A SURVEY AND ANALYSIS OF THE HAEMOPHILIC PARASITES OF MARSH BIRDS

A Thesis

Presented to

the Faculty of Graduate Studies and Research

The University of Manitoba

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

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April 1958

ABSTRACT

An investigation was carried out to determine the incidence of haemophilic parasitism among the adult birds arriving in Manitoba in the spring and the juveniles raised in the Delta marsh area. Five hundred and eighty six birds of the family Anatidae were examined. Fifty were infected with *Leucocytozoon anatis*. One hundred and five were infected with *Haemoproteus nettionis*. Eight ducks were infected with both parasites, and two were harbouring the microfilaria *Ornithofilaria fallisensis*.

There were 59 perching birds examined. Of these two adult crows were found to be infected with *Leucocytozoon ziemanni*. All the other birds were free from haemophilic parasites.

The suspected insect vectors examined were all Diptera. Three families were represented; the Culicidae, the Ceratopogonidae, the Hippoboscidae. One hundred and thirty-three specimens were examined and only Culex mosquitoes were found to be possible vectors for *Haemoproteus nettionis*.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. J. A. McLeod for advice during the course of this study and for critically reading the manuscript. Also for the facilities and equipment used during this project.

Thanks are due to Dr. A. Savage for the technical support and use of photomicrographic equipment. Thanks are also due to the National Research Council for the financial support afforded during the summer months of 1957, and to Mr. A. Hockhaum, Director of the Delta Waterfowl Research Station for their kind cooperation.

Thanks are also due to Mr. Nan Mulder for his assistance in the field and to Mr. Mike Milonski for his advice and suggestions concerning the field techniques.

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CHAPTER I

INTRODUCTION

The haemophilic parasites of birds have in the past been well studied morphologically as well as taxonomically.

Herman in 1938 made a survey of the incidence of these parasites in the birds of Cape Cod, N. B. There had been no significant surveys made among the marsh birds of the prairie provinces.

Two small epizootics of Leucocytozoon were reported in Manitoba. Savage, Isa. (1945) recorded the first major outbreak from the Portage La Prairie area. A poultry farmer of the district lost most of his juvenile turkeys due to infestations with *Leucocytozoon simondi*. The parasites of the genera *Leucocytozoon* and *Haemoproteus* had been reported to occur in juvenile ducks, McTavish (1951). A survey was undertaken to try to establish what ornithophilic insect or insects could play the role of primary host for the two parasites. A small outbreak occurred in 1952 at St. Malo, a small community south-east of Winnipeg. The infected birds were mostly domestic ducklings and goslings.

At this time nothing was known regarding the vectors and the incidence of infections in local birds, and what parasites were present; what vectors were present and to what extent birds became infected in this area.

The majority of specimens collected in the area were teal and mallards, since they are surface feeding ducks and are easier to capture than the diving ducks which tend to nest in an area where the water is deep and fairly open. It should be noted that the greater part of the adult birds were collected during the spring months since the number of parasites in the peripheral blood are in greater numbers at that time than during the late summer and fall months.

Juvenile birds were collected in July and August, prior to and during their first flights. There were a certain number of ducklings which had been raised in the Delta Waterfowl Research Station hatchery that were checked for parasites. In order that no specimens should be checked for parasites and recorded a second time, each captured bird was banded and then released.

Finally, some infected birds were kept in captivity and later filled in order to obtain data on the particular tissue in which schizogony takes place in the infected bird.

CHAPTER II

REVIEW OF THE HISTORY

Danilewski in 1890 was the first to report Haemoproteid parasites of birds. Leucocytozoon was the name given to the new parasite since it was thought to invade the leucocytes. Grassi and Felleti adopted the generic name Haemoproteus (Kruse 1890) Laverania in 1891 but Labbe in 1894 re-named it Haemoproteus. Sergent (1907) refers to the parasite as Haemoproteus. Novy and McNeal studied the gametogenesis of Leucocytozoon in 1904. Ten years later Moldovan reported the asexual cycle in the internal organs of birds. Wickware (1915) described _Leucocytozoon anatis_ in ducks.

The sporogony of Haemoproteus was observed by Sergent (1907), and by O'Roke (1927) in Hippoboscidae. Mezincescu (1909) described the evolution of the ookinetes in the stomachs of mosquitoes. Haemoproteus in the Anatidae were first reported in 1909 by Johnston and Cleland from an Australian teal. Herman (1938) describes Haemoproteus from the black duck (_Anas rubripes tristris_) from Cape Cod. This corresponds to _Haemoproteus hermanni_ from _Anas rubripes_ and finally classified as _H. nettionis_ by Herman (1954).

Fallis and Wood (1957) also named the halter-shaped parasite found in ducks as *H. nettionis*. They also established that the small biting midges, *Culicoides spp* were vectors for the transmission of *Haemoproteus nettionis*. O'Roke (1929), Adie (1924) and Aragao (1907) described the vector of *Haemoproteus columbae* as being pupiparan Diptera, the Hippoboscidae.

O'Roke (1931) and Marten (1932) worked out the asexual cycles of Leucocytozoon which had previously been described by Wickware (1915). Fallis, Anderson and Bennet (1956) substantiated the sporogony cycles of *Leucocytozoon anatis* (simondi) in simuliids with further experiments on domestic ducks.

Huff's (1942) account of the gametogenesis and schizogony of Leucocytozoon in the Anatidae indicated that the gametogenetic cycles occurred mostly in the granulocytes and monocytes.

The third parasite treated in this survey was a microfilaria of the family Dipetalonematidae, *Ornithofilaria fallisensis*. Little was known about these avian filarioids. Anderson (1956) published the life cycle and the transmission of the ornithofilarioids in the Anatidae. Manson in 1878 had recorded a filariid in birds. Anderson (1954) described the microfilaria *Ornithofilaria fallisensis* in ducks.

CHAPTER III

THE PROBLEM

A study of incidence of *Leucocytozoon spp*, *Haemoproteus spp* and other haemophilic parasites in the marsh birds and their probable vector.

STATEMENT OF THE PROBLEM

The purpose of the survey and analysis of the haemophilic parasites of waterfowl and other marsh birds was to: (1) Determine the incidence of the Haemoproteid parasites and other parasites in the adult birds migrating north, and the incidence of infection in the juvenile birds hatched in the Delta marsh area of Lake Manitoba. (2) Try to establish what insect could be the probable primary host for the completion of the sporozony cycle. (3) Try to find the site of the merozoite cycle in the haemopoietic tissues of the infected birds, and the possible pathogenic effects on the host.

CHAPTER IV

THE SPECIMENS

The majority of the collected birds belonged to the order Anseriformes. There were 586 specimens in the family Anatidae; 157 adults and 429 juveniles.

In the Order Gruiformes, five adult of the genus Fulica were examined. In the order Pelicaniformes only one adult was caught and examined.

The order Passeriformes included three juveniles and two adults from the family Corvidae; six juveniles and four adults from the family Icteridae. The order Falconiformes included six adults from the family Accipitridae. The order Strigiformes included only one adult specimen of the genus Strigidae. The order Galliformes included four juveniles and one adult of the family Tetraonidae. The order Charadriiformes included seven juveniles of the family Charadriidae; four juveniles of the family Scolopodidae, two adults and seven juveniles of the family Laridae.

Only two adults of the order Ciconiformes and one adult of the order Podicipitiformes were examined.

The insects examined belong to the order Diptera.

There were only three families represented. Culicidae, Ceratopogonidae, and Hippoboscidae. The Culicidae had three genera. Culex, Aedes and Culiseta. The Ceratopogonidae had one tentatively identified genus, Dasyhela. The Hippoboscidae was represented by one specimen tentatively identified as Ornithomyia.

CHAPTER V

METHODS AND TECHNIQUES

The trap, Figure 1, used to capture the birds, was made of a large wire mesh two by one inches. It was 40 feet long, seven feet wide and four feet high. The trap was covered by a heavy cotton net which was tied to the wire mesh by pieces of thin wire.

The chosen site was on an old railroad bed some distance from the highway and away from possible disturbance from people around the area. Since this was found to be a favorite and extensively used loafing spot for the ducks, the trap was erected with half of the area on land and half in the water. The trap had curved ends. These ends were just wide enough that a duck could pass through the opening. The bait used was chiefly barley obtained from the local farmers. Every morning a bushel was spread around the openings and inside the trap.

After three or four days, the birds were accustomed to the trap on that site and began entering the trap (Figure 2). The trap was visited every morning and the captured birds were removed. This was done by means of a

FIGURE I

THE BIRD TRAP. SHOWING THE BAITED AREA
AND THE CURVED ENDS

FIGURE 2

THE BIRD TRAP WITH CAPTURED MALLARDS. NOTE THE
SHALLOW WATER BEHIND THE TRAP AND THE
REEDS IN FRONT OF IT

side door in the trap and with the aid of a landing net, (Figure 3). The birds were then crated in a large duck crate and brought to the Delta Waterfowl Research Station, where a blood smear of each bird was made.

At first, the drop of blood was drawn from the brachial vein. However, due to the amount of feathers, the strength of the wing muscles and the slowness of the process, the metatarsal vein was chosen as a more practical spot. This new site was very accessible, fairly clean and no haematomas would occur as in the wing.

The blood smears were made in the standard way. A drop of blood near the end of a clean glass slide, then another clean slide was slid to the drop and then pushed away from the drop. This was to insure that the corpuscles would not be crushed. Then the bird was sexed either by using the plumage as a key or if in doubt, by extruding the genetalia of the bird. In this manner, a positive identification was obtained. In the case of birds other than the anseriformes, the plumage was the general basis of identification. Dissection of the genetalia was also used when the bird was dead. After being sexed, the live birds were banded and released.

The dried smears were then placed in methyl alcohol (anhydrous and acetone free) for three minutes, then placed between blotters until dry and then placed in the staining

FIGURE 3

THE BIRD TRAP SHOWING THE SIDE OPENING USED
TO RETRIEVE THE CAPTURED BIRDS

vat for three hours, (Figure 4). The strength of the Giemsa stain used, was one drop of stain to two cc's of distilled water with a slight acidic pH of 6.9. This gave a good stain that was not too light or too dark. Then the slides were removed from the stain and washed in fresh rain water with the pH adjusted to 7.0. The slides were then placed on edge and left to dry in an upright position.

The slides were then examined for 10 to 20 minutes with a 10X objective and a 15X ocular. This was found to be of a sufficient magnification for the identification of the parasitized cells and at the same time a fairly large area of the slide could be examined at a glance. When a parasitized or suspicious looking cell was found, high power and even oil immersion was used for positive identification and the study of the parasite itself if it was present.

Ten infected ducks were kept through the summer for further use. They were kept in an outdoor pen at the Delta Waterfowl Research Station where they were fed daily and where water and some vegetation was always available to them.

The fly trap, (Figure 5) was about 12 inches in diameter and three feet high. A 100 watt bulb was used for illumination and an electric motor with a 10 inch blade was used as a suction mechanism.

In order that only the small, biting midges be caught, a metallic mosquito screen was placed around the opening

FIGURE 4

THE STAINING EQUIPMENT USED. ALSO THE ASPIRATOR IN THE FOREGROUND AND THE STAINING VAT ON THE RIGHT

FIGURE 5

THE FLY TRAP. THE METALLIC SCREEN WAS REMOVED TO SHOW THE LIGHT BULB

of the trap when it was in operation. The insects were collected in a jar placed at the bottom of the cone-shaped inlet of the fly trap which was directly below the light bulb. Between the collecting jar and the mouth of the trap a very fine cheese cloth was used to let the air by, but not the flies. These eventually tired after fighting the strong draught and finally fell into the jar. A cover was always placed over the jar before the machine was turned off. This trap was used during the night for three to six hours. Then the captured flies were transferred by means of an aspirator to an isolation cage, (Figure 6).

The mosquitoes used in the experiment were captured in an open tower that contained some ducks used for another experiment. They were easily caught at night by means of an aspirator and flashlight, since they were hovering over the ducks in droves. It should be noted here that only the mosquitoes that showed no evidence of having had a previous meal were selected. The captured mosquitoes were then transferred to the isolation cage where they were kept for further use.

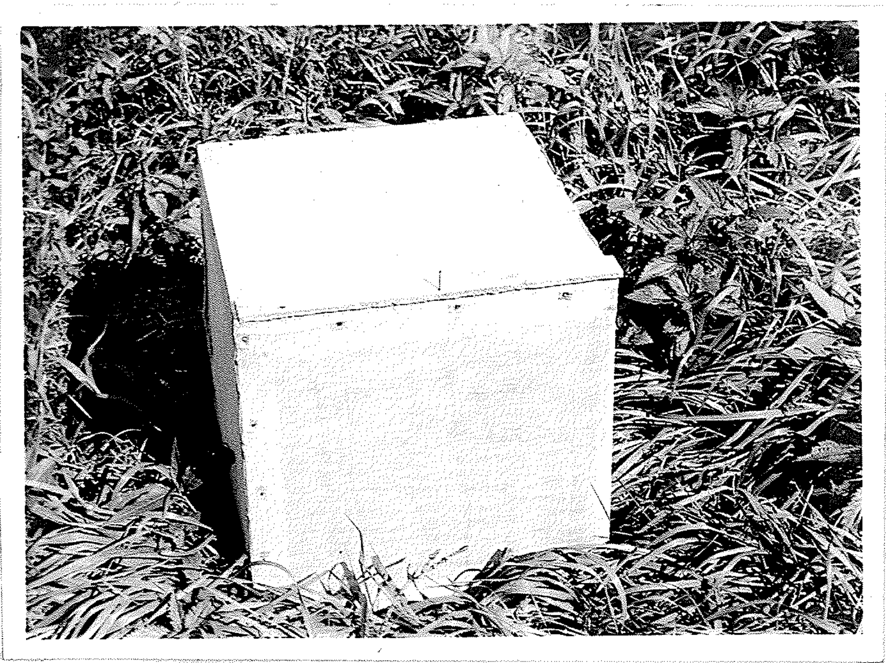
A special transmission cage was made, (Figure 7), and a Haemoproteus infected duck was placed in it. This cage was three feet by three feet and was covered with a very fine cheesecloth that was doubled in order to insure that no small biting midges could get in or out. The cage had a small

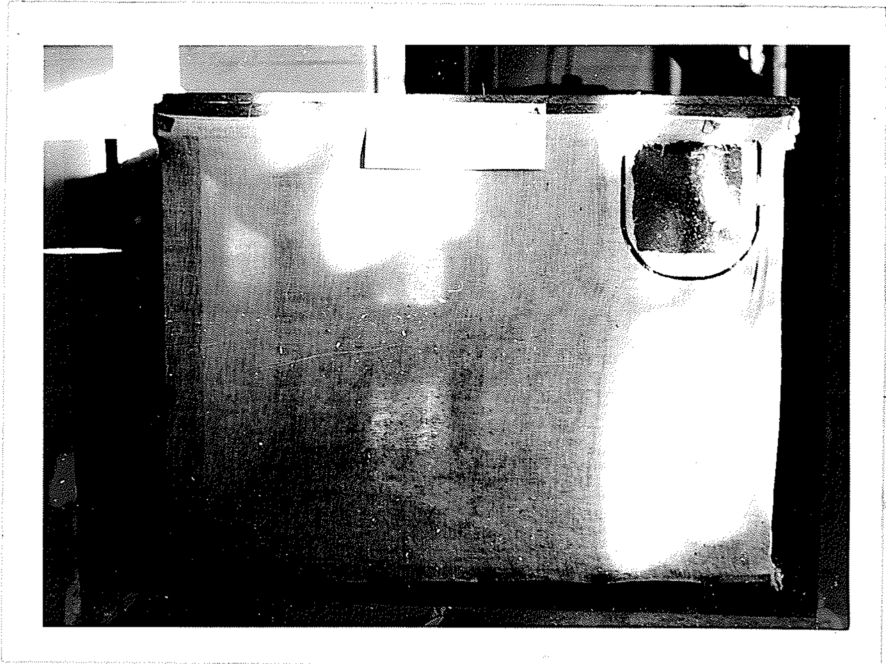
FIGURE 6

THE ISOLATION CAGE

FIGURE 7

THE TRANSMISSION CAGE SHOWING THE PLASTIC WINDOW USED FOR OBSERVING THE DUCK AND FLIES





plastic window so that the insects in the cage could be retrieved with the aspirator without having to use a large opening and risking the possible escape of the insects. Subsequently the biting insects were placed in the cage with the infected duck for a period of three to eight hours. Nightly feeding hours were chosen, since the insects placed in the cage during the day tended to disappear because they were eaten by the captive duck.

Shortly thereafter the duck was removed, the gorged flies recaptured and re-transferred to the isolation cage with the use of the aspirator.

The biting midges and the mosquitoes were kept for three to six days in the isolation box. They were taken out one by one and examined. A binocular microscope was used for the removal of the stomach and the salivary gland. This was done in a petri dish that contained a saline solution. Once removed, the stomach was placed on a clean glass slide and examined under the low power of a microscope 10X ocular and 10X objective. Smears of the contents of the stomach were then made by slitting open the stomach wall and smearing the contents on a dry slide. The dry smears were then placed in acetone free methyl alcohol for three minutes, dried between blotters and then stained for three hours in a dilute Giemsa 1 to 40 stain with a pH of 6.9. The slides were then washed in distilled water and air dried. The stomach contents and

salivary glands were examined under oil immersion using a 10X ocular and 100X objective.

The haemopoietic tissues used were the lung, spleen, and liver of infected birds that were killed for examination purposes. The removed tissues were first washed in saline, then fixed in 10 percent formalin. The imbedding of the tissue was by the cedarwood oil-paraffin procedure. (Gyer, M. F., Animal Micrology, 1953). The slides were stained with Harris alum haematoxylin and counter stained with alcoholic eosin.

The cytological smears were made directly from the removed tissues before they were washed, and the staining procedure was the same as for the blood smears. The histological and cytological smears were examined under oil immersion, 1000X magnification.

CHAPTER VI

RESULTS

The results of the examined blood smears of the collected birds are summarized in Table I. These results showed a fairly high incidence of parasitized adults and juvenile birds by Leucocytozoon and Haemoproteus.

Table II summarized the results of the collected insects. The examined Ceratopogonidae did not show any results whatsoever since they did not feed on the infected duck. The mosquitoes that had fed on the infected duck did show that the parasite could remain for some time in the stomach of the insect and that the sexual cycle could go to completion, since the stomach contents of a *Culex sp* mosquito showed not only the zygote, Figure 8, but also the long vermiform ookinete, (Figure 9). This result was obtained from a mosquito approximately 15 hours after it had fed on the infected duck.

The salivary glands of the Aedes mosquitoes did not show positive results, but the gland of a *Culex sp* mosquito that had been kept for six days after having fed on the infected duck did show minute organisms that could be referred to as sporozoites, (Figure 10).

TABLE I

THE RESULTS OF THE EXAMINED BLOOD SMEARS

Species		No. Caught	Leuco.	Haemo.	Both	Micro-filaria
Anas discors	(Adult)	8	-	1	-	-
(Blue wing teal)	(Juv.)	228	1	62	-	-
Anas Platyrhynchos	(Adult)	102	35	18	4	2
(Mallard)	(Wild Juv.)	82	6	15	-	-
	(Pen-raised Juv.)	67	-	-	-	-
Anas acuta	(Adult)	21	-	-	-	-
(Pintail)	(Juv.)	18	-	3	-	-
Anas carolinensis	(Adult)	1	-	-	-	-
(Green-wing teal)	(Juv.)	4	-	-	-	-
Aythya-americana	(Adult)	9	4	-	-	-
(Red-head)	(Juv.)	10	-	-	-	-
Aythya affinis	(Adult)	2	2	2	2	-
(Lesser-scoup)	(Juv.)	-	-	-	-	-
Aythya collaris (Ring-neck)	(Adult)	1	1	1	1	-
Aythya valesineria (Canvasback)	(Adult)	1	-	-	-	-
Mareca Americana	(Adult)	3	1	1	1	-
(Baldpate)	(Juv.)	3	-	-	-	-
Spatula clupeata (Shoveller)	(Juv.)	7	-	-	-	-
Oxyura jamaicensis (Ruddy duck)	(Adult)	2	-	-	-	-
Aix sponsa (Wood-duck)	(Adult)	3	-	-	-	-
Olor buccinator (Trumpeter-swan)	(Adult)	2	-	1	-	-
Branta canadensis	(Adult)	2	-	-	-	-
(Canada Goose)	(Juv.)	7	-	-	-	-
Branta bernicla (Common brant)	(Adult)	1	-	-	-	-
Chen caerulescens (Blue goose)	(Juv.)	3	-	1	-	-
Fulica americana (Common coot)	(Adult)	5	-	-	-	-
Podiceps grisegena (Red-neck grebe)	(Adult)	1	-	-	-	-
Pelicanus erythrorhynchos (White pelican)	(Adult)	1	-	-	-	-

TABLE I (continued)

Species[x]	No. Caught	Leuco.	Haemo.	Both	Micro-filaria
Botaurus Lentiginosus (Adult) (American bittern)	2	-	-	-	-
Ereunetes pusillus (Juv.) (Semipalmated plovers)	7	-	-	-	-
Lobipes lobatus (Juv.) (Northern phalarope)	4	-	-	-	-
Larus argentatus (Adult) (Herring gull)	2	-	-	-	-
Larus pipixan (Juv.) (Franklin gull)	5	-	-	-	-
Totanus melanolucus (Juv.) (Greater yellow legs)	5	-	-	-	-
Sterna hirundo (Juv.) (Common tern)	2	-	-	-	-
Corvus brachyrhynchos (Crow) (Adult)	2	2	-	-	-
(Juv.)	3	-	-	-	-
Agelaius phoeniceus (Adult) (Red-wing black-bird)	2	-	-	-	-
(Juv.)	4	-	-	-	-
Xanthocephalus Xanthocephalus (Adult) (Yellow-headed black-bird)	2	-	-	-	-
(Juv.)	2	-	-	-	-
Aquila chrysaitos (Adult) (Golden eagle)	3	-	-	-	-
Circus cyaneus (Adult) (Marsh-hawk)	3	-	-	-	-
Pediocetes phasionellus (Adult)	1	-	-	-	-
(Sharp-tail grouse) (Juv.)	4	-	-	-	-
Otus asio (Adult) (Common screech-owl)	1	-	-	-	-
Columba flavirostris (Adult)	2	-	-	-	-
(Red-billed pigeon) (Juv.)	3	-	-	-	-

x A. O. U. Check List

TABLE II

RESULTS OF THE STOMACH AND SALIVARY GLANDS OF THE CAPTURED INSECTS

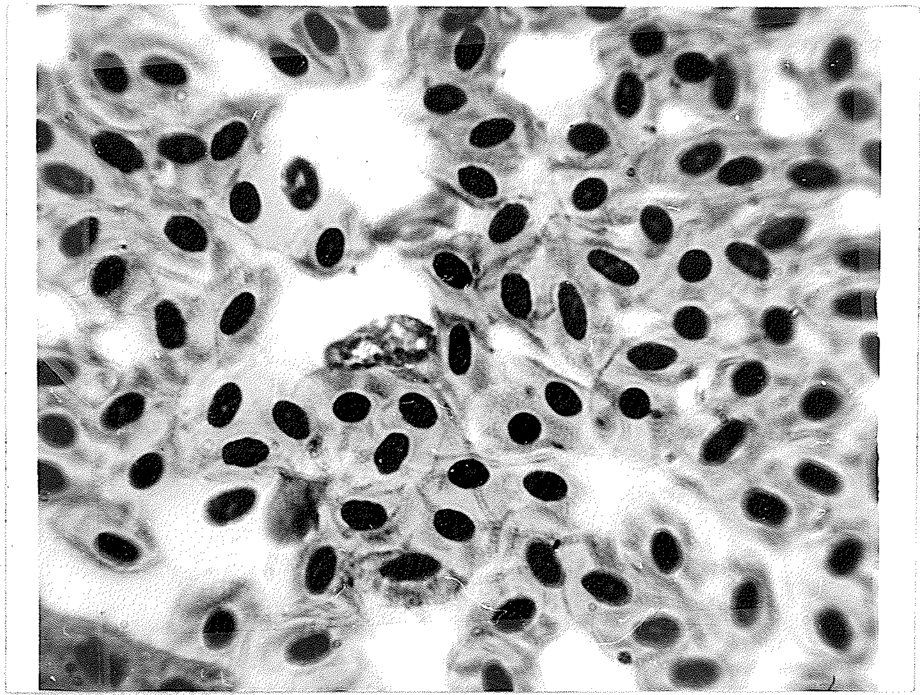
Species	No. Caught	No. Examined	Stomach contents with ookinete or Zygote	Salivary gland with Sporozoites
Dasyhela sp	60	50	-	-
Culex tarsilis	40	30	2	1
Culiseta sp	30	25	-	-
Aedes sp	40	28	-	-

FIGURE 8

THE ZYGOTE IN THE STOMACH CONTENTS OF THE CULEX MOSQUITO MAG. 4500

FIGURE 9

THE OOKINETE IN THE STOMACH CONTENTS OF THE CULEX MOSQUITO. NOTE THE NUMEROUS VACUOLES MAG. 4500



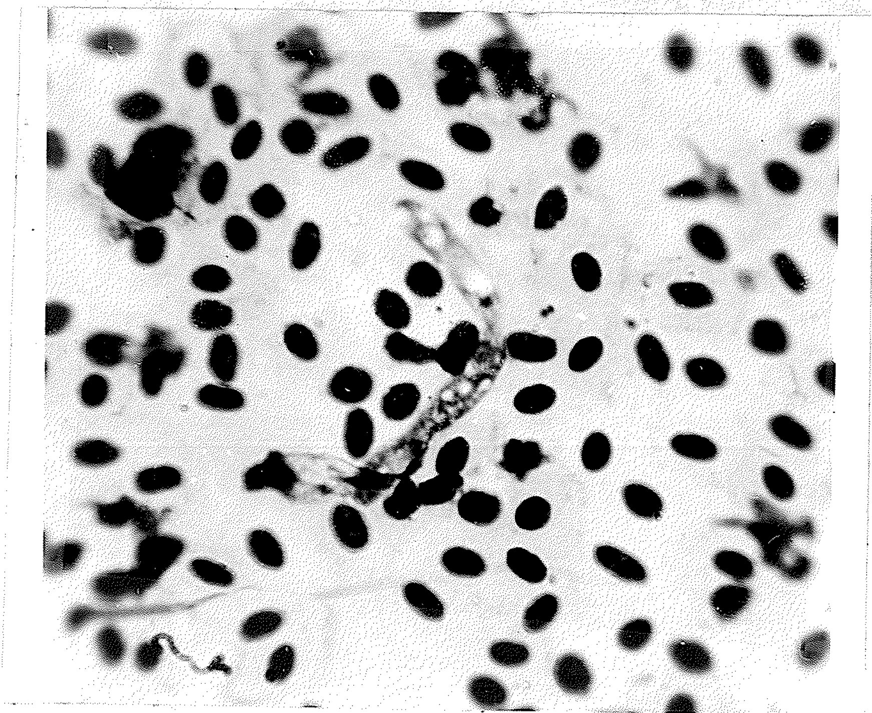
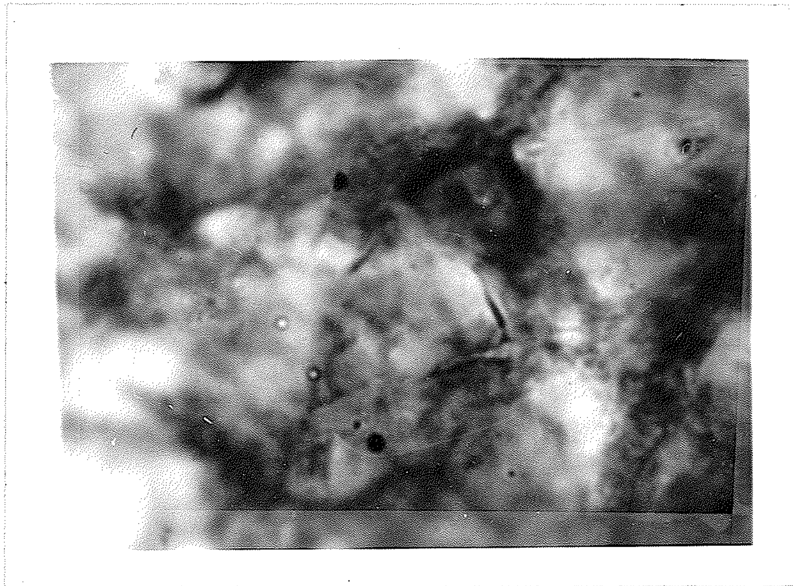


FIGURE 10

SALIVARY GLAND OF CULEX MOSQUITO. NOTE THE FUSIFORM ORGANISM IN THE RIGHT CENTER FIELD MAG. 1800



The results of the blood smears of the perching birds showed no parasites. Only the slides of the two adult crows showed infection by Leucocytozoon sp. This was identified as L. ziemanni, (Figure 11). There was a pronounced spleenomegaly in the infected crows. The normal size of the spleen of the uninfected crows was on the average one cm. long and .7 cm. in diameter. The spleen of the infected crows measured on the average 3.5 cm. long and 1.2 cm. in diameter.

The lung, liver and spleen cytological slides of the blue-goose as well as the blood slide of the trumpeter swan showed a definite merozoite cycle taking place in the tissue and in the erythrocytes (Figure 12 and Figure 13).

The histological slides of the liver, lung and spleen of the infected crow showed the schizonts of Leucocytozoon in the endothelial cells of the tissues and the megalo-schizonts in the lungs (Figure 14).

The blood smear of two adult female mallard ducks contained the microfilaria Ornithofilaria fallisensis, (Figure 15).

As Table I of the Results shows, none of the juvenile ducks raised in the hatchery became infected with Haemoproteus or Leucocytozoon. Yet only a wire screen protected them from the possible vector. The young blue-goose was placed outside before the bulk of the young ducks and it

FIGURE 11

LEUCOCYTOZOON ZIEMANNI IN BLOOD OF A CROW
MAG. 1500

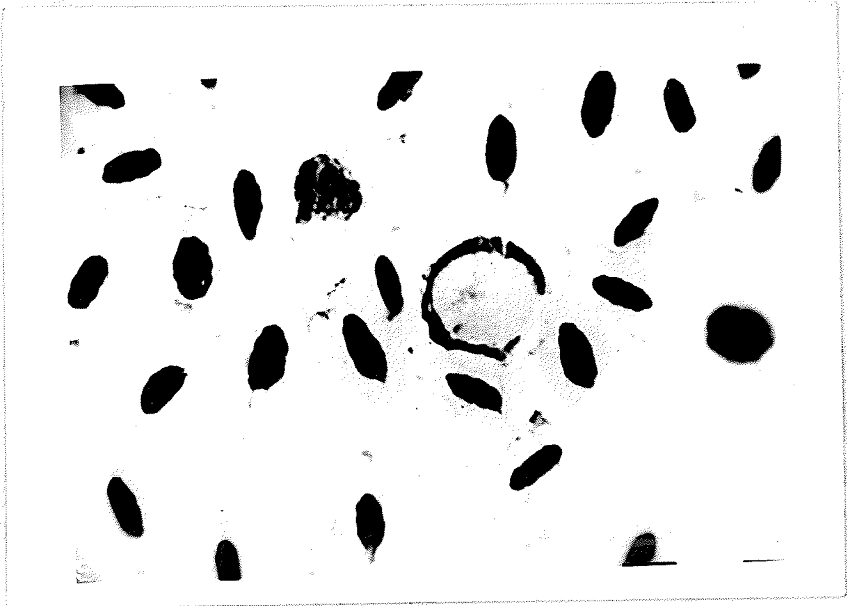
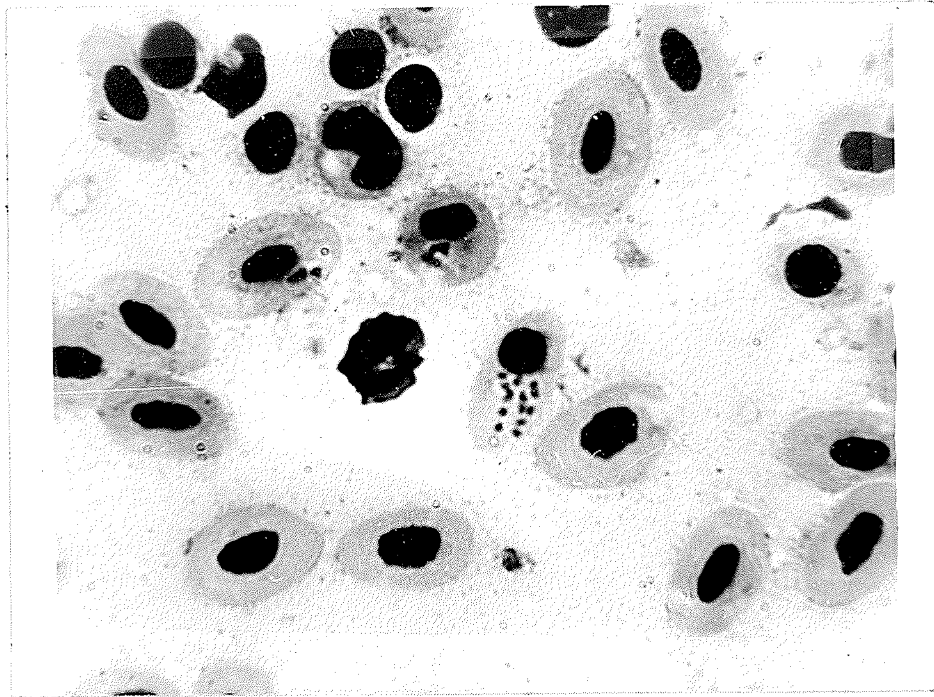


FIGURE 12

SCHIZONTS IN THE BLOOD OF A BLUE-GOOSE. NOTE THE MEROZOITES IN THE CENTRAL ERYTHROCYTE
MAG. 4500

FIGURE 13

SCHIZONT IN THE BLOOD OF A TRUMPETER SWAN. NOTE THE MEROZOITES IN THE RED BLOOD CELL
MAG. 1800



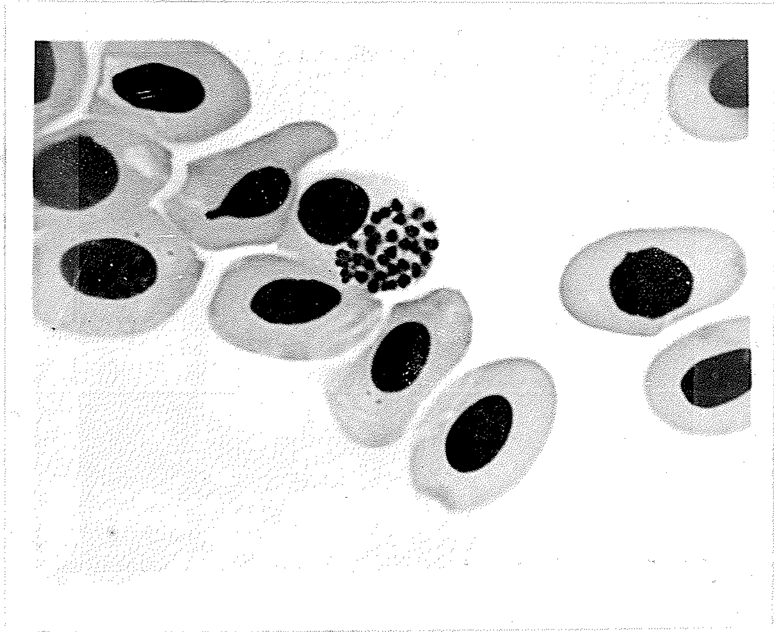
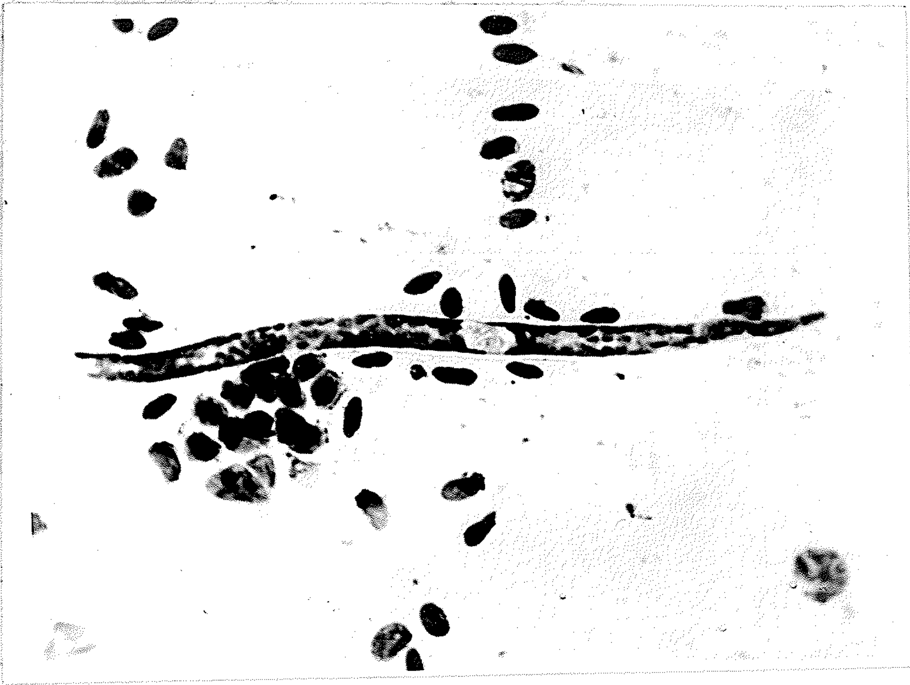


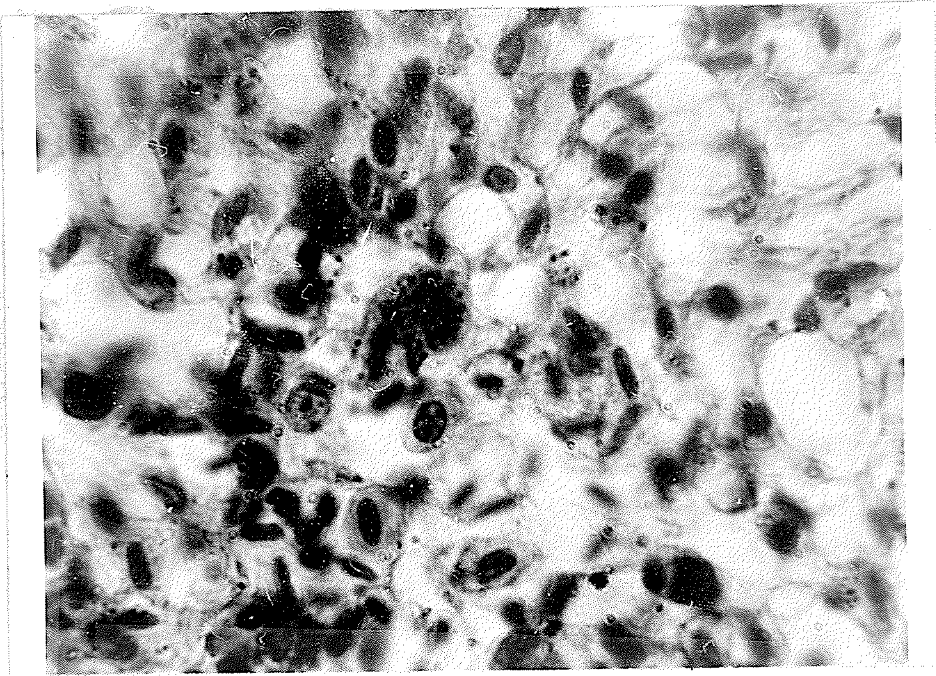
FIGURE 14

MEGALOSCHIZONTS IN LUNG OF CROW. NOTE THE SCHIZONT IN THE CENTER MAG. 4500

FIGURE 15

ORNITHOFILARIA FALLISENSIS IN THE BLOOD OF AN ADULT FEMALE MALLARD MAG. 400





acquired the Haemoproteus parasite.

CHAPTER VII

DISCUSSION OF RESULTS

The blood smears of the adult ducks captured, examined and released show that the adults arriving in the spring have a fairly high incidence of infection with Leucocytozoon anatis. The mallards examined show a 34.5 percent infection with Leucocytozoon anatis and a 17.6 percent infection with Haemoproteus nettionis. The juvenile mallards examined were infected with both parasites, but Leucocytozoon showed only 7.6 percent infection compared to 25.8 percent infection with Haemoproteus. This would indicate that the vector for Leucocytozoon anatis was not very plentiful since only a very small percentage of the young were infected. However, since Leucocytozoon anatis is capable of killing the young ducklings when they are a few days to three weeks old (O'Roke, 1934), this could explain the small number of ducklings showing a Leucocytozoon infection. In the case of Haemoproteus infections, the adult mallards examined in the spring show a 17.6 percent infection and the juveniles raised in the area show only a 18.2 percent infection. This is only an increase of .6 percent. This could indicate that the vector

of Haemoproteus is not abundant or that the ducklings are being destroyed by the parasite. However, according to O'Roke (1927) this parasite did not seem to be detrimental to the young birds. Fallis and Wood (1957) do not mention in their work on the Haemoproteus of ducks that the parasite had any fatal consequences to the infected ducklings. Hence it was assumed that ducklings were not being destroyed in the Delta marsh area. If the ducklings are not being destroyed then the vector must be scarce. But, the blue-wing teal adults show only a 12 percent infection with Haemoproteus compared to a 26 percent infection in the juveniles raised in the same area as the mallards. The vector must be present and plentiful. It is known that the blue-wing teal has a tendency to nest closer to water than the mallard which often nests in stubble fields, headlands and hay fields. If the vector was a mosquito, the closer one is to the water the greater the probability that the parasite would be transmitted.

As far as the Leucocytozoon in the blue-wing teal is concerned, no adults were reported harboring the parasite and only one female out of 228 juveniles did have Leucocytozoon. Fallis, Anderson, Bennet (1956) demonstrated that Leucocytozoon simondi was transmitted by a simuliid. These flies usually occur farther inland than the mosquitoes. Since the teal nests close to water and the mallard nests farther inland or along the banks of creeks, they would be in a better position to

be bitten by the black-fly and acquite the parasite. Only three reports were received on the presence of some tentatively identified simuliids and these three reports were from areas close to creeks or further inland than one would expect ducks to nest.

No other juveniles examined showed any signs of the Leucocytozoon parasite. It should be mentioned here that as the summer progressed and fall arrived, a marked decrease in the number of parasites in the peripheral blood of the infected birds was noticed. One infected duck that was kept throughout the winter was examined at different times and only two infected cells were seen in three carefully examined stained blood smears. Oddly enough, in a doubly infected duck kept throughout the winter, the Leucocytozoon parasite was not detected at this time. Only the Haemoproteus parasite could be found in examined blood smears. In the early spring both parasites became very numerous in the peripheral blood of the captive duck. The disappearance of the parasites in the peripheral blood during late summer and fall would explain the low incidence reported in birds examined at that time.

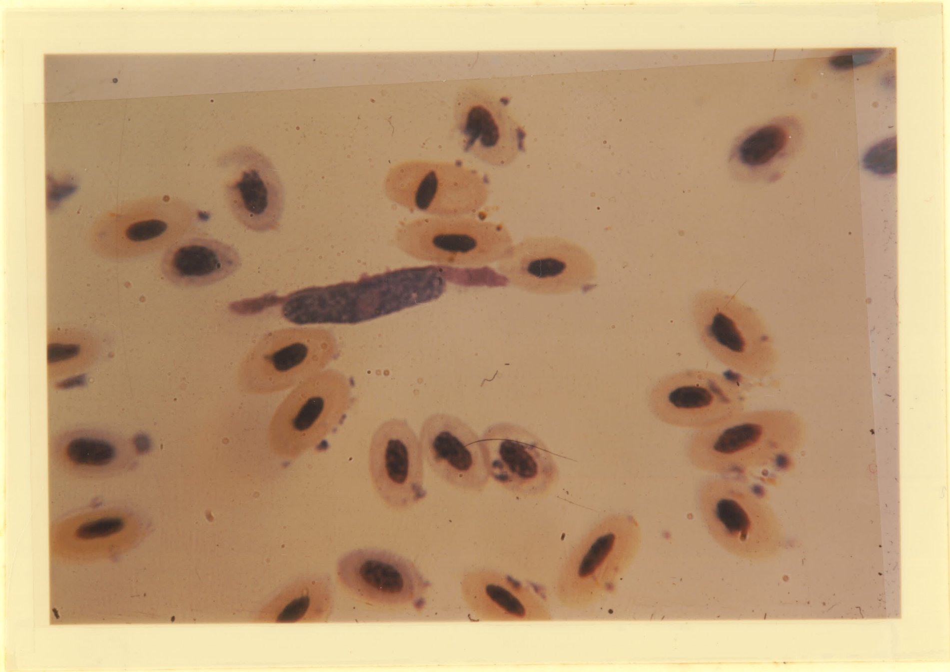
Wenyon (1926), O'Roke (1931), Brumpt (1936), describe Leucocytozoon as an elongate fusiform body devoid of pigment granules. In most cases it was found to be so, (Figure 16 and Figure 17).

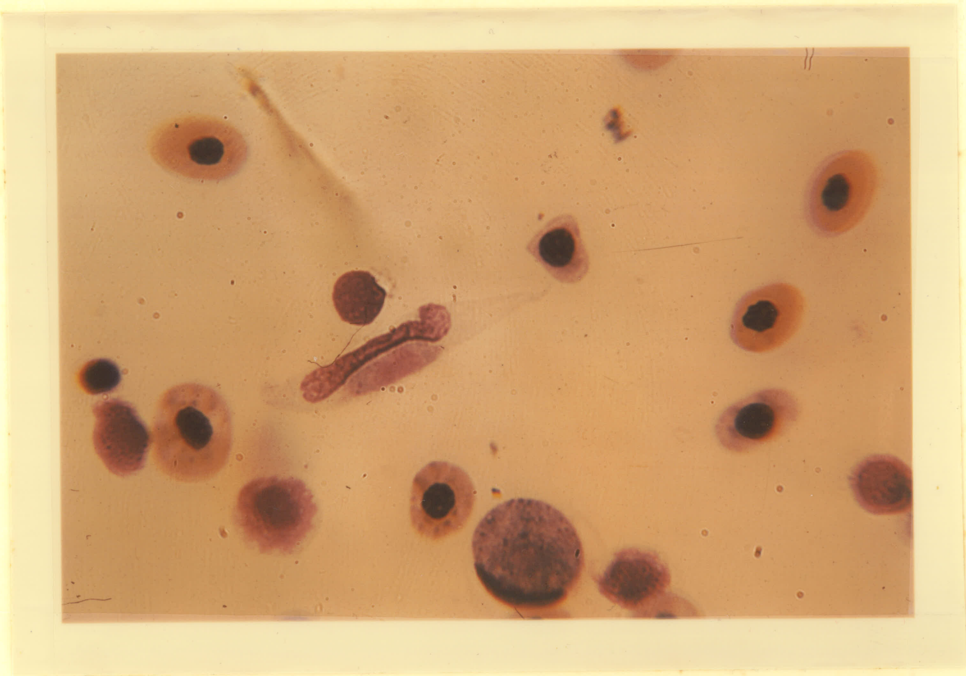
FIGURE 16

LEUCOCYTOZOON ANATIS. MALE GAMETOCYTE WITH NO GRANULES. NOTE THE SIZE OF THE DISPLACED NUCLEUS OF THE PARASITIZED CELL IN COMPARISON TO THE NUCLEUS OF A NORMAL ERYTHROCYTE MAG. 4500

FIGURE 17

LEUCOCYTOZOON ANATIS. FEMALE GAMETOCYTE. NO GRANULES ARE PRESENT MAG. 4500





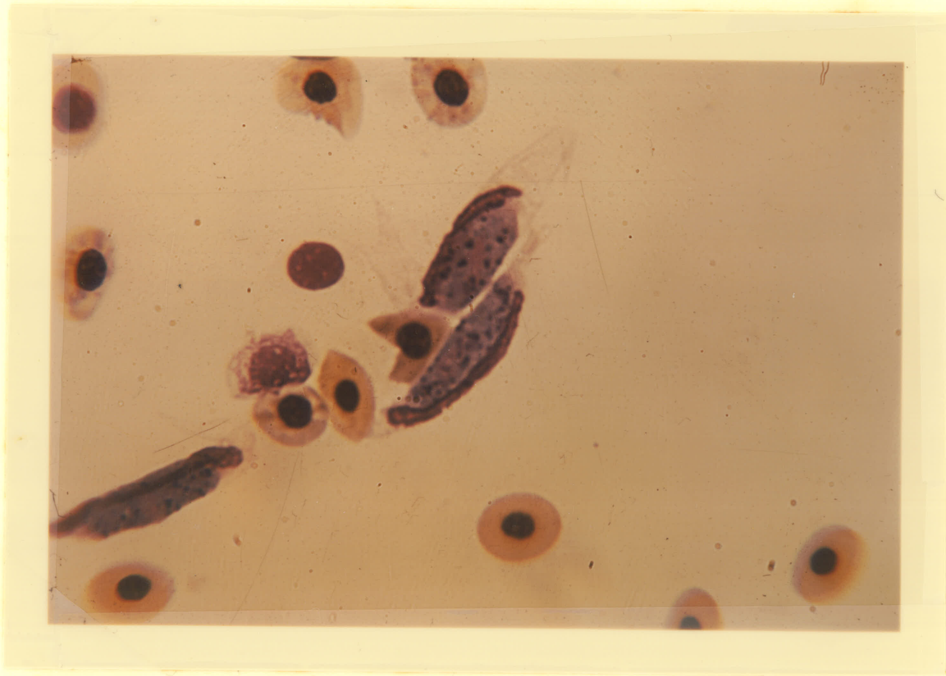
But some parasites in some ducks, notably the mallard (_Anas plathyrhynchos_), did contain some cytoplasmic granules that appeared to be of melanin origin, (Figure 18). These granules could be seen when unstained slides were examined. Laveran and Giovannola observed pigment granules in stained slides of bird blood, but they could not see them in unstained slides. Brumpt (1936) mentions that these granules did not have the same polarized optic properties as the true melanin granules.

Huff (1942) mentions that there were no pigment granules in the _Leucocytozoon anatis_ (_simondi_) with which he worked. The fact that some ducks contained a Leucocytozoon parasite with granules and some without granules could indicate that there might exist a subspecies of Leucocytozoon. However, Huff (1942) shows that the merozoites invade not the erythroblast, but small lymphocytes and monocytes. This could explain the lack of pigment granules since there is no haemoglobin present in these cells.

If what O'Roke (1931), Wenyon (1926) and Wickware (1915) say is correct when they mention that the merozoite invades the immature erythrocyte, then some pigment granules could be formed in the presence of haemoglobin as is the case with the Haemoproteus parasite. Also it was observed that the displaced and distorted nucleus of the parasitized cell is much bigger than the average nucleus of an erythrocyte. This would supplement Huff's theory that the parasitized cells are of

FIGURE 18

LEUCOCYTOZOON ANATIS. FEMALE GAMETOCYTES CONTAINING PIGMENT GRANULES MAG. 4500



either lymphocyte or monocyte origin.

Finally the free gametocyte in the peripheral blood is seen in Figure 19. The remaining cell wall can be seen surrounding part of the parasite.

The Leucocytozoon parasite found in the two adult crows was not the typical L. anatis (Wickware) but more like L. ziemanni, as described by Sergent (1907) found in the blood of the owl (Athene noctua). This is the typical round parasite with the nucleus of the cell forming a crescent around it, (Figure 11). All of the parasitized cells in the crow were alike in this respect.

The second haemophilic parasite found in the blood of ducks was Haemoproteus nettionis. The description that Johnston and Cleland gave of the Haemoproteus found in an Australian teal and the description of Herman (1954) in regards to Haemoproteus nettionis corresponds to the species shown in Figure 20. Some specimens of Haemoproteus nettionis seemed to completely fill the erythrocyte and displace its nucleus to the edge of the cell, (Figure 21). No cells other than the erythrocytes were seen to be parasitized though Grasse (1953) and Huff (1942) report leucocytes containing Haemoproteus gametocytes.

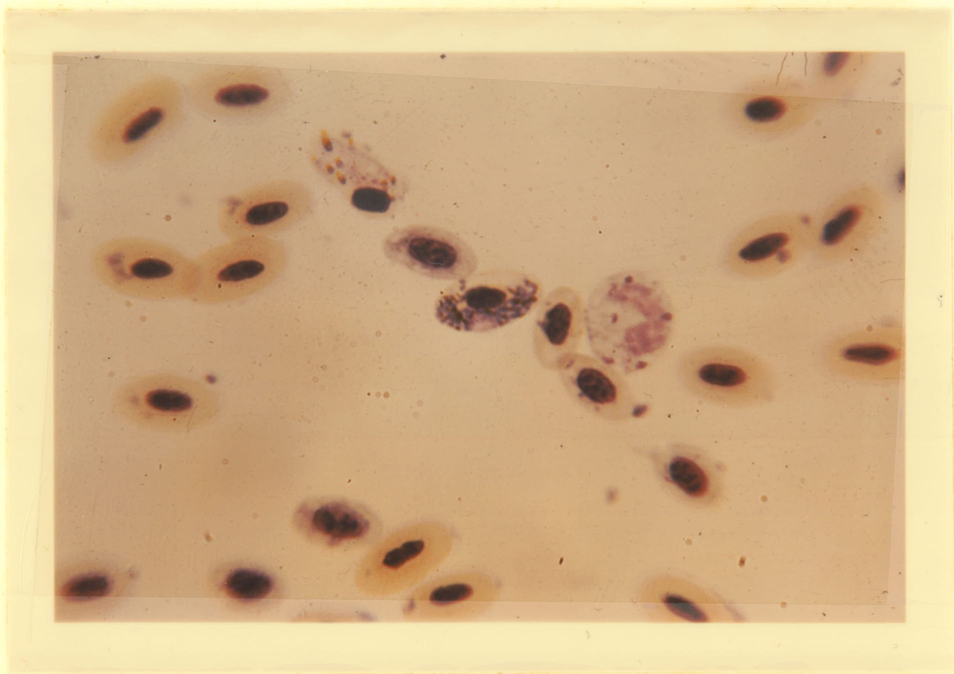
The third parasite recorded in the results was a microfilaria of the family Dipetalonematidae, the genus being identified as Ornithofilaria. Anderson (1954) describes the

FIGURE 19

FREE FEMALE GAMETOCYTE OF *LEUCOCYTOZOON ANATIS DUCK* SHOWING THE REMAINING CELL WALL. NOTE THE PINK NUCLEUS OF THE PARASITE MAG. 4500

FIGURE 20

HAEMOPROTEUS NETTIONIS IN DUCK BLOOD. NOTE NO DISTORTION OF THE ERYTHROCYTE MAG. 4500



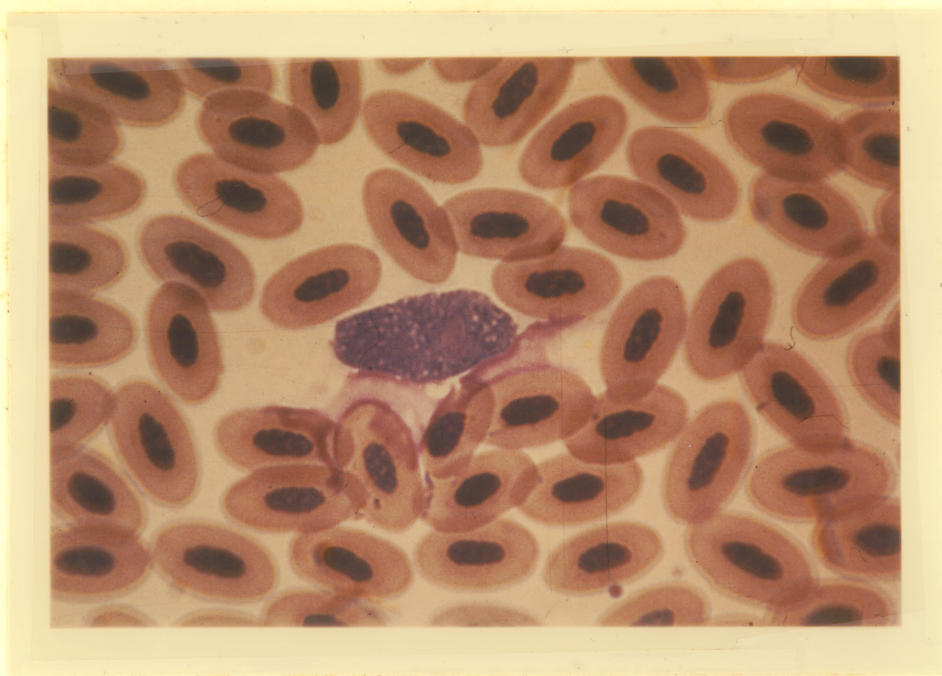
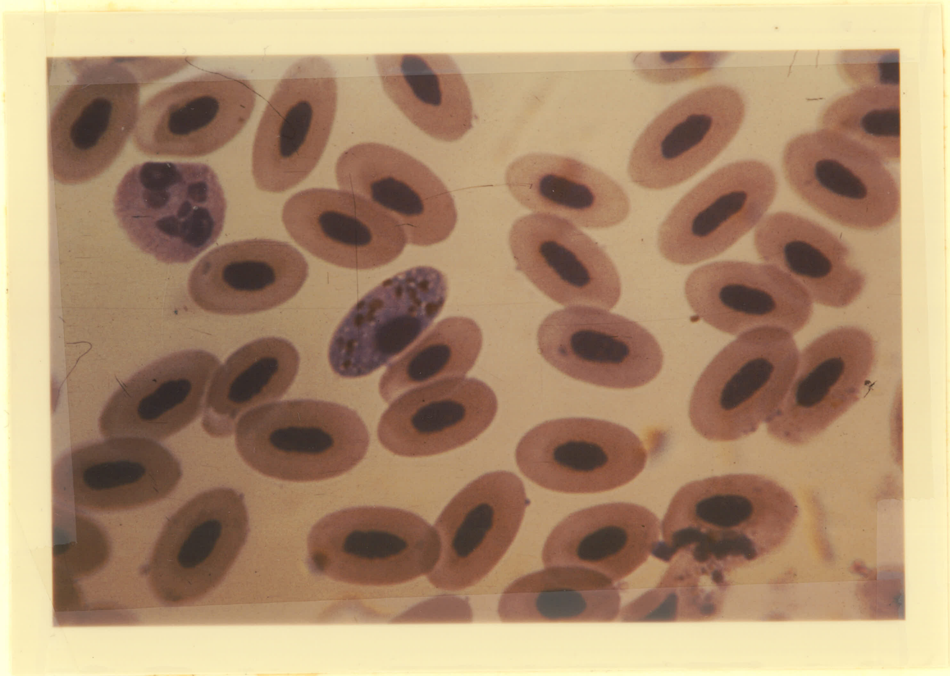


FIGURE 21

MATURE GAMETOCYTE OF HAEMOPROTEUS NETTIONIS IN DUCK BLOOD. NOTE THE DISPLACED NUCLEUS OF THE ERYTHROCYTE MAG. 4500



microfilaria in waterfowl. The large vacuole in the mid-section of the filarioid worm is referred to as the inner body. This inner body is one of the identifying features of the microfilarioid. In most cases there are two; the anterior and posterior inner bodies. (Figure 15 shows only the prominent anterior inner body.) Anderson gives the average length of the microfilaria, Ornithofilaria fallisensis, as being 120 to 131 microns long and four microns wide. The specimens found in two adult female mallards measured 130 microns to 160 microns long and four microns wide. Other specimens which seem to be much older than the one photographed have two inner bodies that are very distinguishable. Though Anderson (1954) reports these filarioids as having a sheath, none could be seen. However, he also mentions the sheath does not show when the parasite is stained with Giemsa.

The histological slides of the lung of the infected crow showed the intercellular schizonts which Huff (1942) refers to as megaloschizonts (Figure 14). No intracellular schizonts could be positively identified. Some schizonts could be seen that seemed to be in an endothelial cell. The megaloschizonts were the typical elongate and sausage-shaped organisms described by Wenyon (1926) and O'Roke (1931). The liver of the Leucocytozoon infected crow contained schizonts which Huff (1942) refers to as hepatic schizonts. These are small globular masses measuring not more than eight microns in diameter. These are in

the hepatic cells. No large megaloschizonts were observed in the spleen of the crow. The examined histological slides of the mallard infected with *Leucocytozoon anatis* did show schizonts in the liver and megaloschizonts in the lungs.

The blue-goose (*Chen coerulescens*) infected with *Haemoproteus spp* was an interesting case. The bird had died from a lung infection and no slides could be made from the peripheral blood. However, blood samples were taken from the deeper organs only one hour after the bird had died. The lungs did show definite schizonts of the Haemoproteus parasite, and merozoites could be seen in some erythrocytes (Figure 12). The numerous granules in the infected cell would represent the multiple infection referred to by Wasilewski and Wulker (1918) and Chandler (1952) when more than one merozoite has invaded a single cell. However, when such a parasitized cell is examined, the young gametocyte shows an area of blue cytoplasm around a purple nucleus. In some of the infected erythrocytes of the blue-goose and trumpeter swan which showed these small granules, blue cytoplasm could not be seen surrounding the nuclei; therefore the granules in the infected cell (Figure 12) are not a representation of multiple infection but a definite schizont.

The infection of the trumpeter swan (*Olor buccinator*) is closely related to the infection in the blue-goose. Here erythrocytes with definite gametocytes that are halter in

shape were seen and are the same as those in the blue-goose and the ducks infected with Haemoproteus (Figure 22). Also the blood smears contained erythrocytes with developing schizonts as in the blue-goose (Figure 13).

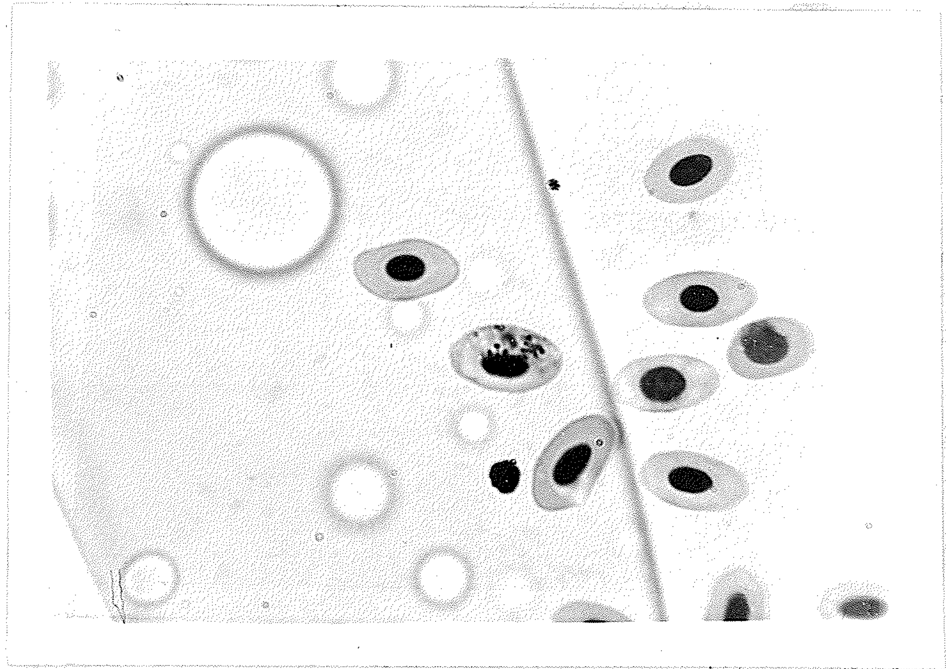
These results would indicate that the parasite observed in the blue-goose and the trumpeter-swan would belong to the family Plasmodiidae instead of Haemoproteidae. But even after an intensive search, no ring stages which identify Plasmodium could be found. The blue-goose seemed to have had a recent infection since no gametocytes were found in the peripheral blood. Only immature gametocytes were found in the cytological smears taken from the liver and lungs.

The insects captured with the fly trap were all caught during the night and belong to the family Ceratopogonidae. The tentatively identified specimens were placed in the genus Dasyhela. The results of the examinations were negative since the flies could not be enticed into feeding on the infected duck. The trials lasted for three weeks and ended when no more flies could be procured. The trials were done during the day, in sunny and shaded areas and at night, but to no avail. It was therefore concluded that the particular species captured did not feed on ducks.

Fallis and Wood (1957) were able to obtain a species of ceratopogonid that fed on ducks at night. They also demonstrated that the sporogony cycle could be completed in

FIGURE 22

HALTER SHAPE PARASITE IN THE BLOOD OF THE TRUMPETER SWAN. NOTE NO DISTORTION OF THE CELL MAG. 4500



these small insects. Their species were of the genus *Culicoides*.

The captured mosquitoes belonged to *Culex spp*, *Culeseta spp* and *Aedes spp*. All three species fed at night when placed in the transmission cage with the infected duck. After the isolation period of three days the engorged mosquitoes were dissected in saline and examined. No oocysts could be seen on the stomach wall of any of the dissected mosquitoes. Only the Culex specimen that had been kept for six days showed organisms in the stained salivary glands. These small organisms could be interpreted as sporozoites since they were not only intracellular but also intercellular. These were spindle shaped or ovoid particles in the cell cytoplasm (Figure 10) and measured about three microns long. However, no definite nucleus could be made out. The stomach contents of a specimen of *Culex tarsalis* that had fed on the infected duck and kept for 15 hours showed definite ookinetes and zygotes. These are vacuolated, vermiform organisms, (Figures 8 and 9). The description of Fallis and Wood (1957) of the ookinete in the Culicoides corresponds to the ones in the mosquito. The ookinetes also did not contain pigment granules which Fallis and Wood say are absent. O'Roke (1927) mentions that the ookinete does contain pigment granules but he was working with *Haemoproteus lophortyx*.

The finding of zygotes and ookinetes in the stomach

of a mosquito would substantiate a theory that the sporogony cycle takes place at least to that stage in local insects. A further study would show if the cycle would go to completion and if *Culex tarsalis* could be the primary host for *Haemoproteus nettionis*.

CHAPTER VIII

SUMMARY

1. Most of the infected birds belonged to the order Anseriformes. Among other groups only the two adult crows were found to harbor a haemophilic parasite. All the other perching birds and marsh birds were free of parasites.

2. The haemophilic parasites encountered in the anseriformes were *Leucocytozoon anatis* and *Haemoproteus nettionis*. Also a microfilaria, *Ornithofilaria fallisensis* and an unidentified Haemoproteus were recorded in the anseriformes. This species of Haemoproteus was found in a blue-goose and a trumpeter swan.

3. The unidentified species of Haemoproteus could be a Plasmodium since merozoites were found developing in the erythrocytes. However no ring stages were found, but erythrocytes containing halter-shaped gametocytes were present. It was therefore concluded that the parasite belonged to the family Haemoproteidae.

4. The specimens of Leucocytozoon found in the two adult crows were not like the species *Leucocytozoon anatis* in the Ahatidae. It was therefore concluded that it was closer to

L. ziemanni.

5. Only the crows infected with *L. ziemanni* showed a conspicuous spleenomegaly. *L. anatis* in the infected ducks did not seem to alter the size of the spleen.

6. The biting midges captured in the Delta marsh area could not be induced to feed on the ducks. Hence they were not counted as a possible vector for Haemoproteus. Of the mosquitoes captured only members of the genus *Culex* were found to be possible vectors since the sporogany cycle was at least completed to the ookinete stage.

7. No oocysts were found on the wall of the stomach of the specimen of Culex examined. However in one specimen kept for six days sporozoites were found in the salivary gland. Hence Culex could be the vector for *Haemoproteus nettionis*.

8. The Haemoproteus parasite in the Anatidae did not seem to have any detrimental effects on the young birds. The low incidence of infected juveniles with Leucocytozoon would indicate either a fairly high mortality among infected young birds in the marsh area or that the vector for *Leucocytozoon anatis* is very scarce.

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