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PARASITOLOGICAL AND HISTOPATHOLOGICAL STUDIES OF THE NATURAL INFECTION WITH *LEUCOCYTOZOON SIMONDI* IN GEESE IN NINEVAH GOVERNORATE

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ABSTRACT

This study is the first report in Mosul for detection of *Leucocytozoon simondi* in geese. Blood smears from ninety-six geese were collected in Nineveh governorate during July 2005 till February 2006 for detection of *L. simondi.* Liver histopathological sections wase also performed. The results showed that 32 geese were positive for *L. simondi*, with infection rate of 22.85% for young birds and 39.34% for adult geese.Males show higher percentage of infection 35.71% versus 30% in females. The infection rates according to the villages were ranged from 16% to 41.66%. Blood smears show the spindle and the spherical form of the haemoparasite. Liver histopathological sections of livers geese show coagulative degeneration and necrosis with spindle and the oval form of the parasite.

دراسة طفيلية ونسجية مرضية للإصابة *LEUCOCYTOZOON SIMONDI* الطبيعية بطفيلي في الوز في محافظة نينوى

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الخلاصة

في هذه الدراسة تم تسجيل أول إصابة طبيعية بطفيلي *Leuocytozoon simondi* في الوز. وتم ذلك خلال الفحوصات المجهرية للشرائح الدموية ل (96) مسحة دموية من طيور الوز التي جمعت من (12) قرية تابعة لمحافظة نينوى للفترة من تموز 2005 ولغاية شباط 2006 للكشف عن الإصابة بهذا الطفيلي . أظهرت النتائج أن 32 من طيور الوز كانت مصابة بالطفيلي وبنسبة 22,85% للأعمار الصغيرة من تلك الطيور و 39,34% في البالغين منها. وان نسبة الإصابة كانت أعلى في الذكور حيث بلغت 35,71% مقابل 30% في الإناث، وتوزعت نسبة الأصابة في مختلف القرى مابين 16 إلى 41,66%. أظهرت المسحات الدموية وجود كلا الشكلين للطفيلي؛ المغزلي والدائري. أما في المقاطع النسجية لأكباد الوزفقد ظهرت تغيرات تنكسية وتنخرية تجلطية مع وجود الشكلين المغزلي والبيضوي للطفيلي.

INTRODUCTION

Leucocytozoonosis is the most important blood protozoan disease of birds, caused by 60 species of a protozoan blood parasite that affect both wild and domestic avian species and transmitted by the bite of a blackfly *Simulium venustum, S. croxtoni, S. euradminiculum,* and *S. rugglesi* (1). *Leucocytozoon smondi,* is one that affect 27 species of ducks and geese in United States, Canada, Europe, and Vietnam (2). In these birds, the development of *L.simondi* progresses through 2 schizogony asexual tissue stages of the vertebrate host, which takes place in internal organs such as liver, brain, spleen and lung , with injecting sporozoites into the birds when the fly feeds. Sporozoites are then enter hepatocytes and develop into small schizonts. Hepatic schizonts then release merozoites and syncytiain a period of 4-6 days . The formed merozoites then either enter erythrocytes or erythroblasts to develop into round gametocytes. Syncytia are phagocytized by macrophages or reticuloendothelial cells through the body primarily found in the spleen, where they develop into megaloschizonts. Megaloschizonts then divide into primary cytomers which multiply into smaller cytomers and finally multiply by schizogony into merozoites. Released merozoites at this stage, will enter lymphocytes and other leucocytes to form elongated gametocytes. The rupture of hepatic schizonts has been observed to occure around post exposure day 5 and rupture of splenic megaloschizonts occure around day 10. A non- infected fly will feed on an infected bird and ingest the elongated gametocytes. The elongated gametocytes become a macrogametocyte(female) and a microgametocyte (male). Sporogeny occurs in the insect vector and may be completed in 3 rd-4 th days. Ookinetes develop following fertilization of the macrogametocyte and may be found in the stomach of the insect within 12 hours after a blood meal. Oocytes form from the ookinetes within the stomach of the invertebrate host and produce sporozoites, which migrate to the salivary glands after emerging from the oocyst, thus starting the life cycle over again (3). The pathogenicity of *L. simondi* in ducks and geese is well documented. An outbreak of *L. simondi* among ducks in Michigan resulted in 35% mortality. Extensive losses of young goslings, attributed to infections of *L. simondi*, were observed annually at Seney Wildlife Refuge, with mortality greater than 70% occurring every 4 years (4). However, not all *L. simondi* infections cause such severe disease. An experimental infection in anatid ducklings caused no mortality and no difference in growth rate. Clinical signs vary with age and the condition of the host. Young ducklings manifest inappetence, weakness, listlessness, dyspnea, and sometimes death within 24 hours. Signs in adults appear less abruptly and consist of listlessness and low mortality. About 60% of fatalities occur 11-19 days post exposure. Some pathologic effects of the disease are anemia, leukocytosis, splenomegaly, and liver degeneration and hypertrophy. Extensive tissue damage was noted in the spleen and heart of ducks carrying megaloschizonts. The greatest number of infections in northern Michigan occurs in July. Gametocytes decrease in number in the blood until midwinter, when they disappear or become scarce and then reappear in the spring (5). Studies to date here, in Ninevah governorate, trailed to elucidate the prevalence of leucocytozoon spp. only in turkey and pin tailed sand grouse, being 14% and 13.8% respectively (6, 7) but no attempt was carried out to investigate the presence of Leucocytozoon spp. in geese, and this was the aim of study.

MATERIALS AND METHODS

This study was carried out between July, 2005 , and February ,2006, involving 96 geese, 35 young and 61 adults, of which 56 males, and 40 females, from Hamdania locality in Ninevah governorate. The geese was sampled from 12 villages and brought to the laboratory of pathology in the college of veterinary medicine, Mosul University. Blood smears, were prepared from each goose after sacrifice. The age and sex of geese were determined by necropsy. It is generally accepted that the complete development of the left ovary in females and testicles in males is achieved at the end of the 5 month of life. Therefore, the geese under 5 months were considered young and above 5 months were considered adults. Blood smears were air dried and fixed by methyl alcohol and stained with 5% giemsa stain and examined by immersion. Histopathological studies of liver lesions were performed by fixing affected portions in 10% neutral buffered formalin. Fixed tissues were trimmed, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin stain (8). Tissue sections were then examined microscopically. Morphomertric measurements of parasite developmental stages were performed using ocular micrometer. Gametocytes of *L. smondi* and histopathological sections were photographed.

Results

The villages, total number, age and sex of geese examined are presented in table 1.

Table 1: Total number, age and sex, of geese examined in villages in Nineveh governorate.

Village	Number of geese	Age		Sex	
		Young	Adult	Female	Male
Bartilla	24	9	15	5	19
Bazkertan	5	3	2	1	4
Shakoli	3	1	2	2	1
Tahraoa	6	2	4	1	5
Karamles	2	1	1	2	0
Karakosh	13	3	10	8	5
Kharab Sultan	7	2	5	5	2
Balawat	5	2	3	3	2
Ali Rash	3	1	2	2	1
Khazna	15	6	9	7	8
Basakhra	6	2	4	2	4
Kabarli	7	3	4	2	5
Total	96	35	61	40	56

Of the 96 geese, 32 were found to be infected with *L. smondi.* When the infection rate was reassessed according to age (young-adult), and sex, it varied from 22.85 % (8/35) in young geese to 39.34 % (24/61) in adults (Table 2), it was 30 % (12/40) in females and 35.71% (20/56) in males (Table 3). Infection rates were also calculated according to the villages where the geese were obtained. In

addition, the highest infection rate were observed in geese from Khazna 60% (9/15) and Bartilla, Bazkertan 41.66% and 40%(10/24 and 2/5) respectively. Similar percentage was recorded in Shakoli, Ali rash and Basakhra 33.33% (1/3, 1/3, and 2/6, respectively).The lowest percentages were observed in Karakosh and Kharab Sultan, 30.67% and 28.57% (4/13 and 2/7) respectively. No infection was encountered in Karamles, Balawat and Kabarli (Table 4).

Table 2: Infection rate of *Leuocytozoon simondi* according to age.

Examined geese		Infected geese	
Age	Number	Number	%
Adult	61	24	39.34
Young	35	8	22.85
Total	96	32	33.33

Table 3: Infection rate of *L. simondi* according to sex.

Examined geese		Infected geese	
Sex	Number	Number	%
Female	40	12	30
Male	56	20	35.71
Total	96	32	33.33

Table 4: Infection rate of *L. simondi* according to locality.

Villages	Number of geese	Infected geese	
		Number	%
Bartilla	24	10	41.66
Bazkertan	5	2	40
Shakoli	3	1	33.33
Tahraoa	6	1	16.66
Karamles	2	0	0
Karakosh	13	4	30.76
Kharab sultan	7	2	28.57
Balawat	5	0	0
Ali rash	3	1	33.33
Khazna	15	9	60
Basakhra	6	2	33.33
Kabarli	7	0	0
Total	96	32	33.33

The measurements of *L. simondi* in blood smears are represented in table 5.

Table 5: Measurements of *L. simondi* developmental stages in blood smears.

Shape of the parasite					
Spindle			Round		
Length µm			Diameter µm		
Mean	Range	Standard deviation	Mean	Range	Standard deviation
17.6	13.6-20.8	2	15.3	14.4-16	0.7
Width µm					
Mean	Range	Slandered deviation			
4.7	4.1-5.6	0.6			

The measurements of the *L. simondi* development stages are illustrated in table 6.

Table 6: Measurements of *L. simondi* developmental stages in histopathological liver sections.

Shape of the parasite					
Spindle			oval		
Length µm			length µm		
Mean	Range	Standard deviation	Mean	Range	Standard deviation
89.8	83-91.3	3.1	49.2	41.9-49.8	2.1
Width µm			Width µm		
Mean	Range	Standard deviation	Mean	Range	Standard deviation
31.5	24.9-33.2	3.3	26.2	24.9-33.2	3.1

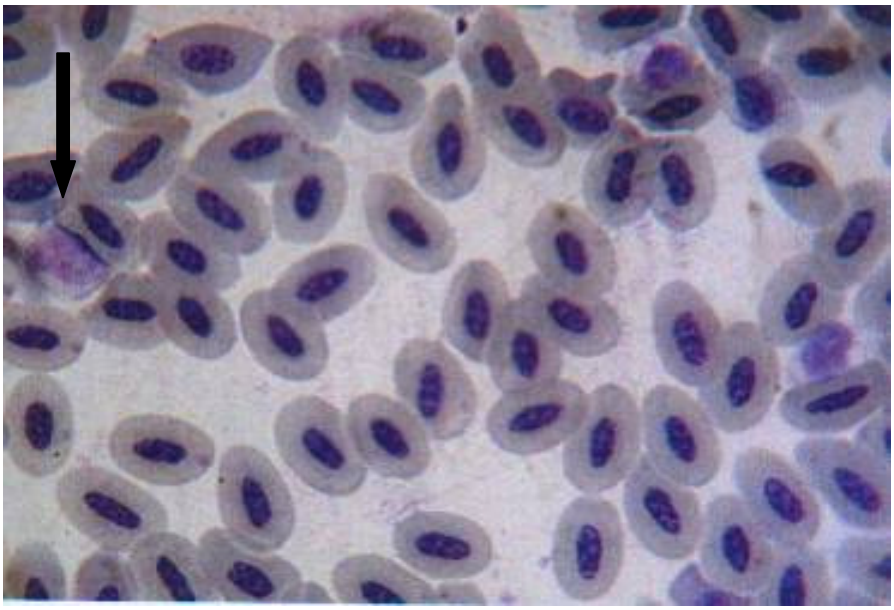

Figure 1: blood smear of goose showing the round shape of *L. simondi.*

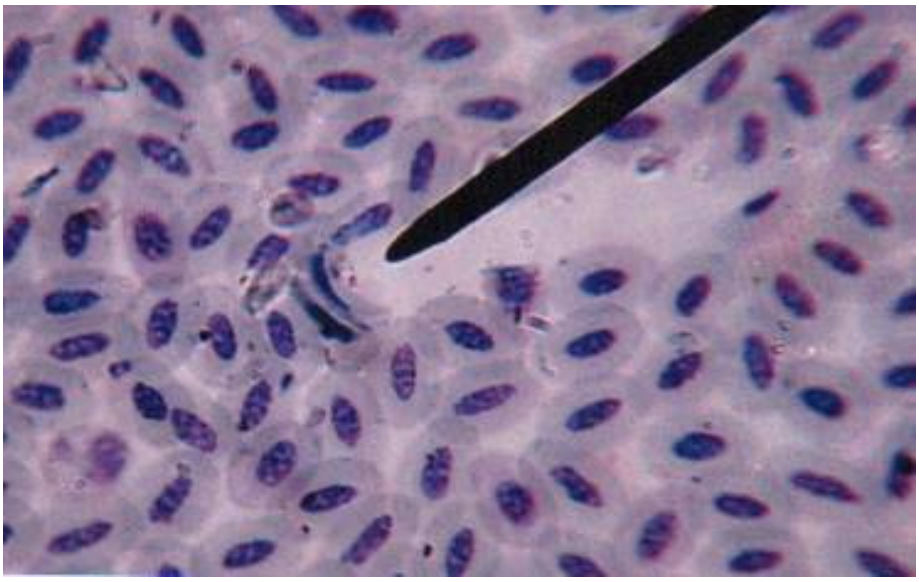

Figure 2: blood smear of goose showing the spindle shape of *L. simondi.*

Clinical signs of affected geese manifest inappetance, weakness, listlessness and anemia. The histopathological section of livers geese is presented in figures (3, 4) shows coagulative degeneration and necrosis, infiltration of lymphocytes and macrophages with oval and the spindle form of the parasite.

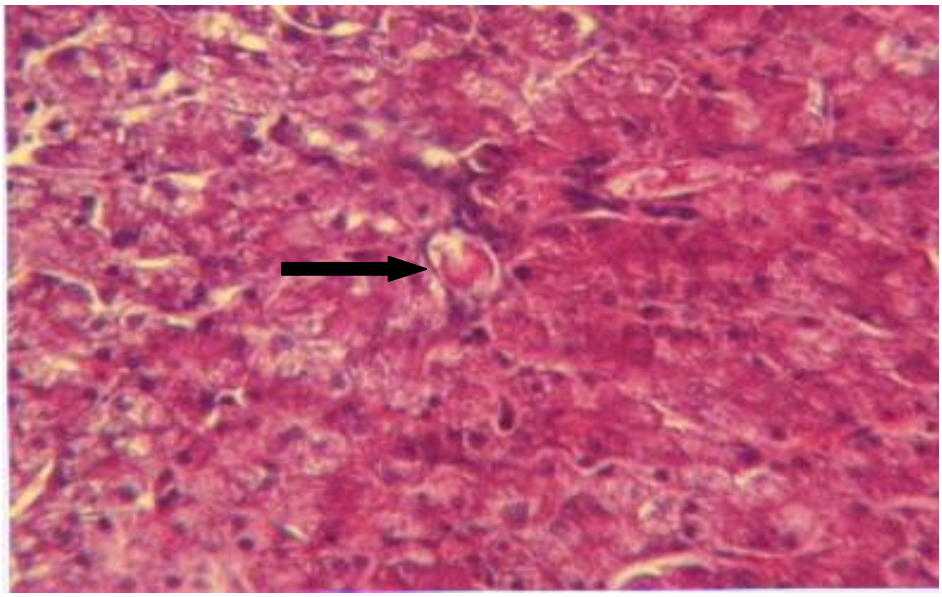

Figure 3: Histopathological section of goose liver showing the oval shape of schizont for *L. simondi* .X40.

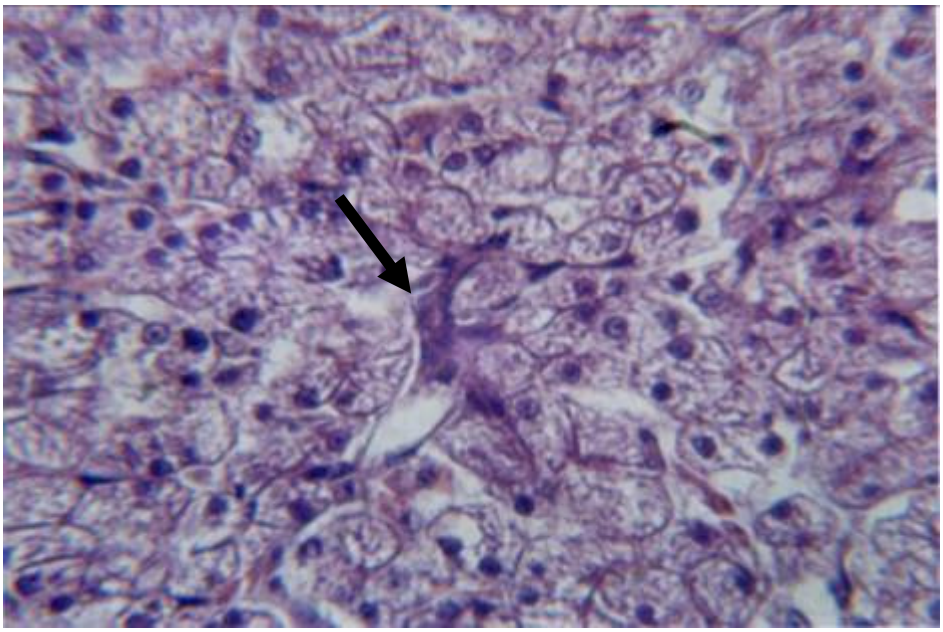

Figure 4: Histopathological section of goose liver showing the spindle shape of *L. simondi* .X100.

DISCUSSION

Our findings of *L. simondi* is the first report of a blood parasite in Mosul geese and the third record of avian hematozoan after that reported of *leucocytozoon* in *Pterocles alchata* (6) and that reported in turkey (7). *Leucocytozoon* is one of the reported avian hematozoa in Mosul governorate. Other avian hematozoa isolated in turkey by (7) were *Aegyptianella moscoviski*; *plasmodium spp* and *Haemoproteus spp*, while that isolated in Pterocles alchata by (6) was Haemoproteus spp. Bennett et al. (9) reported 42 geographically wide spread species of ducks and geese as hosts of this parasite. *Leucocytozoon spp*. are transmitted by black flies (simuliidae) vectors that are active during the spring and summer in northern regions (10). Infective stages of some *leucocytozoon spp*. Increase in number in the peripheral blood during the spring, which enhances parasite transmission when suitable vectors are present (11). During the collection, we observed black flies near the breeding sites of the examined geese. Some of the insects may be potential vectors of leucocytozoonosis and local transmission of parasites may occur. It is probable that the infected male acquired the *Leucocytozoon* infection on the breeding grounds. *L.simondi* had been implicated in disease and metabolites especially in juvenile birds (10, 12). Anemia has accompanied *L.simondi* infections in ducks (13) and a large – scale mortality of Canada goose (Branta Canadensis) goslings on the breeding grounds in upper Michigan was attributed to *L. simondi* (14). All the forms and measurements of parasite developmental stages are in agreement with the most authors (15, 16, 17). Because little is known about avian blood parasites in Northern Iraq especially here in Mosul governorate, the possibility of *Leucocytozoon* transmission in the breeding area is of interest and warrants monitoring of this species for disease and mortality.

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