873					

The results in table 3.2 showed that: In 3,000 chickens were tested blood samples, there were 619 chickens infected with *Leucocytozoon* protozoan, the average prevalence in two provinces was 20.63%; vacillating from 14.80% to 24.85% in each province. In particular,

chickens in Thai Nguyen infected with a proportion of 20.55% and 20.88% in Bac Giang.

About the intensity of infection: chickens in Thai Nguyen and Bac Giang mainly infected protozoa in mild and moderate intensity (50.08% and 31.18% respectively), high prevalence was 18.74%.

3.1.2.2. *The prevalence and intensity of infection of Leucocytozoonosis in chickens according to topographical terrain*

Table 3.3: The prevalence and intensity of infection of *Leucocytozoonosis* in chickens according to topographical terrain

Topographical terrain	Tested chickens	Infected chickens	Prevalence (%)	Intensity of infection (% RBC containing protozoa)					
				≤ 5		> 5 - 10		> 10	
				n	%	n	%	n	%
Mountainous	139	41	29.50 ^a	19	46.34	13	31.71	9	21.95
Midland	142	27	19.01 ^b	15	55.56	8	29.63	4	14.81
Delta	138	14	10.14 ^c	9	64.29	4	28.57	1	7.14
Total	419	82	19.57	43	52.44	25	30.49	14	17.07

Note: According to the vertical, the prevalence bearing in different letters differs statistically significant ($P < 0.05$)

Chickens in mountainous region infected with *Leucocytozoon* protozoa at highest prevalence (29.50%), following by chickens in the midland region (19.01%) and lowest in delta region (10.14%). Comparing the statistic data, prevalence of *Leucocytozoon* between midland and mountainous region, between midland and delta region has significant difference ($P < 0.05$); the prevalence between mountainous and delta was clearly different ($P < 0.001$).

3.1.2.3. *The prevalence and intensity of infection of Leucocytozoonosis in chickens according to seasons*

The prevalence and intensity of infection of *Leucocytozoonosis* in chickens were different in each season. *Leucocytozoon* infected chickens were much in spring and summer, less in autumn and winter.

Causes of mentioned different prevalence were due to season: the weather in spring and summer was hot, humid and rainy, favorable to

blackflies reproduce and increase their activity. Therefore, chickens usually infect *Leucocytozoonosis* at high prevalence and intensity of infection at this time.

Table 3.4: The prevalence and intensity of infection of *Leucocytozoonosis* in chickens according to seasons

Season	Tested chickens	Infected chickens	Prevalence (%)	Intensity of infection (% RBC containing protozoa)					
				≤ 5		> 5 - 10		> 10	
				n	%	n	%	n	%
Spring	140	31	22.14 ^a	15	48.39	10	32.26	6	19.35
Summer	154	43	27.92 ^a	19	44.19	14	32.56	10	23.26
Autumn	141	26	18.44 ^{ab}	15	57.69	8	30.77	3	11.54
Winter	137	14	10.22 ^b	9	64.29	4	28.57	1	7.14
Total	572	114	19.93	58	50.88	36	31.58	20	17.54

Note: According to the vertical, the prevalence bearing in different letters differs statistically significant ($P < 0.05$)

3.1.2.4. The prevalence and intensity of infection of *Leucocytozoonosis* in chickens according to age

Table 3.5: The prevalence and intensity of infection of *Leucocytozoonosis* in chickens according to age

Age (month)	Tested chickens	Infected chickens	Prevalence (%)	Intensity of infection (% RBC containing protozoa)					
				≤ 5		> 5 - 10		> 10	
				n	%	n	%	n	%
≤ 2	118	13	11.02 ^a	8	61.54	4	30.77	1	7.69
> 2 - 4	127	24	18.90 ^{ab}	13	54.17	7	29.17	4	16.67
> 4 - 6	124	28	22.58 ^b	13	46.43	9	32.14	6	21.43
> 6	113	31	27.43 ^b	14	45.16	10	32.26	7	22.58
Total	482	96	19.92	48	50.00	30	31.25	18	18.75

Note: According to the vertical, the prevalence bearing in different letters differs statistically significant ($P < 0.05$)

In 482 tested chickens, there were 96 *Leucocytozoon* infected chickens, represented 19.92%. The prevalence increased in accordance with age of chickens. Lowest prevalence in chickens was at 2 months of age (11.02%) and highest was at 6 months of age (27.43%). Thus, the prevalence of chickens at less than 2 months of age and 6 months of age was significantly difference ($P < 0.001$). The reason was explained due to chickens under 2 months of age which were cared well, guaranteed hygienic cages.

3.1.2.6. The prevalence and intensity of infection of Leucocytozoonosis according to veterinary hygienic conditions in poultry husbandry

Table 3.7: The prevalence and intensity of infection of *Leucocytozoonosis* according to veterinary hygienic conditions

Status of veterinary hygienic conditions	Tested chickens	Infected chickens	Prevalence (%)	Intensity of infection (% RBC containing protozoa)					
				≤ 5		> 5 - 10		> 10	
				n	%	n	%	n	%
Good	152	15	9.87 ^a	10	66.67	4	26.67	1	6.66
Moderate	149	30	20.13 ^b	16	53.33	9	30.00	5	16.67
Poor	143	42	29.37 ^b	19	45.24	13	30.95	10	23.81
Total	444	87	19.59	45	51.72	26	29.89	16	18.39

Note: According to the vertical, the prevalence bearing different letters differs statistically significant ($P < 0.05$)

Chickens in poor veterinary hygienic condition infected protozoa at high prevalence (29.37%); in opposite, chickens in good veterinary hygiene have low prevalence (9.87%).

Thus, chickens in good veterinary sanitation have prevalence and intensity of infection of *Leucocytozoon* protozoa were much lower than chickens in poor hygienic condition, because poor veterinary hygiene created favorable condition for blackflies reproducing and increasing their activities.

3.1.3. Study on some characteristics of blackfly activities - intermediate vector transmitted *Leucocytozoonosis* for chickens.

3.1.3.1. Identification of biting blackfly specie - transmitted *Leucocytozoonosis* for chickens at localities.

The result of blackfly identification in 10 districts, cities, towns in Thai Nguyen and Bac Giang province showed that: in Thai Nguyen and Bac Giang there were 3 species - intermediate vectors transmitted *Leucocytozoonosis* for chickens, in these, *Culicoides arakawa* and *Simulium slossonae* specie were widely distributed, the appearance frequency was 100%. and 80.00%, respectively. *Culicoides odibilis* specie was more narrow distribution (30.00%).

Table 3.10: Specie composition and appearance frequency of biting blackflies at studied regions

Localities	Result of blackfly classification			Encountered specie	
	<i>Culicoides arakawa</i>	<i>Culicoides odibilis</i>	<i>Simulium slossonae</i>		
<i>Thai Nguyen</i>	Song Cong	+	-	+	2
	Pho Yen	+	-	+	2
	Đông Hy	+	+	+	3
	Phu Binh	+	-	+	2
	Vo Nhai	+	+	+	3
	Đinh Hoa	+	-	+	2
<i>Bac Giang</i>	Yen The	+	-	-	1
	Tan Yen	+	-	-	1
	Son Đông	+	+	+	3
	Luc Ngan	+	-	+	2
Appearance frequency(%)	100	30.00	80.00	3	

Note: (+): Appeared

(-): Not appeared

3.1.3.2. The proportion of blood biting blackflies containing *Leucocytozoon*

In 250 individual blackflies were collected in Thai Nguyen and Bac Giang province, there was 12.80% individual blackflies containing *Leucocytozoon*.

Table 3.11: The individual proportion of blood biting blackflies containing *Leucocytozoon* protozoa

Localities	Tested blackflies	<i>Leucocytozoon</i> protozoal blackflies	Proportion (%)	
Thai Nguyen	Song Cong	25	2	8.00
	Pho Yen	25	2	8.00
	Đong Hy	25	3	12.00
	Phu Binh	25	5	20.00
	Vo Nhai	25	5	20.00
	Đinh Hoa	25	3	12.00
	Total	150	20	13.33
Bac Giang	Yen The	25	2	8.00
	Tan Yen	25	3	12.00
	Son Đong	25	4	16.00
	Luc Ngan	25	3	12.00
	Total	100	12	12.00
Total	250	32	12.80	

The above results allow us to comment that: i) Blackflies are insects that bite and suck blood in chickens, also *Leucocytozoon* infected chickens; ii) Once *Leucocytozoon* protozoa liberate from red blood cells, the protozoa continue to reproduce in meiosis form into blackflies; then reach up to salivary gland in blackflies; iii) When blackflies bite and suck blood of healthy chickens, due to gastric contraction and saliva secretion *Leucocytozoon* protozoa are transmitted to blood of healthy chickens.

3.1.3.3. Activity rule of blood biting blackflies

* Activity rule of blood biting blackflies according to year

The results in table 3.12 showed that: In November, December, January, February the weather was cold, dry therefore blackflies were

not inactive. From April to September, when the weather was warm, drizzle, humid creating favorable conditions for growth and development of blackflies, so in this time increased their activity, especially from May to July. Poor veterinary hygiene also contributed to increase number and activity of blackflies.

Table 3.12: Activity rule of blood biting blackflies according to months

Localities	Months of year											
	1	2	3	4	5	6	7	8	9	10	11	12
Thai Nguyen												
Song Cong	-	-	-	+	++	++	++	+	+	-	-	-
Pho Yen	-	-	+	++	++	+++	++	+	+	-	-	-
Đong Hy	-	-	+	++	+++	+++	+++	++	++	+	-	-
Phu Binh	-	-	+	++	+++	+++	+++	++	++	+	-	-
Vo Nhai	-	-	++	+++	+++	+++	+++	++	++	+	-	-
Đinh Hoa	-	-	+	+	+++	+++	+++	++	++	+	-	-
Bac Giang												
Yen The	-	-	+	++	+++	+++	+++	+	+	-	-	-
Tan Yen	-	-	-	++	++	+++	+++	++	++	+	-	-
Son Đong	-	-	+	++	+++	+++	+++	++	++	+	-	-
Luc Ngan	-	-	+	++	+++	+++	+++	++	++	+	-	-

Note: Observed in 2 years (2013-2014)

(+++): *Intense active*

(++): *Medium active*

(+): *Less active*

(-): *Not active*

* *Activity rule of blood biting blackflies according to days*

The results in table 3.13 showed that: In May, June, July and August, blackflies active from 8 am to 16 pm when light intensity was high; from 6 am to 8 am and 16 pm - 18 pm blackflies diminished activity cause by low light intensity. In March, April, September and October, it's cold, dry, low light intensity and temperature, blackflies decreased their activity.

Table 3.13: Daily activity rule of blood biting blackflies

Months	Daily activity (hour)				
	6 – 8	8 – 11	11 - 13	13 - 16	16 - 18
March	-	+	++	+	-
April	+	+	++	++	+
May	++	+++	+++	+++	++
June	++	+++	+++	+++	++
July	++	+++	+++	+++	++
August	++	+++	+++	+++	++
September	+	+	++	+	+
October	-	+	++	+	-

Note: Observed in 2 years (2013 - 2014)

(+++): Intense active

(++): Medium active

(+): Less active

(-): Not active

3.2. Study on *Leucocytozoonosis* in chickens in Thai Nguyen and Bac Giang

3.2.1. Identify *Leucocytozoon* specie in chickens in Thai Nguyen and Bac Giang

Table 3.14: *Leucocytozoon* pathogenic species in chickens in Thai Nguyen and Bac Giang

Province	Districts, towns, cities	<i>Leucocytozoon</i> species			Identified species
		<i>L. caulleryi</i>	<i>L. smithi</i>	<i>L. sabrazeis</i>	
Thai Nguyen (3 species)	Pho Yen	+	-	-	1
	Dong Hy	+	-	-	1
	Phu Binh	+	-	+	2
	Đinh Hoa	+	+	-	2
	Vo Nhai	+	+	+	3
	Song Cong	+	-	-	1
Bac Giang (3 species)	Tan Yen	+	-	-	1
	Yen The	+	-	-	1
	Luc Ngan	+	+	+	3
	Son Đông	+	+	-	2
Appeared frequency (%)		10/10 = 100	4/10 = 40.00	3/10 = 30.00	3

There was identified 03 protozoal species of the genus *Leucocytozoon* infected to chickens in Thai Nguyen and Bac Giang province were *L. caulleryi*, *L. sabrazesi* and *L. smithi*. *L. caulleryi* distributed widely and popular, appearing in 100% of studied localities. *L. sabrazesi* appeared in 40% of studied localities and *L. smithi* appeared in 30% of studied localities.

3.2.2. Symptoms of *Leucocytozoon* infected chickens

Table 3.15: The proportion and clinical symptoms in *Leucocytozoon* infected chickens

Infected chickens	Symptomatic chickens	Proportion (%)	Results		
			Mainly clinical symptoms	Chickens Proportion (%)	
619	184	29.73	Pale crest	184	100
			Thinness	119	64.67
			Mournful,	151	82.07
			sluggish movement		
			Anorexia	129	70.11
			Diarrhoea,	116	63.04
			green feces		
			Dyspnea	69	37.50
Neurological symptoms	14	7.61			

Table 3.15 reported that: in 619 *Leucocytozoon* infected chickens, there were 184 clinically symptomatic chickens, occupied 29.73%. Mainly symptoms were often encountered in *Leucocytozoon* infected chickens were: anemia, pale crest, mournfulness, anorexia, thinness, green feces.

3.2.3. Study on hematological indicators of *Leucocytozoon* infected chickens in comparison with healthy chickens

3.2.3.1. The change of hematological indicators of *Leucocytozoon* infected chickens

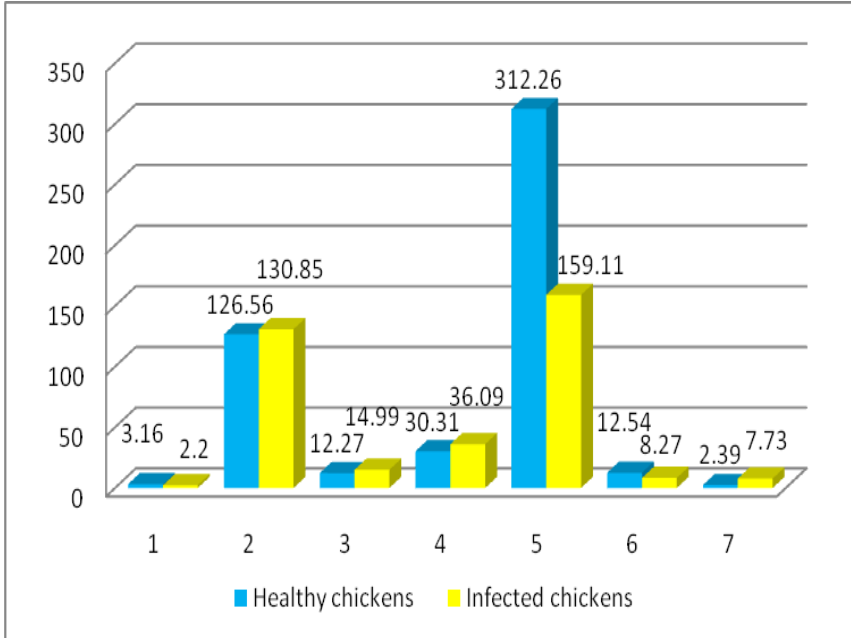


Figure 3.10: The change of hematological indicators in *Leucocytozoon* infected chickens.

- Note:** 1. Red blood cells (million/ mm^3 blood)
 2. Mean corpuscular volume (fl)
 3. Red distribution width (%)
 4. White blood cells (thousand/ mm^3 blood)
 5. Platelets (thousand/ mm^3 blood)
 6. Hemoglobine (g%)
 7. Clotting time (minutes)

3.2.3.2. *The change of white blood cell formula of Leucocytozoon infected chickens in comparison with healthy chickens*

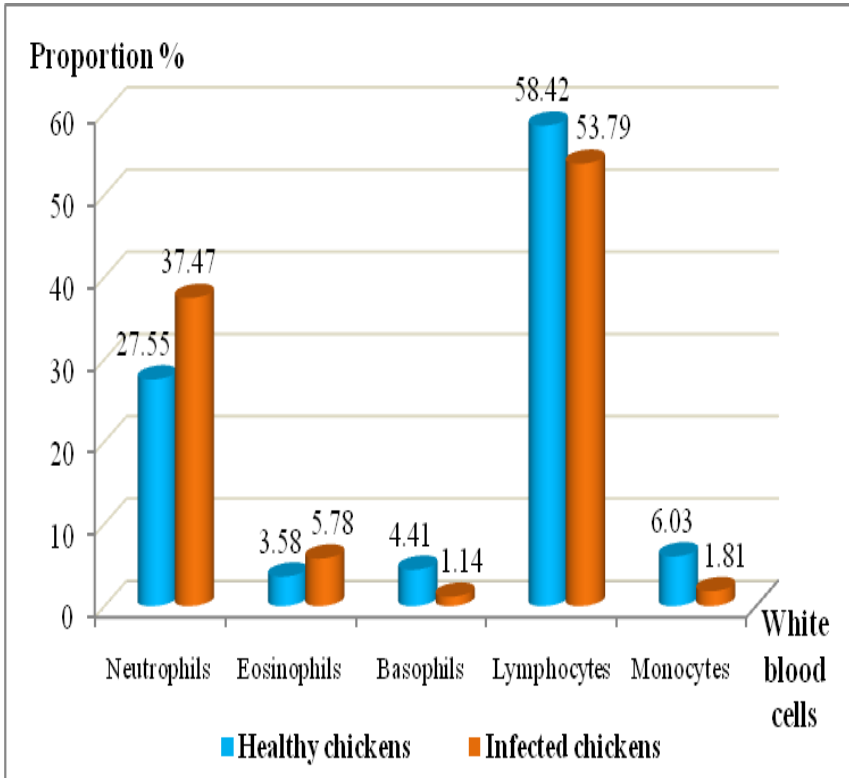


Figure 3.11: The change of white blood cell formula of *Leucocytozoon* infected chickens in comparison with healthy chickens

From the result in figure 3.10 and 3.11, we commented that *Leucocytozoon* infected chickens have significantly change about hematological indicators in comparison with these of healthy chickens. Hematological indicators reflected health status of the body. The above change demonstrated significantly pathogenic change caused by parasitic protozoa in chickens.

3.2.4. Macroscopic lesions caused by *Leucocytozoon* protozoa in chickens

Table 3.18: Macroscopic lesions caused by *Leucocytozoon* protozoa in chickens

Necropsy chickens	Lesional chickens	Proportion (%)	Observed results		
			Mainly macroscopic lesions	Chickens	Proportion (%)
184	116	63.04	Hemorrhagic in chest, thigh muscle	89	76.72
			Hemorrhagic liver	64	55.17
			Congestion, limp, fragile liver	72	62.07
			Aqueous and difficult coagulant blood	116	100
			Congestion and hemorrhagic kidneys	63	54.31
			Congestion and Hemorrhagic spleen	49	42.24
			Hemorrhagic pancreas	21	18.10
			Hemorrhagic lung	33	28.45
			Hemorrhagic intestinal mucosa	16	13.79
			Green feces	116	100

Necropsying 184 *Leucocytozoon* infected chickens, there were encountered 116 chickens with macroscopic lesions, occupied 63.04%. Main lesions focused on phenomenon of aqueous and difficult coagulant blood, congestion and hemorrhagic kidneys, hemorrhagic thigh and chest muscle, congestion and hemorrhagic spleen, hemorrhagic liver.

3.2.5. Microscopic lesions caused by *Leucocytozoon* protozoa in chickens

3.2.5.2. Microscopic lesions caused by *Leucocytozoon* protozoa in abdominal organs in chickens

Table 3.21: Microscopic lesions in abdominal organs caused by *Leucocytozoon* protozoa in chickens

Organ template	Lesional template	Observed result		
		Mainly microscopic lesion	Templates	Proportion (%)
Heart	12	- Hemorrhagic heart tissue	12	100
		- Infiltrated inflammatory cells	9	75.00
Liver	15	- Degenerated hepatic tissue	13	86.67
		- Hemorrhagic hepatic tissue	15	100
Spleen	15	- Extended spleen cavity, containing full blood	11	73.33
		- Hemorrhagic and degenerated spleen tissue	14	93.33
Kidneys	15	- Hemorrhagic renal tissue	15	100
		- Widening renal tube	8	53.33
Lung	11	- Hemorrhagic lung tissue	10	90.91
		- Hemorrhagic bronquial tissue	6	54.55

Thus, most of abdominal organs in chickens have been damaged by *Leucocytozoon* protozoa.

3.3. Study on preventive and treatment measures *Leucocytozoonosis* in chickens

3.3.1. Treatment measure of *Leucocytozoonosis*

3.3.1.1. Testing 3 treatment regimens of *Leucocytozoonosis* in chickens in the laboratory

Conducting to experimental treatment for 15 *Leucocytozoon* infected chickens with an average intensity of infection was from 9.73 to 10.07 (% RBC containing protozoa). After 10 days of medical application, their blood was taken to examine, there were encountered 14/15 chickens have no *Leucocytozoon* protozoa into red blood cells.

Thus, definitively treatment efficacy of all 3 above regimens in chickens in the laboratory obtained from 80 to 100%.

3.3.1.2. Efficacy and safety of three regimens in treating *Leucocytozoonosis* for chickens in the field

The experimental result of *Leucocytozoonosis* in chickens in the field showed that: The regimen 1 and 2 can be used in treating *Leucocytozoonosis* for chickens. The treating efficacy was from 93.33 to 96.67%. In those, the regimen 1 with Daimentol soda (2 grams / liter of water) was more effective. This result was lower than the treatment result in the laboratory.

3.3.2. Approve and recommend integral preventive and treatment measures of *Leucocytozoonosis* in chickens

3.3.2.3. The prevalence and intensity of infection of *Leucocytozoonosis* in chickens after 3 months trial

After 3 months trial, the prevalence of *Leucocytozoon* protozoa in the control group was significantly higher than 12.5% in experimental group ($P < 0.01$).

Thus, the application of integral preventive measure in 3 months was significantly reduced the prevalence and intensity of infection of *Leucocytozoonosis* in experimental chickens comparing with control chickens.

3.3.2.4. The corporal weight of chickens in the control and experimental group after 1, 2 and 3 months trial

Table 3.30: The corporal weight of chickens at each experimental time

Lotes	Number of chickens/ lote	Corporal weight of chicken ($\bar{X} \pm m_{\bar{x}}$) grams			
		1 day of age	1 month of age	2 day of age	3 day of age
Experimental	100	34.16 ± 0.18	529.90 ± 4.46	1075.30 ± 10.60	1932.30 ± 20.40
Control	100	34.36 ± 0.14	510.10 ± 7.17	1021.10 ± 13.70	1815.20 ± 26.90
P	-	P > 0.05	P < 0,05	P < 0.01	P < 0.01
Experimental/ Control		99.42%	103.88%	105.31%	106.45%

Note: At 2 and 3 months trial, experimental lote has 99 chickens and 97 chickens in control group

The application of integral preventive measure of *Leucocytozoonosis* has been reduced the prevalence and intensity of infection, increasing corporal weight of experimental chickens in comparison with chickens of control group, thereby contributing to increased poultry productivity.

3.4. Recommend preventive measure of *Leucocytozoonosis* for chickens

3.4.1. Recommend preventive measures

1. Eliminate *Leucocytozoon* protozoa in chickens
2. Eliminate intermediate vector transmitted *Leucocytozoonosis*
3. Hygiene stables and backyards
4. Good performance principle "All in, All out", evacuate cages after each sale, be clean, carefully disinfected before introducing new flock.
5. Strengthen care, nurturing poultry flocks.

3.4.2. Recommend to apply preventive and control measures of *Leucocytozoonosis* in chickens.

We have delivered integral preventive and control measures of blood protozoan disease to chickens in Phu Binh district in Thai Nguyen province and Yen The district in Bac Giang province. Through inquests, we have encountered that the procedure application was obtained positive results: the *Leucocytozoon* infected and dead chickens caused by this disease in 2015 had markedly decreased, thereby contributing to increase poultry productivity in two these districts.

CONCLUSIONS AND RECOMMENDATION

1. Conclusions

1. Epidemiological characteristics

The current status of poultry parasitic prevention in general and *Leucocytozoonosis* in particular in localities of Thai Nguyen and Bac Giang province was poor.

The prevalence of *Leucocytozoon* protozoan in the nature in chickens in Thai Nguyen province was 20.55%, with an intensity of infection was 20.68%, in Bac Giang province the prevalence was 20.80%, with an intensity of infection was 14.90% .

The prevalence and intensity of infection of *Leucocytozoonosis* increased from delta regions up to midland and mountainous regions (10.14%, 19.01% and 29.50%).

The prevalence and intensity of infection of *Leucocytozoonosis* in summer and spring were higher and heavier than these in Autumn and Winter (27.92% and 22.14% in comparison with 18.44% and 10.22 %).

The prevalence and intensity of infection of *Leucocytozoonosis* were lower in backyard chickens with fences than chickens without fences (18.85% compared with 21.05%).

The prevalence and intensity of infection of *Leucocytozoonosis* increased in accordance with age of chickens and reach the highest in chickens of above 6 months of age (27.43%).

Chickens were raising in good veterinary hygiene conditions obtained the prevalence and intensity of infection lower and softer than chickens in poor veterinary hygiene conditions.

The prevalence and intensity of infection of *Leucocytozoonosis* in chickens with a density of 5 - 6 chickens/ m² were higher than chickens with the density of 3 - 4 chickens/ m² and 1 - 2 chicken/ m² in the same conditions.

The sex of chickens was not affected to the prevalence of *Leucocytozoonosis*.

We have identified three blackfly species: *C. arakawa*, *C. odibilis* and *S. slossonae* as intermediate vector inoculating, transmitting *Leucocytozoon* protozoa to chickens in Thai Nguyen and Bac Giang province.

There was 12.80% of individual blackflies collecting in Thai Nguyen and Bac Giang containing *Leucocytozoon* protozoa into.

Blackflies were highest active from May to July and from 8 am to 16 pm.

2. Disease caused by Leucocytozoon protozoa in chickens

There were identified 3 protozoal species parasitizing pathogenic cause in chickens in Thai Nguyen and Bac Giang were *L. caulleryi*, *L. sabrazesi* and *L. smithi*.

High fever, anemia, pale crest, thinness, cheerless, anorexia, diarrhea, green feces were main symptoms of *Leucocytozoon* infected chickens .

Red blood cells, platelets và hemoglobin concentration decreased; white blood cells especially eosinophils and neutrophils of *Leucocytozoon* infected chickens increased markedly in comparison with healthy chickens.

Leucocytozoon infected chickens have lesions of hemorrhagic liver, spleen, kidneys, lungs, heart, pancreas, intestine, thigh and chest muscle; swelling and hemorrhagic liver, kidney and spleen.

The abdominal organs of infected chicken have markedly microscopic lesions as follows: hemorrhagic heart, pulmonary and bronchial tissue, widening spleen cavity, degenerated and hemorrhagic hepatic tissue, hemorrhagic renal tissue.

3. Preventive and treatment measures of Leucocytozoonosis in chickens.

Treatment efficacy of *Leucocytozoonosis* in 3 regimens reached 85 - 100%. In these, the regimen 1 with daimentol soda (2 grams/ liter of water) combined with TA. liver - kidney - spleen antidote and doxyvit – thai obtained highest efficacy, also very safe for chickens.

The application of preventive and treatment measures have significantly reduced the prevalence and intensity of infection of *Leucocytozoonosis* also increased the corporal weight of experimental chickens in comparison with chickens in control group after 3 months trial.

2. Recommendation

Localities should use the regimen 1 in treating *Leucocytozoonosis* for infected chickens or susceptible chickens.

Apply integral preventive measures of *Leucocytozoonosis* for chickens widely in Thai Nguyen, Bac Giang province and to other provinces.

**LIST OF PAPERS PUBLISHED
RELATING TO DISSERTATION**

1. Nguyen Thi Kim Lan, **Duong Thi Hong Duyen** (2011), “Study on *Leucocytozoonosis* in chickens in some localities of Thai Nguyen province”, *Journal of husbandry science and technology*, 8 (149), pp. 35 - 39.

2. Nguyen Thi Kim Lan, **Duong Thi Hong Duyen** (2012), “Study on some related factors and impacts of *Leucocytozoon* in chickens”, *Journal of Agriculture and rural development*, pp. 124 - 128.

3. N. T. K. Lan, M. A. Khoa and **D. T. H. Duyen** (2012), “Studying on *Leucocytozoonosis* in chickens at Thai Nguyen Province, Viet Nam”, *Proceedings of the 15th AAAP Animal Science Congress*, pp. 1827 - 1837.

4. **Duong Thi Hong Duyen**, Nguyen Thi Kim Lan, Le Van Nam, Nguyen Dinh Hai (2015), “Prevalence of *Leucocytozoon* spp. protozoa in chickens in Thai Nguyen, Bac Giang province”, *Journal of Agriculture and rural development*, pp. 273: 88 - 92.