FURTHER EVIDENCE OF WESTERN ENCEPHALITIS INFECTION IN SASKATCHEWAN MAMMALS AND BIRDS AND IN REINDEER IN NORTHERN CANADA

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Introduction

In an attempt to determine the host range of western encephalitis (WE) virus in Saskatchewan, samples of vertebrate blood were collected and tested for serum neutralizing antibodies during the years 1964–1969. Most of these were from the agricultural area of the province, but some samples were received from the forested region of northern Saskatchewan and from the Northwest Territories. Previous reports included hosts identified within the agricultural area (1, 2, 3, 8, 9, 16). These were predominantly wild or domestic birds and smaller wild vertebrates such as ground squirrels, mice, muskrats, frogs and garter snakes. Associated studies (8, 9) indicated that nestling birds are the primary hosts in which multiplication of the virus occurs during the summer months and that man and horses become infected via mosquitoes, from the bird-mosquito-bird infection cycle.

The role of vertebrates, other than birds, in the epidemiology of WE is still not clear. The susceptibility of a wide range of mammals to WE infection has been tested (14) but the course of infection with WE virus has been followed in relatively few mammals. Available evidence indicates that in Saskatchewan, rodents, garter snakes, frogs and other vertebrates that acquire the infection do so from the midsummer bird-mosquito-bird infection cycle. Some of these species might subsequently serve as over-wintering hosts of the virus (2, 9). With the exception of some very young mammals and birds, man and Equidae are the only species which exhibit clinical symptoms following peripheral inoculation of the virus. Following peripheral inoculation, some of the smaller species of vertebrates, although they exhibit no clinical symptoms, do develop sufficiently high titers of viremia to infect arthropods (18, 19) although the duration of viremia is short in some species (4). In general, it appears that the larger the vertebrate, the lower the titer of viremia that can be produced and the shorter the duration. In south temperate regions there is little evidence that larger mammals such as cattle and horses are of any importance in the epidemiology of WE (14). On the other hand, in north temperate regions larger animals are attacked by hordes of *Aedes* mosquitoes. Some of these *Aedes* species can be readily infected with WE virus in the laboratory. In Saskatchewan we have found higher WE virus infection rates in *Aedes* mosquitoes than have been encountered in the United States (10). This paper reports additional evidence of WE infection in some of the larger vertebrates. The evidence is based on the results of serum neutralization tests. Since WE is the only group A arbovirus known to occur in Western Canada, the possibility of cross reactions is remote.

Methods

Source of Blood and Serum Samples

Reindeer blood samples were collected from the herd at Atkinson Point, N.W.T., about 130 miles northeast of Inuvik (Lat. 69°30′ N), which is well inside the Arctic Circle. The samples were taken at the time of slaughter under the direction of the Department of Indian Affairs and Northern Development and the sera were later shipped to this laboratory. Bison blood samples were obtained from a herd in Prince Albert National Park (about Lat. 53°55′ N) and were supplied to us by the Health of Animals Division, Canada Department of Agriculture. The pintail duck bloods were taken from wild, male adults in July 1968 during a banding drive on the Kutawagan Marsh, about 100 miles southeast of Saskatoon. Blood samples were collected from pigs July 17, 1964 to September 10, 1964 at abattoirs in Saskatoon, Prince Albert and Regina. The samples were from pigs born

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This forms part of a broader study of Western encephalitis supported by research grants from the Department of National Health and Welfare (No. 607-7-59), the Canada Department of Agriculture EMR Contract No. 159, the Defence Research Board (No.'s 6801-27 and 6801-30) and the United States Public Health Service (No. A107314).

the preceding January and February. Turkey blood samples were also collected at killing plants and were from birds hatched in 1964. All other samples were collected during our routine field studies.

Serum Neutralization Tests

Neutralizing tests were done by preparing 10-fold dilutions of virus and adding equal volumes of undiluted serum; a control test was prepared at the same time using undiluted normal rabbit serum for calculating the log neutralization indices. All sera were inactivated at 56°C for 30 minutes. Two strains of WE virus were used; either R1 or 1540–1544–66 (Ottawa), which were both of human origin. Serum-virus mixtures were incubated for one hour at 37°C before inoculating 10-day old chick embryos with 0.1 ml amounts. Tests were first done using three embryos for each of at least three dilutions per test and a second test was then carried out using five embryos per dilution when there was any indication of antibody reaction. Tests were incubated at 36°C for 48 hours, at which time results were recorded. Neutralization indices were calculated according to the method of Reed and Muench (13). Serum samples capable of neutralizing at least 2.00 logs of WE virus were considered positive. Some of the samples were tested 12–14 days after being collected but most were held at −20°C for some time before the tests were done. The reindeer sera had been frozen and thawed twice before being examined at this laboratory.

Results and Discussion

Blood specimens giving WE serum neutralizing titers of 2.00 logs or greater are listed in Table I. The blood samples from pigs were from animals born in the January and February preceding collection and the turkeys were hatched in 1964, hence infections in the pigs and turkeys must have been acquired in the summer of 1964. Since the pintail ducks were adults of unknown age, their exposure to the virus could have occurred in the preceding, or some earlier year. The skunk blood collected on August 15, 1966 at Aberdeen was a single specimen of doubtful significance because seven blood samples taken from skunks caught near North Battleford in July 1968 were all negative. The record of naturally acquired antibodies, indicating previous infection, in the bison is the most northerly in Saskatchewan. However, the disease in horses has been reported much farther north in Alberta, at Edmonton and in the Peace River District (Lat. 56°14′ N) (11). The record of antibodies in the reindeer sera appears to be the most northerly record of naturally acquired WE infection in North America.

In 1964 a total of 144 chickens in six sentinel (indicator) flocks (12) were exposed in six different localities in the province. The infection rate in the sentinel chickens was 11.8%. However, the infection rate in the pigs (17.6%) suggests that they might be a more sensitive indicator of virus activity in nature than either the turkeys (9.8%) or the chickens.

The mosquito, *Culex tarsalis*, is considered to be the principal epidemic transmitter of WE virus in Western Canada (10, 15) but the northern limits of its distribution are not well known. It is present at St. Walburg, Saskatchewan (Lat. 53°38′ N) but it is not abundant there (10). It is also present in the Edmonton, Alberta district (Lat. 53°19′ N) but again apparently not abundant (20). Two specimens of *C. tarsalis* were found at George Lake (Lat. 53°57′ N) in 1966 (6) and it has been recorded from Banff, Alberta in the Central Rocky Mountains at an altitude of about 4583

TABLE I

Western Encephalitis Serum Neutralizing Antibodies in the Blood of Some Birds and Mammals in Saskatchewan and the Northwest Territories, Canada

Common Name	*Host* Scientific Name	Date Collected	Location	*Number Positive** Number Tested
Pig	*Sus scrofa*	July 17–Sept. 10/64	Central & Eastern Saskatchewan	46/260
Bison	*Bison bison*	December 15/64	Waskesiu, Sask.	5/9
Turkey	*Meleagridis gallopavo*	Aug. 14–Sept. 8/64	SE Saskatchewan	10/104
Skunk	*Mephitis mephitis*	Aug. 15/66	Aberdeen, Sask.	1/1
Bison	*Bison bison*	Nov. 27/68	Waskesiu, Sask.	4/8
Reindeer	*Rangifer spp.*	Aug./68	Atkinson Point, NWT	8/160
Pintail Duck	*Anus acuta*	July 10/68	Kutawagan Marsh, Sask.	43/301
Red Fox	*Vulpes fulva*	May 22/68	Fairmount, Sask.	1/3

*Log_{10} Serum Neutralizing Index 2.00 or greater.

feet (17). It was not taken in several years' work at the junction of the Pembina and Athabasca Rivers (about Lat. 54°43′ N) or in other piece surveys extending as far north as Yellowknife (Lat. 62°28′ N) in the Northwest Territories (B. Hocking, personal communication). The most northerly record for *C. tarsalis* in Canada is at Norman Wells (Lat. 65°17′ N) in the Northwest Territories (5) and it is unlikely to be abundant there (7). Hence, we must assume that transmission of the WE virus in these more northerly areas is accomplished either by *Culiseta inornata* (10) or by the species of *Aedes* whose occurrence in hordes is notorious in the northern forests and tundra. In the absence of another group A arbovirus, these findings lend support to the theory that the WE virus is indigenous to the wildlife of Western and Northwestern Canada.

Summary

By means of serum neutralization tests, additional vertebrate hosts of the WE virus have been identified in Saskatchewan and the Northwest Territories. Infection rates indicate that pigs might be more sensitive indicators of virus activity in nature than domestic poultry. The known northern distribution of *Culex tarsalis* is reviewed. *C. tarsalis* is the principal epidemic transmitter of WE in Western Canada. Although this mosquito has been recorded as far north as Norman Wells, N.W.T., it is not abundant north of the prairie farmlands. It is concluded that transmission of the virus to wildlife in these northern areas must be accomplished by *Culiseta inornata* or the hordes of pest *Aedes* mosquitoes.

Résumé

A l'aide des tests de neutralisation du sérum, on a identifié de nouveaux vertébrés pouvant servir d'hôtes au virus de l'encéphalite de l'ouest (W.E.), en Saskatchewan et dans les Territoires du Nord-Ouest. Les taux d'infection indiquent que les porcs pourraient bien être des indicateurs plus sensibles de l'activité du virus que la volaille. On rappelle la zône septentrionale de distribution connue de *Culex tarsalis*. Il s'agit là du principal vecteur lors des épidémies d'encéphalite dans l'ouest canadien. Bien que l'on ait rapporté la présence de ce moustique aussi haut que Norman Wells, T. N.-O., il ne se rencontre pas en grand nombre au nord des terres cultivées des prairies. On conclut donc que le virus peut se transmettre à la faune des régions septentrionales par l'intermédiaire de *Culiseta inornata* ou par les hordes de moustiques du genre *Aedes* qui y abondent.

Acknowledgments

We wish to express our appreciation to Dr. L. P. E. Choquette, Canadian Wildlife Service, Department of Indian Affairs and Northern Development, Ottawa for contributing the blood samples from reindeer; Dr. I. M. Dryden, Regional Supervisor, Health of Animals Branch, Canada Department of Agriculture, for obtaining the samples from bison, pigs and turkeys; Mr. T. Sterling of Ducks Unlimited, Dr. B. Gollop and Mr. A. Dzubin of the Canadian Wildlife Service for their cooperation and assistance in making the collection of duck blood samples possible. Dr. C. H. Bigland and Dr. T. T. Kramer reviewed the manuscript and made helpful suggestions.

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BOOK REVIEW

Lactogenesis. Edited by M. Reynolds and S. J. Folley. Published by University of Pennsylvania Press, Philadelphia. 1969. 269 pages. Price $12.50.

Perhaps no aspect of physiology exemplifies the advantages of multidisciplinary research as does the study of lactation. Histology, molecular biology, cardiovascular physiology, immunology, behavioural studies, neurophysiology, endocrinology and biochemistry have been brought to bear, in a most fruitful manner, on the elucidation of the processes involved in lactogenesis. This book is a summary of a symposium held in August 1968 at the University of Pennsylvania School of Veterinary Medicine and constitutes an excellent summary of recent findings on the factors involved in the onset of lactation.

The first portion of the book is devoted to a series of papers by various authors on the microscopic and ultrastructural changes in the mammary gland before, during and after parturition. The subcellular elements involved in the formation of various milk constituents are given detailed descriptions. Biochemical processes and their control by hormonal substances are outlined for various species on the basis of both *in vitro* and *in vivo* studies.

Of particular interest are the results of studies on the dramatic shifts in blood flow between the uterus and the mammary gland at the time of parturition. Cowie has provided an excellent summary of the multihormonal theory of the endocrine control of lactation and more confirmatory evidence suggesting that the rabbit may depend principally on prolactin and thus be an exception. The complexities involved in proving or disproving the separate identities of prolactin, somatotrophin and placental lactogen, especially in man, are admirably discussed in several papers.

This book will appeal most directly to those who are involved in lactation and dairy science. For the veterinarian associated with vivariums there is much useful information pertaining to laboratory animals. The large animal practitioner in a dairy area will benefit particularly from the chapters by Cowie on hormonal controls, Reynolds on hemodynamics, Kronfeld on metabolic changes, for they are important in parturient paresis and ketosis, and the chapter by Lascelles on immunoglobulin secretion into ruminant colostrum. *R. M. Liptrap*