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PRELIMINARY SURVEY OF BLOOD PARASITES
IN UPLAND GAME BIRDS (AND SOME OTHER SPECIES)
IN THE PROVINCE OF ALBERTA
DURING THE WINTER OF 1947-1948

- by -

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A THESIS

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ABSTRACT

The present paper reports the relative incidence of protozoan blood parasites in our economically important wild game birds (and some other species) in the Province of Alberta, during the winter of 1947-1948. Prior to this paper, no parasitological investigations in this field had been made in this Province. A total of 566 blood smears, representing 343 birds, comprising 32 species, were examined in this survey. The smears were obtained through the kind co-operation of the Provincial Game Inspectors, Forest Rangers, sportsmen and hunters in 23 localities throughout the Province. The smears were air-dried when received by the laboratory, and were stained with Wright's, Giemsa's or Leishman's Blood Stains.

Since two of the protozoan blood parasites are but little known, it was deemed advisable to include a short review of the life histories of the three protozoan blood parasites, Plasmodium, Haemoproteus and Leucocytozoon, and to include reports of literature relevant to this paper. A comprehensive review has been made of Plasmodium, and the other two have been referred to only in their essential differences from the Plasmodium.

The results of the investigation and the localities represented in the investigation are illustrated by Table 1 and a map.

Special note has been made of the occurrence of what is believed to be the most northerly recorded instance of microfilaria in a Northern Sharp-Tailed Grouse. A table of some of the characteristics of the microfilaria noted in this survey is included (See Table 11).

In this report is contained a discussion based on the effects of temperature and humidity on insect vectors in Alberta, the possible role of insects in relation to the health of birds, and a report of observations recorded at the peak year of 1934.

The paper concludes with a brief summary of my statistical data and the conclusions arrived at by me, based on existing knowledge.

INTRODUCTION

The numbers of many species of animals and birds are known to fluctuate widely through definite intervals of time.

The fact that game birds have gradually increased in numbers plus the knowledge that they will, after a few years of increase, again disappear, has raised the question of the possibility of protozoan blood parasites as a contributing factor to their sharp decline, indicating specifically the crash of the ten-year cycle.

The preservation of Alberta's upland game birds is of prime economic importance. The present paper was planned in view of the fact that very little has been done in the way of general bird-blood parasite surveys in Canada; it was also planned to see if there could be any possible connection between the incidence of blood parasites and the rise and fall of bird populations in the Province during this dramatic ten-year cycle. This can only be made possible by actual statistical records of the blood parasites occurring in our resident birds. This I have attempted to show in the statistical data presented

in the pages to follow.

The study of bird-blood protozoology began in 1885 with the discovery by Danilewsky of Plasmodium in birds. Since then, researchers have found Plasmodium in almost all vertebrates, Haemoproteus in reptiles and birds, and Leucocytozoon in birds alone. However, the literature available on the morphology and physiology of the parasites in birds is, at the present moment, scattered, obscure, uncorrelated and often contradictory.

In an effort to examine all the original literature on the subject, I have consulted other libraries as well as our own. All those relevant to this paper are included and indicated thus Smith and Smith (1931). (Additional papers that have been examined are included only in the bibliography).

This paper consists of two major discussions: first, that of the life cycles of parasites observed in this survey (including a review of important relevant literature) and second, the statistical data obtained during the survey.

ACKNOWLEDGMENTS

The writer wishes to take this opportunity of thanking Dr. W. Rowan, under whose supervision this work has been persued, for his help, encouragement and advice. In addition, the writer is grateful to Dr. Cockburn for the time devoted by him to checking many of the slides. He is appreciative also of the assistance of all those friends whose interest and helpful suggestions have made each stage of this study less arduous, and the co-operation of those persons who contributed the specimens upon which this study was based. A great debt is acknowledged to the Medical Research Fund, whose patronage made this study possible.

PART 1

REVIEW OF LITERATURE

CLASSIFICATION

Phylum Protozoa
 Sub-phylum Plasmodroma
 Class Sporozoa
 Sub-class Coccidiomorpha
 Order Coccidiida
 Sub-order Haemosporidiidea
 Family Haemoproteidae
 Genus Haemoproteus
 Genus Leucocytozoon
 Family Plasmodiidae

FAMILY PLASMODIIDAE

Life Cycle in the Bird:

The similarity of the avian and human malaria cycle is most strikingly comparable in many respects. The cycle in the bird is as follows: Sporozoites present in the salivary glands of certain species of mosquitoes (Culex, Aedes, Theobaldia, and Anopheles) are injected into the blood of the bird while the insects are biting. The sporozoites now undergo a pre-erythrocytic phase (the pre-patent period). Huff and Coulston (1944) using massive doses of sporozoites of P. gallinaceum investigated this pre-erythrocytic phase. They found that the sporozoites invade the macrophages at the site of inoculation and later the capillary walls in the spleen. After 36 hours, when this first of cryptozoic generation is mature, the parasite is a large multinucleate

organism occupying almost the whole cell. The cell ruptures, liberating a large number of merozoites which have the ability of infecting other endothelial cells but not the erythrocytes. The generations following the cryptozoites are known as the metacryptozoic generations, the first of which is now found in cells of the lymphoid-macrophage system and endothelial cells. The metacryptozoites resemble the cryptozoites in their morphology and in their maturation in 36 hours. After 60 hours, metacryptozoites are observed in the inoculation site, spleen, heart, kidney and brain. On reaching maturity, the metacryptozoite liberates its merozoites, which may now invade the erythrocytes initiating the well known trophozoite cycle, or they may parasitize other cells in the spleen, heart, kidney, brain, lung, liver, bone-marrow or muscle, perpetuating the exoerythrocytic stages of the infection. The first parasite in the red blood cells is known as a trophozoite (ring stage), which enlarges within the red cell, displaces the host cell nucleus in many cases, and becomes either a segmenter or a gametocyte; the former representing the asexual division stage, the latter the sexual stage which undergoes further development in the insect host. The products of the division of each segmenter, known as merozoites, escape and penetrate

new erythrocytes. The haemoglobin within the red cell is apparently digested and pigment granule by-products are deposited within the parasite.

Cycle in the Mosquito:

The gametocytes are of two types: macrogametocytes (female) and microgametocytes (male). When a mosquito ingests blood containing gametocytes further development takes place. Each microgametocyte undergoes exflagellation producing 6 to 8 flagellated bodies (male gametes) in the stomach of the mosquito while the female gametocyte undergoes a maturation process to become a female gamete. Fertilization takes place and the resulting zygote becomes an elongate, motile ookinete that penetrates the stomach wall of the mosquito and comes to rest just beneath the outer epithelium, where it grows at the expense of the surrounding tissues. Within the resulting spherical oocyst large numbers of minute elongate cells develop, called sporozoites, which eventually escape into the body cavity and find their way to the salivary glands. When the mosquito next bites, some of the sporozoites are inevitably injected into the vertebrate host where the asexual cycle begins anew.

THE MALARIAL CYCLE

(Schematic)

Wenyon (1926) reports that Sargent (1919) carried out experiments pertaining to the development of P. praecox in C. pipiens; he found that the optimum temperature lay between 20°C and 30°C. Mosquitoes after being exposed to 12°C for the first 6 hours following the "infecting feed" rarely became infective on being transferred to favorable temperatures. If after feeding, mosquitoes were kept at a temperature varying between 17.5°C and 24°C, development took place very slowly, sporozoites not appearing in the salivary glands till after the expiry of 2 months. (Wenyon, 1926, obtained complete development, including infection of the salivary glands, in less than 5 days although 7 to 10 days is the generally accepted time today).

Symptomatology and Pathology:

The impression one gets from the available literature is that the question as to whether Plasmodium infections cause as high a degree of mortality in wild game birds as in laboratory birds is nothing more than a supposition. Herman (1938) states that Plasmodium infections probably do not cause a high mortality in game birds, the disease tending greatly to lower the

resistance of the infected individual and thus making it vulnerable to other diseases or natural enemies. This assumption was based on observations on a Red-wing which had sustained an acute infection in nature. The bird did not seem to be suffering any noticeable ill effects; the only indication of the disease was the great number of parasites to be seen in its blood smear. On the other hand, canaries inoculated with the infected blood sustained high mortality (more than 50%).

Hewitt (1940) reports observations made by Danilewsky (1885-1890) as follows: Out of 300 captured birds kept under observation for weeks or months only 4 or 5 died from what he considered to be the effects of the parasite. In describing symptoms, Danilewsky noted that the temperature of infected birds rose moderately (from 1 to 1.5°C or more). The birds lost their appetite, they became apathetic, their plumage became ruffled, they suffered convulsions and they lost weight. These symptoms were found to occur at the time of sporulation of the parasites. In general the sickness gradually subsided within 4 to 6 days, after which recovery followed, the parasites disappearing eventually from the blood and the

condition of the bird becoming once more normal. The spleen and liver were also noted to enlarge and contain enormous amounts of pigment. Huff (1939), however, demonstrated that there was no rise in temperature even when greatest segmentation of the asexual forms took place. Subsequent investigations have largely confirmed these observations.

Huff and Bloom (1935), Porter (1942), Simpson (1944), Coulston, Cantrell and Huff (1945), Taliaferro and Bloom (1945) and others, noted in addition that exoerythrocytic stages occurred in lung, bone-marrow, brain, heart, kidney, ovary, lymph nodes, thymus and inoculation site. Relapses are known to occur in experimentally infected canaries, but whether they occur in the same frequency in wild birds is not known.

Species and Host Index:

Hewitt (1940) compiled a list of birds (taken chiefly from the extensive check-lists given by Wenyon (1926), Coatney and Roudabush (1936) and Giovannola (1939) in which 258 species of birds are listed) in which plasmodia have been described. Since then supplementary lists have been published (the latest of which was by Herman in 1944).

In 1942, the Committee on Terminology of Strains of Avian Malaria recognized 14 species of plasmodia including 38 strains.

Russell and Mohan (1942) published a list of mosquito hosts to the avian plasmodia with special reference to P. gallinaceum. They noted that in Florida (at 25 to 31 degrees latitude) that 32 species of mosquitoes were capable of transmitting P. gallinaceum into various bird hosts. Since then several new mosquito hosts have been recorded. The evidence from the literature suggests that at higher latitudes the range of mosquito vectors becomes correspondingly restricted. Herman (1938) and Russell and Mohan (1942) noted that mosquitoes are specific as transmitters not only for particular species of bird malaria, but also possibly for certain strains of the same species or perhaps that there is an immunological difference in mosquitoes collected from different localities.

Results of Current Experimental Work:

The fact that Plasmodium passes its asexual stages in the circulating blood cells, and as such is easily transmitted from one susceptible host to another, has made the

investigation on the malarial parasite one of the most fruitful fields in the study of protozoan diseases. It is one characteristic of bird malaria not to be strongly host-specific; consequently, most (not all) of the malarial parasites found in wild birds can be grown in the common laboratory birds -- the canary, chick and duck. For example, P. lophurae isolated from the Fire-back pheasant is inoculable into the canary, duck and chicken. On the other hand, Simpson (1944) has noted that P. durae, isolated from the common turkey, is not inoculable into the duck, and is, according to present knowledge, a strongly host-specific species. Huff and Bloom (1935) found that P. elongatum, in contrast to other malarial parasites, is capable of living in all circulating blood cells and hemopoietic cells of the canary, and is unique in that it includes granular leucocytes. Pigment formation, however, was confined only to those parasite forms found in cells containing haemoglobin. Coulston, Cantrell and Huff (1945), in an extremely interesting experiment, showed that infection of the bird takes place in less than one minute after the pierce of the infected mosquito. They

also demonstrated that not all the sporozoites left the site of inoculation, because amputation of the site of inoculation could by subinoculation produce an infection in another vertebrate host. Bloom and Taliaferro (1938) showed that birds with extensive infarcts during acute malaria, succumbed in greater numbers than those without infarcts. They also showed that the spleen is able to recover and regenerate and again become effective in combating malaria with its phagocytes. Taliaferro, Taliaferro and Simmons (1945), showed that irradiation of the bird host by X-rays of sufficiently high magnitude caused increased parasitemia and a decrease in the survival time of the host. Studies on relapse conditions were made by Hegner (1937) and Gajewski and Tatum (1944); relapses were produced by the use of insulin, epinephrine, low oxygen content of the air, ultra-violet radiation, glucose, blocking of the macrophages, and by natural conditions such as acute infections of other sorts, extreme cold, exhaustion and change from low to high altitudes. Hegner and Hewitt (1937) noted that more young red blood cells are susceptible to the parasite than are old red blood cells, even though the proportion is 6 to 94. They also noted that cells which

were parasitized with more than 1 parasite died in 21 hours. They suggested that this would account for most of the decrease in the number of parasites that is known to take place during an asexual generation. Taliaferro and Taliaferro (1940) demonstrated that infections with small numbers of parasites had a slow development, low intensity and usually no crisis, in contrast to infestations with large numbers of parasites in them. They also showed that birds show a high degree of immunity to superinfection by the same species of parasite. They also noted that young chicks suffered heavier mortalities than did adult birds. Gingrich (1941-42) illustrated that phagocytosis is not merely an adjunct to acquired immunity but an active process and a major factor in the parasitocidal mechanism of immunity. He further noted that natural immunity is manifested especially during the acute rise of infection with P. cathemerium when approximately 2/3 of the parasites fail to survive (note Hegner and Hewitt above). He states that the mechanism of acquired immunity is primarily cellular and consists of an increase in the number of macrophages (and the phagocytosis by them of parasitized red blood cells) chiefly in the spleen, liver and to a

lesser extent in the bone marrow. A deficiency of protein or Folic acid or the addition of glucose to the diet of the bird caused a heavier infection in the host. (Seeler and Ott (1945), Hegner (1937) respectively). Bennison and Coatney (1945) demonstrated that the female chicks were more susceptible to malaria than males. They showed a higher parasite count, and experienced an earlier endothelial invasion.

FAMILY HAEMOPROTEIDAE

Genus Haemoproteus

Cycle in the Bird

Haemoproteus, of which there are many species in birds, is interesting because its gametocytes closely resemble those of some species of malaria, even in having the same type of pigment. Although the sexual forms are found in red blood cells, the asexual ones are not. The latter, however, are morphologically similar to the exoerythrocytic forms of the plasmodia and occur in similar locations in the host. As in the case of Plasmodium, if more than one merozoite

enters a red blood cell, the excess is either expelled or the red cell dies. The gametocyte when fully formed is "halter shaped" and is frequently called a Halteridium. As increase in size takes place, the organism grows around one side of the host cell nucleus; host cell and nucleus retain approximately their normal shape. On the other hand, the gametocyte may enclose the host cell nucleus entirely or push it to the side, but it does not distort it, and the parasite still encloses the nucleus like a halter, as does P. circumflexum, P. lophurae (and others). It is difficult to distinguish these species of Plasmodium from Haemoproteus if segmenting forms are not present. Haemoproteus is so much like Plasmodium that there is but one way of telling the difference... this is by subinoculation into a parasite-free host. Because gametocytes are incapable of developing in the vertebrate body, subinoculation of the suspected parasite, if Haemoproteus, will give no infection; but if it is Plasmodium, an infection will result.

Cycle in the Fly

Subsequent development of the gametocytes takes place in the invertebrate host; in all cases where these are known, the hosts are the Hippoboscids blood-sucking flies. It is

not necessary to give a complete outline of the development here, since it is similar to the Plasmodium cycle in the mosquito. Such important factors as effect of temperature and humidity on the development of the parasite in the fly have yet to be determined. The above cycle takes place within 10 to 12 days (Hegner, 1938).

Check List and Host Index.

Coatney (1936) published a check-list and host index of the genus Haemoproteus in which are listed 45 specific names, most of which are described in birds. He also lists 648 species of birds known to be affected by this parasite. Herman (1944) published a supplementary list, in which several new specific names are added in new bird hosts. The genus occurs widely in the species of birds, including the owls, flickers, woodpeckers, ducks and passerine birds.

As Herman (1944) has noted, "the inability to transmit these parasites by simple blood transfusions makes it difficult to investigate their host-specificity and thus clarify the validity of named species".

Symptomatology and Pathology

Identical to that of Plasmodium. In passing it is worth noting that Plasmodium and Haemoproteus are never

transmitted by the same invertebrate host, yet both produce the same pathological conditions.

Results of Current Experiments

Very little in the way of experimental work or observations has been done with this parasite since no method has yet been devised to study latent cases.

Herman (1938) records a case where a black duck, kept in captivity from September, 1936 until its death in May, 1937, was observed to have parasites (Haemoproteus) in its blood during the entire period. Similar persistence of the parasite was found by Huff (1942) to be present in mallard ducks. Clarke (1938) found very few of the grouse in Ontario to be infected with Haemoproteus, the scarcity being possibly due to the fact that Hippoboscid flies have not been found commonly on Ontario grouse.

Huff (1942) suggests that in areas where birds are known not to be attacked by Hippoboscid flies, the Simuliid fly may serve as the vector of Haemoproteus as well as of Leucocytozoon. He also noted that there is a striking parallel between the schizonts of Haemoproteus and Leucocytozoon, i.e., the same characteristic clefts and forms of merozoites.

Herman (1938) noted that a number of Chipping Sparrows which were examined several times during the summer exhibited a high level of infection in each subsequent examination. Thus the maintenance of a high gametocytic level in the blood of infected birds throughout the summer and the presence of a low grade infection throughout the winter (as compared with the rapid course of the infection with most species of Plasmodium) may account for the higher incidence of Haemoproteus reported in most of the literature.

Only 2 species, H. maccallumi and H. sacharovi, found in doves, have been successfully transmitted by Huff (1932) to domestic pigeons, the definitive host in both cases being the Hippoboscids blood-sucking flies.

On the whole, very little has been done in the study of the parasite, and much is still in doubt.

FAMILY HAEMOPROTEIDAE

Genus Leucocytozoon

Life Cycle in the Bird

The genus Leucocytozoon is somewhat difficult to define; nevertheless, it is fundamentally very close to

Plasmodium and Haemoproteus, and it occurs only in birds.

It is similar to Haemoproteus in its asexual life cycle; however, the young gametocytes enter immature red blood cells.

There is considerable doubt concerning the true nature of the host cell, and it may in all probability be a leucocyte or a lymphocyte. The host cells containing adult parasites are so distorted by the infection that their identification no longer becomes possible. During growth of the gametocyte, the cell is profoundly altered, and in most cases the cell becomes an elongate spindle. Leucocytozoons do not form much pigment. Wenyon (1926) working with L. neavei noted that, whereas in fresh blood preparations all the parasites were in spindle-shaped cells, in dried films, especially if made some time after the death of the bird, there was a much greater diversity of shape, many of the parasites being spherical, while the cells appeared to have lost their tail-like prolongations.

Cycle in the Fly

The vectors of Leucocytozoon are apparently flies of the genus Simulium, principally the Black Fly. The gametocytes undergo fertilization and development in the

same manner as the Plasmodium in the mosquito. Observations by O'Roke (1934) indicate that the development of L. anatis (= L. simondi) in the female of the blood sucking black fly, Simulium venustum, can be completed in 5 days or less (although this is not necessarily the case for all species). The minimum temperature, the humidity, and the time required for the different stages in the sexual cycle of the parasite in the fly has not as yet been determined.

Symptomatology and Pathology

Birds harboring Leucocytozoa as a rule appear to be little affected by the parasite with the exception of certain species of birds, in which the Leucocytozoon disease is particularly pathogenic, and is the cause of heavy mortality. O'Roke (1934) noted mortality of 35 per cent in an outbreak among young ducks in Michigan. Mallards and Black Ducks of all ages were susceptible, and death usually occurred on the twelfth day after infection. L. bonasae Clarke from the ruffed grouse has been shown by Clarke (1935) to be associated with cyclical mortality in the grouse population and was considered by him as a possible cause of the cycle. Oliger (1940) noted that young Hazel grouse were attacked most violently

and produced most losses. Domestic turkeys are very susceptible to Leucocytozoon parasites and heavy mortalities in various parts of the world have been recorded. Macroscopic lesions are as follows: acute swelling of the spleen, the normal color changing to a light grey; enlargement of the liver, the surface covered with large light areas of degeneration; the kidneys enlarged and congested; bilateral edema of the lungs; hemorrhagic enteritis throughout the length of the intestine, and petechial hemorrhages in the heart and the brain substance. In turkeys there appear to be three types of infection:

(1) the per-acute form in which apparently healthy birds die suddenly following a few convulsive seizures; (2) the less acute form with weakness, progressive emaciation, and moderate turbidity of the humors of the eye; (3) the chronic form, characterized by arthritis, periartthritis, distortion of the hock joint, lameness and marked turbidity of the humors of the eyes.

Leucocytozoon infections are noted to be cyclic in nature by Clarke (1938), Huff (1942) and other investigators. Birds surviving an epizootic Leucocytozoon disease show a decrease in number of schizonts and gametocytes throughout the fall and winter months until April of the following year, at

which time there is a reappearance of the schizonts in the tissues followed by an appearance of gametocytes in the blood stream again.

Species and Host-Index

Leucocytozoon study is a relatively new zoological field, and a great deal has yet to be done towards clearing up their classification. Leger and Leger (1914) suggested that Leucocytozoon has a definite host-specificity, and that morphological characteristics tend to be dependent on the order (or group) of hosts infected. Most investigators have assumed that these parasites are extremely host-specific and many have thus been named on their first discovery in a previously unreported host. The inability to transmit these parasites by simple blood transfusion makes it difficult to investigate the host-specificity, and so to clarify the validity of a named species. Coatney (1937) has catalogued and host-indexed all species known up to that year. Sixty-eight specific names appear in his list, but there may be synonyms among these. Of the 68, only 58 were found in birds... the other 10 described in animals, and since Leucocytozoon occurs only in birds, Coatney claims that they were probably

Hepatozoons, Haemogregarines and Leishmanias. The host-index list has now been greatly increased, and Herman (1944) has published a paper to supplement the check-lists, thus bringing up to date the host distribution of blood protozoa in the Western Hemisphere.

Results of Current Experiments

Very little experimental work (e.g. relapses etc.) has been carried out on this genus (Leucocytozoon), as most investigators have hitherto concentrated on the direct life cycle of the parasite. Studies are now being conducted by Starr and West (1940) to discover further possible invertebrate host vectors in L. smithi disease of turkeys. This work was begun during an outbreak in a turkey flock when no *Simulium* flies could be observed; nor were there any local streams suitable for the breeding of these insects, whose normal range of dispersal is limited, existed in the district. It was noted, however, that lice of the genus *Mallophaga* infested the turkeys, and that, when remedial measures were instituted, no further losses were encountered, and blood smears failed to reveal any further infection. However, the supposition that lice are vectors still remains unproved.

P A R T 11

OBSERVATIONS AND CONCLUSIONS

MATERIALS AND METHODS

The present study has consisted of an examination of the blood smears for protozoan blood parasites. Although it is desirable to make the microscopic examination as soon as possible after death of the host, this was found to be impractical because of the large number of localities investigated. The material for this study was received from the game inspectors, forest rangers and sportsmen hunters of the Province. To avoid sacrifice of the edible birds, the collectors were requested to make smears of the peripheral blood and of the heart on clean glass slides provided, as soon after the death of the bird as possible. Dabs of liver, spleen and brain were later also requested. Thus, barring unforeseen circumstances, the smears were all made in the field.

On account of the fact that in the collection of the material for this study I was dependent, to a great extent, on the generosity and opportunity of others, this method of collection introduced some difficulties. The collection was, therefore, very irregular.

Blood smears of captive birds in the Zoological Parks

were made by piercing the brachial vein on the under side of the wing with a surgical needle.

The smears were air dried and stained in the laboratory with either Wright's, Giemsa's or Leishman's Blood Stains, from one to several days after preparation. Diagnosis for the presence or absence of parasites was based on a 15 to 20 minute examination of a single blood smear: 1 - 5 minutes with low power (10X oculars) and 15 minutes with oil immersion lens (10X oculars). Tabulation of results was made on the basis of individual birds.

NUMBERS OF BIRDS RECEIVED

The distribution of the specimens received is as follows:

Edmonton	185
Calgary	37
Edson	7
Hay Lakes	12
Whitford Lake	20
Ardrossan	3
Uncas	2
Bogues Lake	3
Sullivan Lake	1
Bruderheim	34
Kirkpatrick Lake	1
Brooks	38
Chin Lake	1
Coaldale	1
Lethbridge	1

C O L U M B I A

B R I T I S H

S A S K A T C H E W A N

OUTLINE MAP OF ALBERTA

SCALE IN MILES

0 50 100 150 200

UNITED STATES

St. Paul	2
Pincher Creek	3
South Cooking Lake	2
Wembley	3
Ellerslie	1
Lac-la-Nonne	2
Rich Valley	1
Fort Chipewyan	1
North West Territories	11

Included in the above numbers are 84 adult birds from the Edmonton Zoological Park and 37 adult birds from the Calgary Zoological Park.

Mr. Heustis, Fish and Game Commissioner of the Province of Alberta, directed his deputies to collect samples of blood especially for this investigation, and since this was a closed season, the regular shooting season having ended, a good number of the specimens were obtained in this way.

All samples were of apparently healthy adult birds.

OBSERVATIONS

A total of 566 smears, representing 343 birds, comprising 32 species was examined. Of these, 36 birds (or 10.5 per cent) were found to be parasitized by one or more parasites, a total of 42 infections in all.

The parasites observed included examples of Plasmodium, Haemoproteus, Leucocytozoon and microfilaria. The data in Table I summarizes the distribution of these parasites based on a study of their morphology in the blood films.

Success in finding parasites readily in smears is in some measure dependent upon the quality of the blood smears received. Unfortunately, on account of the large area represented in this report and the necessity of mailing the smears to the laboratory, many of the smears were contaminated by artifacts, bacteria or moulds, thus masking the presence (if any) of the parasites. In addition, many of the smears were made in freezing weather, and this seemed to have a destructive effect on the blood cells, particularly the monocytes.

As observed in an earlier part of this paper, the parasites disappear from the blood stream in a relatively short time and are (in the winter months especially) extremely difficult to find or to identify. This fact is amply illustrated in this investigation where, for the most part, the infections were very light. No attempt was made to assign the parasites to species, as too few parasites were found to permit average measurements and comparison with described species.

TABLE I -- DISTRIBUTION OF PARASITES

HOSTS		PARASITES					
Classification and Name	Common Name	Number Examined	Number Infected	P	H	L	M
<u>Order Galli</u>							
Bonasa u. umbelloides	Ruffed Grouse	11	3	1	1	1	0
Pedioecetes phasianellus	Sharp-tailed Grouse	8	1	1	0	0	0
Campestris							
Pedioecetes p. phasianellus	Northern Sharp-tailed Grouse	1	1	0	0	1	1
Canochites canadensis	Spruce Partridge	9	1	0	0	0	1
Perdix perdix	Hungarian Partridge	9	3	1	1	1	0
Phasianus c. torquatus	Ring-neck pheasant	3	0	0	0	0	0
Lagopus lagopus albus	Willow Ptarmigan	11	2	0	0	2	0
Phasianus colchicus	Pheasant	53	3	1	0	3	1
Meleagris gallopavo	Domestic Turkey	1	1	0	0	1	0
<u>Order Anseres</u>							
Mareca americana	American Wigeon	10	4	2	2	1	1
Chaulelasmus streperus	Grey Duck	1	0	0	0	0	0
Nettion carolinense	Green-winged Teal	2	0	0	0	0	0
Spatula clypeata	Shoveller	5	0	0	0	0	0

TABLE I (Cont'd)

Classification and Name	Common Name	Number Examined	Number Infected	P	H	L	M
<u>Order Anseres (cont'd)</u>							
Anas p. platyrhynchos	Mallard	64	6	1	3	2	2
Defila acuta tzitzihua	Pintail	10	0	0	0	0	0
Melanitta perspicillata	Scoter	1	0	0	0	0	0
Cairina moschata	Muskovy	1	0	0	0	0	0
	Pekin	2	0	0	0	0	0
Nyroca affinis	Lesser Scaup	3	0	0	0	0	0
Chen h. hyperborea	Snow Goose	1	0	0	0	0	0
Branta c. canadensis	Canada Goose	14	2	0	1	0	1
Cygnus buccinator	Trumpetor Swan	2	0	0	0	0	0
Cygnus columbianus	Whistling Swan	1	0	0	0	0	0
<u>Order Columbæ</u>							
Columba livia domestica	Domestic pigeon	57	3	3	0	0	0
<u>Order Accipitres</u>							
Circus hudsonius	Marsh Hawk	1	1	0	0	1	0
<u>Order Striges</u>							
Nyctea nyctea	Snowy Owl	1	0	0	0	0	0

TABLE I (Cont'd)

Classification and Name	Common Name	Number Examined	Number Infected	P	H	L	M
<u>Order Striges (cont'd)</u>							
Bubo v. subarcticus	Horned Owl	1	1	0	0	1	0
Bubo v. virginianus	Great Horned Owl	1	1	0	0	0	0
<u>Order Passeres</u>							
<u>Family Corvidae</u>							
Pica pica hudsonia	Magpie	3	1	1	0	0	0
Perisoreus c. canadensis	Canada Jay	1	0	0	0	0	0
<u>Family Paridae</u>							
Penthestes h. columbianus	Chickadee	1	0	0	0	0	0
<u>Family Fringillidae</u>							
Passeres d. domesticus	English Sparrow	54	3	0	3	0	0
TOTAL:-		343	36	11	11	14	7

LEGEND:

P - Plasmodium
H - Haemoproteus

L - Leucocytozoon
M - Microfilaria

PLASMODIUM

Parasites of the genus Plasmodium were found in 11 birds of 8 species, or 26.2 per cent of the infected birds. All were of an undetermined type, showing only a few trophozoites which might have belonged to any one of several species.

Chronic cases of avian malaria are often impossible to detect by peripheral blood smears, or smears from internal organs (Huff, 1943) especially in those species in which the parasite level is extremely low after the crisis is past. To determine more accurately the extent of avian malaria in nature, blood inoculations should be made into canaries from birds suspected or harboring the malarial organism, or better into clean birds of the same species. As this procedure was impossible in this survey, these figures for Plasmodium infections represent only an estimate of the probable infection in the number of birds examined.

Of the 11 Plasmodium infections found in this study, 3 were found in Pigeons, 2 in American Wigeons, and one each in Ruffed Grouse, Sharp-tailed Grouse, Hungarian Partridge, Pheasant, Mallard and Magpie. It is of interest

that these samples were obtained in late September and early October, just after the disappearance of the mosquito vectors.

HAEMOPROTEUS

Parasites of the genus Haemoproteus were found in 11 birds of 6 species or 26.2 per cent of the infected birds.

Of the 11 Haemoproteus infections found in this study, 3 were from Mallards, 2 from American Wigeons, 3 from English Sparrows and one each from Ruffed Grouse, Hungarian Partridge and Canada Goose. As with Plasmodium, the infections were very light and no attempt was made to allocate these to the species.

LEUCOCYTOZOON

Parasites of the genus Leucocytozoon were found in 14 birds of 10 species, or 33.3 per cent of the infected birds.

The basis for classification of the Leucocytozoon is the change in the morphology wrought by the parasite in the host cell, rather than the morphology of the parasite itself. Generally, birds of the large orders are parasitized by the type of Leucocytozoon which produces fusiform or

TYPES OF LEUCOCYTOZOON PARASITES
OBSERVED IN THE STUDY

1.

2.

3.

4.

5.

- 1 - Leucocytozoon sp. in Willow Ptarmigan
- 2 - 3 - Leucocytozoon sp. in Mallards
- 4 - Leucocytozoon sp. in Northern Sharp-Tailed Grouse
- 5 - Leucocytozoon smithi in Domestic Turkey

spindle - shaped host cells, while the smaller birds are infected with the round host cell type.

In only one case was positive identification of the Leucocytozoon possible. This was L. smithi which was found in the domestic turkey.

Of the 14 Leucocytozoon infections found in this study, 2 were from Willow Ptarmigans, 3 from Pheasants, 2 from Mallards and one each from Ruffed Grouse, Northern Sharp-tailed Grouse, Hungarian Partridge, domestic Turkey, American Wigeon, Marsh Hawk and Horned Owl.

MICROFILARIA

These parasites were found in 7 birds of 6 species, or 14.3 per cent of the infected birds.

Little is known of the pathogenicity of the avian types. They have been observed in large numbers at autopsy, but what their relation to the cause of death may be is not known. Because some are periodic in appearance, in the blood stream, the presence of a large number in a bird may be regarded as a periodic "shower" (Boughton, Byrd and Lund, 1938).

A few of the characteristics of some of the forms

TABLE II

MICROFILARIA, MEASUREMENTS AND CHARACTERISTICS

Host and Locality	Number of Forms Measured	Average Length in Microns	Average Width in Microns	Ant. End.	Post. End	Sheath
Spruce Partridge Edson	8	123.4	5.1	Blunt	Pointed	Sheathed
Mallard Hay Lakes	2	98.5	5.3	Blunt	Pointed	None
Mallard Lethbridge	3	104.7	6	Blunt	Pointed	Sheathed Striated
American Wigeon Whitford Lake	1	150	7	Blunt	Pointed	Sheathed
Pheasant Edmonton	2	140	4	Blunt	Blunt	None
Canada Goose Kirkpatrick Lake	3	83	3	Blunt	Pointed	Sheathed Striated
Northern Sharp- Tailed Grouse Fort Chipewyan	3	155	4	Blunt	Tapered	None

found are recorded in Table II. It is believed that the occurrence of microfilaria in the Northern Sharp-tailed Grouse obtained at Fort Chipewyan is the most northerly case on record.

MULTIPLE INFECTIONS

As is evident from a glance at Table I, on comparing numbers infected and total types of parasites indicated, one finds that a few birds harboured more than one parasite. Where only one infection in a bird was found, the table indicates the type of parasite. The organisms occurring in mixed infections were Haemoproteus (H), Leucocytozoon (L) and microfilaria (M). By using the letters indicated above, the complete figures for the birds with 2 or more genera of parasites is indicated in this manner: LM in 1 Northern Sharp-tailed Grouse; HLM in 1 Mallard; HL in 1 Mallard.

SAMPLES OF MAMMALIAN BLOOD

Incidental to the study of protozoan blood parasites in birds, smears were received and examined of 9 Snowshoe Rabbits, 4 Squirrels, 3 Weasels and 1 Moose. In one

smear from a Bush rabbit microfilaria was found. Four sheathed forms were measured. The average measurements were 180 microns by 7.2 microns. The microfilaria were characterized by blunt ends.

DISCUSSION

In closing this paper and drawing conclusions from the available printed material and the survey just completed, I believe the following represents a reasonable discussion of the controversial points.

Although Plasmodium malaria is usually classified as a tropical disease, it is also widely prevalent in temperate regions, such as the Southern United States. Many have expressed doubt as to the probability of malaria in Alberta because of the temperature and humidity involved in the parasite's sexual cycle in the invertebrate hosts. As was noted earlier in this paper, the parasites will develop in the invertebrate hosts at temperatures between 20°C and 30°C (68°F and 86°F). A drop in temperature below 20°C for several hours after ingestion of the parasites by the invertebrate host is sufficient to slow

TABLE III

ANNUAL METEOROLOGICAL SUMMARY

Edmonton, Alberta, 1947

<u>Month</u>	<u>Mean Maximum</u>	<u>Mean Minimum</u>	<u>Relative Humidity</u>
July	78.5	51.4	64
August	67.2	47.0	75
September	59.3	39.1	77
October	54.4	33.0	68

down the rate of development to a very large degree. Concerning relative humidity, it may be said that, if the average or mean monthly humidity is less than 60 per cent (provided the temperature is suitable) it is not likely that malaria transmission will take place. A glance at Table III would seem to indicate that this belief is justifiable. However, the fact that Plasmodium was observed in this, a winter survey, in some of the slides examined by myself, and also that Plasmodium has been observed in Sparrows by Dr. Cockburn (1948) at Lamont, Alberta, proves that Plasmodium can and does exist in Alberta. As it can only be transmitted in the summer time, these winter cases are evidently latent. Concerning the presence of Plasmodium in Canadian birds, Fallis (1946) found a strain of P. circumflexum in ruffed grouse in Ontario, which he describes as apparently non-pathogenic and concludes that for this reason this Plasmodium is probably not a factor in causing periodic dying-off of the ruffed grouse in Ontario.

All reports cited as to the pathogenicity of Haemoproteus and Leucocytozoon, though apparently substantiated by sufficient evidence, are nevertheless open to doubt. None of the reporters appear to take into account the nature of the invertebrate vector -- not as a carrier of the parasite, but for its own sake. Simuliid flies (Black Flies) are known

to have an extremely vicious bite, hanging on by means of the short mouth parts for an indefinite period. Reports from Saskatchewan show that economic losses in domestic animals have run into thousands of dollars as a result of this pest. Even if a blood parasite is suspected, the vertebrate host dies long before the cycle of a parasite could be suspected of completion. No reports were found regarding the direct affect of the flies on domestic poultry, but one may provisionally infer that a sufficient infestation of the fly alone might similarly induce a mortality in birds.

The suggestion set forth by Starr and West (1940) that species of the genus ~~Mallophaga~~ might be implicated in the transmission of Leucocytozoon disease in turkeys is to date without factual basis. Turkeys attacked by these lice, and showing no signs of the blood parasite, develop a nervous diarrhea and finally die. The presence of a high Leucocytozoon level in the blood could have been as a result of an immediate infection or the result of a relapse of a latent infection. Regarding the statement that no suitable breeding place for the Simuliid fly could be found within their normal flying range, it should be noted that it has

been recorded by Herms (1939) that wind is able to carry the fly from its original breeding place for a distance of 90 miles.

Rowan (1934) records the fact that the peak year of 1934 in Hungarian Partridges would often find a female starting off with a brood of 20 chicks, but early summer saw a gradual dying off of the young birds until perhaps only 2 chicks were left to each female. Old birds were not affected. The next year the birds were scarce--the cycle was complete. Autopsy of birds revealed no observable parasites, but at the time no blood smear examinations were made. The possibility exists that a contributing factor in the sharp decline of the number of the young could have been the presence of protozoan blood parasites, but it does not rule out the possibility of other diseases such as pullorum. There can be no doubt that there are some species and strains of blood parasites that are extremely pathogenic in their normal bird hosts. On the other hand, there are many more species and strains that are non-pathogenic to any bird host. The mere presence of the parasites cannot, therefore, be considered a cause for bird mortality until their actual pathogenicity in any specific bird can be demonstrated.

SUMMARY

In the present survey, 343 birds of 32 species were examined. Thirty-six individuals (or 10.5 per cent) representing 20 species (or 6.2 per cent) were found to be infected.

Plasmodium was observed in 11 birds of 8 species.

Haemoproteus was found in 11 birds of 6 species.

Leucocytozoon was found in 14 birds of 10 species.

Microfilaria were found in 7 birds of 6 species, including a possible new host record -- the Northern Sharp-tailed Grouse.

Mixed infections were found in 3 birds of 2 species.

CONCLUSION

I believe I am justified in being cautious in applying to Canadian and particularly Albertan birds and invertebrate host populations the deductions arrived at by other workers in other regions. The Department of Entomology of the University of Alberta has confirmed the fact that species of invertebrate hosts known to be susceptible in other parts of the world are present in this

Province, but regarding their ability to transmit the more virulent species or strains of parasites at our latitudes cannot be taken for granted on the strength of investigations carried on elsewhere.

On the basis of the results tabulated in this report, nothing definite can be stated as to whether blood parasites could exert a deleterious effect on local bird populations. The fact, however, that these parasites have been demonstrated elsewhere to infect young birds, causing fatal infections, indicates that their presence may well be a menace in Alberta. The Province harbours the vectors, the hosts and the parasites. Whether the parasites belong to virulent species (or possibly strains) cannot be answered until intensive research is conducted in this field, particularly during the summer months.

This winter survey, however, has demonstrated the presence of latent infections in avian hosts and justifying the assumption that there is a potential resident reservoir, independent of migratory species, which might lead to widespread summer infections.

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