AN INVESTIGATION OF THE HYPOTHESIS THAT HAEMOPROTEUS IS NOT TRANSMITTED IN THE DELTA MARSH AREA WITH RELATED NOTES ON LEUCOCYTOZOON

A Thesis

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John Neil McArthur

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ABSTRACT

During the summers of 1959 and 1960 evidence was collected supporting the concept that, while *Haemoproteus* and *Leucocytozoon* are present as parasites of ducks in the Delta Marsh area, transmission does not occur locally. This was substantiated by two facts - firstly the absence of suitable insect vectors and secondly the lack of transmission to experimental animals raised in the hatchery and transported to various locations around the marsh.

ACKNOWLEDGMENTS

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Mr. H.A. Hochbaum, Director of the Delta Waterfowl Research Station, gave freely of his time, advice and encouragement during the two summers I spent at Delta. Dr. F. McKinney, Mr. P. Ward and Mr. N. Mulder were the source of both information and material necessary to the study. I wish to thank the aforementioned and the rest of the staff at Delta Waterfowl Research Station for their co-operation.

Dr. A.M. Fallis, of the Ontario Research Foundation, spent an afternoon in December, 1959 discussing various aspects of this problem which he has been interested in for several years. This discussion was of great assistance in directing the work in 1960.

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CHAPTER I

THE PROBLEM

Haemoproteus nettionis Johnston and Cleland is a malaria-like parasite of ducks that has as its primary host the biting midge *Culicoides* sp. (Diptera: Ceratopogonidae or Heleidae) Fallis (1957). Unlike malaria, only the gametocytes appear in the blood stream of the intermediate host and a period of approximately three weeks is necessary for them to form from the time of infection. Certain factors are prerequisite for the disease to become noticeable in a population, namely:

1. the causal organism of the disease;
2. the transmitting organism; and
3. the host (in this case intermediate) to act as a reservoir.

Any one of these three being absent would cause a cessation of the disease. The same conditions apply to *Leucocytozoon simondi* Mathis and Leger, the transmitting organism in this case being a fly of the family Simuliidae.

Before artificial controls by man came into existence in the case of human malaria, the above mentioned limitations were in effect. Thus definite malarious areas have been designated on maps surrounded by fringe areas, that would fluctuate from year to year depending on certain variables such as temperature, rainfall, humidity, etc. These natural areas have been greatly reduced by the efforts of man toward the ultimate goal. On the other hand, due to the relative importance of the malaria-like parasites of waterfowl to mankind, only a beginning has been made at mapping the extent of the parasites. The actual effect on population fluctuation is little understood, and only recently have the actual transmitting organisms been discovered.

As indicated by the literature, many surveys have been done in various locations to determine the extent of the infestations geographically, but to date no indication has been made as to whether transmission is coincident with infestation of the waterfowl or much more restricted. It was the purpose of this study to determine whether or not transmission occurred in the Delta Marsh area. This area is ecologically different from known transmission areas, but the parasites were present in the waterfowl. On the other hand, the area is ecologically similar to the regions farther west known to be free of the parasites.

From work done in the summer of 1959, a tentative conclusion was reached that the Delta Marsh area was either a fringe area for transmission, or lay completely outside the usual transmission areas. To this end the work of the summer of 1960 was directed.

Chapter II will outline the history of the topic; Chapters III and IV will describe the equipment used, methods of study, and results obtained during the summer of 1959; Chapters V and VI will be similar to the previous two, but cover the summer of 1960; and Chapter VII will give conclusions drawn from the work of both summers, along with a discussion of the conclusions.

CHAPTER II

REVIEW OF THE HISTORY

The Haemoproteidae type of blood parasite as found in birds was first described from the species found in the grey crow. It was called Haemoproteus danielewskyi Kruse, in 1890. Plimmer (1912), while conducting a general survey of blood parasites, listed one hundred and eighteen species of birds, representing several families, as having H. danielewskyi present in the blood stream, implying that this one species was cosmopolitan as to the type of bird host. Since this article appeared, two trends are evident in the literature concerning the parasite. The first is the use of the generic name only, and the second is the assigning of new specific names to the organism, some of which have been maintained, others which have proved to be duplicates have been discarded.

Herman (1938) reported the first evidence of Haemoproteus found in North American waterfowl, but declined to give the organism a specific name. In 1951, however, he examined over a thousand waterfowl in California and referred to the parasite as H. hermani, and concluded that all previously reported species from waterfowl were of this type. Wetmore (1941) examined the parasites of ducks and reported finding three different types. The first was of the H. danielewsky type, in which the parasite surrounded the nucleus but did not alter the shape of the host's blood cell. The second was of the H. columbae Celli and Sanfelice, type which displaced the nucleus of the host cell, and the third H. lophortyx O'Roke, type which grew on one side of the nucleus only without displacement. Levine and Hansone (1953) reviewed the literature and concluded that the name H. nettionis should be adhered to for the parasite infecting waterfowl unless proven otherwise by cross-transmission experiments.

Surveys as to the prevalence of this parasite have been conducted in many areas of North America, but relevant to this study a survey made by Burgess (1957) is the most important. During 1954 and 1955 he took blood samples from birds caught by banding crews working in Saskatchewan and southwestern Manitoba, and found that only four of seven hundred and two birds were infested with Leucocytozoon simondi and none was infested with Haemoproteus sp.

Actual work on the transmission of the

Haemoproteus parasite was first done by Sergents and Aragao in the early part of the century, who proved that a hippoboscid was responsible for the transmission of *H. columbae* of pigeons. The same type of vector was found to be responsible for the transmission amongst quails in California by O'Roke in 1930. Fallis and Wood (1957) working in the Algonquin Park area of Ontario found *Culicoides sp.* to be responsible for transmission amongst ducks. Fallis has been able to demonstrate the developmental stages in this insect.

The confusion that was prevalent in the genus *Haemoproteus* was avoided in the genus *Leucocytozoon*, in that the original name, *L. simondi*, has been adhered to fairly consistently in respect to waterfowl infestations. This concept has been confirmed by the work of Fallis, Pearson and Bennett (1954), who found that both by artificial and natural ways *L. simondi* could be transmitted to goslings and ducklings but not to grouse, chickens, turkeys or pheasants. Transmission in all cases of infestation in the Class Aves has been accorded to the family Simuliidae, or blackflies. Surveys of *Leucocytozoon* are generally run concurrently with those of *Haemoproteus*, and again the most pertinent survey in Western Canada is the one conducted by Burgess

(1957). Of the four infestations found, only one was necessarily the result of a local transmission, since it was in a flightless juvenile bird.

CHAPTER III

THE MATERIALS USED AND TECHNIQUE

SUMMER, 1959

The Delta Marsh is situated on the southern edge of Lake Manitoba. It is bordered on the south by flat cultivated land and on the north by a treed ridge. Ducks arrive as early as the end of March, with the main migration arriving in the middle of April. Water may be laying in the fields to the south of the marsh in early April, but the spring breakup in the marsh does not occur until the last quarter of April. The ice moves off the lake in early May. Except during the spring run-off, the water in the area is generally stagnant, with no creeks or rivers running. Mosquitoes may appear in late April or early May, depending on the weather. High winds of thirty to forty miles per hour are not infrequent and sudden drops of temperature may occur. This area is ecologically representative of much of the prairie breeding area of waterfowl in Western Canada and the results obtained here should be applicable to a large portion of the summer habitat..

The facilities at Delta used for collecting birds for banding were used to obtain specimens for examination. Since surveys of the incidence of the disease had been done (La Fleche 1958), no attempt was made to establish further figures in this regard. After the birds were banded by Mr. Mulder, a blood sample was taken by piercing the metatarsal vein in the leg. The slides were first examined directly, using a fifteen power ocular and four mm. objective. If there was absolutely no indication of any infested corpuscles, the bird was released. Later the slides were stained, using absolute methyl alcohol, followed by Giemsa stain, and re-examined as a check on the validity of the first examination. On the other hand, if an infestation was suspected during the first examination, the slide was stained using Wright's triple stain and then re-examined. The bird was then released or held, depending on the results. Other sources of birds were also used, such as the birds in the flight pen. Tests were run continuously from the latter half of May to the middle of August, depending on the availability of specimens.

A negative blood smear does not signify that a bird is uninfested since in the early stages no gametocytes are formed. Therefore uninfested birds for transmission experiments were obtained from the hatchery as soon as they were able to survive away from the brooding lamps and placed in the cages. In this way a possible source of error was eliminated. These birds were to be paired with infested wild birds in cages. Insects collected in the area were then to be released into the cages and later checked as to feeding habits. Some of the feeding insects were to be removed and dissected while others were to be left. The originally uninfested ducks could at the end of the season be examined for infestation.

Anas platyrhynchus, the common mallard, was used, with one exception being Marila americana, the redhead duck. For this type of experimentation the mallard was best for two reasons. Firstly, it appeared to be the most susceptible to infestation as shown by the figures of La Fleche (1958) and secondly, it was less prone to sickness and death from handling and confinement in the cages.

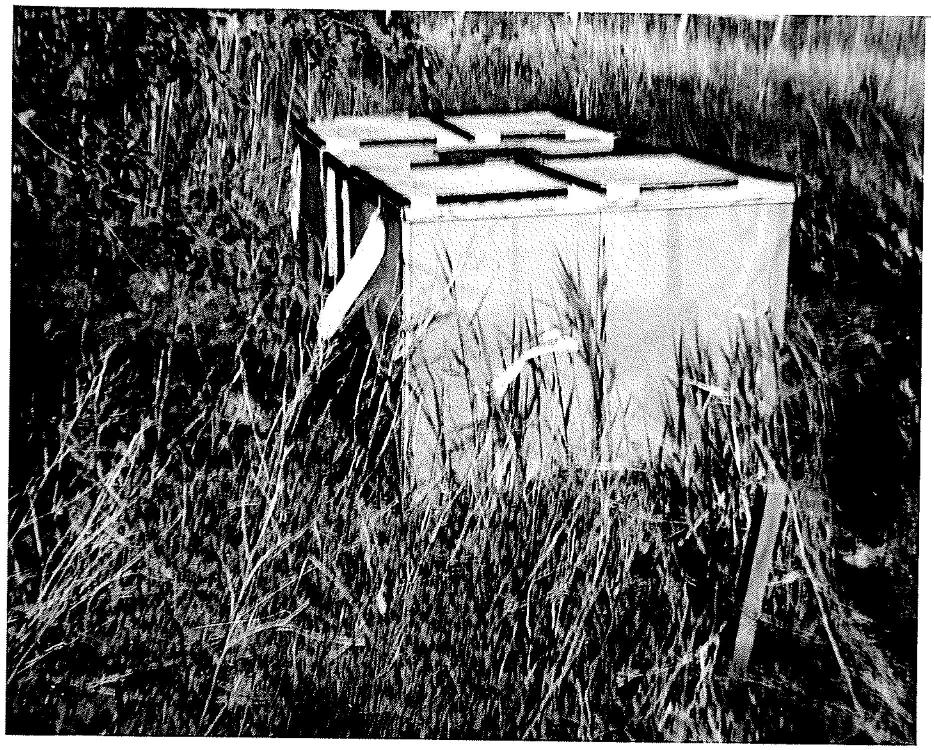
An interesting phenomena was observed when placing ducks together in confined quarters. Some pairs seemed compatible while in other cases a definite "peck order" was established almost immediately, with the result that they had to be separated to avoid injury.

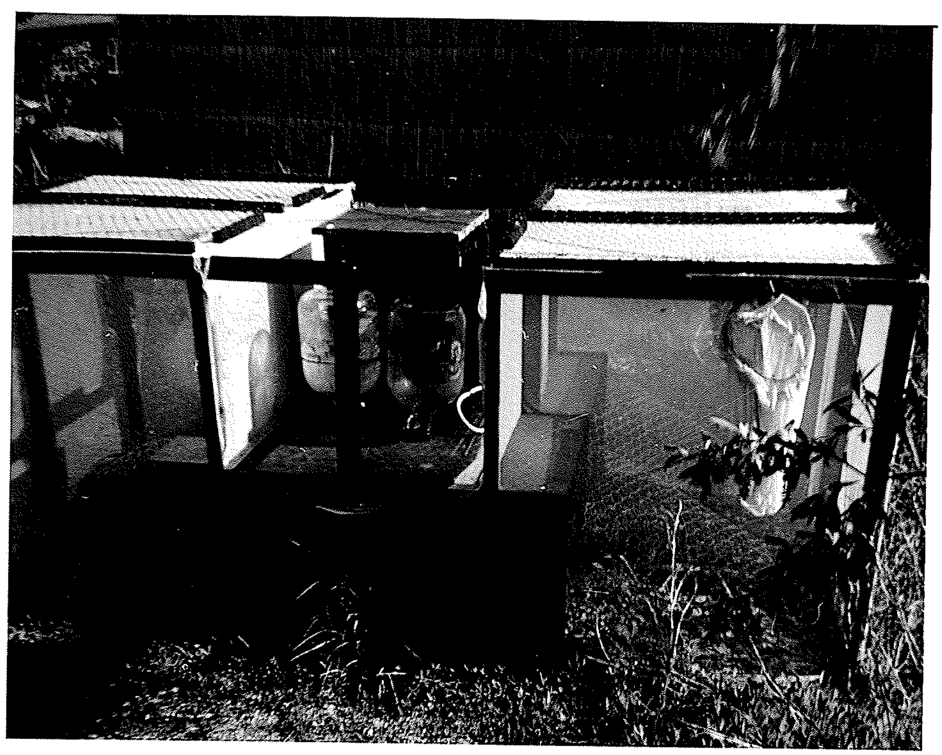
Two factors seemed to be involved. One was the age, size and natural aggressiveness of the birds, and the other was the previous duration of confinement. For example, a newly captured juvenile was at a definite disadvantage to one which had been confined for some time, but this could be counterbalanced if the newly captured specimen was an adult.

Four separate cages were built as one unit, each unit holding two ducks (Figure 1). The complete unit measured seven feet by four feet by three and one-half feet high, and was divided lengthways and crossways into four. The areas in which the ducks were free to move were enclosed half by plywood and half by one inch chicken wire. Food and water were supplied by a hopper and four one gallon narrow necked bottles. The hopper was centrally located over all four cages and the flow of grain was controlled by the depth already in the trays. The four bottles were fitted with two-holed rubber stoppers, from which a short and long rubber tube ran to the water trays. The shorter of the two acted as an air line and controlled the level of the water. The bottles could be removed, refilled and replaced with very little loss, and lasted for approximately three

FIGURE 1

THE CAGES. END VIEW AND SIDE VIEW SHOWING THE FOUR UNITS AND POSITION OF CENTRAL FEEDER, WATER BOTTLES ETC.



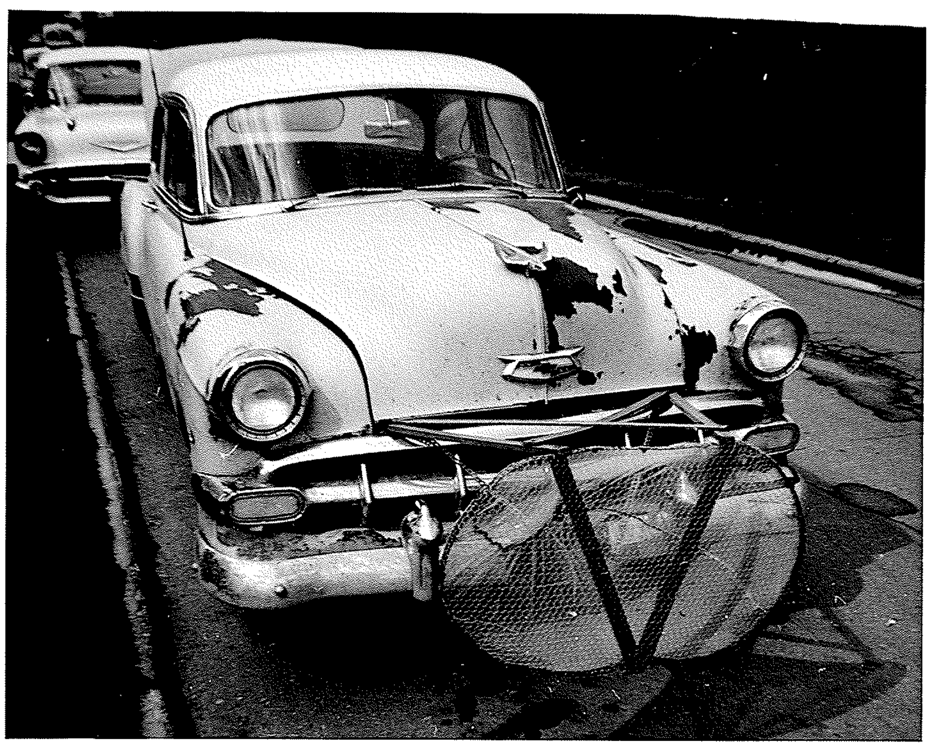


days. Both the food and water trays were covered with heavy wire screening, with an opening in each just large enough to allow the ducks to feed and drink, but to prevent them from sitting in the trays and ruining the food and water. Each cage was completely insect proof. Terylene netting was used with thirty-five by forty threads to the inch. Sleeves of the same material were sewn into the side of each cage for access to insert and withdraw insects. The sleeves were weighted at the ends by heavy wire to stop the wind from opening them. Recessed doors allowed access to place ducks into the cage. The top of the cage was covered by one-half inch chicken wire, raised one inch above the netting to prevent passerine birds from ripping the cloth when landing on the cage.

Insects were collected in several ways. The first and simplest method was by capping the desired species directly with a plastic bottle when it alighted. The second method was with the use of a hand net, with or without a flashlight. The third method was by use of a device on the front of an automobile (Figure 2). An oval hoop was made of one-half inch metal strapping and covered with one-half

FIGURE 2

THE INSECT COLLECTOR. FRONT AND SIDE VIEWS.



inch chicken wire, through which insects would pass easily but larger objects would be stopped. This was held out approximately two and one-half feet in front of the automobile by plywood braces. From this hoop, a cone of the terylene fabric previously mentioned passed backward and was wired to the ring top of a quart sealer. The quart sealer itself was held in place by a ring of metal strapping attached to the lower wooden support. The sealers used could be interchanged quickly, and the whole apparatus could be placed on, or removed from, the automobile in seconds. It was used both day and night by driving at approximately fifteen miles per hour. In this manner, much greater areas could be covered than by any other means. An aspirator was used to withdraw the desired insects from the bottle.

In late August an attempt was made to obtain insects in the Whiteshell area of Manitoba. This area is in the pre-Cambrian Shield and has the usual land configuration of small lakes, streams, evergreens, poplars, and large outcroppings of rock. The same methods as in the Delta Marsh area were used, concentrating on sweeping by automobile. An entomologist from the Department of Agriculture, University of

Manitoba, working in the area, had an ample supply of blackfly pupae. These were reared in a laboratory at the University, and by taping them to a duck and to the writer's wrist in a plastic vial their feeding habits were determined. The clear plastic vial was one inch in diameter and one and one-half inches high, the lower two-thirds of which was covered by an opaque material.

CHAPTER IV

RESULTS AND CONCLUSIONS

SUMMER, 1959

Testing of the ducks for Haemoproteus was started in late May and continued through to August. At first the aim was to obtain birds with an obvious and heavy infection, but as time progressed a compromise was made where a duck showing any signs whatsoever would be used. Since, as mentioned previously, no attempt was being made to survey the incidence, no account was kept of the number tested, the primary purpose being only to obtain four infested birds for the cages. As a rough estimate, however, approximately one hundred to one hundred and fifty wild birds were checked, of which five were infested. About forty captive birds in the flight pens at Delta Research Station were also examined but none was infested.

Major population movements by waterfowl are not confined to the early migration of the birds from their winter habitats in southern United States. This first movement is in search of proper areas for breeding and raising the young. After breeding has occurred, males and unsuccessful females move to areas suitable for moulting and feeding. This may entail a flight of only a few or many miles depending on the species and climatic conditions. While the first movement is thought to be governed by the female returning to her place of birth, the directive nature of the second is unknown.

The first occurrence of the infestation in the wild adult birds of the Delta area corresponded to two possible factors: first the transmission time as found by Fallis (1957) and second the post-breeding migration to the moulting grounds. However, if the disease was being transmitted locally, then the birds from which any insects could become infested should have been present previous to this time.

The insects collected during June and July were not of the species suitable for the transmission of *Haemoproteus*. The original reason for the four separate cages containing one infested and one uninfested duck was to place various specific types of biters in three of the cages and use the fourth cage as an insect-free control. As it turned out, only mosquitoes were caught in the area and no blackflies or biting midges were obtained. For this reason one cage was used for mosquitoes only, one for all types of insects collected and two left free of insects, one of which could be used for either midges or blackflies if they appeared.

In August a few days in the Whiteshell area proved fruitless in the search for any biting insects as the season for them by this time was fairly well over. However the area on examination seemed to present ideal conditions for transmission. Ducks were seen loafing in the roadside ditches and marshy areas. Reports of both midges and blackflies were received from the Forest Biology Station, and reports from local people indicated that both insects occurred over a wide range, in fairly large numbers and quite regularly.

It was in this same area that the blackfly pupae were obtained. After being shipped to the University, they were stored in a cold room where they would remain indefinitely in the pupa stage. Within three or four hours after removal from the cold room the pupae hatched and the adults would take a blood meal. Duplicate experiments were run by capping the blackflies in a vial on the shaved neck of a duck and on the author's wrist. While feeding occurred on a human, it did not occur on a duck. However it was noted that feeding only occurred on the hairless inner part of the wrist and arm, and not on the outer part, where the blackflies seemed to be completely incapable of coping with the slightest obstruction. Thus it would be invalid to say that this species was not ornithophilic.

From these observations, a very tentative conclusion was drawn for which further evidence would be gathered in the summer of 1960. The evidence seemed to the author to lead to the conclusion that the Delta area of Manitoba was similar to the area covered by Burgess in western Manitoba in that no transmission occurred but that the picture was complicated by infested ducks moving in from some other area. Since the Whiteshell area is somewhat similar to the area Fallis worked in, and the type of insects present there were of the type that could be vectors, it would seem more plausible to change to this area in the summer of 1960. This, however, would mean necessarily neglecting the Delta area, and it was decided by the author to attempt to obtain more evidence of non-transmission in the Delta area rather than shift to a strange new area.

CHAPTER V

THE MATERIALS USED AND TECHNIQUE

SUMMER, 1960

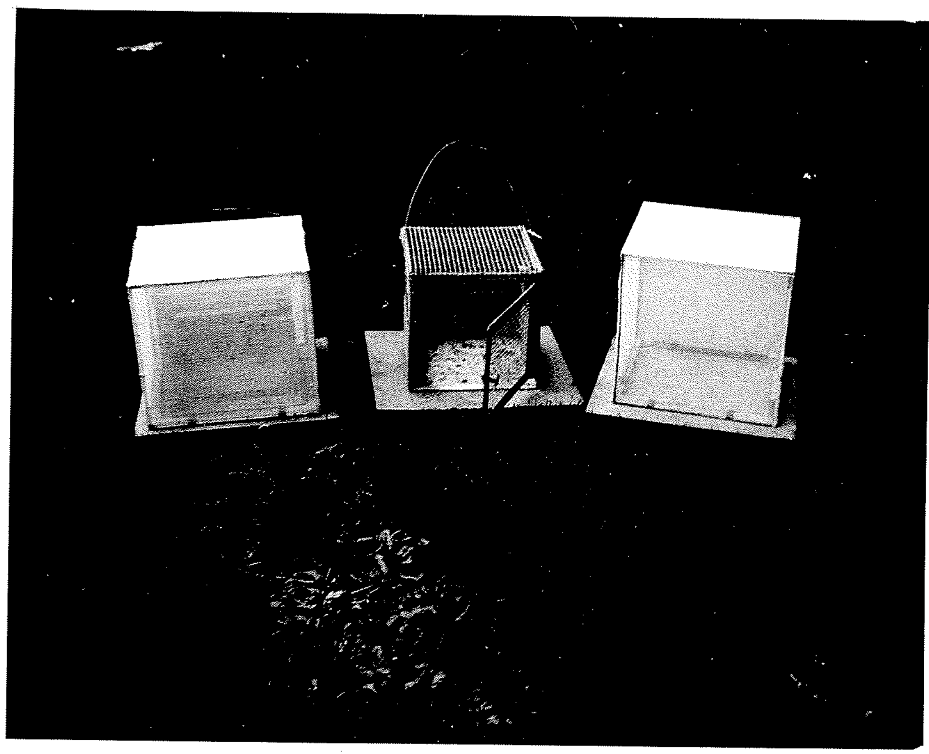
Due to the experience gained and the very tentative conclusion reached during the previous summer and the advice received from Dr. A.M. Fallis, the equipment used was greatly altered. The aspirator was changed from the type used by drawing air through with the mouth to one employing a rubber suction bulb. The cages were discarded and replaced by new units. The insect collector was discarded.

The cages used were similar to those which Fallis used in his experiments (personal communication). Rat cages, thirteen inches, cubed with half inch wire mesh sides and door, were nailed to three-eighth inch plywood twenty inches by twenty inches (Figure 3). The solid metal tops were removed and replaced by half inch wire mesh. Heavy wire handles were made for transporting the cages which could be removed when the cage was in use.

The insect hoods used for capping the cages were made of strips of galvanized iron three-quarters of an inch wide and approximately sixteen inches long,

FIGURE 3

THE CAGES AND HOODS. ON THE LEFT, CAGE WITH HOOD IN PLACE; CENTRE, CAGE WITH CARRYING HANDLE; RIGHT, HOOD ON PLYWOOD FOR TRANSFERRING.



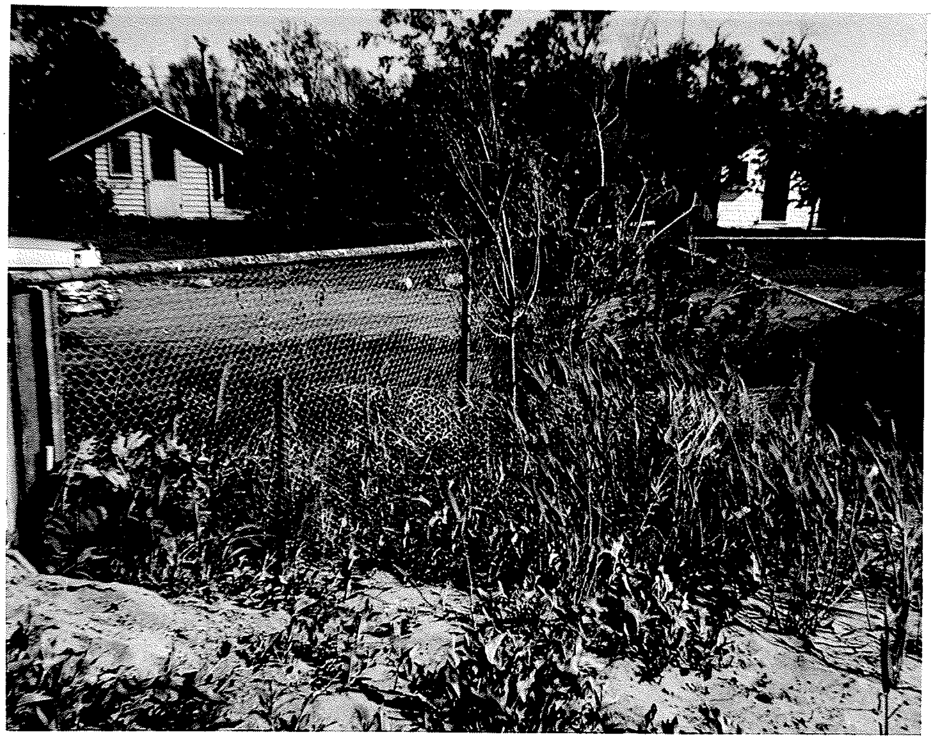
bent lengthways at right angles (Figure 3). Twelve of these strips were soldered to form a cube. These frames were covered with a fine mesh netting, one of approximately fifty by fifty threads to the inch and the other with material of thirty by thirty threads to the inch. Lead weights were attached to the lower sides of the cages to add weight to them, otherwise winds would shift them during experimentation.

The aspirator was made out of glass and rubber tubing, a glass bottle approximately six inches by one and one-half inches, a suction type rubber bulb, and a two-holed rubber stopper. The rubber tube leading from the bulb to the bottle was three feet long and the glass tubing leading from the bottle to collect the insects was approximately eighteen inches and bent at a thirty degree angle. These modifications on a standard aspirator allowed greater ease in manipulation under the insect hood.

Three holding pens were built of thirty inch chicken wire with a one inch mesh (Figure 4). One pen was at the station and used for holding the ducks in between experiments. The other two pens were out in the marsh and ducks could be left in them for any desired length of time.

FIGURE 4

THE HOLDING PENS. TOP - MAIN HOLDING PEN AT STATION. BOTTOM - A SMALL PEN ON A SLOUGH.



A total of fourteen ducks was used during the experiments. Ten were juvenile hatchery-raised mallards, two were hatchery-raised shovellers, Spatula clupeata, and two were wild adult mallards, one infected with Leucocytozoon sp. and one with Haemoproteus sp. The ducks were all colour banded and a record kept as to the number and type of experiments conducted on each. Two of the juvenile mallards and the two shovellers were obtained at a later date and used exclusively in the Whiteshell area.

Records were kept of temperature, rainfall, humidity, wind direction and velocity by means of wet and dry bulb thermometers, and a rainfall gage. The direction and velocity of the wind were estimated in terms of nil, light, medium, heavy, north, northwest, etc.

Experiments were conducted in selected positions around the marsh and some distance from the marsh. Two juvenile ducks were placed in each of two cages and taken to the site selected. Sometimes this routine was varied by replacing two juveniles in one of the cages by an infested adult. During the afternoon the Leucocytozoon infested adult was used, and the Haemoproteus infested adult was used in the evening.

One-half hour after positioning, the hood was placed over one of the cages and left for approximately twenty-five minutes. It was then removed and any insects that were caught in the hood were removed by means of the aspirator. It was then placed over the other cage. Except during the interval of positioning or removing the hood, it was kept on a sheet of plywood to reduce the chance of insects entering the hood while transporting it. When the insects were being withdrawn by means of the aspirator, the hood was drawn off the plywood only enough to allow the aspirator to be inserted. At night a hand spotlight powered by an automobile battery was used for examination of the cage and removal of the insects.

Once in the aspirator, the insects were immobilized by cigarette smoke. This was found to be the best way to examine the insects, as the effect lasted about fifteen minutes. After this time, the insects could either be preserved in seventy per cent alcohol or placed in a cage where they revived. No ill effects seemed to occur from this treatment, and recovery was complete. This was established by immobilizing some mosquitoes that had fed on humans three times and then keeping them for from forty-eight to seventy-two hours alive. On releasing them, they flew away normally. There was a loose relationship between the size of the insects and the time both for the smoke to take effect and the length of time the effect lasted. A dragon-fly (Order Odonata) approximately two inches long took approximately one-half hour to immobilize, and it did not recover for four or five hours, whereas a small insect such as a blackfly required only two or three minutes to immobilize and ten to fifteen minutes to recover. This is reasonable if the effect is via the trachieds and circulatory system.

The effectiveness of recovery was checked by releasing specific insects into the hood just before placing it over a cage and leaving them in during two or three transfers. By this means it was demonstrated that actual loss of insects was negligible during transfer of the hood. In the same manner, the feeding habits of specific insects in relation to ducks were determined. Insects were caught and released into the cages and left there during a number of tests to see if feeding occurred.

The positions chosen for experiments were selected in such a way as to give coverage of the marsh and the surrounding areas in respect to the various micro-environments. The types of positions selected were as follows:

1. the immediate vicinity of the station itself, which is situated immediately north of the ridge;
2. five miles east of the station on the ridge, and close to the beach of Lake Manitoba;
3. along the sides of some small sloughs in the marsh east of the station;
4. near the edge of larger bodies of water in the marsh;
5. along the edge of Portage Creek, which is approximately seven miles southeast of the Station and drains into Lake Manitoba through the marsh. After the initial run-off in the spring, water may flow either way in the creek, depending on the direction of the wind;
6. on the banks of the Assiniboine River near Portage la Prairie, twenty miles south of the Station.

When the ducks were not being used, they were kept in the holding pens. Here also transmission could take place, as no precautions were taken to protect them from insects. Thus the ducks were exposed continuously from late May. Little use was made of the pens in the marsh due to the number of predators present in the area. These included cats, weasels, skunks and raccoons. The main holding pen was in the Station enclosure, and therefore protected to a greater degree from predators.

One hundred wild birds were examined and a record kept as to species, sex, and date examined. Two of these were kept, one with *H. nettionis*, and one with *L. simondi*. Periodically a series of blood tests was taken on the ten birds used during the experiments in the Delta Marsh.

After sufficient evidence had been gathered in the Delta Marsh area, four more juveniles were obtained and taken to the Whiteshell area, where the same experimental procedure was followed.

CHAPTER VI

RESULTS

SUMMER, 1960

From May 31st to July 2nd one hundred and sixty-two tests were run, using the insect hood and caged ducks in the places previously described. In general, the number of insects attracted to ducks was few in both type and number, and actual feeding took place on very rare occasions. Many of the insects trapped could be considered to be due to chance dispersion of insects and nothing to do with an ornithophilic nature. Other insects would be attracted by the fecal material in the cages.

Blackflies appeared sporadically for a day or two and then disappeared. They first appeared for one day on May 31st, then again on June 8th, when feeders were caught in the hood between the hours of four and seven P.M. They did not appear again until June 23rd. On this date their numbers were very light but they built up in numbers and then disappeared again on June 26th. One coincidence that was noted was the time of appearance of the flies after a day or two of medium or heavy south or southeast winds. For this reason, areas to the south of Delta were checked and, while blackflies were found to appear and disappear at Portage Creek at the same time as at Delta, they were never found in the area of the Assiniboine River.

It has been established that blackflies may travel miles in search of food and be carried up to forty miles by winds. Their sporadic appearance this year and their complete absence last year and during La Fleche's study suggested that they were not a normal inhabitant of the Delta area.

Mosquitoes of the genus Culex and Aedes were found around the ducks fairly regularly, but only two feeders were actually collected. The marsh area of Delta is very heavily infested with mosquitoes and, since the author could not use any insect repellant because of possible contamination of the equipment, similar hooding experiments run on man consecutively with those of the ducks would have netted hundreds of feeders. For this reason it was felt that humans or mammals were the preferred hosts of the mosquitoes in the area.

Only two Ceratopogonidae were collected during the tests in the area and neither of these had fed. The author at no time was bitten by a midge in the area to his knowledge.

During the time spent in the Whiteshell area, blackflies, biting midges and mosquitoes were collected around the ducks. Of the insects collected, only mosquitoes of the genus Culex showed signs of having fed. A total of thirty-seven tests was run in the area on the weekends of July 23rd and July 30th.

The results of the blood tests taken during the year for _Haemoproteus sp._ are shown in Table I. The percentage of infestation is lower than that obtained by La Fleche (1958) but a fluctuation is to be expected, as shown by the previous year. On June 18th blood tests were taken on the eight juveniles used in the experiments, and all were negative for both _Haemoproteus sp._ and _Leucocytozoon sp._ On July 2nd they were again tested, along with the two infested birds, and again they showed negative in respect to the two parasites. The two infested birds still showed evidence of heavy infestation. On July 31st a similar series of tests was run, and again the result was negative as far as the juveniles were concerned. The adult bird with _Haemoproteus sp._ now appeared to be free of this parasite in the blood stream. Approx-

TABLE I

RESULTS OF BLOOD SMEARS, 1960

Species	No. Tested	No. Infected Haemoproteus	Percentage
Anas platyrhynchus (Mallard)	47	9	19
Aix sponsa (Wood-duck)	12	1	8
Aythya affinis (Scaup)	6	1	16
Aythya americana (Redhead)	11	1	9
Spatula clupeata (Shoveller)	6	0	0
Anas acuta (Pintail)	9	0	0
Others	9	0	0
TOTAL	100	12	12

imately ten minutes were spent examining the slide, with no positive indication. The adult bird infested with Leucocytozoon sp. showed only a very light infestation remaining. Many fields had to be examined before any infested cells were seen. Of the four ducks taken to the Whiteshell area, one died from unknown causes, and the remaining three proved negative to both diseases.

Tables II and III show the comparative figures regarding weather between the Algonquin Park area and Delta, as obtained from the Dominion Weather Bureau. The mean temperature, humidity etc. are given for the months of May and June in the Delta area, as well as the long term averages for the months in both areas. While these figures do not show anything as far as the day to day variations and the more limited confines of the actual working area are concerned, they do show that the two areas are similar with reference to the over-all features of weather, and that the summer of 1960 was not unusual.

TABLE II

COMPARATIVE WEATHER FOR MAY

ALGONQUIN PARK - DELTA

Factor	Delta 1960	Delta Average	Algonquin Average
Rain (inches)	1.34	1.99	3.52
Wind Velocity (M.P.H.)	12.3	13.8	10.7
Sunshine (hours per month)	325	238	-
Temperature (Degree F.)	55.4	52.4	49.5
Humidity (relative)			
Midnight	63	72	71
6 A.M.	72	79	78
12 Noon	45	52	58
6 P.M.	40	52	64

TABLE III

COMPARATIVE WEATHER FOR JUNE

ALGONQUIN PARK - DELTA

Factor	Delta 1960	Delta Average	Algonquin Average
Rain (inches)	2.18	2.64	3.22
Wind Velocity (M.P.H.)	12.9	12.7	10
Sunshine (hours per month)	260.5	248.7	-
Temperature (Degree F.)	61.5	62.0	60
Humidity (relative)			
Midnight	73	78	83
6 A.M.	79	84	81
12 Noon	54	57	60
6 P.M.	52	56	65

CHAPTER VII

DISCUSSION AND CONCLUSIONS

The two species of parasites were not transmitted to the eight ducks used during the summer, even though they were exposed continuously and in different areas during June and July. The two infested birds did not become doubly infested during the same period. La Fleche (1958) found that, while adult wild mallards showed a thirty-four and eighteen per cent infestation for Leucocytozoon and Haemoproteus respectively, hatchery raised juveniles showed no infestation at all. On testing wild juveniles, he found that six out of eighty-two had Leucocytozoon and fifteen had Haemoproteus. He does not state, however, whether these juveniles were flightless or full winged, or at what time of year the tests were taken. Thus, from work done in the area from 1956 to 1960, there is positive evidence that neither of the parasites invaded a host guaranteed to have been in the area during its life span. This is in direct contrast to a known transmission area such as Fallis worked in. Fallis (1957) states:

"Many of the ducks that were placed

> out-of-doors died as a result of infection with *Leucocytozoon*, before *Haemoproteus* was detected. *H. nettionis* developed in most of those that survived."

The same comparison can be made between areas as far as insects are concerned. La Fleche found no simuliids or biting midges as feeders. During 1959 neither of these were found by the author, and in 1960 the simuliids that did appear and feed were very sporadic. Since a certain time is necessary for the infective stage of the parasite in the insect to be reached, transmission could not occur in the short time they were present. Fallis further stated that:

> "In 1955 many simuliids of the subgenus *Eusimulium* fed on ducks in the latter part of May and early June and *S. rugglesi* fed commonly on them in June and early July."

In respect to biting midges and mosquitoes, he stated:

> "It seemed more likely to be a biting midge as hundreds of them were feeding on ducks compared to a few score of mosquitoes."

From the evidence collected during 1959 and 1960, supported by findings of previous workers in the area, it appears to the author that only one conclusion can be drawn, namely, that *Haemoproteus nettionis* is not transmitted in the Delta area under normal circumstances, and that the Delta Marsh area lies outside the fringe area referred to on page 2.

Due to the occasional appearance of simuliids in the area, the conclusions concerning L. simondi cannot be as definite. It appears to the writer that it would be possible for transmission to take place during an exceptional season, but that of the ten per cent infestation found by La Fleche very little, if any, could be accounted for by local transmission. Thus the Delta Marsh area could be designated as being in the fringe area for transmission of L. simondi.

The fact remains that the parasites were present in the area. The first thing to be determined in any future work is where the parasites are coming from. The survey by Burgess (1957) shows that to the west of Delta infestations are almost absent. The terrain to the south is quite similar, leaving east and north of Delta as the more likely prospects. The actual locations could be determined by building large pens in selected positions which would require a minimum of attention. Ducks left in these could be tested periodically. On a chance basis, following this procedure, the centre or centres of transmission could be located. The fact that the proper families of insects were found to be present by the author in the Whiteshell area would suggest that this area could prove productive, but no conclusions other than this can be drawn from the author's limited work in this area.

The results of any future study in conjunction with this research would be of interest both from the ecological and game management points of view. *Haemoproteus* does not appear to have any effect on waterfowl but under certain conditions *Leucocytozoon* does. If a duck is reared from birth in an area where *Leucocytozoon* is prevalent, an immunity is acquired due to continual reinfestation with the sporozoites, but if a previously uninfested duck moves into a transmission area it quickly succumbs to the parasite (C.F. Bennett, personal communication). Therefore loss due to the parasite would be negligible in an area of high transmission in comparison to an area where ducks may move in and out of a transmission zone. Since from this study it appears that Manitoba is the latter type of area, the results may be meaningful in guiding the localities to be maintained for the preservation of waterfowl.

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