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

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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.

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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. daniel-ewsky 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.