

PROBABLE RESERVOIRS OF CHLAMYDIA FOR PEOPLE
OF NORTHERN CANADA

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ABSTRACT

PROBABLE RESERVOIRS OF CHLAMYDIA FOR PEOPLE OF NORTHERN CANADA

Chlamydial group antibodies have been recognized in the people of northern Canada for many years. This project has been concerned with a determination of some of the probable reservoirs of the chlamydia which are producing the antibodies in the people.

Initial examination of snow goose (Chen caerulescens caerulescens) eggs have revealed inclusion and elementary bodies by May Grunwald-Giemsa and immunofluorescent staining. Snow goose yolk sac, embryo fibroblast and tissue were subsequently passaged in mice, and smears from the tissues of the autopsied animals also demonstrated inclusion and elementary bodies. Chlamydial antibodies were also found in the mouse sera after inoculation. Serological surveys of barren-ground caribou (Rangifer tarandus groenlandicus) showed chlamydial antibodies in 22 out of 106 sera, of which 51 of the remainder were anticomplementary. Mouse passage of caribou tissue also demonstrated chlamydial antibodies after inoculation. Serological surveys of reindeer (Rangifer tarandus) showed that 1 out of 18 sera possessed chlamydial antibodies (10 of the remainder were anticomplementary).

The experimental results of the snow goose specimens as well as the descriptive findings obtained from field observations indicated that snow geese probably serves as a reservoir of chlamydia

for the people. Similarly, the experimental and descriptive findings for caribou and reindeer provided evidence that these species are additional reservoirs. Considering the assembled evidence from our research as well as from others, it may be concluded that the human antibodies are caused by exposure of the humans to snow geese, caribou, reindeer and several other bird and mammal species which harbor chlamydial agents. Northern Canada, thus, appears to be an endemic area of chlamydial infections wherein chlamydial agents circulate reasonably freely among the various animal inhabitants.

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INTRODUCTION

It has been known since 1957 that many people of northern Canada possess antibodies towards the chlamydia. This project was designed at determining some of the probable reservoirs of chlamydia for the people. Particular emphasis was directed at the isolation of a chlamydial agent from the most likely birds and mammals in the vicinity of the human settlements. It was considered that such an isolation from some bird or mammal would provide evidence that the species might serve as a reservoir of chlamydial infections for the people.

REVIEW OF THE LITERATURE

A. EPIDEMIOLOGY OF CHLAMYDIA IN NORTHERN CANADA

a) Introduction

Over the years, sera from different human populations have revealed relatively high prevalences of antibodies to chlamydia. Beeson et al (1944) showed that 33% of coloured people and 10% of Caucasians from the U.S.A. possessed chlamydial group antibodies. It was later thought that the lymphogranuloma venereum agent might be responsible for the majority of the infections. Fløystrup et al (1950) demonstrated chlamydial antibodies in 46 of 308 (15%) Danes, many of whom had atypical respiratory infection. Later, Dane (1955) showed that 132 of 607 (22%) healthy Australians possessed chlamydial group antibodies (titres of 5 and greater). It was concluded that the antibodies represented past or present infection with chlamydia, rather than due to non-specific causes. Trachoma, inclusion blennorrhoea and lymphogranuloma venereum were excluded as possible causes because of rarity of clinical symptoms. Since the people have close contacts with birds, Dane suggested ornithosis would be the most likely causative agent of the antibodies.

b) Chlamydial investigations in northern Canada

In 1957 blood samples were obtained from Eskimos of Baffin Island by Hildes et al (1958), and 15% demonstrated chlamydial antibodies. As with the other human populations, it was unknown

which chlamydial agent (Table I) was responsible, since an antigen common to the group had been used in the test to detect the antibodies. The possibilities were raised that the antibodies reflected human infections, and also that a new reservoir for the chlamydia had been found. The situation in northern Canada appeared endemic since a sampling in Winnipeg by Wilt et al (1959) of 1792 persons with suspected respiratory disease only revealed 18 (1%) with chlamydial antibodies. The interest initially focused on the cause of the antibodies in these northern people.

The feline pneumonitis agent was excluded as a possible cause of the human antibodies since cats occur infrequently in the North and lymphogranuloma venereum was eliminated because of absence of clinical disease. Hildes et al (1958) eliminated the bacterium Treponema palladum, the causative agent of syphilis, because of negative Wasserman tests on 39 Eskimo sera which previously demonstrated chlamydial antibodies. Surveys in Keewatin settlements in 1957 by Willis demonstrated a considerable number of persons with corneal scarring. This finding was later confirmed by Hildes (1958), and was also demonstrated by Van den Berg in the central Arctic. A survey by Reed et al (1959) in the Keewatin District, however, indicated that most of the corneal scarrings were caused by phlyectenular keratoconjunctivitis, a manifestation of tuberculosis. Wilt et al (1959) therefore concluded that trachoma and inclusion conjunctivitis did not exist in clinical forms in the North, and were probably not

TABLE I
THE CHLAMYDIA*

Agent	Tissue Tropism
psittacosis	respiratory tract
ornithosis	respiratory tract
animal pneumonitis	respiratory tract
meningopneumonitis	meninges, respiratory tract
human pneumonitis	respiratory tract
trachoma	eye
inclusion conjunctivitis	eye
	genital tract
lymphogranuloma venereum	genital tract

* From Meyer (1965)

responsible for the human chlamydial antibodies.

Wilt et al (1959) subsequently reported chlamydial group antibodies in humans of settlements across the North (Table II). Some of the positive sera were reacted with mumps, adenovirus, influenza, enterovirus, herpes simplex, brucella and tularensis antigens, but no relation was found between the prevalence of antibodies to these agents and antibodies to the psittacosis-L.G.V. group antigen.

Some genetically determined protein was considered by Wilt and his co-workers to be acting as the chlamydial group antibody or an anticomplementary agent. It was thus expected that one race in the North would possess a much higher antibody prevalence than other races. The prevalence of antibodies among Indians (Old Crow), however, was approximately the same as the prevalence among Eskimos (Cambridge Bay). Changes in the antibody titres in the same individual over short time intervals also made this genetic hypothesis seem doubtful.

Hildes et al (1965) reported that snow and blue and Canada geese possessed antibodies to chlamydia. Snow and blue goose, Canada goose, eider duck, red loon, ptarmigan, raven, and snow bunting material (tissues, droppings and egg albumin) were subsequently collected, but attempts failed at the isolation of an agent. In 1969 14 out of 68 (20%) snow and blue geese sera demonstrated chlamydial antibodies.

Spalatin et al (1966) succeeded in the isolation of chlamydial

TABLE II
PREVALENCE OF CHLAMYDIAL GROUP ANTIBODIES AMONG PEOPLE
OF NORTHERN CANADA*

Location	Year of Sampling	Number Tested	Population	Percentage Prevalence**
Frobisher Bay	1957	42	480	14
Kivitoo	1957	6	40	16
Broughton Island	1957	15	70	7
Durban Island	1957	9	30	33
Padloping	1957	15	30	0
Scott Inlet	1957	11	50	18
Sam Ford Fiord and Cape Christian	1957	5	20	0
Clyde River	1957	21	100	0
Pond Inlet	1957	43	250	25
Arctic Bay	1957	10	170	10
Lake Harbour	1957	22	280	27
	1959		280	26
Keewatin District	1958	88	1400	34
Cambridge Bay	1958	54	1400	81
Old Crow	1958	105	180	74
	1960	112	180	81
	1961	69	180	41
Pangnirtung	1959	42	300	86
Garry Lake	1959	21	50	14
Eskimo Point	1963	150	350	30
	1967	100	500	88

* From Hildes *et al* (1958), Wilt *et al* (1959) and Wyman *et al* (1969).

** Titres (expressed as reciprocal of serum dilutions) = 4 or greater.

agents from two muskrats (Ondatra zibethicus spatulatus) and two snowshoe hares (Lepus americanus) from Saskatchewan, Canada. Experimental infection of muskrats and snowshoe hares by Iversen et al (1970) suggested that chlamydial infections are enzootic in muskrats and epizootic in snowshoe hares. Chlamydial group antibodies were detected in 3 of 69 (4.3%) muskrats from the Yukon and the MacKenzie River delta, and in 2 of 15 (13%) muskrats from Saskatchewan. Antibodies were also found in 3 of 15 (20%) snowshoe hares from Saskatchewan, but of 398 snowshoe hares from Alberta all were negative.

Iversen et al (1970) also found chlamydial antibodies in 3 of 10 sera from conservation field personnel who contacted various animals, including muskrats. Mallard ducklings were artificially exposed to the muskrat agent (M56), which was later recovered from the feces. Isolation of M56 from Haemaphysalis leporispalustris ticks feeding on experimentally infected snowshoe hares suggested the possible involvement of a vector in the transmission of the agent.

Subsequent to the finding by Hildes and Wilt of chlamydial antibodies in Eskimos, Eddie et al (1966) collected 314 sera from the Eskimos and Caucasians of the Pribilof Islands and found 191 (61%) had chlamydial antibodies (titres of 2 and greater). Proceeding further, these researchers showed that 103 (45%) out of 233 fur seals (Callorhinus ursinus) from the islands possessed chlamydial

antibodies and succeeded in isolating a chlamydial agent from the spleens of two seals. A fulmar and gull were also found to possess chlamydial antibodies, but isolation of an agent from these species was not attempted.

Wyman et al (1969) reacted psittacosis and meningopneumonitis "specific" antigens with Eskimo sera that had demonstrated chlamydial antibodies and obtained negative reactions; an ornithosis "specific" antigen was also tried but proved to be anticomplementary when tested. Pasteurella tularensis, Brucella abortus, Proteus OX19, Mycoplasma pneumonia and Coxiella burneti were then reacted with positive Eskimo sera. No relation was found, however, between the prevalence and titres of antibodies to these antigens and those to the psittacosis-L.G.V. group antigen. No decrease in antibody titre was reported when Eskimo sera with chlamydial antibodies were adsorbed with N. meningitidis, N. sicca, H. influenza, M. tuberculosis, and Proteus spp, and then again reacted with the psittacosis-L.G.V. group antigen. Wyman concluded that a new, unisolated chlamydial agent was probably responsible for the human antibodies. Wyman suggested that the most likely chlamydia would be ornithosis, human pneumonitis, or animal pneumonitis. Seals, snow and blue geese, and caribou were cited as likely reservoirs of chlamydia for the people.

B. THE HOST SPECTRUM OF CHLAMYDIA

a) Introduction

Meyer (1967) has recently surveyed the host species parasitized by the chlamydia, and thereby demonstrated the remarkable variety and range of hosts. The list, consisting of over two hundred species, has been condensed into one table for this thesis (Table III).

b) Hosts of chlamydia

i) Class: Aves

Psittacosis, as Meyer (1965) stated, was first recognized in Switzerland in 1876 as a disease in humans transmitted through contact with parrots. In 1928-1929 psittacosis outbreaks occurred in many countries due to importation of parrots and related birds from the tropics. It was thus considered for many years that the order Psittaciformes was the only reservoir of psittacosis.

Haagen et al (1938), however, soon found that petrels of the order Procellariiformes were causing a psittacosis-like illness among inhabitants of the Faroe Islands. Isolation of the causative agent prompted researchers to assign the organism to the same group as the psittacosis agent.

Since the discovery of Haagen and associates, many hosts for ornithosis agents have been found. Coles (1940) was the first to discover that pigeons were naturally infected with ornithosis. It was later found by Meyer et al (1942a) that pigeons were reservoirs for human infection. Reservoirs of ornithosis in poultry were first

TABLE III
THE HOST SPECTRUM OF CHLAMYDIA*

Phylum	Class	Order	Common Name	
Arthropoda	Arachnida	Acarina	mite, tick	
	Insecta	Mallophaga	chewing lice	
		Anoplura	sucking lice	
		Siphonaptera	flea	
Chordata	Pisces			
	Aves	Podicipediformes	grebe	
		Procellariiformes	fulmar, muttonbird	
		Ciconiiformes	grey heron, egret, paddy bird	
			Anseriformes	goose, duck
		Falconiformes	vulture	
		Galliformes	turkey, pheasant, partridge, fowl	
			Charadriiformes	lapwing, willét, gull
		Columbiformes	pigeon, dove	
		Psittaciformes	lory, conure, parrot parrotlet, parakeet	
			Cuculiformes	cuckoo
		Apodiformes	hummingbird	
		Passeriformes	oriole, jay, magpie, finch, sparrow	
			Mammalia	
			Marsupialia	opossum
				Primates
			Lagomopha	hare, rabbit
			Rodentia	mouse, rat, muskrat, woodchuck, ground squirrel, gopher
				Carnivora
			Pinnipedia	seal
		Artiodactyla	pig	
	Ruminantia	cow, goat, sheep		

* From Meyer (1967)

reported in chickens by Meyer et al (1942b), and later in ducks by Meyer et al (1952). Irons et al (1951) demonstrated that turkeys were infected with chlamydia and Boney et al (1952) indicated that turkeys constituted reservoirs for human infection. Chlamydia were first isolated from domestic geese by Trojan et al (1955), and later suspected as being reservoirs by Strauss (1957). Transmission of ornithosis through the egg has been demonstrated for ducks and the black-headed gull by Illner (1962) and Lehnert (1962). Transovarian transmission has been suspected for several other species, but has never been proven. Gulls were found to be infected by Strauss et al (1957), and have often been suspected as sources of infection for various species. More recently isolates have been obtained from penguins by Cameron (1968) and grey herons by Myers et al (1969). Egrets, in addition, have been shown to be reservoirs for human infections. Meyer (1967) reported that one hundred and thirty species of birds are hosts for psittacosis and ornithosis agents and a substantial number are reservoirs for transmission to man.

ii) Class: Mammalia

Despite the predominance of birds, an increasing number of mammals have also been found as hosts of chlamydia. The first mammalian strain to be isolated was the meningopneumonitis agent from mice by Francis et al (1938); this was soon followed by the isolation of pneumonitis agents from mice and cats. Researchers

have suspected but never proven that cats are a reservoir for man.

Lymphogranuloma venereum was found by Findlay (1938) to be the first chlamydia to have man as a reservoir. Trachoma and inclusion conjunctivitis have been known to be human parasites for many years, but have only recently been propagated and identified with the chlamydia.

In 1949 Roca-Garcia (1949) isolated chlamydia from the common and wooly opossum; the role of opossums as a reservoir, however, has not yet been shown. In the 1950's Stamp et al (1950) isolated a chlamydia from sheep; this was followed by isolation from cattle by Menges et al (1953), and goats by Omori et al (1957). Barwell (1955) has reported an infection of a laboratory worker with the agent of enzootic abortion of ewes, but it is not known whether ewes constitute a natural reservoir. An organism was isolated by Barnes et al (1964) from a rancher in contact with cattle possessing epizootic bovine abortion, but it was not shown if the isolate was a mammalian strain. Chlamydial infections have been suspected in dogs for years. Groulade et al (1954) found chlamydial antibodies among dogs and Philip et al (1954) has reported initial bodies in smears from diseased dogs. Debbie (1967) found that 200 out of 500 (40%) deer from New York and southern Quebec demonstrated chlamydial antibodies. Eddie et al reported the first isolation of chlamydia from an aquatic mammal (the northern fur seal) near Alaska.

Rodents were first discovered to be infected by the isolation of a chlamydia from a neotropical water rat by Downs, as reported by Meyer (1967). Stoenner et al (1959) and Sidwell et al (1964) have reported chlamydial antibodies in jack rabbits, deer mice and wood rats, but have not isolated an organism. Recently an isolate was reported from muskrats by Spalatin et al (1966), but there has been no conclusive evidence that any rodent is a reservoir of chlamydia for man.

iii) Classes: Arachnida and Insecta

There have been reports of isolation of chlamydia from both classes of arthropods (Arachnida and Insecta) by Terskikh et al, Eddie et al (1962), and Eddie et al (1969). Arthropods, however, so far have not been considered generally to constitute reservoirs or vectors for the natural transmission of chlamydia.

iv) Class: Pisces

In studying canine diseases on the west coast of the United States, Philip (1955) suspected salmon as being involved in transmission of chlamydia between trematodes and diseased dogs. More recently, Wolke et al (1970) has tentatively identified a chlamydia, largely on a morphological basis, as responsible for epitheliocystis disease in the gills of striped bass and white perch.

MATERIALS AND METHODS

A. COLLECTION OF SPECIMENS FOR ISOLATION

a) Introduction

It is known that in many infections of animals and birds in nature, chlamydia produce inapparent infections. Under certain stressful conditions to the host, Austin (1957), and Stewart (1960) reported that chlamydia may be activated and thus easier to detect. It was therefore decided to collect the specimens during periods of stress to the selected species, since the chances of isolation would be enhanced.

b) Goose tissues (Table IV)

A period of considerable stress to wild geese occurs between spring migration and hatching of the eggs, as described by Dr. A. Hochbaum (Delta Research Station, Delta, Manitoba). Prior to egg hatch, adult geese were shot and dissected under sterile conditions. Portions of the liver, spleen, lung, kidney, and ovaries were removed, placed in sterile containers and frozen at -17°C at the settlement. The specimens were later transported to Winnipeg in dry ice and kept at -65°C until tested.

c) Goose eggs (Table IV)

Soon after the eggs were laid, a number were collected, and carefully sent to Winnipeg. Upon arrival, the eggs were candled and then incubated at 37°C for 14-18 days.

TABLE IV
SPECIMENS FOR SEROLOGY AND ISOLATION OF CHLAMYDIA

Test	Specimen	Collector or Contributor	Collection Area	Date of Collection	Number of Specimens
Isolation	Snow goose eggs	Mr. P. Wilt	Eskimo Point*	June 1970	118
	Snow goose tissues	Mr. P. Wilt	Eskimo Point	July 1970	18
	Canada goose eggs	Mr. C. Toni and Mr. M. Greene	Churchill**	June 1970	2
	Caribou tissues	Mr. P. Wilt	Eskimo Point	Sept. 1970	23
		Mr. E. Land	Baker Lake*	Feb. 1971	5
Serology	Eskimo sera	Dr. A. Ronald	Baker Lake	June 1969	128
				June 1970	97
	Caucasian sera	Dr. J. Hildes and Dr. N. Choi	Churchill	July 1970	16
		Dr. A. Ronald	Baker Lake	June 1969	41
		Mr. P. Wilt	Churchill	May 1970	4
			Watson Point**	Sept. 1970	4
				May 1970	4

* Northwest Territories

** Manitoba

TABLE IV (CONTINUED)

Test	Specimen	Collector or Contributor	Collection Area	Date of Collection	Number of Specimens
	Indian sera	Dr. J. Hildes and Dr. N. Choi	Churchill	July 1970	147
	Caribou sera	Dr. L. Choquette	Brochet**	April 1968	20
			Rankin Inlet*	June 1968	62
			(Unknown)	June 1970	4
		Mr. P. Wilt	Eskimo Point	Sept. 1970	23
	Reindeer sera	Dr. L. Choquette	Reindeer Grazing Preserve*	March 1969	14
			(Unknown)	(Unknown)	4

d) Caribou tissues (Table IV)

A stressful period for caribou occurs during fall migration and before rutting, as described by Kelsall (1968). In September of 1970, liver, lung, and spleen caribou samples were collected, and then placed in sterile bottles containing streptomycin (150 mg/ml) to avoid excess bacterial contamination. Upon arrival at the settlement, the specimens were frozen at -17°C until transport to Winnipeg. In addition, some caribou tissues were collected in February of 1971 and sent frozen to Winnipeg. All samples remained frozen at -65°C until analysis.

B. ISOLATION PROCEDURES

a) Screening of material for isolation

i) Introduction

Most of the material for isolation was first screened for the presence of chlamydia. The specimens that were positive on the screen test were then used for isolation.

ii) Goose tissues

The goose tissues were first thawed, cut into small fragments, and then impressed onto slides and coverslips for examination.

iii) Goose eggs

The eggs were opened, pieces of the yolk sac and embryo removed, and smears made. The yolk sacs and embryos were subsequently placed in sterile petri dishes with 0.85% saline and minced into small fragments with sterile scissors. The fragments were then

removed using sterile pipettes and placed over a layer of chicken plasma (Grand Island Biological Company; Grand Island, New York) inside 2 ounce culture bottles. The bottles were incubated at 37°C for approximately 15 minutes for complete coagulation of the plasma and then layered with 4 ml of medium 199 with 10% inactivated calf serum and streptomycin (200 ug/ml). The bottles were reincubated at 37°C for about two weeks, changing the media every 4-5 days. Smears were made from the pellets after low speed centrifugation of the collected media for staining and microscopic examination.

b) Preparation of material for isolation

i) Goose tissues

The geese tissue specimens were minced into small fragments with sterile scissors and ground using sterile sand and mortar and pestles. Brain heart infusion broth or medium 199 with 10% inactivated calf sera and streptomycin (200 ug/ml) was added to make a 5-10% emulsion. The emulsions were aseptically poured into sterile tubes and centrifuged at 800 rpm for 10 minutes. The supernatants were then removed and used as the inocula.

ii) Eggs

The embryo fibroblast material was minced and digested with trypsin before grinding in mortar and pestles, while the yolk sacs

were ground without mincing. The ground material was then emulsified and prepared for inoculation as described for goose tissues.

iii) Caribou tissue

The caribou tissues were minced into small fragments with sterile scissors and ground in a Sorval homogenizer with sterile, closed containers. The ground material was then emulsified and prepared for inoculation as described above.

c) Mouse passages

i) Animals

Approximately 21 day old ICR female mice (Canadian Research Animal Farms; Bradford, Ontario) were employed for isolation.

ii) Inoculations

Material (0.5 ml) was inoculated intraperitoneally into a group of 3-4 mice. The animals were kept for 7 days and then autopsied and compared with the organs of control animals. Spleen and lung impression and peritoneal (scrapings) smears were made. In some instances, peritoneal washings were performed using saline (pH = 7.2). The washings were then centrifuged at 800 rpm for 10 minutes, and smears made from the pellets and stained.

iii) Controls

Parallel to each passage, a number of control mice were kept in a separate room for about seven days. The mice were then sacrificed and their organs processed as described above.

d) Tissue culture passages

i) Cells

The L-cells (mouse fibroblast) used were originally obtained from Dr. A. Holloway (Manitoba Cancer Research Foundation) and were since then routinely maintained in our laboratory using medium 199 with 10% calf serum and streptomycin (200 ug/ml).

ii) Inoculations

Spleen and lungs from mice (previously inoculated with goose material) were minced, ground and filtered using a Swinnex 13, Millipore 2 (HAWP 01300, HA = 0.45 μ , 13 mm) filter. The filtrates were removed aseptically and inoculated into a number of tubes in which L-cell monolayers on coverslips had been placed. The tubes were placed at 37°C and the coverslips removed at predetermined intervals for staining and examination.

C. IDENTIFICATION OF THE AGENT

a) May Grunwald-Giemsa staining

The May Grunwald-Giemsa staining method was routinely performed.

b) Immunofluorescent staining

i) Method

Immunofluorescence was performed using the indirect method as described by Coons (1950).

ii) Sera

Sera from human psittacosis patients and commercial (Markham Laboratory, Chicago, Illinois) antisera prepared in humans against

psittacosis were used as well as commercial preparations (Grand Island Biological Company, Grand Island, New York) of goat anti-human globulin and goat anti-human gammaglobulin antisera (fluorescein conjugated).

iii) Antigens

The commercial psittacosis-human pneumonitis group antigens (Markham Laboratory, Chicago, Illinois) as well as a psittacosis 6BC antigen from this laboratory were used. The 6BC strain was originally obtained from Dr. J. Moulder (Department of Microbiology, University of Chicago, Chicago, Illinois).

iv) Examination

Immunofluorescent slides were examined using a Zeiss fluorescent microscope equipped with an Osram HB) 200 mercury vapor lamp. A Schott 4 mm, BG 12, blue excitor filter and a K470 or K440 and K650 barrier filters were used.

c) Serology

The infected and control mice used for isolation were bled from the tail and the sera used for serology.

D. SEROLOGY

a) Specimens for serology

The human, caribou and reindeer sera used for serology have been listed in Table IV.

b) Separation of sera

The blood samples were allowed to stand at room or atmospheric

temperature immediately after collection. After clotting had occurred, the blood clots were rimmed using applicator sticks and then placed in a refrigerator (4°C) for about 18 hours. The animal sera were then removed and frozen at -17°C; the human samples were first centrifuged before removal of the sera. The sera were stored at -20°C until the serological analysis.

c) Testing of sera

i) Tests

The sera were tested by a direct and indirect complement fixation test by the procedures of Kabat et al (1961). The two methods of these tests were the test tube method of Bordet et al (1901) and the microplate method described by Kabat (1968). The sera were tested by Dr. H. Sayed and Mr. W. Stackiw (Medical Microbiology Department, University of Manitoba, Manitoba).

ii) Antigens

The antigen used in all tests was a commercial (Markham Laboratory, Chicago, Illinois) psittacosis-human pneumonitis group antigen.

iii) Antisera

Antisera prepared in humans against psittacosis and meningo-pneumonitis were utilized.

iv) Complement, sensitized erythrocytes, and buffer

Guinea pig complement (Flow Laboratories, U.S.A.) which originally contained 256 CH₅₀ units was titrated to 4 CH₅₀ units for the

test. Sheep erythrocytes in Alsevers solution (National Biologics, Canada) were washed several times before being sensitized by a commercial rabbit hemolysin (Markham Laboratory, Chicago, Illinois). A 4% suspension of sensitized cells were used for the tube test and a 2% suspension for the microplate assay. The diluent in all complement fixation procedures was veronal buffer with CaCl_2 and MgCl_2 added.

RESULTS

A. DESCRIPTIVE PHASE

a) Introduction (Tables V, VI and VII)

There is a very wide variety of animal life which inhabits northern Canada, as described by Godfrey (1966) and Burt et al (1964). Since the host spectrum for chlamydia is so varied, as described in the literature review, all of the mammals and birds in northern Canada were considered as possible reservoirs for the people. To determine which species were the most probable reservoirs, two trips were arranged to a northern settlement to intensively study the epidemiological situation of chlamydia in the North.

b) Visits to Eskimo Point

Eskimo Point was chosen for study mainly because a high prevalence of chlamydial antibodies was found in the human population three years previously (Table II). The settlement was also chosen because of convenient transportation. Since most northern communities are coastal, Eskimo Point was considered by the author as being a fairly representative northern settlement.

The first visit to Eskimo Point was in the spring and summer (May 19 - July 20) and the second in the fall (August 27 - September 21) of 1970.

c) The human population of Eskimo Point

Eskimo Point, located on the west coast of Hudson Bay in the Keewatin District, is comprised of approximately 600 Eskimo and 50

TABLE V
CLASSIFICATION OF THE ANIMAL LIFE
OF NORTHERN CANADA*

Phylum	Class
Arthropoda	Arachnida
	Insecta
Chordata	Pisces
	Aves
	Mammalia

* From Hickman (1966)

TABLE VI
BIRDS OF NORTHERN CANADA*

Order	Family	Common Name
Gaviiformes	Gaviidae	loon (4)**
Procellariiformes	Procellariidae	fulmar (1)
Anseriformes	Anatidae	swan (1), goose (5), duck (16)
Falconiformes	Accipitridae	hawk (4)
	Falconidae	eagle (2), falcon (4), caracara (1)
Podicipediformes	Podicipedidae	grebe (2)
Galliformes	Tetraonidae	grouse (4), ptarmigan (3)
Gruiformes	Gruidae	crane (2)
Charadriiformes	Charadriidae	plover (4), turnstone (1)
	Scolopacidae	snipe (1), sandpiper (9)
	Phalaropodidae	phalarope (2)
	Stercorariidae	jaeger (3)
	Laridae	gull (6), tern (1)
Strigiformes	Strigidae	owl (6)
Coraciiformes	Alcedinidae	kingfisher (1)
Piciformes	Picidae	woodpecker (4)
Passeriformes	Tyrannidae	flycatcher (5)
	Alaudidae	lark (1)
	Hirundinidae	swallow (5)
	Corvidae	jay (1)

* From Godfrey (1966)

** Number of species

TABLE VI (CONTINUED)

Order	Family	Common Name
	Paridae	chickadee (2)
	Turdidae	thrush (4), solitaire (1)
	Sylviidae	kinglet (1)
	Motacillidae	pipit (1)
	Bombycillidae	waxwing (1)
	Laniidae	shrike (1)
	Parulidae	wood warbler (8)
	Icteridae	blackbird (1)
	Fringillidae	grosbeak (1), finch (3), sparrow (10), bunting (1)

TABLE VII
MAMMALS OF NORTHERN CANADA*

Order	Family	Common Name
Insectivora	Soricidae	shrew (5)**
Chiroptera	Vespertilionidae	plainnose bat (4)
Carnivora	Ursidae	bear (3)
	Mustelidae	weasel (7), skunk (1)
	Canidae	dog (1), wolf (1), fox (2)
	Felidae	cat (1)
Pinnipedia	Odobenidae	walrus (1)
	Phocidae	hair seal (6)
Rodentia	Sciuridae	squirrel (6)
	Castoridae	beaver (1)
	Muridae	muskrat (1)
	Dricetidae	mouse (1), lemming (3), vole (8)
	Zapodidae	jumping mouse (2)
	Erethizontidae	porcupine (1)
Lagomorpha	Ochotonidae	pika (1)
	Leporidae	hare (2)
Artiodactyla	Cervidae	deer (4)
	Bovidae	goat (1), musk ox (1), sheep (1)
Cetacea	Monodontidae	white whale (1), narwhal (1)

* From Burt et al. (1964)

** Number of species

Caucasians, as stated by Father Ducharme. The author's trips to the settlement demonstrated some of the changes occurring in Eskimo life. The greatest factors producing this change, in the author's opinion, have probably been improved transportation, the influx of southern commodities, and the creation of settlement jobs. Despite changes, however, a substantial number of Eskimo are still engaged in hunting.

The Caucasians at the settlement consist of primarily school teachers, nurses, administrators, store personnel, and ministers. As reported by Father Ducharme, the majority of these persons stay for approximately 1-3 years and have little or no contact with animal life.

d) The contact between the animals and the people of Eskimo Point

i) The contact between snow and blue geese and humans

Snow and blue geese, as reported by Mr. F. Bailey of the Game Management Department (Churchill, Manitoba) and Father Ducharme, are hunted and eaten by the Eskimo Point people. The geese are hunted every spring and fall by the male adults and occasionally by the women, adolescents and children. The geese are generally plucked by the male adults and brought to the settlement, where the female adults prepare the meat for cooking. Contact therefore exists between most of the humans and snow and blue geese.

ii) The contact between caribou and humans

In the opinion of Mr. F. Bailey and Father L. Ducharme, the people of Eskimo Point hunt, and thus contact, more caribou than any other species. These observations were confirmed by the author's

study of the settlement.

As observed during the visit of the author to Eskimo Point, the greatest numbers of caribou were hunted during spring and fall. The hunters consisted predominantly of male adults, and rarely of women and children. The caribou were shot and then immediately skinned and butchered. The skins and meat were brought back to the settlement, and left to dry for several days or weeks. Large quantities of meat were frozen immediately. The adult women prepared the meat for cooking as well as made clothing and blankets from the skins. Little contact apparently existed between children and adolescents and the caribou. In summary, the greatest contact occurs between caribou and the male and female adults of Eskimo Point.

iii) Contact between other animals and humans

The Eskimo also hunt and have contact with a great many other mammals, as described by Father Ducharme. Every spring and fall, the male adults hunt great numbers of seals. After returning to the settlement, the seals are skinned by the males and then generally sold to the local craft shop for export. There apparently seems to be little contact between seals and the women, adolescents and children. Wolves and foxes are other mammals hunted in great numbers. The male adults, as usual, do the hunting and skinning. The meat is generally discarded, while the skins are cleaned by the adult women for export. Other animals which Eskimo hunt to a lesser degree are Canada geese, ptarmigan, walruses, and polar bears. The contact

between the people and these species, however, is still considerable.

B. ANALYTICAL PHASE

a) Introduction

This section is concerned with an analysis of data in an attempt to gain information concerning northern chlamydial reservoirs. The data for the analysis was from serological surveys performed in northern Canada; the purpose of which was to determine the prevalence of chlamydial antibodies in the human inhabitants. Many of the sera were collected by Hildes, Wilt and associates during the period 1957-1967 (Table II), while some were collected more recently by various persons as shown on Table IV. Figure 1 illustrates the location of most of the human settlements which were sampled, the year of collection and the percentage prevalence of chlamydial antibodies.

b) Analysis of serological data

i) Prevalence of chlamydial antibodies among humans of different age and sex groups

The descriptive findings demonstrated that adult males and females apparently have greater contact than children and adolescents with the surrounding wildlife. It was thus hypothesized that adult males and females would have a higher prevalence of antibodies than any other age and sex groups. The human serological data from eight settlements was separated into age and sex groups and plotted (Figure 2). The prevalence of chlamydial antibodies among the groupings was statistically tested using the chi-square contingency test (Appendix). As

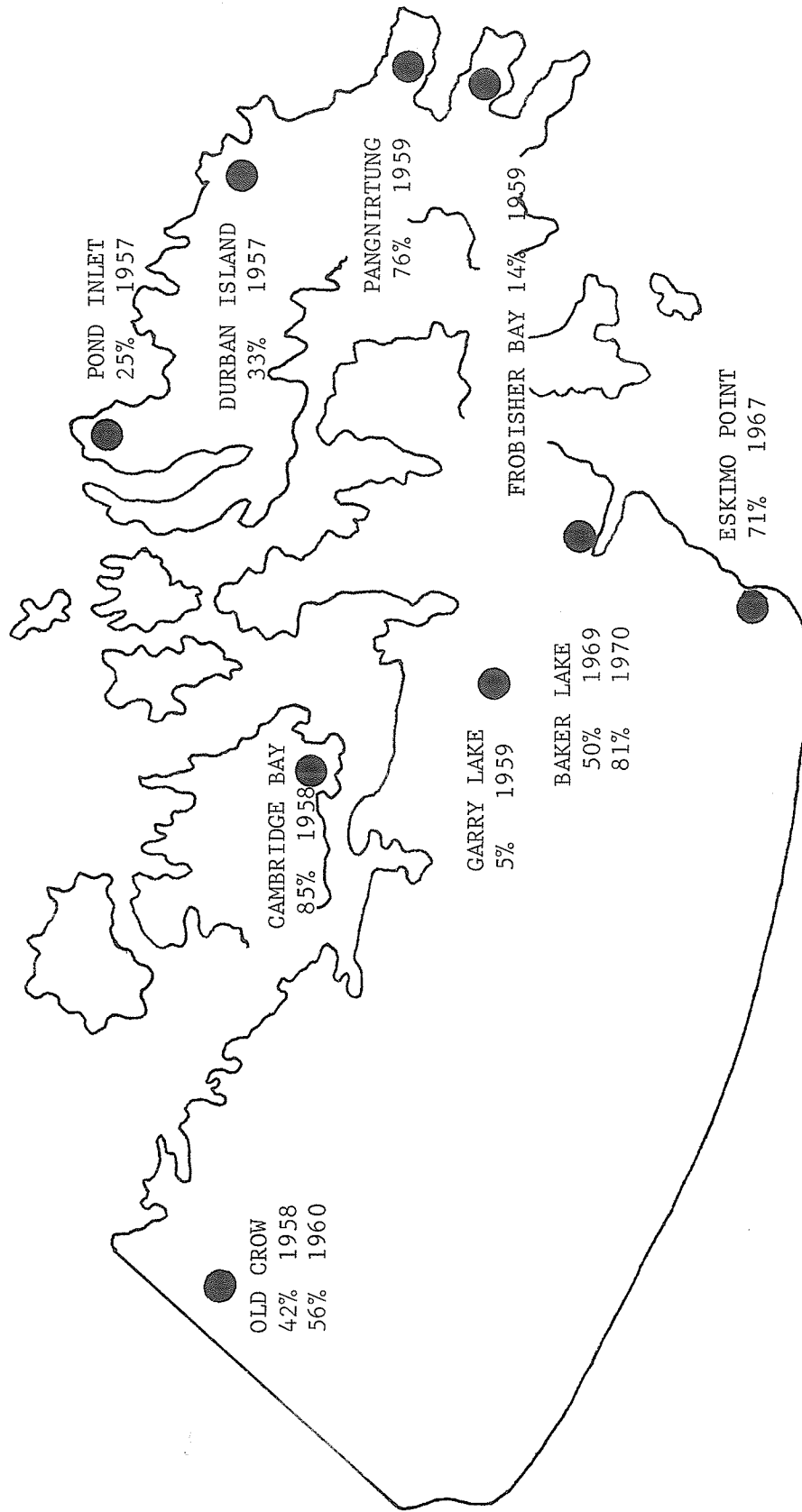


FIGURE 1
PREVALENCE OF CHLAMYDIAL GROUP ANTIBODIES
AMONG PEOPLE OF NORTHERN CANADA

From Hildes et al (1958),
Wilt et al (1959) and Wyman et al
(1969)

Titres = 8 or greater

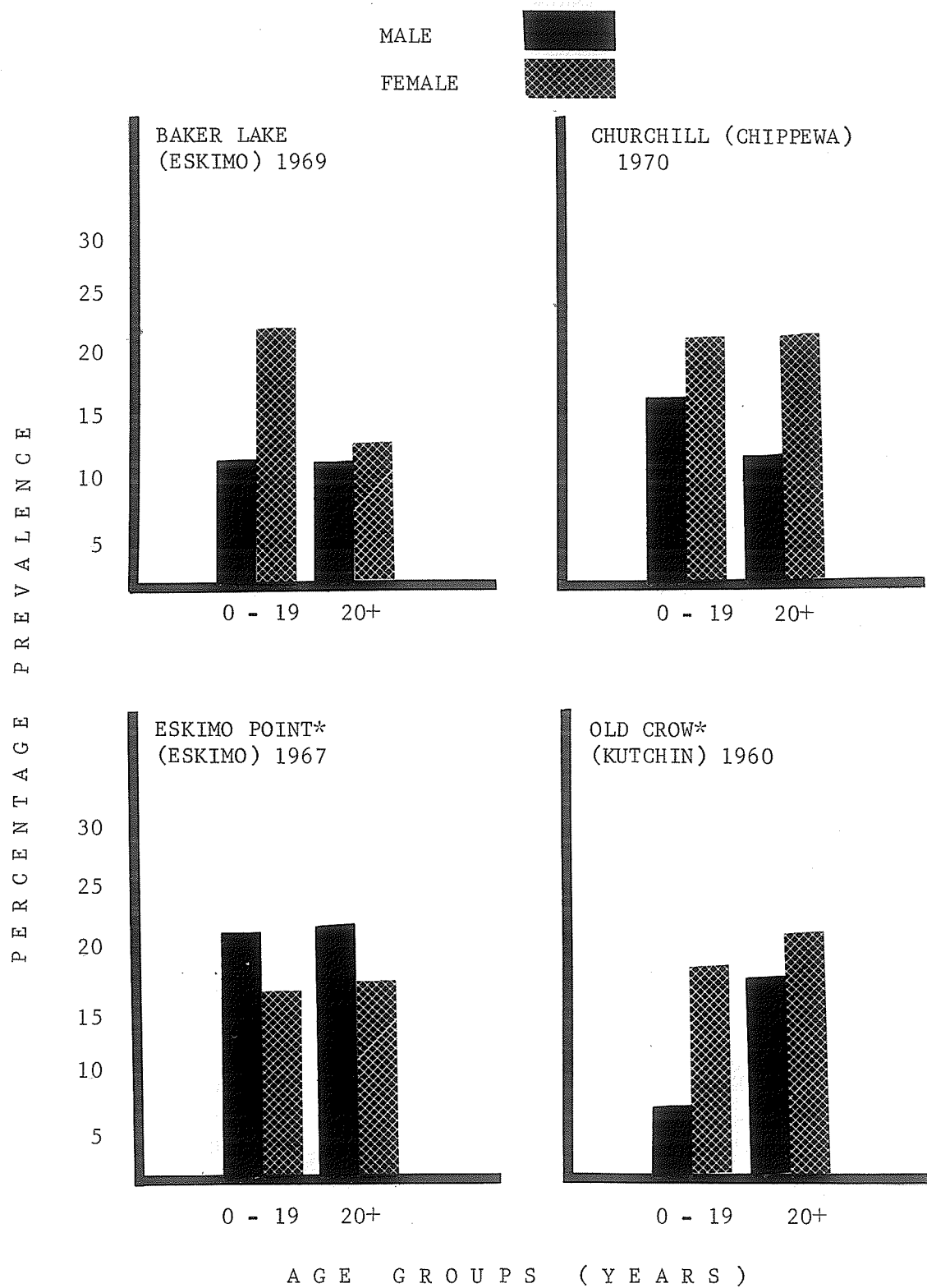


FIGURE 2

PREVALENCE OF CHLAMYDIAL GROUP ANTIBODIES AMONG
AGE AND SEX GROUPS OF NORTHERN PEOPLE

*Serological data
from Wilt *et al* (1959)
and Wyman *et al* (1969)

Titres = 8 or greater

MALE



FEMALE

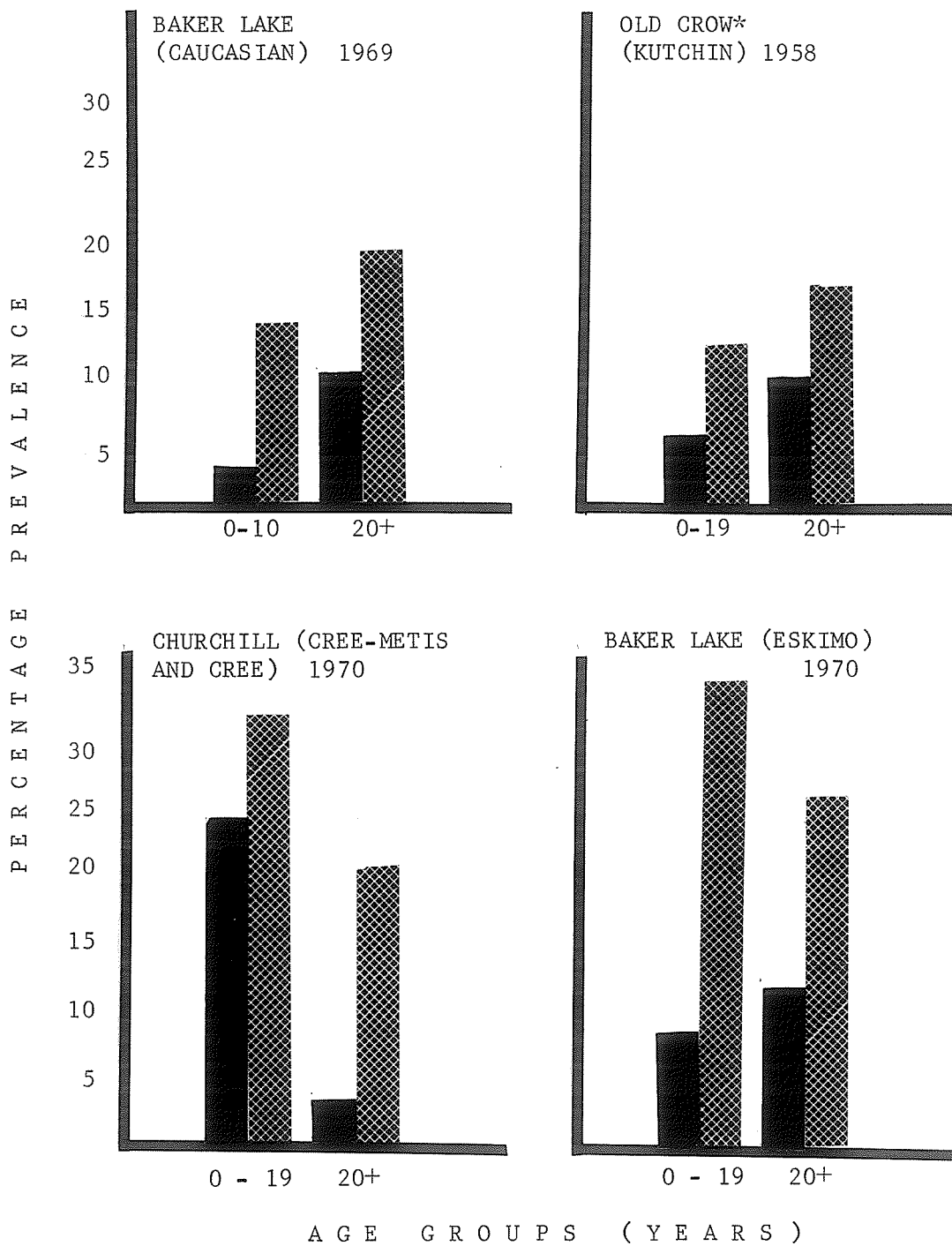


FIGURE 2 (CONTINUED)

shown, a statistical difference was observed among the age and sex groups of humans in only one settlement. The statistical data suggested that a higher prevalence of antibodies were found among female and male adults. These results indicated that adults may be more exposed to chlamydia than children or adolescents. More data would be required to correlate prevalence of antibodies with age and sex groupings of humans.

ii) Prevalence of chlamydial antibodies among humans of coastal
and inland settlements

As illustrated by Godfrey (1966) and Burt et al (1964), there are great variations in the distributions of birds and mammals in northern Canada. From a study of animal distributions with prevalence of chlamydial antibodies, it was thought possible to gain some information concerning reservoirs. It was hypothesized that some differences in antibody prevalence might occur between inland and coastal settlements, since aquatic reservoirs of chlamydia might exist at the latter communities which would be absent in the former. A comparison was thus made between the prevalence of antibodies among Eskimos of a coastal (Pangnirtung) and an inland (Garry Lake) settlement (Figure 3). A chi-square contingency test (Appendix) demonstrated a higher antibody prevalence among the coastal settlement. This finding suggested that persons of coastal settlements might be more exposed to chlamydial reservoirs than persons of inland settlements. Additional comparisons were then made as shown in Figure 3. This data was not tested statistically, however, because an additional variable (racial group) was present in the comparisons. Further data should be obtained before

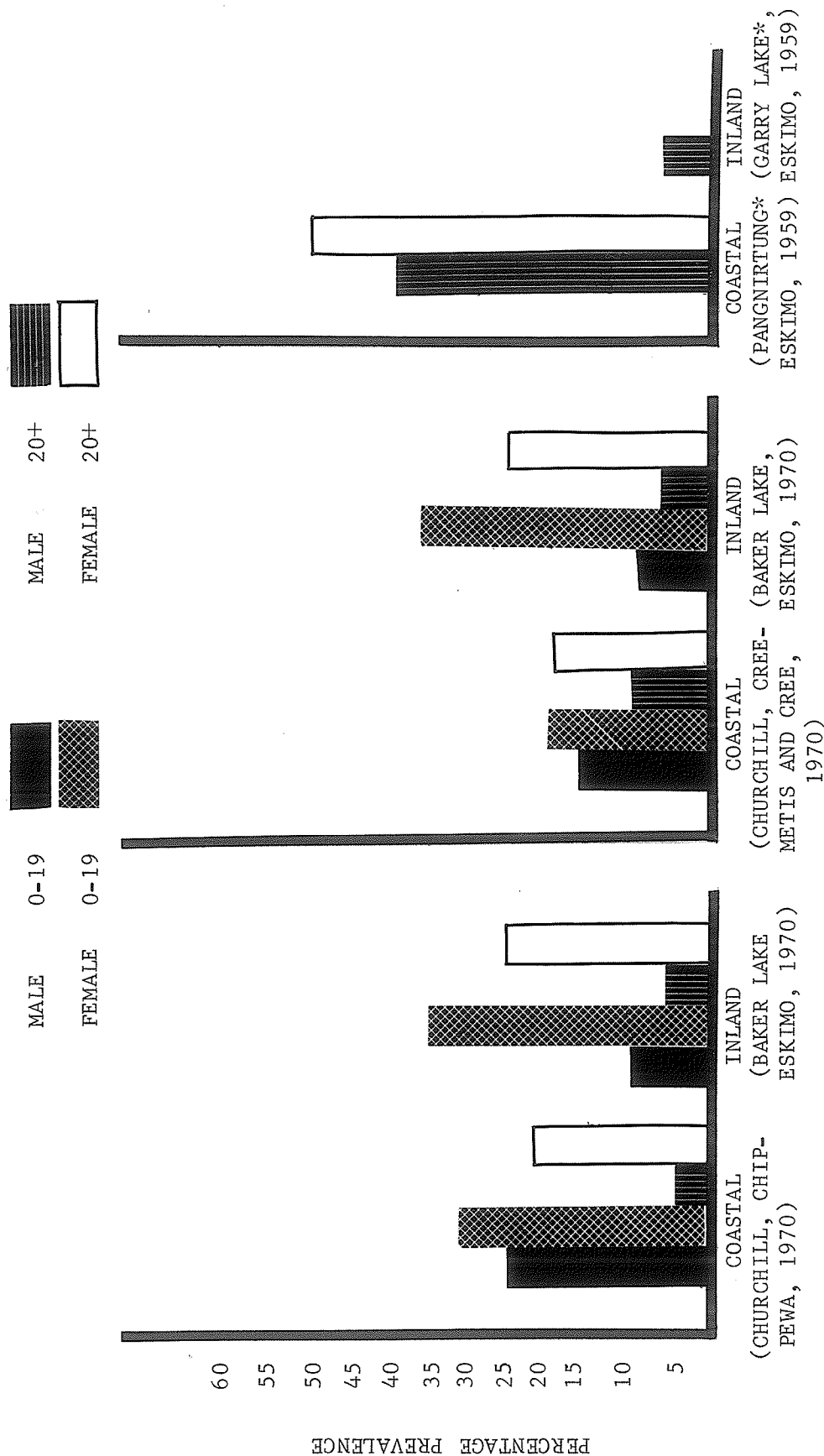


FIGURE 3

PREVALENCE OF CHLAMYDIAL GROUP ANTIBODIES IN THE PEOPLE OF COASTAL AND INLAND SETTLEMENTS

*Serological data obtained from Wilt et al (1959)

Titres = 8 or greater

any definite correlation is made between antibody prevalence and geographical location.

iii) The prevalence of chlamydial antibodies among humans of different racial groups

As discussed in the literature review, many hypotheses have been formulated to explain the presence of chlamydial antibodies among the people of northern Canada. One hypothesis, proposed by Wilt et al (1959), suggested that a genetically determined protein was acting as the chlamydial antibody or an anticomplementary agent. To investigate this hypothesis, comparisons were made between the prevalence of antibodies among different racial groups of humans in the North. As shown in Figure 4, chlamydial antibodies are displayed by all racial groups. This finding suggested that a genetically determined protein was not responsible for the chlamydial antibodies.

C. FORMULATION OF HYPOTHESES

The detection of chlamydial group antibodies by Hildes et al (1965) in sera from snow, blue and Canada geese provided the first evidence of chlamydial infection in these species. As illustrated in Figures 5 and 7 the distribution of geese is extremely wide in northern Canada; overlapping most of the settlements whose inhabitants have displayed chlamydial antibodies. Hildes and associates thus hypothesized that snow, blue and Canada geese were possible reservoirs of chlamydia for at least some of the northern people.

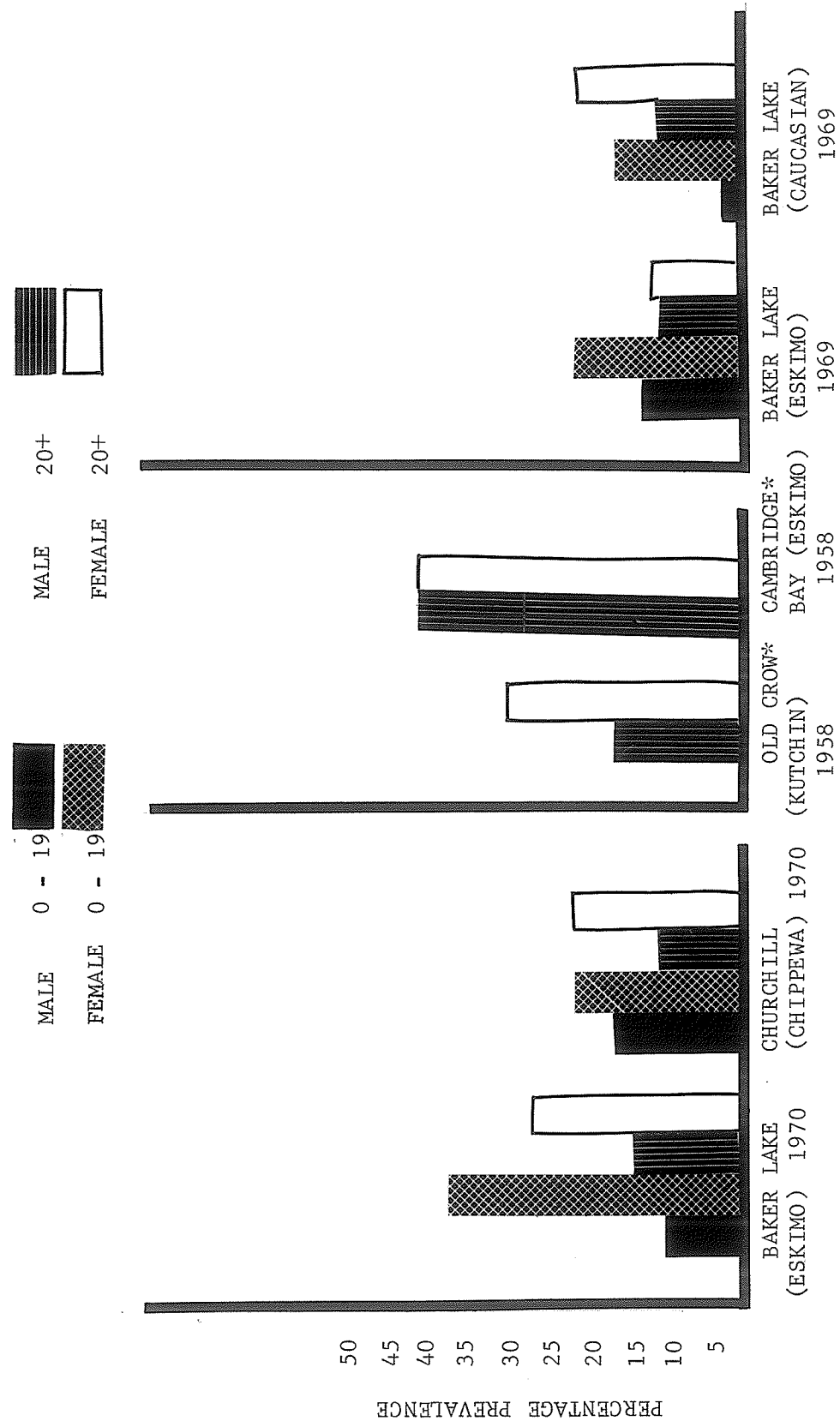


FIGURE 4
PREVALENCE OF CHLAMYDIAL GROUP ANTIBODIES
AMONG RACIAL GROUPS OF THE NORTHERN PEOPLE

*Serological data obtained from Wilt et al (1959)

Titres = 8 or greater



FIGURE 5

DISTRIBUTION OF SNOW GEESE IN NORTHERN CANADA

From Godfrey (1966)

A few years later, chlamydia were isolated from muskrats and snowshoe hares by Spalatin et al (1966) and from seals by Eddie et al (1966). Considering the wide distributions of these mammals in northern Canada (Figures 8 and 9), these researchers hypothesized that muskrats, snowshoe hares and seals were possible chlamydial reservoirs for the northern people.

Wyman et al (1969) hypothesized that caribou as well as seals and snow and blue geese were possible northern reservoirs of chlamydia. This hypothesis was based mainly on descriptive findings by Wyman after a visit to Eskimo Point. Figure 6 illustrates the extremely wide distribution of caribou across northern Canada. It was thus realized that caribou might serve as reservoirs of chlamydia for people across these northern regions.

The descriptive findings of the author indicated that snow and blue geese and caribou were two likely reservoirs of chlamydia for the Eskimo Point people. This hypothesis was primarily supported by the abundant contact between humans and these species. It was fully realized however, that many other animal species in the vicinity of the settlement might also be involved as reservoirs for the people.

D. EXPERIMENTAL PHASE

a) Screening of specimens for agent isolation

i) Snow goose tissues

Examination of snow goose tissues by May Grunwald-Giemsa

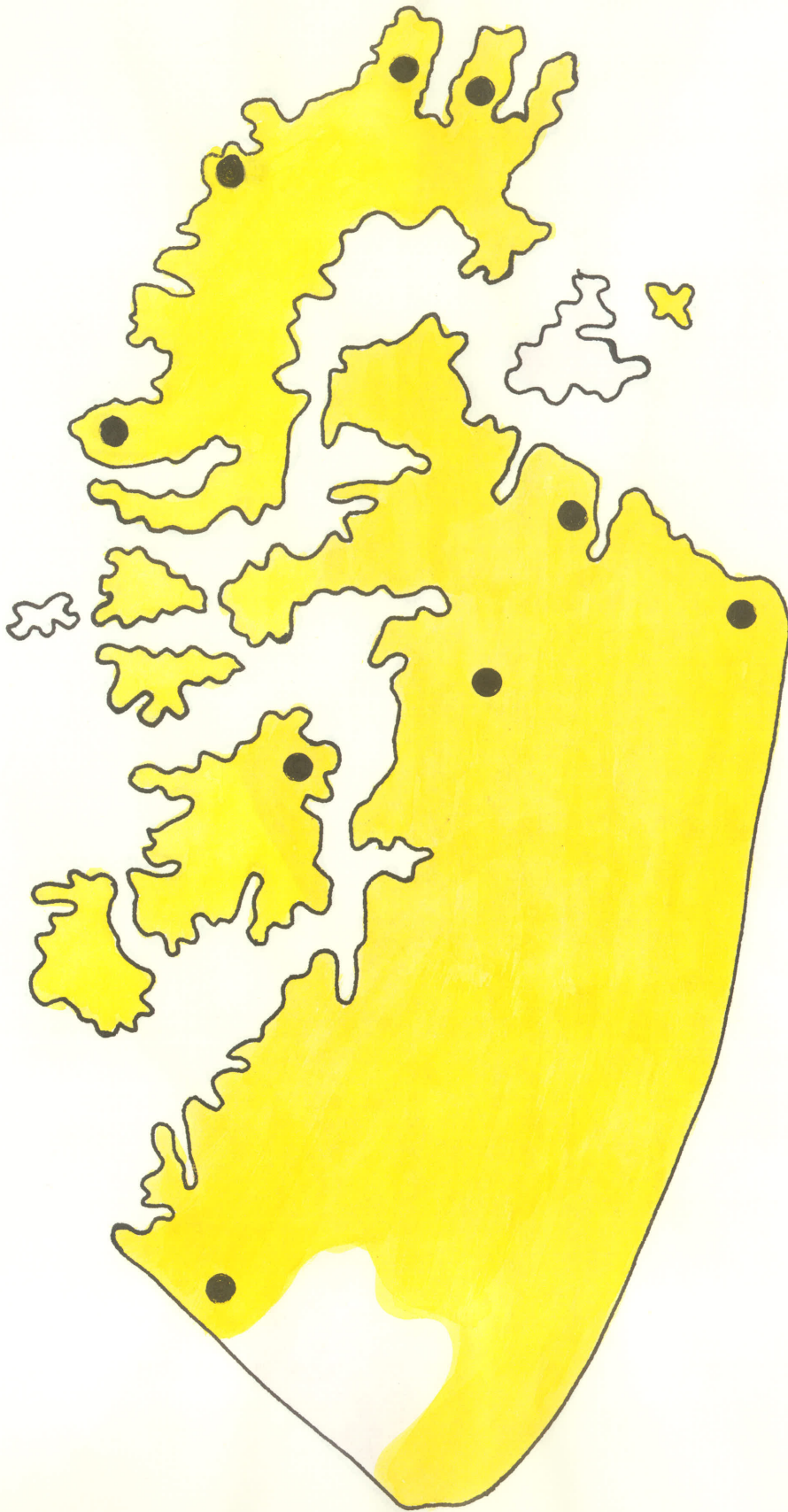


FIGURE 6

DISTRIBUTION OF CARIBOU IN NORTHERN CANADA

From Burt et al (1964)

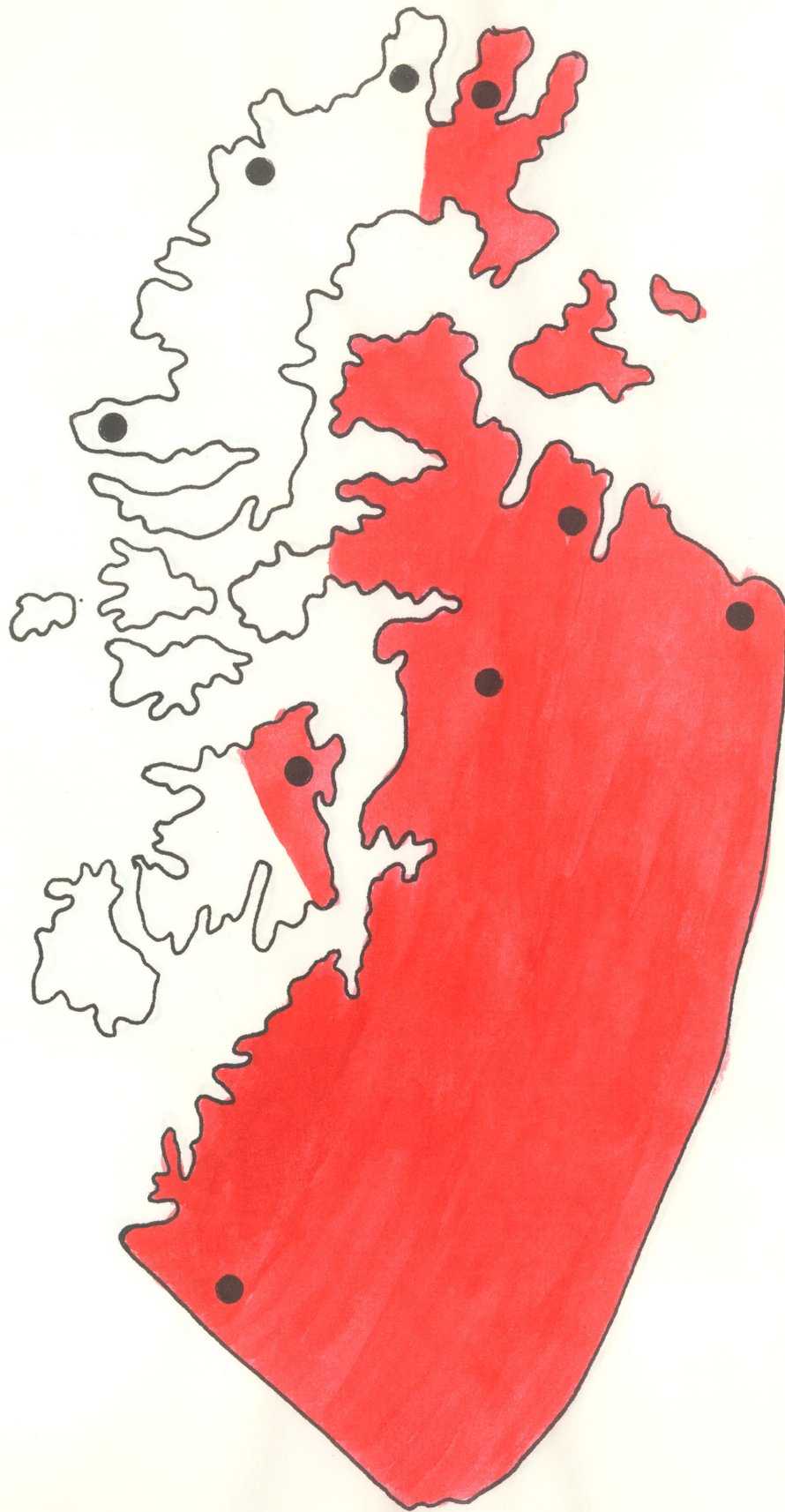


FIGURE 7

DISTRIBUTION OF CANADA GEESE IN NORTHERN CANADA

From Godfrey (1966)

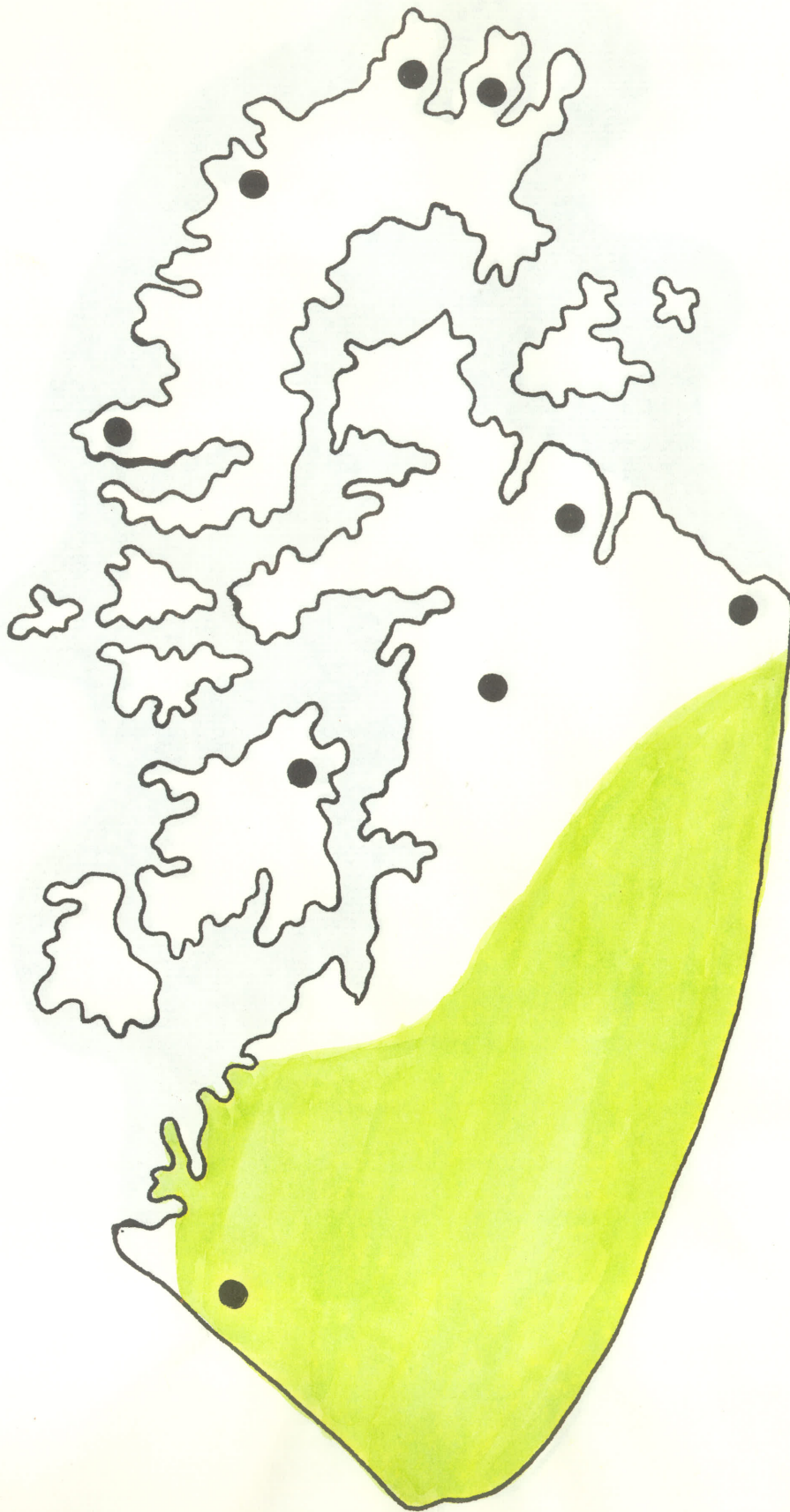


FIGURE 8

DISTRIBUTION OF MUSKRATS AND SNOWSHOE HARES IN NORTHERN CANADA

From Burt et al (1964)

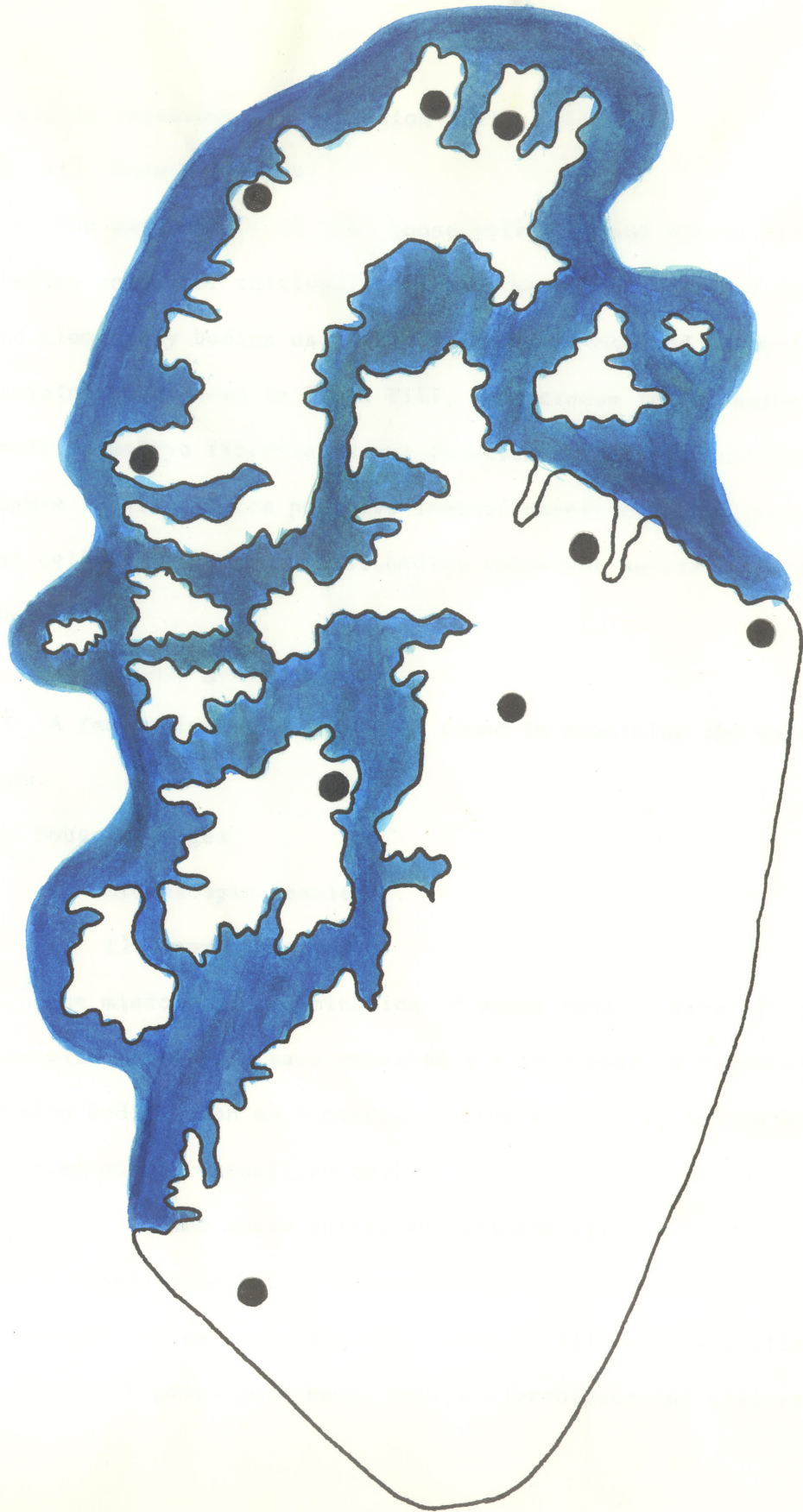


FIGURE 9

DISTRIBUTION OF SEALS IN NORTHERN CANADA

From Burt *et al* (1964)

staining revealed some inclusion bodies.

ii) Snow goose eggs

The examination of snow goose yolk sacs and embryo fibroblasts (before and after cultivation of this material) revealed inclusion and elementary bodies using May Grunwald-Giemsa and immunofluorescent staining. As shown in Table VIII, an increase in the numbers of positive embryo fibroblasts was observed during the cultivation. Figure 10 illustrates positive immunofluorescence of a goose yolk sac cell. The large, bright bodies represent specific chlamydial inclusions.

iii) Canada goose eggs

A few inclusion bodies were found in examining the Canada goose eggs.

b) Mouse passages

i) Microscopic results

1) Snow goose yolks

The microscopic examination of mouse tissues after inoculation of mice with goose yolk sacs revealed a fair number of intracellular inclusion bodies upon each passage. Some elementary bodies were also observed within vacuolized cells.

2) Snow goose yolks, embryos and tissues

A moderate number of intracellular inclusion bodies and occasional elementary bodies were generally found in tissue smears after inoculation of mice with goose yolk sacs, embryo fibroblasts and tissues. Some in-

TABLE VIII
SCREENING OF EMBRYONATED SNOW GOOSE EGGS

Test	Before Cultivation*		After Cultivation**	
	Yolk Sac	Embryo Fibroblast	Yolk Sac	Embryo Fibroblast
Giemsa	18/25*** (72%) (++)***	11/25 (44%) (++)	11/14 (78%) (+,++)	7/13 (53%) (++)
Immunofluorescence	17/22(77%) (++)	6/25 (24%) (+)	6/9 (66%) (++)	7/13 (53%) (++,+++)

- * Initial examination
 ** Examination after in vitro cultivation
 *** Number positive / total number examined
 **** Average number of organisms: +, few; ++, moderate; +++, many

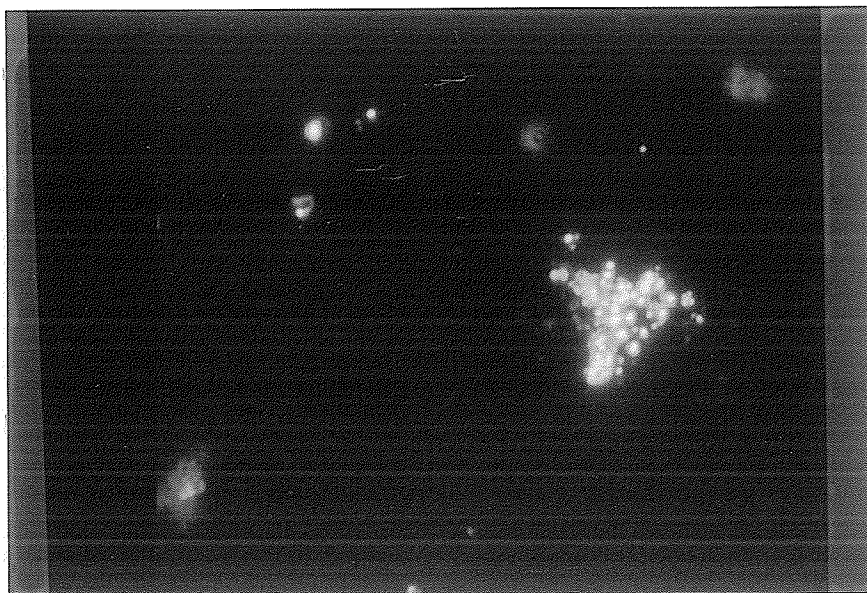


FIGURE 10

IMMUNOFLUORESCENT CHLAMYDIAL INCLUSION BODIES
IN GOOSE YOLK SAC CELL

crease in the number of inclusions was observed during passage.

3) Control mouse tissues

Only a very few inclusion bodies were seen in tissues from control (non-inoculated) mice. No elementary bodies or vacuolized cells were found.

ii) Serological results

The serological results for the mouse passages of snow goose and caribou material have been summarized in Tables IX and X respectively. The tables indicate much higher percentages of positive (showing chlamydial antibodies) serum pools for the inoculated than for the non-inoculated (control) mice. In addition, passage of non-inoculated mouse tissues in mice revealed no chlamydial antibodies (Table XI).

c) Tissue culture passages

Some inclusion bodies were found in L-cells after inoculation with mouse tissues (obtained from mice inoculated with goose yolk sacs, as described). Some vacuolization of cells was also seen.

d) Serological surveys of caribou and reindeer

Table XII has summarized the results of serological testing of caribou and reindeer sera for chlamydial antibodies.

e) Serological surveys of human transients to northern Canada

The complement fixation results of sera from individuals before and after visits to northern Canada have been shown on Table XIII. As shown, four of the students developed chlamydial antibodies during their visits to Churchill (Manitoba), signifying that exposure had occurred in northern Canada. No illnesses were reported among any of the students.

TABLE IX
 COMPLEMENT FIXATION RESULTS FOR PASSAGE OF SNOW GOOSE MATERIAL IN MICE

Specimen for Passage	Type of Mice	Number of Pools	Total Number of Mice	Serum Pools with Chlamydial Group Antibodies	
				Number	Percentage Average Titres*
Goose yolk	Non-inoculated**	11	44	2	18
	Inoculated	4	48	3(3rd passage)	75
Goose yolk, embryo and tissue	Non-inoculated	43	129	1	2
	Inoculated	5	65	5(3rd passage)	100

* Titres are expressed as reciprocal of serum dilution

** Control mice

TABLE X
COMPLEMENT FIXATION RESULTS FOR PASSAGE OF CARIBOU MATERIAL IN MICE

Specimen for Passage	Type of Mice	Number of Pools	Total Number of Mice	Serum Pools with Chlamydial Group Antibodies	
				Number	Percentage
Caribou tissue	Non-inoculated**	7	28	0	0
	Inoculated	6	24	2(1st passage)	33

* Titres are expressed as reciprocal of serum dilution
 ** Control mice

TABLE XI
COMPLEMENT FIXATION RESULTS FOR PASSAGE OF CONTROL MOUSE MATERIAL IN MICE

Specimen for Passage	Number of Pools	Total Number of Mice	Serum Pools with Chlamydial Group Antibodies	
			Number	Percentage
Mouse tissue	3	12	0(2nd passage)	0

* Titres are expressed as reciprocal of serum dilution

TABLE XII

PREVALENCE OF CHLAMYDIAL GROUP ANTIBODIES AMONG CARIBOU AND REINDEER

Common Name	Location	Date of Collection	Number Tested	Number AC*	Number Positive	Complement Fixation Titres**						
						8	16	32	64	128	256	512
Caribou	(unknown)	(unknown)	4		2			2				
	Brochet	April 1968	20	2	9		2	6	1			
	Rankin Inlet	June 1968	62	44	3		3					
	Rankin Inlet	June 1970	4	2	1		1					
	Eskimo Point	Sept. 1970	16	3	7		1		3	1	1	1
Reindeer	Reindeer	(unknown)	4									
	Grazing Preserve	March 1969	14	10	1		1					

* Anticomplementary

** Titres are expressed as reciprocal of serum dilution

TABLE XIII

CHLAMYDIAL GROUP ANTIBODIES AMONG HUMAN
TRANSIENTS TO NORTHERN CANADA

(SUMMER, 1970)

Location	Individuals	Complement Fixation Titres*		
		Pre-Trip**	Post-Trip (1)***	Post-Trip (2)****
Churchill, Manitoba	S.S.	0	16	-
	C.T.	0	16	-
	M.G.	0	8	-
	P.W.	0	8	0
LaPeruse Bay, Manitoba (Snow goose colony, Watson Point)	P.M.	-	16	-
	J.P.	-	8	-
	B.G.	-	8	-
	L.L.	-	0	-

* Titres are expressed as reciprocal of serum dilution

** 1-3 weeks before trip

*** 1-4 weeks after trip

**** 6 months after trip

DISCUSSION

The objective of this investigation has been to determine some of the probable reservoirs of chlamydia for people of northern Canada. To achieve this aim, attention has been directed towards the isolation of a chlamydial agent from birds and mammals in the vicinity of the human settlements. The selection, collection and transport of specimens have received special consideration in the project.

As indicated in the literature review, birds throughout the world have been identified as hosts and reservoirs of chlamydia. Many of these birds, moreover, have served as reservoirs for transmission of chlamydia to man, and have resulted in many diseases. The birds in northern Canada, therefore, attracted considerable attention as possible chlamydial reservoirs for the people.

The species which has received the greatest emphasis as a probable reservoir has been the snow and blue goose (Chen caerulescens caerulescens). The main reasons for the selection of these birds were their tremendous numbers and wide distribution in northern Canada. Various opportunities for transmission of the organisms to humans were considered possible. In our experiments, evidence has been found that snow and blue geese not only contact chlamydia, as previously shown, but also harbor and transmit the organisms transovarially. This evidence has demonstrated that the former serological reactions in snow and blue geese sera were specific. In accordance with reports

by Myers et al (1969) and others as well as correspondence with R. Nichols (Department of Microbiology, School of Public Health, Harvard University, Boston) concerning avian strains of chlamydia, the snow and blue goose chlamydial agent appeared avirulent upon mouse passage.

The Canada goose (Branta canadensis) is another avian species which has received attention in this study. Like snow and blue geese, Canada geese are very numerous in northern Canada and have contact with the human population. The observation of inclusion bodies in a few goose eggs provided some evidence that Canada geese harbor chlamydia. As previously discussed, evidence has been presented for the existence of a snow and blue goose reservoir of chlamydia in the North. Since the habitats of snow and blue and Canada geese overlap one another, as shown by Godfrey (1966), it seemed likely that a Canada goose reservoir exists in northern Canada.

It has been described by Meyer (1967) and others that mammals often harbor chlamydia, but it has been indicated that they less frequently serve as reservoirs for transmission. It has been suggested by Eddie et al (1966), Spalatin et al (1971) and others, however, that mammals probably play a more important role in the transmission of chlamydia to humans than was originally thought. Mammals have received major

emphasis in this project because of the great number, wide distribution and close contact with the human population.

Among the various mammals in northern Canada, the caribou (Rangifer tarandus groenlandicus) has received the greatest consideration. These mammals are very abundant and are distributed throughout the North. Since caribou migrate and thus contact a great variety of animals, it was considered possible that they would be involved in the transmission of chlamydia. Serological tests demonstrated that caribou had been exposed to chlamydia, and the detection of chlamydial antibodies in sera of mice after passage of caribou tissue suggested that caribou also harbor the organisms. Since no deaths or pathological symptoms were observed after the mouse passages, the caribou chlamydial strain seems avirulent to mice.

Reindeer (Rangifer tarandus), a closely related species to the caribou, was another mammal investigated as a possible reservoir of chlamydia. The serological evidence presented has demonstrated that reindeer have been previously exposed to chlamydial agents. These mammals, however, are not widely distributed in northern Canada and have little contact with the human population. Reindeer, therefore, do not appear to play a major role in transmission of chlamydia to the people.

The isolation of chlamydia from the muskrat (Ondatra zebethicus spatulatus) by Spalatin et al (1966) from Saskatchewan added another species as a possible reservoir of chlamydia in northern Canada.

A few years later, Iversen et al (1970) provided serological evidence that muskrats from northern Canada were also exposed to chlamydial organisms, and Spalatin et al (1971) suggested these mammals as possible chlamydial reservoirs for some of the people of northern Canada.

Another possible reservoir of chlamydia in northern Canada, which has received great interest in this study, is the snowshoe hare (Lepus americanus). Isolated from these mammals in Saskatchewan by Spalatin et al (1966), the snowshoe hare chlamydia appears epizootic in these mammals and seems to be closely related to the previously discussed muskrat chlamydial strain. Snowshoe hares, therefore, seem to be more important as sources of infection than as reservoirs.

The isolation by Eddie et al (1966) of chlamydia from northern fur seals (Callorhinus ursinus) near the Pribilof Islands provided the first indication that aquatic mammals might be additional reservoirs of chlamydia. The demonstration of chlamydial antibodies in a Crabeater and Weddell seal by Sladen (1962) and Moore et al (1969), respectively, supported Eddie's finding of chlamydial infection in fur seals. The presence of chlamydial antibodies among the humans of the Pribilof Islands suggested the possible role of seals in the transmission of chlamydia to man. The wide distribution of seals and the great contact between seals and humans in the North further implicated seals in the transmission of chlamydia in northern Canada.

It is hypothesized that the high prevalence of chlamydial antibodies among the people of northern Canada is the result of continual contact with various bird and mammal chlamydial reservoirs, some of which have been identified in this thesis. Additional likely animal reservoirs have been identified by other workers. The specific animal reservoirs for the human inhabitants of the North may vary considerably among different settlements.

The experiments performed in this investigation have demonstrated the avirulence of snow goose and caribou chlamydial agents upon passage in laboratory mice. It was observed that no deaths or overt diseases occurred among the snow geese and caribou populations that were sampled for this study. It is thus suggested that the snow goose and caribou chlamydial agents might be avirulent within their respective host species. As discussed, Wilt and Hildes have continually detected low titre chlamydial antibodies among humans of northern Canada without finding any clinical disease. The present results showed that several Caucasians developed low titre chlamydial antibodies during visits to northern Canada, and again no disease was found. On the basis of these studies among inhabitants and transients, it might be speculated that the chlamydial agents which circulate in northern Canada are avirulent for man. It is well known, as described in Horsfall *et al* (1965), that most microbes circulate in nature as avirulent strains and that pathogenic strains are rather unusual. Chlamydial research over the past two decades has verified the prophetic words of Nocard, who admitted, while vigorously denying the existence of psittacosis, "La psittacose n'existe pas, si elle existe, elle est partout" (Storz, 1971).

SUMMARY

As summarized in Table XIV, evidence has been presented to identify certain species of birds and mammals as probable reservoirs of chlamydia for the people of northern Canada. This evidence has suggested that the agents are widespread in the wildlife of the North, and that many more animals are involved in transmission of chlamydia to the human inhabitants.

TABLE XIV
 PROBABLE RESERVOIRS OF CHILAMYDIA FOR PEOPLE
 OF NORTHERN CANADA

Evidence	Snow Goose	Canada Goose	Caribou	Reindeer	Muskrat*	Snowshoe Hare*	Seal**
Serology	●	●	●	●	●	●	●
Microscopic detection	●				●	●	●
Isolation	●				●	●	●

* From Spalatin et al (1966) and Iversen et al (1970)

** From Eddie et al (1966)

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APPENDIX

TABLE I

STATISTICAL ANALYSIS OF CHLAMYDIAL GROUP ANTIBODIES

AMONG HUMANS OF DIFFERENT AGE AND SEX GROUPS*

(P = 0.05)

Settlement	Year	Racial Group	χ^2 Value	Result
Baker Lake	1969	Eskimo	0.58	accept Ho**
Churchill	1970	Chippewan	0.66	accept Ho
Eskimo Point	1967	Eskimo	0.02	accept Ho
Old Crow	1960	Kutchin	3.01	accept Ho
Baker Lake	1969	Caucasian	0.83	accept Ho
Old Crow	1958	Kutchin	0.31	accept Ho
Churchill	1970	Cree-Metis and Cree	4.49	reject Ho
Baker Lake	1970	Eskimo	0.47	accept Ho

*Chi-square contingency test

**Ho (null hypothesis) = no difference between prevalence of antibodies among humans of different ages and sexes.

TABLE II
 STATISTICAL ANALYSIS OF CHLAMYDIAL GROUP ANTIBODIES
 AMONG HUMANS OF COASTAL AND INLAND SETTLEMENTS*
 (P = 0.05)

Settlement	Geographical Area	Year	Racial Group	χ^2 Value	Result
Pangnirtung	Coastal	1959	Eskimo	11.15	reject Ho**
Garry Lake	Inland	1959	Eskimo		

*Chi-square contingency test

**Ho (null hypothesis) = no difference between prevalence of antibodies among humans of coastal and inland settlements.