WEST NILE IN THE COMMUNITY:

Report of the 2004 West Nile Virus Seroprevalence Studies in Saskatchewan

Five Hills Health Region &
Blood Donors in Regina and Saskatoon

***Culex tarsalis* feeding Source: Agriculture and Agri-food Canada Saskatoon Research Centre**

A collaboration of

Saskatchewan Health,
Five Hills Health Region
Canadian Blood Services

August 2005

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November 2004

Prepared by:
The West Nile Virus Investigation Team:

Five Hills Health Region
Tara Schellenberg
Mark Vooght

Saskatchewan Health
Maureen Jackson
William Osei
Alexia Campbell
Ross Findlater/Eric Young
Brandace Winquist

ISBN 1-55157-050-5

ACKNOWLEDGMENTS

This survey was done in collaboration with the Five Hills Health Region, Canadian Blood Services, Saskatchewan Health and Health Canada. Saskatchewan Health and Health Canada are gratefully acknowledged for providing the funding support, which made this research possible. The Five Hills Health Region, Saskatchewan Health and Health Canada all provided significant in-kind contributions, for which we are very grateful. We thank Canadian Blood Services for arranging and providing the resources for the successful completion of this crucial serosurvey for blood donors in Regina and Saskatoon.

The authors thank: Dr. Eric Young for contributing to the study concept and design as well a for securing funding; Phillip Curry, Provincial West Nile Virus Coordinator for expert entomological advice; Chris Vohland, Assistant Laboratory Manager, Moose Jaw Union Hospital, for overseeing serological samples for shipment to NML; Dr. Greg Horsman, Medical Director of the Saskatchewan Provincial Laboratory for expert laboratory advice; Dr. Harvey Arstob and Dr. Michael Drebot, National Microbiology Laboratory (NML) for providing testing and results, as well as expert advice on laboratory methods; Helen Bangura , CD Epidemiologist for review of the manuscript; Victorian Order of Nurses, for providing blood letting services; Interview and data entry staff: Alan Easton, Kevin Klassen, Tricia Neustader, Valerie Peters, Colin Schellenberg, and Elaine Wotherspoon. We especially thank all of the study participants in the Five Hills Health Region, for generously donating their time and blood samples, and without whom the study would not have been possible. Thank you!

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Executive Summary

Background: In the summer of 2003 there was an outbreak of West Nile virus (WNV) in the province of Saskatchewan. The Five Hills Health Region, situated in the south central area of Saskatchewan, reported the highest number of WNV cases. A serologic survey was undertaken in the Five Hills Health Region to assess the seroprevalence (cases confirmed by a blood test) of the virus and the knowledge, attitudes and behaviours of the residents.

Methods: Study participants were contacted by telephone in March of 2004. All were asked to complete a questionnaire and to have a sample of blood drawn. Respondents had to be at least 18 years of age, and have been residents of the Five Hills Health Region between July 1st and September 15th, 2003. Blood samples were tested at the National Microbiology Laboratory (NML). All samples were screened for flavivirus immunoglobulin using a WNV IgG ELISA. Sera identified as positive and/or equivocal were further tested using a plaque reduction neutralization test (PRNT) to confirm the presence of WNV specific antibody. Descriptive analyses related to respondents' demographics, knowledge, attitudes, behaviours, and seropositivity were performed. Odds ratios were used to assess risk of WNV infection.

Results: In total there were 619 questionnaire respondents, of whom 501 (81%) donated a blood sample. The seroprevalence rate of WNV in the Five Hills Health Region was 9.98% (95% C.I. = 7.37-12.59%). Rural areas had a seropositivity of 16.8% compared to 3.2% in the urban area. Most (97%) of participants thought WNV was an important health issue. Forty-eight percent (48%) of the participants used DEET containing insect repellents always or most of the time. There was an overall good knowledge regarding the risk of WNV transmission by "mosquitoes" (99%), "blood transfusions" (70%), "organ transplants" (48%), and "contact with dead birds" (65%). There were good levels of knowledge regarding prevention of the spread of WNV: wearing protective clothing (96%); applying DEET containing insect repellents (95%); & avoiding going outside during peak mosquito times (96%). Rural residents were approximately six times more likely to be positive for WNV, compared to urban residents (OR=6.13, 95%C.I. = 2.82 – 13.34). Additionally, self-reported previous WNV diagnosis was an accurate indicator for laboratory confirmed seropositivity (OR=78.9, 95% C.I. = 17.45 – 362.81).

Interpretation: This is the highest seroprevalence rate of West Nile virus recorded in North America thus far. There were many factors that could have potentially influenced this outbreak such as the type of eco-region, early prolonged periods of hot weather, level of mosquito control programs, differences between urban and rural communities, occupation, and personal protective behaviours. Study limitations must be considered when interpreting study findings.

1.0 INTRODUCTION

Saskatchewan experienced its first ever outbreak of West Nile virus (WNV) during the summer of 2003. As of April 15th, 2004 there were 935 clinically confirmed cases of West Nile virus reported from the 2003 outbreak. According to information obtained from the 2003 West Nile Virus Human Surveillance database, 836 cases manifested in West Nile Fever (WNF), 62 in the more serious West Nile Neurological Syndrome (WNNS), 10 were asymptomatic, and 26 cases were categorized with unknown manifestation. Additionally, these cases included six deaths, of which West Nile was reported as having been causative in four of the deaths and contributory in the remaining two.

The Five Hills Health Region had the highest number of cases of WNV in Saskatchewan during the summer of 2003. A total of 223 cases of West Nile virus were reported. There were 205 cases of WNF, 13 cases of WNNS, two individuals were asymptomatic, and the clinical manifestation is unknown for three infected persons. In total, 32 hospitalisations were attributed to West Nile infection.

The West Nile Virus Serological Survey was undertaken as a joint project between the Five Hills Regional Health Authority, Saskatchewan Health, and Health Canada. This serological survey, the first of its kind in Saskatchewan, describes the population health impact of the WNV outbreak, WNV distribution in the population, and risk and protective factors associated with infection.

The objectives of this study were three fold: 1) to identify the prevalence of West Nile virus in select areas of the Five Hills Health Region in Saskatchewan in 2003; 2) to estimate the level of knowledge, attitudes, behaviours and practices in relation to West

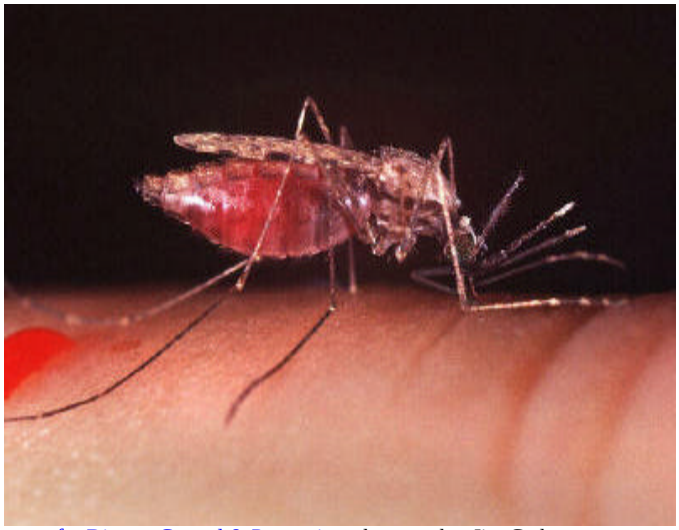
Nile virus infections and 3) to identify WNV risk and protective factors in the Five Hills Health Region.

This report will provide background information about the virus, study methods, and the survey results and conclusions.

2.0 BACKGROUND

2.1 WHAT IS WEST NILE VIRUS?

West Nile virus has recently emerged as an important human pathogen. It is an arbovirus from the *Flavirodae* family of viruses, and belongs to the Japanese Encephalitis sero-complex. Included in this sero-complex are other mosquito-borne viruses, such as St. Louis encephalitis virus, Murray Valley encephalitis virus, and the Kunjin virus. West Nile virus is also closely related to the viruses that cause Dengue and Yellow fever (1,2). Previously limited to Africa and the Mediterranean, West Nile has emerged as a novel threat to public health in Europe and North America only in the last few years. It infects humans, birds, and mammals including horses.

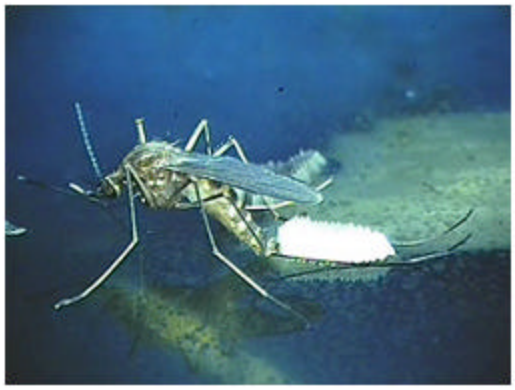


Source: Centers for Disease Control & Prevention, photographer Jim Gathany
This female *Anopheles gambiae* mosquito is feeding. You can see the blood swelling her abdomen.

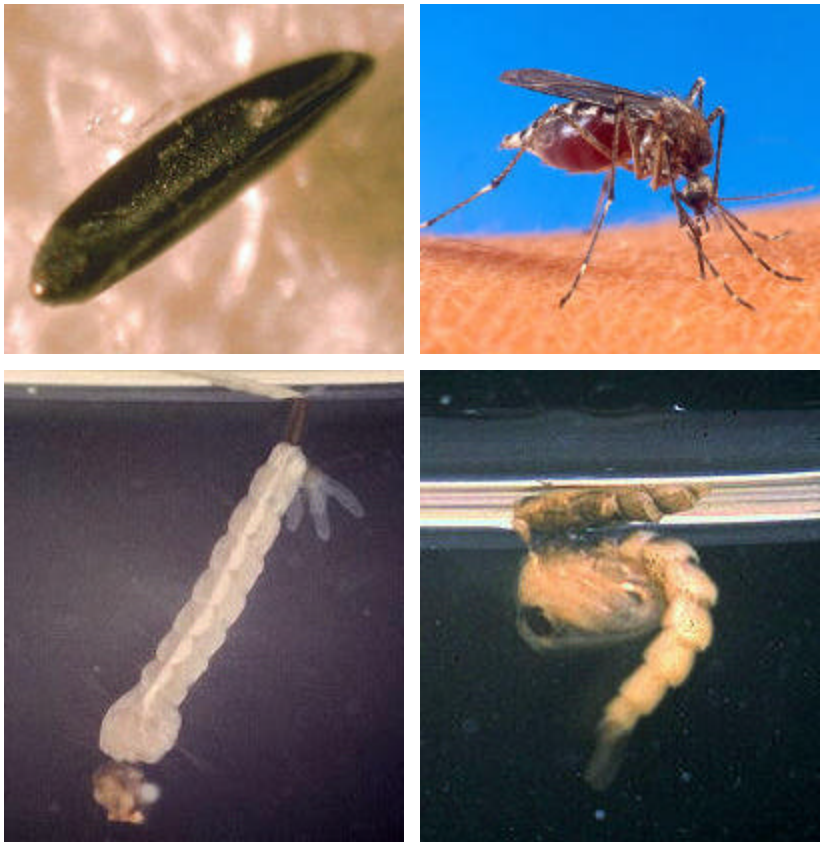
2.2 HISTORY OF WEST NILE

West Nile virus was first isolated in 1937 from the blood of a patient who was experiencing clinical encephalitis in the West Nile province of Uganda. Since 1937, infrequent human outbreaks, mainly associated with mild febrile illnesses, were reported mostly in groups of soldiers, children, and healthy adults in Israel and Africa (3). Since the mid-1990s, outbreaks of West Nile virus encephalitis have occurred in Algeria-1994, Romania-1996, Czech Republic-1997, Russia-1999, the United States 1999-2003, and Israel 2000 (2,4,5,6,7).

In 1999 West Nile made its first appearance in North America (8,9,10,11,12,13). This outbreak resulted in 59 cases hospitalized and seven deaths attributed to West Nile meningoencephalitis (8). In 2001, the first infected dead bird in Canada was identified in Southern Ontario (9). By 2002, the virus had spread extensively across the U.S. with 46 States reporting either human cases of WNV or mammalian, avian, or mosquito infection (14). During this same year Ontario and Quebec reported their first ever human cases. The outbreak expanded to Ontario, with 319 confirmed human cases and 18 deaths were attributed to West Nile (9,10,11). Cases of infected birds were reported in Nova Scotia, Quebec, Manitoba, and Saskatchewan for the first time in 2002 (13,15).



Culex mosquito laying eggs. Source: Centers for Disease Control & Prevention.
(View enlarged image.)

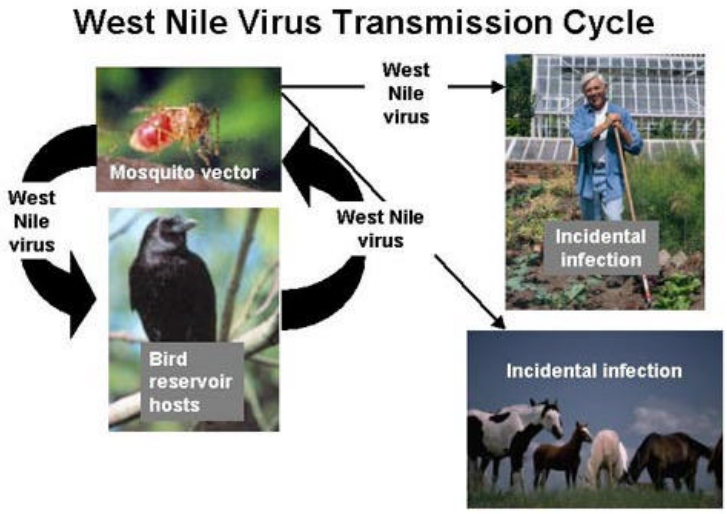


Life cycle of the yellow-fever mosquito (*Aedes aegypti*):
An egg (upper left) laid on the surface of the water hatches into an aquatic larva (lower left). The larva changes into an aquatic pupa (lower right), which then changes into a free-flying adult (upper right). The adult female bites a human to gather blood for laying eggs. Source: Centers for Disease Control & Prevention and USDA/ARS (upper right)

2.3 HOW IS WEST NILE VIRUS SPREAD?

Infected adult mosquitoes carry virus particles in their salivary glands and infect susceptible bird species during blood feeding meals. High West Nile antibody seroprevalence has been reported for several bird species. Competent bird reservoirs will sustain an infectious viraemia (virus circulating in the bloodstream) for approximately 1 to 4 days after exposure, after which time the bird host often develops immunity to the virus (16). During this period mosquitoes will continue to feed on infected birds. This ensures that the transmission cycle continues (3,16). Humans, horses, and other mammals only serve as incidental or "dead-end" hosts (see Figure 1)

FIGURE 1



Source:http://www.cdc.gov/ncidod/dvbid/westnile/birds&mammals.htm

2.4 MOSQUITOES AND WEST NILE VIRUS IN SASKATCHEWAN

Mosquitoes are one of the most familiar insects in Saskatchewan and can make outdoor activity during the summer quite a challenge when female mosquitoes go searching for a blood meal. A number of Saskatchewan mosquito species are host to several endemic arboviruses similar to West Nile, ranging from the innocuous Cache Valley and Flanders Hart Park viruses to more serious viruses such as St. Louis encephalitis and Western Equine encephalitis (17).

Unlike the outbreaks that have occurred in urban settings (i.e. New York, 1999, and Southern Ontario, 2002) in which the *Culex pipiens* mosquito is believed to have been the primary vector for disease transmission (18,19), in Saskatchewan West Nile virus has been isolated from three different mosquito species: *Culex tarsalis, Culex restuans*, and *Culiseta inornata* (17).

Culiseta. inornata is the most numerous of the three species, however it prefers its blood meals from large mammals and usually takes only one making viral transmission to

other mammals and humans relatively rare. *Culex restuans* is an efficient enzootic (animal) and bridge vector for WNV. While their numbers were once believed to be relatively low in Saskatchewan, unpublished mosquito trap data suggests that the species is more prevalent than originally assumed. *Culex tarsalis* on the other hand is believed to be the primary vector for transmitting West Nile virus from birds to humans since they are the most opportunistic of biters, will bite both birds and mammals, and have the greatest propensity to bite humans (20). *C.ulex tarsalis* adult biting mosquitoes are active chiefly during dawn and dusk, and remain relatively quiet during daylight hours (21).

2.5 CLINICAL FEATURES OF WEST NILE

An estimated 80% of West Nile viral infections are asymptomatic (5). In cases of apparent illness, the incubation period is approximately 3-14 days. According to the Canadian National Case Surveillance Tool for West Nile Virus with Saskatchewan modifications (CNCST), clinical manifestations are categorized into three subgroups: West Nile Fever, West Nile Neurological Syndrome, and West Nile virus Asymptomatic Infection. Epidemiological surveys in several countries suggest the once infected, approximately 1 in 5 people develop fever, and 1 in 150 develop central nervous system symptoms (22). The following are the clinical criteria for both the milder West Nile fever (WNF) and the more severe West Nile Neurological syndrome (WNNS), as adapted from the CNCST.

WNF (West Nile Fever) Clinical Criteria

Onset of fever and at least one of the following:

- myalgia (muscle pain)
- arthralgia (joint pain)
- headache
- fatigue
- photophobia (sensitivity to light)
- lymphadenopathy (swollen lymph glands)
- rash

WNNS (West Nile Neurological Syndrome) Clinical Criteria

Onset of fever and at least one associated neurological syndrome consistent with a diagnosis of

- encephalitis (inflammation of the brain) or meningoencephalitis (inflammation of the brain and lining of the brain and spinal cord)
- viral meningitis (inflammation of lining of the brain or spinal cord)
- acute flaccid paralysis (poliomyelitis-like syndrome or Guillain-Barré-like syndrome) (quick onset of limb weakness or paralysis)

2.6 RISK FACTORS FOR ACQUIRING WEST NILE VIRUS

Outdoor activity without the use of personal protective clothing, particularly during the high-risk periods of dawn and dusk, and exposure areas that provide a conducive environment for mosquito breeding, have been shown to be important risk factors for contracting West Nile virus.

A serosurvey conducted in New York City after the outbreak of West Nile encephalitis in 1999, found that individuals who spent more than two hours outdoors after dusk or dawn (peak biting periods for the Culex mosquito genus), and persons who did not use the insect repellant diethyltoluidine (DEET) had the highest rates of West Nile

virus seroprevalence (5). They also found that having seen a dead bird in one's neighbourhood was associated with a higher rate of infection. A 1996 study in Romania concluded that risk of infection was significantly higher for apartment dwellers with flooded basements (4,22).

Get double protection: wear long sleeves during peak biting hours, and spray repellent directly onto your clothes.

Drain standing water from around your home

Source: http://www.cdc.gov/ncidod/dvbid/westnile/prevention_info.htm

3.0 STUDY RATIONALE

Although the 2003 mosquito season ended in September, cases of West Nile virus from this outbreak continued to be reported from the Saskatchewan lab until as recently as March 12, 2004. As of April 15, 2004, 223 clinically confirmed cases of West Nile virus were recorded from the Five Hills region into the human WNV surveillance database. With a population of 55,246 the corresponding rate of illness was 404 per 100,000 (0.4%), which was the highest in Saskatchewan. In Canada, 17.5% of all West Nile virus infections for 2003 were in the Five Hills Health Region.

As it has been documented that approximately 80% of all WNV infections are asymptomatic[6], it stands to reason that the actual seroprevalence rate of the virus is much higher than the actual number of cases tested in 2003.

In areas where West Nile has only recently been introduced such as Romania in 1996, New York in 1999, and Ontario in 2001, seroprevalence of the WNV antibodies were found to be 4.1%, 2.6%, and 3.1% respectively (4,5,9).

This survey was carried out to estimate the highest possible infection rate likely to have occurred in Saskatchewan and to determine the knowledge, attitudes, behaviours, and practices associated with West Nile virus infection of residents in this area.

4.0 METHODS

4.1 THE STUDY

A cross-sectional study design was used to estimate the prevalence of WNV in the Five Hills Health Region (FHHR). A sample was drawn randomly from telephone directories for each community. Situated in south central Saskatchewan, FHHR has a population of approximately 55,000. Owing to its high number of reported WNV cases in 2003, the region was chosen in order to estimate the likely highest WNV seropositivity in Saskatchewan.

4.1.1 TARGET POPULATION

All residents of selected areas of the Five Hills Health Region who lived in the area between July 1, 2003 and September 15, 2003, who were 18 years or older were eligible to participate in the study. A previous diagnosis of WNV did not exclude potential subjects from participating in the study.

4.1.2 DATA COLLECTION

A questionnaire to elicit knowledge, attitudes and beliefs regarding WNV was administered; this questionnaire was modified slightly from Manitoba Health's instrument (31, 32). Following completion of the telephone administered questionnaire, nursing staff booked an appointment with the participant to have a sample of blood drawn.

The seroprevalence of West Nile virus antibodies previously found in other studies ranged from 2% to 6%. For the present study, we assumed a prevalence of 4%, with a 2% precision, and significance level of 0.05. Based on this estimate, the minimum sample size was calculated to be 369. The study aimed to collect information from 400 to 600 individuals.

In total, 619 subjects responded to the questionnaire, and of those, 501 provided a blood sample. An accurate response rate to the questionnaire cannot be calculated, as the number of participants who refused to answer the survey was not collected. However, it is estimated that 20% of those contacted participated in the questionnaire, and of these respondents, 81% provided a blood sample. All non-responses, whether there was no answer to the telephone number or the person answering the phone refused to participate, were grouped together.

A pilot study was conducted to assess the questionnaire responses, wording and process. Pilot study subjects were recruited outside of the study areas in the Five Hills Health Region; the pilot participants were only given an interview, no blood was collected. Figure 2 shows the study areas chosen within the Five Hills Health Region.

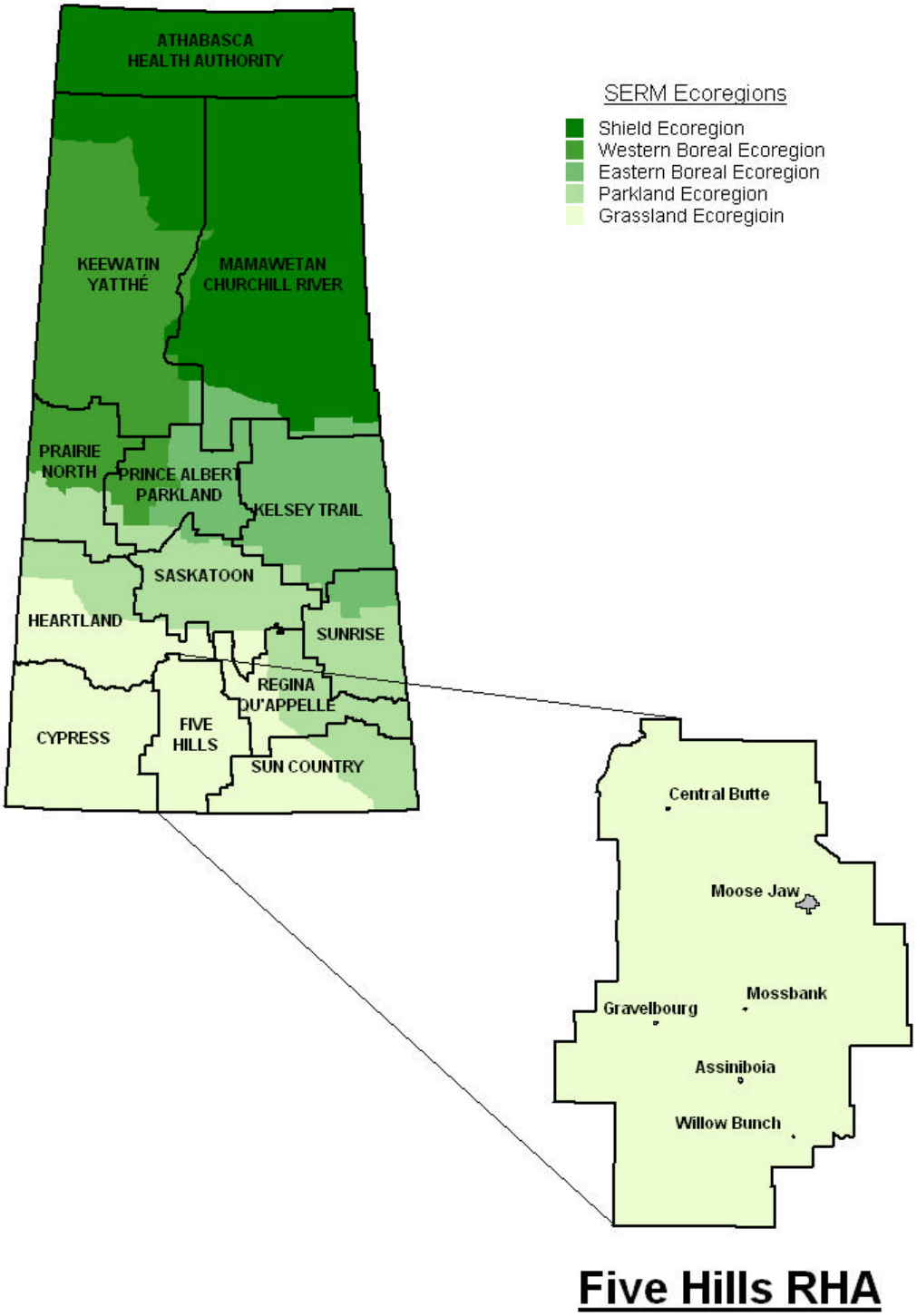
Blood samples were tested at the National Microbiology Laboratory (NML) in Winnipeg. All sera were screened for flavivirus IgG using a WNV antibody capture ELISA as described previously (23). Reactive samples were confirmed for the presence of WNV specific antibody by carrying out a plaque reduction neutralization test (PRNT). The standard PRNT was performed essentially as described by Beaty *et al.* (24). In brief, mixtures of virus (200 plaque forming units) and dilutions of human sera were incubated at 37^0 C for 1 hr, and added to six well plates containing Vero cell monolayers. After a 37^0 C incubation of 1 hr a nutrient-agar overlay was added, and the plates were placed in a CO_2 incubator for approximately 3 days after which a second overlay was added containing neutral red as a vital stain. Plates were then checked periodically over the next few days for plaque formation. The highest serum dilution with a plaque reduction of at least 90% was defined as the titration end point.

4.1.3 STATISTICAL ANALYSIS & ETHICS

Data were entered into Epi Info 2002 and analysis was completed using Epi Info and SPSS, Version 11.5, SPSS Inc., Chicago, IL.

The study applied for and obtained ethics approval from the Biomedical Ethics Board at the University of Saskatchewan, as well as from the Ethics Boards in both the Five Hills Health Region and Health Canada.

Figure 2: Map of Five Hills Health Region, Study Sites and Eco-Region



4.2 TARGET POPULATION

Potential study participants were contacted in three study areas chosen to reflect the size and effectiveness of their levels of mosquito control programs. These programs emphasized larval development site identification, mosquito source reduction, and larviciding. It was questioned whether or not WNV infection might vary according to the level and intensity of mosquito control programs. Gravelbourg, Mossbank and Willow Bunch were areas with low levels of mosquito control programs; Assiniboia had medium and Moose Jaw had a large and intensive mosquito control program. Gravelbourg, Mossbank, Willow Bunch and Assiniboia (Areas I and II) were grouped into the "rural" category, and Moose Jaw was considered "urban" (Area III) (see Table 1).

Area I- Gravelbourg, Mossbank & Willow Bunch- low mosquito control program
Area II- Assiniboia- medium mosquito control program
Area III- Moose Jaw- large and intensive mosquito control program

Table 1. Mosquito control programs, population and area of the three study regions in the Five Hills Health Region

	Mosquito Control Program Rating	Larval Development Site ID	Mosquito source reduction	Larvaciding	Pop.	Area Km^2
Area 1	Low	-	-	-	2,109	5.02
Area 2	Medium	x	x	x	2,710	3.95
Area 3	High	x	x	x	34,185	47.17

Source: Population and area figures from Corporate Information Technology Branch, Saskatchewan Health.

Based on information provided by the Provincial West Nile Coordinator/ Entomologist, Area II and III both engaged in about the same level of mosquito management activities. However, there were no mosquito control measures implemented within a 3-km radius surrounding either area. Due to lack of pest control in this 3-km

buffer zone, the potential for in-migration of mosquitoes from property adjacent to either area existed. Moose Jaw's larger size means that the percentage of treated area relative to the untreated 3-km buffer zone surrounding the city was much higher than that of Assiniboia. Infected mosquitoes could effectively penetrate much further into Area II (Assiniboia) than they could into area III (Moose Jaw). In addition, people living outside of the towns in either Area I or II would not benefit from their respective mosquito control programs, while most Area II residents would. Hence, Area II was given a medium mosquito control program rating while Area III was ranked as a large and intensive mosquito control program.

Dipping for mosquito larvae Source: Phil Curry

4.3 DATA COLLECTION

Potential study subjects were contacted using the reverse telephone directory, names and addresses were removed and numbers were randomly assigned to one of four telephone interviewers. Telephone interviews were conducted Monday through Friday between 1730 and 2130 hours and Saturday between 0900 and 1530 hours. Originally, all valid prefixes in the three areas were entered into an excel format and the last four digits of the phone numbers were randomly generated. Utilizing this method during the pilot study, which was conducted in Central Butte, however, yielded a high percentage of not-in-service numbers, business numbers, and fax lines. Due to time constraints surrounding data collection it was decided to contact only individuals with directory listed telephone numbers for the study. The phone numbers were scrambled to make the list more random. Of the available telephone numbers, 766 numbers were dialled in Area I, 884 dialled in Area II, and 2,257 dialled in Area III.

Telephone interview testing for Knowledge, Attitude, Behaviour/Practices (KAB/P) occurred between March 6th and April 1st, 2004. Interviews were approximately fifteen minutes in length. Please see Appendix A for a copy of the questionnaire used in the analysis. Interviewers filled out hard copy questionnaires, which were entered by a data entry clerk into Epi Info 2002 (version 3.2. The Five Hills epidemiologist routinely checked data for accuracy. After administration of the telephone survey, participants were contacted by the Victorian Order of Nursing (VON) to set up a location for blood drawing.

Blood samples were processed at the Moose Jaw Union Hospital, sent to and stored at four degrees Celsius in the Provincial Laboratory in Regina, and subsequently

shipped to the National Microbiology Laboratory (NML) in Winnipeg. All samples were screened for flavivirus immunoglobulin using a WNV IgG ELISA; sera identified as positive and/or equivocal were further tested using a plaque reduction neutralization test (PRNT) to confirm the presence of WNV specific antibody. Titres of antibody greater than or equal to 1:40 dilution following PRNT were considered positive.

4.4 STATISTICAL ANALYSIS

Analysis of the data proceeded in two stages. First, descriptive level analysis of variables in the study was completed. This stage of the analysis focused on survey responses only, serology results were not analyzed until testing at the NML was complete. The second stage of the analysis included serology results. Odds ratios and 95% confidence intervals were calculated to examine factors associated with WNV infection.

5.0 RESULTS

5.1 DESCRIPTIVE ANALYSIS

5.1.1 FIVE HILLS RESIDENTS

Survey respondents were predominantly female (70%), which indicates there was an underrepresentation of men in this study. The age distribution of the study sample reflected the age distribution of the health region, with the exception of the 20-29 year old population, which was under-sampled in this study. The majority of the study sample (52.2%) had completed at least some post-secondary education. Respondents were equally divided between rural and urban residences (50.4% and 49.6% respectively). This differs somewhat from the population distribution between rural and urban areas in

the Five Hills Health Region (38% and 62% respectively). The most common occupations cited were: office, factory, retail, and restaurant in the urban region; and farming in the rural areas. Overall the sample contained a wide range of participants, however non-responders and the sampling method may have led to the overrepresentation of some groups and underrepresentation of others. Potential biases are discussed in the limitations section. The detailed demographic characteristics of the study sample are available below in Table 2.

TABLE 2: CHARACTERISTICS OF SURVEY RESPONDENTS (N=619) BY STUDY AREA

	Rural n=312 (%)	Urban n=307 (%)	Total n=619 (%)
Sex			
Male	88 (28.2%)	96 (31.3%)	184 (29.7%)
Female	222 (71.2%)	211 (68.7%)	433 (70.0%)
N/A	2 (0.6%)	0 (0%)	2 (0.3%)
Age			
18-29	14 (4.5%)	61 (19.9%)	75 (12.1%)
30-39	39 (12.5%)	48 (15.6%)	87 (14.1%)
40-49	77 (24.7%)	82 (26.7%)	159 (25.7%)
50-59	75 (24.0%)	61 (19.9%)	136 (22.0%)
60-69	45 (14.4%)	27 (8.8%)	72 (11.6%)
70+	60 (19.2%)	28 (9.1%)	88 (14.2%)
N/A	2 (0.6%)	0 (0%)	2 (0.3%)
Mean age	53.6	45.7	49.7
Education			
Grade School	80 (25.6%)	45 (14.7%)	125 (20.2%)
High School	76 (24.4%)	90 (29.3%)	166 (26.8%)
Post Secondary	153 (49.0%)	170 (55.4%)	323 (52.2%)
N/A	3 (1.0%)	2 (0.7%)	5 (0.8%)
Indoor/Outdoor Job			
Mainly Indoor	127 (40.7%)	179 (58.3%)	306 (49.4%)
Mainly Outdoor	102 (32.7%)	70 (22.8%)	172 (27.8%)
Unemployed	78 (25.0%)	57 (18.6%)	135 (21.8%)
N/A	5 (1.6%)	1 (0.3%)	6 (1.0%)
Specific Job Type			
Farming	69 (22.1%)	16 (5.2%)	85 (13.7%)
Government	7 (2.2%)	14 (4.6%)	21 (3.4%)
Education	24 (7.7%)	12 (3.9%)	36 (5.8%)
Health Care	18 (5.8%)	34 (11.1%)	52 (8.4%)
Trade	22 (7.1%)	24 (7.8%)	46 (7.4%)
Parks & Recreation	4 (1.3%)	3 (1.0%)	7 (1.1%)
Office, factory, retail& restaurant	48 (15.4%)	81 (26.4%)	129 (20.8%)
Other	4 (1.3%)	5 (1.6%)	9 (1.5%)

The geographic distribution of WNV varied across the region. Of the 501 sera tested, 50 were found to be positive for WNV specific antibody. The overall WNV seroprevalence for the Five Hills Health Region was 9.98% (95% C.I. =7.37-12.59%). Rates of infection were higher in the rural areas than the urban (See Table 3 below).

TABLE 3: SEROPREVALENCE BY URBAN-RURAL AREAS

Area	WNV Seroprevalence
Rural	
Assiniboia	15.3%
Gravelbourg	20.8%
Mossbank	18.8%
Willow Bunch	14.7%
Urban	
Moose Jaw	3.2%

The questionnaire shows that residents of the Five Hills Health Region have a good understanding and awareness of WNV. Most respondents felt that West Nile virus was an important health issue (96%). Almost half of the respondents used DEET containing insect repellents at least most of the time (48%). A majority of participants recognized that WNV is transmitted by "mosquitoes" (99%), "blood transfusions" (70%), and "organ transplants" (48%). However, nearly two-thirds (65%) of those surveyed thought WNV could be contracted by "contact with dead birds", which is not the case. Some other erroneous assumptions regarding the possible means of contracting WNV, included: "shaking hands with a WNV positive patient" (6%); "having sex with a WNV positive patient" (6%); "being in the same room as a WNV positive patient" (3%); and "drinking infected water" (28%). There were good levels of knowledge regarding how to

prevent the spread of WNV, including, “wearing protective clothing” (96%), “applying DEET containing insect repellents” (95%), and “avoiding going outside during peak mosquito times” (96%). However, many believed “washing hands regularly” (51%), and “using a bug zapper” (72%), were effective preventive measures. Table 4 below includes further details.

TABLE 4: KNOWLEDGE, ATTITUDES, AND BEHAVIOURS OF SURVEY RESPONDENTS BY RESIDENCE (N=619)

	RURAL N=312 (%)	**URBAN N=307 (%)**	**TOTAL N=619 (%)**
Has heard of WNV	309 (99%)	306 (99.7%)	615 (99.4%)
Believes WNV can be contracted through:			
Mosquito bites	306 (98.1%)	305 (99.3%)	611 (98.7%)
Blood transfusions	205 (65.7%)	229 (74.6%)	434 (70.1%)
Organ transplants	141 (45.2%)	157 (51.1%)	298 (48.1%)
Contact w/ dead birds	201 (64.4%)	200 (65.1%)	401 (64.8%)
Believes WNV can be contracted through:			
Sexual contact w/ WNV case	16 (5.1%)	22 (7.2%)	38 (6.1%)
Same room as WNV case	12 (3.8%)	6 (2.0%)	18 (2.9%)
Drinking infected H2O	76 (24.4%)	94 (30.6%)	170 (27.5%)
Shaking hands w/ WNV case	20 (6.4%)	18 (5.9%)	38 (6.1%)
Believes WNV to be an important health issue	298 (95.5%)	294 (95.8%)	592 (95.6%)
Believes repellents w/ DEET are worth using	256 (82.1%)	262 (85.3%)	518 (83.7%)
Supports adulticiding	241 (77.2%)	225 (73.2%)	466 (75.3%)
Believes the following behaviours prevent WNV:			
Wearing long-sleeved/ protective clothing outdoors	299 (95.8%)	298 (97.1%)	597 (96.4%)
Avoid outdoors during peak mosquito hours of dusk & dawn	296 (94.9%)	297 (96.7%)	593 (95.8%)
Applying DEET	298 (95.5%)	292 (95.1%)	590 (95.3%)
Applying Off-botanicals	93 (29.8%)	93 (30.3%)	186 (30%)
Removing standing H2O	301 (96.5%)	298 (97.1%)	599 (96.8%)

Table 4 cont'd	**RURAL N=312 (%)**	**URBAN N=307 (%)**	**TOTAL N=619 (%)**
Believes the following behaviours prevent WNV:			
Washing hands	164 (52.6%)	152 (49.5%)	316 (51.1%)
Apply repellent w/ ingredients other than DEET or citronella	102 (32.7%)	94 (30.6%)	196 (31.7%)
Wearing a mask	52 (16.7%)	38 (12.4%)	90 (14.5%)
Using a bug zapper	235 (75.3%)	210 (68.4%)	445 (71.9%)
Always, or most of the time practiced the following behaviours:			
Avoid mosquito areas	113 (36.2%)	104 (33.9%)	217 (35.1%)
Restrict outdoor activity	29 (9.3%)	26 (8.5%)	55 (8.9%)
Wear long sleeves or pants	154 (49.4%)	101 (32.9%)	255 (41.2%)
Apply repellent – DEET	145 (46.5%)	153 (49.8%)	298 (48.1%)
Apply repellent – Non-DEET	15 (4.8%)	23 (7.5%)	38 (6.1%)
Inspected screens in home during summer 2003	197 (63.1%)	186 (60.6%)	383 (61.9%)
Repaired screens in home during summer 2003	78 (25%)	76 (49.4%)	154 (24.9%)
Found sources of standing H2O on property	159 (51.0%)	162 (52.8%)	321 (51.9%)
Reduced source(s) of standing H2O on property	83 (52.2%)	113 (69.8%)	196 (61.1%)
Always or most of the time used DEET by Age Category			
18-59	112 (35.9%)	128 (41.7%)	240 (38.8%)
60+	33 (10.6%)	25 (8.1%)	58 (9.4%)

Odds ratios were used to calculate the association between WNV infection and survey respondent characteristics. Only two variables were statistically significant, rural residence and a self-reported previous WNV infection. Rural residents were approximately 6 times more likely to be WNV positive compared to urban residents (OR=6.1, 95%CI=2.82 – 13.34). And, those who self-reported a previous WNV diagnosis were 79 times more likely to be positive for WNV, compared to those who did

not report a previous diagnosis of WNV. (OR= 78.9, 95% CI=17.45 – 362.81). Please see Table 5 for more details.

TABLE 5: ASSOCIATIONS BETWEEN WNV BLOOD RESULT AND DEMOGRAPHIC CHARACTERISTICS (N=501)

Variable	POSITIVE N=50 (10%)	NEGATIVE N=451 (90%)	O.R.	95% C.I.
Age				
0-59	33 (66.0%)	328 (72.7%)	1.37	0.74 - 2.56
60+	17 (34.0%)	123 (27.3%)		
Sex				
Male	14 (28.0%)	129 (28.6%)	1.03	0.54 – 1.97
Female	36 (72.0%)	322 (71.4%)		
Education (N=499)				
≤ high school	29 (58.0%)	205 (45.7%)	1.64	0.91 – 2.97
Post secondary	21 (42.0%)	244 (54.3%)		
Residence				
Rural	42 (84.0%)	208 (46.1%)	6.13*	2.82 – 13.34
Urban	8 (16.0%)	243 (53.9%)		
Job Type (N=378)**				
Outdoor				
Indoor	19 (46.3%)	107 (31.8%)	1.86	0.96 – 3.58
	22 (53.7%)	230 (68.2%)		
Avoid areas where mosquitoes were likely to be a problem†				
Yes				
No	14 (28.0%)	171 (37.9%)	1.57	0.82 – 3.00
	36 (72.0%)	280 (62.1%)		
Restricting outdoor activity†				
Yes				
No	2 (4.0%)	46 (10.2%)	2.73	0.64 – 11.59
	48 (96.0%)	405 (89.8%)		
Wore long and long pants when outdoors†				
Yes	23 (46.0%)	185 (41.0%)	0.82	0.45 – 1.47
No	27 (54.0%)	266 (59.0%)		
Applied insect repellents containing DEET†				
Yes	19 (38.0%)	214 (47.5%)	1.47	0.81 – 2.69
No	31 (62.0%)	237 (52.5%)		
Applied insect repellents containing non-DEET†				
Yes	3 (6.0%)	28 (6.2%)	1.04	0.30 – 3.54
No	47 (94.0%)	423 (93.8%)		

Table 5 cont'd	POSITIVE N=50 (10%)	NEGATIVE N=451 (90%)	O.R.	95% C.I.
Used two or more personal protective measures†				
Yes	17 (34.0%)	196 (43.5%)	1.49	0.81 – 2.76
No	33 (66.0%)	255 (56.5%)		
Self reported previously diagnosed with WNV				
Yes	13 (26.0%)	2 (0.44%)	78.89*	17.45 – 362.81
No	37 (74.0%)	449 (99.6%)		

* Statistically significant
** Excluded unemployed, retired and refused responses
† Performed behavior always or most of the time

As rural residents were shown to be at greater risk of being WNV positive, we conducted a separate analysis, in order to look more closely at the risk of WNV infection for Saskatchewan farmers. Respondents were asked, 'what type of job do you have'? A list of possible professions was not read to respondents; those answering farming were grouped into the farming occupation category. Among total questionnaire respondents, 85 people (13.7%) were farmers. The majority of farmers (81%) lived in a rural area, but only 22% of rural residents in this sample farmed. Twelve (19%) farmers tested positive for WNV. **Farmers were approximately two and a half times more likely to be positive for WNV, compared to non-farmers** (OR=2.48, 95% CI=1.14 – 5.30, p=0.01). However, because only one occupation per participant was recorded, and a number of farmers will have multiple occupations, some farmers may have been classified as having other occupations. Future analyses of this data should include regression models to control for confounding factors and to determine predictive variables of WNV infection.

TABLE 6: DEMOGRAPHIC CHARACTERISTICS, FARMERS VS. NON-FARMERS

	Farmers n=85 (%)	Non-Farmers n=534 (%)	Total n=619 (%)
Sex			
Male	40 (47.1%)	144 (27.0%)	184 (29.7%)
Female	44 (51.8%)	389 (72.8%)	433 (70.0%)
N/A	1 (1.2%)	1 (0.19%)	2 (0.3%)
Age			
18-29	3 (3.5%)	72 (13.5%)	75 (12.1%)
30-39	12 (14.1%)	75 (14.0%)	87 (14.1%)
40-49	28 (32.9%)	131 (24.5%)	159 (25.7%)
50-59	21 (24.7%)	115 (21.5%)	136 (22.0%)
60-69	11 (12.9%)	61 (11.4%)	72 (11.6%)
70+	9 (10.6%)	79 (14.8%)	88 (14.2%)
N/A	1 (1.2%)	1 (0.19%)	2 (0.3%)
Mean age	50.8	49.5	49.7
Education			
Grade School	21 (24.7%)	104 (19.5%)	125 (20.2%)
High School	24 (28.2%)	142 (26.6%)	166 (26.8%)
Post Secondary	39 (45.9%)	284 (53.2%)	323 (52.2%)
N/A	1 (1.2%)	4 (0.75%)	5 (0.8%)
Residence			
Rural	69 (81.2%)	243 (45.5%)	312 (50.4%)
Urban	16 (18.8%)	291 (54.5%)	307 (49.6%)
Blood Results (n=501)			
Positive	12 (19.0%)	38 (8.7%)	50 (10.0%)
Negative	51 (81.0%)	400 (91.3%)	451 (90.0%)

5.1.2 CANADIAN BLOOD SERVICES (CBS) AND WEST NILE VIRUS

Although WNV statistics often capture those people who become ill, report their symptoms to a physician, and are tested for WNV; another approach is to examine blood collected routinely from those without symptoms. This method can provide a clearer picture of those who are positive (and may not be aware) and those who are not positive for WNV in the community.

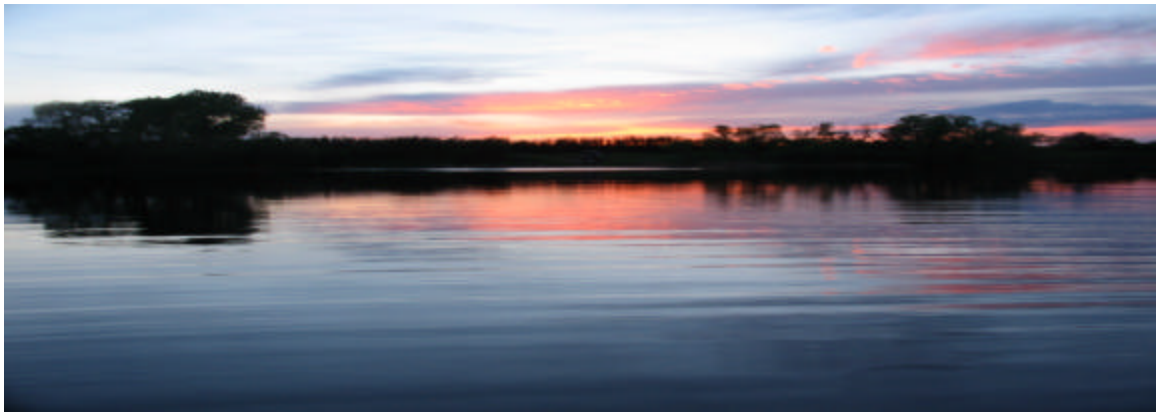
Similar to the above study done in the Five Hills Health Region, the testing of blood collected through Canadian Blood Services (CBS) provides us with information on

the prevalence of WNV in the general populations of Regina and Saskatoon. It is perhaps helpful and interesting to compare rates identified in the three regions.

From the period June 10 to June 18, CBS recruited willing participants in Regina and Saskatoon blood donation clinics to agree to a WNV serological test, and respond to a questionnaire regarding West Nile virus. The questionnaire elicited responses on previous WNV infection history, residence, sex and age.

A blood sample from each respondent was tested for WNV at the National Microbiology laboratory in Winnipeg. All samples were tested for WNV Immunoglobulin (IgG ELISA); positive and/or equivocal results subsequently underwent plaque reduction neutralization confirmatory testing (PRNT). Titres of antibody greater than or equal to 1:40 dilutions following PRNT were considered positive.

In total, 596 persons completed the questionnaire and agreed to have their blood tested for the study, and of these participants, 595 provided blood samples for serology. **The overall WNV infection rate was 1.0% of blood donors,** with Regina and Saskatoon reporting similar rates. Rates differed significantly from those identified in the Five Hills Health Region study**.** Table 7 has detailed demographic information of study participants.



Culex mosquitoes are most active at dusk in Saskatchewan and are more abundant near water sources. Source: Brandy Winquist, 2005.

Table 7: Demographic characteristics of Blood Donors according to WNV Serological Status, SK 2004 (n=595)

Variable	Frequency n=595 (%)	
	WNV positive (n=6)	WNV negative (n=589)
Sex		
Male	2 (33.3%)	299 (50.8%)
Female	4 (66.7%)	290 (49.2%)
Total	6 (1.0%)	589 (99%)
Age*		
18-29	0 (0.0%)	114 (19.4%)
30-39	1 (16.7%)	89 (15.1%)
40-49	0 (0.0%)	204 (34.6%)
50-59	4 (66.7%)	132 (22.4%)
60-69	1 (16.7%)	49 (8.3%)
70+	0 (0.0%)	1 (0.2%)
Mean age	54	43
Total	6 (1.0%)	589 (99%)
Clinic Location		
Regina	3 (50.0%)	297 (50.4%)
Saskatoon	3 (50.0%)	292 (49.6%)
Total	6 (1.0%)	589 (99%)
Residence		
Regina Qu'Appelle Health Region	3 (50.0%)	292 (49.6%)
Saskatoon Health Region	2 (33.3%)	277 (47.0%)
Other	1 (16.7%)	20 (3.4%)
Total	6 (1.0%)	589 (99.0%)

* as of July 1st, 2004

Age (50 years versus older than 50 years) was the only variable for which there was a statistically significant difference between those who tested positive for WNV compared to those who tested negative. The odds of a person over 50 testing positive for WNV were 11 times greater, compared to a person aged less than 50 years old (OR=11.2, 95%CI=1.23-530.1). However, it is worth noting that the numbers are small, which makes it difficult to know if this age group is truly at an increased risk for WNV infection.

Table 8: Infection rate of WNV among blood donors, SK 2004 (n=595)

Variable	Infection rate of WNV (rate per 1,000)
Sex	
Male	6.6 per 1,000
Female	13.6 per 1,000
Age	
18-29	0
30-39	11.1 per 1,000
40-49	0
50-59	29.4 per 1,000
60-69	20.0 per 1,000
70+	0
Clinic Location	
Regina	10.0 per 1,000
Saskatoon	10.2 per 1,000
Residence	
Regina Qu'Appelle Health Region	10.2 per 1,000
Saskatoon Health Region	7.2 per 1,000
TOTAL infection rate	10.1 per 1,000

In the summer of 2003, when Saskatchewan experienced an outbreak of West Nile virus, the rates of reported disease were higher in Regina Qu'Appelle Health Region (RQHR) compared to the Saskatoon Health Region (SHR). During the outbreak RQHR reported 256 cases of WNV (1.1 cases per 1,000). In SHR there were 62 reported cases of disease (0.22 cases per 1,000).

During the 2003 outbreak, 11 blood donors from Regina and Saskatoon donor clinics were reported WNV positive. Following this outbreak, the Canadian Blood Services (CBS) and Saskatchewan Health collaborated to conduct a WNV serosurvey on prospective blood donors in order to compare the WNV positive cases reported in blood clinics during the outbreak. The 2004 serosurvey was similar to the community WNV survey conducted in the Five Hills Health Region (as described above).

In the present analysis using 2004 CBS data, there were three positive cases among blood donors in Regina Qu'Appelle Health Region and two in Saskatoon Health Region. Regina had a rate of 10.2 per 1,000 cases of WNV, while Saskatoon had rate of 7.2 per 1,000 blood donors (Table 8).

During the 2003 outbreak of WNV in Saskatchewan, the Regina Qu'Appelle Health Region had a much higher rate of disease compared to SHR. In this sample of blood donors, however, there is not a large difference between the two regions. Caution must be taken when making broad interpretations, however, as the numbers of positive cases is very small in the CBS sample. Additionally, we are unable to control for potential confounding variables, such as whether or not blood donors worked outside or inside. Please see Table 9 for more information.

Table 9: Sex, Age and Seropositivity among blood donors by region of residence, SK 2004 (n=574)

Variable	Frequency n=574 (%)	
	Regina Qu'Appelle Health Region (n=295)	Saskatoon Health Region (n=279)
Sex		
Male	137 (46.4%)	153 (54.8%)
Female	158 (81.0%)	126 (45.2%)
Total	295 (100%)	279 (100%)
Age		
18-29	54 (18.3%)	59 (21.1%)
30-39	48 (16.3%)	37 (13.3%)
40-49	107 (36.3%)	88 (31.5%)
50-59	61 (20.7%)	70 (25.1%)
60-69	25 (8.5%)	24 (8.6%)
70+	0 (0.0%)	1 (0.4%)
Mean age	43	43
Total	295 (100%)	279 (100%)
Seropositivity		
Positive	3 (1.0%)	2 (0.7%)
Negative	292 (99.0%)	277 (99.3%)
Total	295 (100%)	279 (100%)

6.0 STUDY LIMITATIONS

There are several limitations to the study, based on the methodology employed. It is important to bear these limitations in mind when reading the results of the study.

Study subjects who had been previously diagnosed with WNV were not excluded from participating in our serosurvey. Out of 501 participants who provided a blood sample, 15 (3%) self reported a previous diagnosis with WNV. In our sample, there were 50 individuals who tested positive for WNV, including 13 of the 15 who had self-reported a previous WNV infection. It is possible, that previously diagnosed WNV patients were more likely to volunteer for our serosurvey, introducing selection bias. If the 13 previously diagnosed subjects are removed from the seroprevalence calculation, the seroprevalence rate is 7.4% (as opposed to 10%). These subjects may be artificially increasing the seroprevalence rate to a number greater than what would be expected in a random sample of Five Hills Health Region residents.

Other limitations of the study include the use of telephone administered questionnaire using numbers from the reverse directory of the phone book. Those without telephones, use cell phones exclusively, or have an unlisted number would not have had an opportunity to participate in the study. According to SaskTel, the primary telephone service provider in Saskatchewan, approximately 1 in 20 phone numbers are unlisted (25).

Females were over sampled in this study (70% of sample), as we did not employ sex-specific selection methods. The first person to answer the phone that met the eligibility criteria was invited to participate, which is most likely why there has been an overrepresentation of women. Although it is not certain how this would influence the

study findings, it is possible that those who responded spent less time outside than those missed. If true, this may have led to an underestimate of WNV.

Recognizing that this survey was administered over seven months after the West Nile virus outbreak, recall bias could also have had an impact on survey responses. Participants were telephoned in March and April 2004. However, the questionnaire was designed to recall participants' behaviour during the summer of 2003. The extent of over- or under-reporting bias on behaviours, practices, and exposure is unknown. Furthermore, we cannot determine whether the level of knowledge that respondents had at the time of the questionnaire was the same as it was during the outbreak.

There were no reported laboratory confirmed human cases of WNV detected in 2002. Although WNV was present in corvids and horses during 2002, which makes it is possible that 2003 was not the first year WNV was introduced in the human population. If true, then the seroprevalence rate could be a cumulative rate of WNV over two years (or more), instead of a point prevalence estimate for 2003. However, given that there were no reported human cases in 2002, it is more likely that our study is measuring the 2003 point prevalence of WNV.

There was a difference of 118 between the number of surveys completed and the number of blood samples collected. This difference was attributed mainly to the timing of blood collection and the start of the farming season. As blood collection extended into the month of April, seeding in rural areas may have prevented some individuals from attending a blood collection clinic. This could have accounted, at least in part, to the 118 individuals who were lost to follow up for blood collection; these individuals would have been mainly from rural areas and the influence on serology results is unknown.

It is important to bear in mind that any statistically significant relationships outlined in the report are indicative of association only. It is difficult to claim causation given the cross-sectional nature of this survey. Multivariate statistical analysis is also needed to ensure that other confounding factors have not skewed the association between those risk factors identified and the WNV outcome.

7.0 DISCUSSION

In 2004, West Nile virus seroprevalence in the Five Hills Health Region in Saskatchewan was found to be approximately 10%. To date, this is the highest seroprevalence rate for WNV in North America (5,9,18). The province of Saskatchewan experienced the highest number of WNV cases in Canada during the summer of 2003 (12). Approximately 80% of all WNV infections are asymptomatic (5,26); therefore a high WNV survey seroprevalence was to be expected.

The Five Hills Health Region was selected for study, as it had the highest number of reported cases in 2003. Limited resources were concentrated in one geographical area of the province, where the highest seropositivity rate was likely to be measured. Other regions of the province had fewer or no reported cases

In our questionnaire, respondents were asked, "before today, were you ever previously diagnosed with WNV?". Fifteen (15) respondents answered yes to this question. Following serological testing, thirteen (13) of those fifteen blood samples were found to actually be positive for WNV. Individuals who self-reported a previous WNV diagnosis were ~80 times more likely to actually be WNV positive, compared to those who did not self-report a previous WNV diagnosis (OR=78.0, 95% C.I.=17.45 – 362.81).

Therefore, self-reported WNV infection was a fairly accurate measure for laboratory confirmed WNV positivity, in our study sample. This finding could be useful for subsequent questionnaires that rely on self-reported WNV infection alone.

7.1 BIRD & MOSQUITO FACTORS RELATED TO SEROPREVALENCE OF WNV

While the Saskatchewan rate of WNV was reportedly the highest in the country during the 2003 outbreak, high rates of testing likely also contributed to the elevated number of WNV-positive cases in Saskatchewan. In the three-month period covering July to September 2003 inclusive, 4861 serum specimens from Saskatchewan health regions were analyzed by the Provincial Laboratory, with 937 positive cases (in this period). Thus the yield was approximately one positive case for every 5 WNV serological tests, over the 3-month period.

Birds and horses are also susceptible to West Nile virus. In birds, species of the family Corvidae are very susceptible to the virus, and have a high fatality rate (27,28). Infected corvids usually exhibit mortality before, or early into the onset of human cases (27), which makes them a good sentinel for the virus. In 2003 there were 157 documented cases of dead corvid species positive for WNV in the FHHR (29).

Several other contributing factors relating to the weather in 2003, the landscape ecology of the region, and presence of capable mosquito vectors can help explain the high seroprevalence rate. These factors include above normal temperatures in the spring and summer period, prolonged periods of hot weather and warm nights, and an extended frost-free period, which all led to a long WNV exposure period, particularly in southern prairie grassland areas. Other factors included a pronounced enzootic amplification in

birds and other animals, with the resultant increased transmission by "bridging" mosquito vectors[1] of the virus to humans.

The primary mosquito vector driving the outbreak in birds and humans in Saskatchewan was *Culex tarsalis*.

The Missouri Coteau area of the Five Hills Health Region is an area of rolling hills located south of Moose Jaw. It is located in the mixed grassland ecoregion of the prairie ecozone and is characterized hilly topography, many sloughs, potholes, numerous creeks and coulees. *Culex tarsalis* is widely distributed throughout the area. The region is consistently hotter and drier than other areas. As a result, *Culex tarsalis* usually starts earlier in the season and there are more generations and higher numbers of adult mosquitoes than in more northerly parkland areas.

The *Culex tarsalis* species is more numerous in southern prairie grassland ecoregion where there are numerous wetlands, hotter temperatures and more open grassland habitat that is home to numerous bird and other wildlife species. Although overall populations were low, *Culex tarsalis* was more numerous in southern grassland ecoregions than in the parkland or boreal transition ecoregions. The range of average counts in New Jersey mosquito traps placed in 34 different communities in July and August 2003 were 16.8 to 56.6 for grassland habitat and 6.4 to 29.9 for parkland habitat (29,30).

[1] A vector that carries the disease from one animal group – birds to another group – humans

Table 10: Effects of Size of Community and Intensive Larviciding on Culex tarsalis Counts – Saskatchewan – July-August, 2003

	Large Communities over 5,000 people			Small Communities under 5,000 people
	Intensive larviciding			No larviciding or applied sporadically
n=17	*Cx. tarsalis*		n=17	*Cx. tarsalis*
Mean	**11.9 ***			**32.5 ***

*** Average seasonal catch of *Culex tarsalis* (P=0.000129)**

Rural communities, due to their small size and close proximity to abundant *Culex tarsalis* habitat could not employ intensive larviciding programs over large areas of the landscape. They are literally small "islands in a sea of habitat". In addition, they are easily within the flight range of adult *Culex tarsalis* mosquitoes, which can quickly migrate into areas treated with larvicide. Their mosquito control programs focus on source reduction, public education, and personal protection. This coupled with the fact that rural residents are outdoors more often and for longer periods of time, the warmer weather in 2003, the abundance of habitat for *Culex tarsalis* and the eco-system of the region, may help to explain the difference in the distribution of seropositivity rates in Saskatchewan.

7.2 KNOWLEDGE, ATTITUDE & BEHAVIOUR REGARDING WNV

In terms of behaviour and knowledge regarding WNV, this study revealed a number of interesting findings. Study subjects felt DEET containing insect repellents were worth using, but half of them did not use them at least most times while outside. Persons who were over the age of 60 were less likely to use insect repellents containing DEET at least most of the time, compared to respondents 18-59. This finding has possible implications for public messaging regarding WNV risk.

In terms of knowledge, many respondents believed that WNV could be contracted through drinking infected water, indicating that public messaging regarding the link between WNV and standing water may be misunderstood by the public. In terms of prevention of WNV, many respondents felt that washing hands and using a bug zapper prevented WNV. If participants are using washing their hands as a sole means of prevention, this should be addressed in public messaging. Ironically, use of a bug zapper may attract more mosquitoes than it actually captures, thus increasing the risk of exposure and possible infection.

Culex mosquito development sites
Source: Phil Curry, Saskatchewan Health

In this study, it was discovered that the odds of being positive for WNV were six times higher for rural residents compared to urban residents. As the seroprevalence of WNV is considerably higher in rural as compared to urban respondents, it is important to ask what factors contribute to this increase in infection among rural residents. For example, are differences in rates between urban and rural people due to differences in their knowledge, attitudes or behaviours? The data shows that while rural respondents were slightly less likely to apply DEET containing repellent and to reduce sources of standing water on their property, they were much more likely to wear long sleeves or pants and slightly more likely to avoid mosquito areas. No other notable differences appeared between the knowledge, attitudes and behaviours of rural as compared to urban people that would contribute to the substantially higher rates of WNV in rural residents. However, rural communities had lower levels of mosquito control programs, compared to the urban community; this, coupled with the fact that rural residents are outdoors more often and for longer periods of time, and the eco-system of the region, may help to explain the difference in the distribution of seropositivity rates in Saskatchewan.

Mosquito floodwater development site Source: Phil Curry, Saskatchewan Health

8.0 CONCLUSIONS

With the aforementioned limitations in mind, this serological survey of WNV in Saskatchewan found 10% (95% C.I. 7.37 – 12.59%) of survey respondents had antibodies to WNV. This is a striking result and one that requires further investigation, in order to fully elicit contributing factors.

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APPENDIX A

WEST NILE VIRUS SEROLOGICAL SURVEY:

Questionnaire

FIVE HILLS REGIONAL HEALTH AUTHORITY,

SASKATCHEWAN HEALTH &

Health Canada

Population Health Branch
Saskatchewan Health

Questionnaire for Saskatchewan Health/Health Canada

Seroprevalence and Case Control Study

Hello my name is _____ and I am calling on behalf of the Five Hills Health Region, Saskatchewan Health, and Health Canada to invite you to take part in a 15-minute survey on West Nile Virus. As you may be aware there was a large outbreak of West Nile Virus in the region this past summer. What we are trying to understand is why some people became infected and others did not.

There are two parts to this study. If you agree to participate we will do a phone interview today, as well as take your first name, address and verify your phone number so a nurse can contact you. This nurse will arrange a time for a small sample of your blood to be taken. The reason for taking your blood is so that we can get an estimate of the number of people who came in contact with West Nile Virus last summer.

Any information you provide will remain strictly confidential. By participating you will be helping us to better respond to potential problems that West Nile may cause your community in the future.

A. Would you like to participate in this study? *(If yes go to B, Thank them first before continuing)*

(If first answer is NO ask if there is a better time in which you could call back, If no again, Thank respondent and say goodbye)

No - Thank-you. Good-bye.

B. Did you live in your area between July 1st and September 15th , 2003 (within 3km)? *(If YES GO TO C)*

No - Unfortunately, you do not meet the criteria required for this study, thank you for your time, Good-bye.

C. Are you at least 18 years of age? *(If respondent is not 18, ask if there is anyone else in the household who would be willing to take part in the survey. If so go back and reread intro)*

No- Unfortunately, you do not meet the criteria required for this study, thank you for your time, Good-bye

D. Before today have you ever been diagnosed with West Nile Virus?
(*If Yes ask)* When were you diagnosed? _____ (Month/Year).
And what type did you have? _____(Asymptomatic, WVF, WNM/E)

KNOWLEDGE SECTION

1. Before today, have you ever heard of West Nile Virus?
    1. YES
    2. NO *(Go to Q # 8)*
    3. DK/ DR *(Go to Q# 8)*

2. Are you aware of how humans contract West Nile virus? Yes / No

(*If "NO" skip to question 4)*

3. How is it spread to humans?

(Check all that apply)

(Do not read this list)

____ mosquito bites
____ through blood
____ by birds
____ by handling dead birds
_____ by mosquitoes infected by biting birds
____ by close contact with a person who has West Nile virus
____ by Deer mice
_____ from horses
_____other

4.

Please tell me if you agree with the statement that you can get West Nile virus in the following ways.		**Yes**	**No**	**Don't know**
A	Mosquito bites	3	2	1
B	Sexual contact with someone who has an active case of the WNV	3	2	1
C	Being in the same room with someone who has an active case of the WNV	3	2	1
D	Drinking infected water	3	2	1
E	Blood Transfusions	3	2	1
F	Organ transplants	3	2	1
G	Contact with dead birds	3	2	1
H	Shaking hands with someone who has an active case of the WNV	3	2	1

5.

Please tell me if you agree with the statement that the following actions will prevent West Nile virus infection.		Yes	No	Don't know
A	Wearing long-sleeved shirts or other protective clothing outdoors	3	2	1
B	Avoiding going outside during the peak mosquito hours of dawn or dusk	3	2	1
C	Washing hands regularly	3	2	1
D	Applying a repellent containing DEET such as "Off" or "Muskol"	3	2	1
E	Applying a repellent containing *p-menthane, 3 diol* such as "Off-botanicals"	3	2	1
F	Applying mosquito repellent containing active ingredients other than DEET or citronella	3	2	1
G	Wearing a mask	3	2	1
H	Removing standing water from places where water collects	3	2	1
I	Using a bug zapper	3	2	1

6. This past summer, do you remember receiving any information about West Nile virus and/or about how to avoid mosquito bites from the following sources (*READ EACH LINE AND CHOICES):*

Source:	**1.Yes**	**2. No**	**3. DK/DR**
Newspaper			
Radio			
TV			
Internet			
Neighbours/friends/acquaintances			
Doctors/health care professionals			
Posters / Brochures			
Child's School			
Health Line			

***IF RESPONSE IS NO OR DK/DR FOR ALL OF #6 SKIP TO QUESTION #8)*

7. In your opinion, which source of information was the most useful to you in finding out about West Nile virus and/or about how to avoid or prevent mosquito bites? *(SAME LIST, BUT DO NOT READ)*

Source:	**Check if listed:**
Newspaper	
Radio	
TV	
Internet	
Neighbours/friends/acquaintances	
Doctors/health care professionals	
Postures / Brochures	
Child's School	
Health Line	

The next few questions have to do with your outdoor activity. Please take your time to think carefully about your general pattern of being outdoors. This may include when you're around your home, at the park or at work, or any other time spent outdoors.

8a) Would you classify your job as mainly indoor or mainly outdoor?

1. Mainly indoor *(less than 1 hour outside while at work)*
2. Mainly outdoor (*1 or more hours outside while at work*)
3. Unemployed or retired (*go to Q#9)*
4. Refused *(Go to Q #9)*

8b). What type of job do you have *(Do not read Choices*)?

1. Farming
2. Government
3. Education
4. Health Care worker
5. Tradesperson
6. Parks & Recreation
7. Office, factory, retail or restaurant worker
8. Other, please

specify:_____

9. On a **typical WEEKDAY** this past summer, how often did you go outdoors during the following time periods? *(4-8 AM; 8AM-5PM MEANS AFTER 8AM, i.e., 8:15am, etc.)*

a) Early morning (4-8AM) 1 Always 2 Sometimes 3 Rarely 4 Never
b) Daytime (8AM-5PM) 1 Always 2 Sometimes 3 Rarely 4 Never
c) Evening (5PM-9PM) 1 Always 2 Sometimes 3 Rarely 4 Never
d) Night time(9PM-4AM) 1 Always 2 Sometimes 3 Rarely 4 Never

10. On a **typical WEEKEND** when you were not on vacation how often did you go outdoors during the following time periods?

a) Early morning (4-8AM) 1 Always 2 Sometimes 3 Rarely 4 Never
b) Daytime (8AM-5PM) 1 Always 2 Sometimes 3 Rarely 4 Never
c) Evening (5PM-9PM) 1 Always 2 Sometimes 3 Rarely 4 Never
d) Night time (9PM-4AM) 1 Always 2 Sometimes 3 Rarely 4 Never

11. What effect did West Nile virus have on your summer plans this year? Would you say it had…
    1. A big effect- we changed our plans
    2. A medium effect - we considered it when making plans
    3. No effect - it didn't influence our plans
    4. Don't know / didn't have any plans

12. HOW OFTEN DID YOU DO ANY OF THE FOLLOWING ACTIVITIES THIS PAST SUMMER?

a) Avoid areas where mosquitoes are likely to be a problem?
1. Always 2. Most of the time 3. Sometimes 4. Rarely 5. Never

b) Restrict outdoor activity
1. Always 2. Most of the time 3. Sometimes 4. Rarely 5. Never

c) Wear long sleeves, long pants when outdoors

1. Always 2. Most of the time 3. Sometimes 4. Rarely 5. Never

d) Apply insect repellent containing DEET, when outdoors

1. Always 2. Most of the time 3. Sometimes 4. Rarely 5. Never

e) Apply non-DEET insect repellent when outdoors (i.e., p -menthane 3, diol)

1. Always 2. Most of the time 3. Sometimes 4. Rarely 5. Never

f) Did you inspect screens in your home last summer

1. Yes
2. No
3. DK/DR
4. Refused

g) Did you repair screens in your home last summer

4. Yes
5. No
6. DK/DR
4. Refused

EXPOSURE ASSESSMENT

13A) Were you bitten by any mosquitoes this past summer?

1. Yes
2. No *(GO TO Q14)*
3. DK/ DR *(GO TO Q14*)
4. Refused *(GO TO Q14)*

13B) When do you think you were bitten most? Please give the month or months (*i.e., July, August…*)_____

14. During this past summer (July1st –Sept15), did you see a dead crow, magpie, grey jay, or blue jay in your area of residence, recreation, or work (*THESE ARE DIFFERENT TYPES OF BIRDS*)?

1. Yes
2. No
3. DK/DR

15A). Did you find any sources of standing water within 100 m or yards of your home, including on your property?

1.Yes
2. No (*GO TO Q #16)*
3. DK/ DR (*GO TO Q #16)*
4. Refused (*GO TO Q #16*)

15B). Did you take action to reduce the source or sources of standing water on your property?

1. Yes
2. No
3 DK / DR
4 Refused

16a) Did you spend more than one week at a time living away from your home this summer between July 1st and September 15th?

1.Yes
2. No (*GO Q# 16d)*
3. DK/ DR (*GO to Q# 16d)*
4. Refused (*GO to Q# 16d)*

16b). Please indicate how many weeks that were spent living away from your home between July 1st and September 15th, 2003.

_____ Number of weeks

16c) Where was this time spent (*they can give several answers, and ask for specific name of location*)

_____

Next we would like to ask you a few questions about your health

17. Were you ill with a fever anytime between July 1st and September 15th, 2003?

1. Yes
2. No
3. DK / DR
4. Refused

18A). Do you have any medical conditions for which you require regular medical care and/or treatment (i.e., diabetes)?

1. Yes
2. No (*Skip to Q#19)*
3. DK (*Skip to Q#19)*
4. Refused *(Skip to Q#19)*

18B) Please tell me if you have any of the following conditions? (*They should answer either "yes", "no", or "refuse to tell")*

___Cancer
___Diabetes
___Other (Specify)
___Heart Disease
___High Blood Pressure
___REFUSED TO SPECIFY

19) Did you have a blood transfusion anytime between July 1st and Sep 15th?

1. Yes
2. No
3. Don't Know
4. Refused

20a) Have you ever been an organ or bone marrow donor?

1. Organ donor
2. Bone Marrow donor
3. Organ & Bone
4. No

20b) Have you ever been an organ or bone marrow recipient?

1. Organ recipient
2. Bone Marrow Recipient
3. Organ & Bone
4. No

21) Are you taking any medication on a continual basis that may affect your ability to fight infection? *(This may include steroids like prednisone or cortisone; chemotherapy treatments for cancer or other diseases)? (NOTE TO SURVEYOR—STEROID PUFFERS DO NOT COUNT)*

1. Yes
2. No
3. DK
4. Refused

ATTITUDE & BELIEFS

22. In your opinion, how important is West Nile virus as a health issue

1. Not at all important
2. Somewhat important
3. Very important
4. Don't know
5. Refused

23a) Do you feel that common Insect repellants containing DEET are worth using?

1.Yes (Go to Q#24)
2. No
3. DK *(Go to Q#24)*
4. Refused *(Go to Q#24)*

23b) If you did not use DEET repellents, why not? *(CHECK ALL THAT APPLY, DO NOT READ CHOICES)*

___ Not applicable, did use DEET
___Perceived very low risk of West Nile infection
___Concern over pesticides
___Did not see any mosquitoes
___Too much trouble
___Concern over interaction with sunscreen
___Other (specify):_____

23c) The most common approach to fighting the spread of West Nile Virus is to use a type of pesticide in bodies of water to kill mosquito larvae. If this is not effective, the other option is to use a different type of pesticide that is sprayed in the air to kill adult mosquitoes. This type is sprayed, usually at night or early in the morning. Would you strongly support, somewhat support, somewhat oppose or strongly oppose the use of spraying to kill adult mosquitoes in your area to fight the spread of West Nile Virus?

1. Strongly Support
2. Somewhat support
3. Somewhat oppose
4. Strongly oppose
5. DK
6. Refused

We just have a few more questions that will help us to categorize your information.

24A) Just for confirmation, your telephone number is_____.

(Ask if this is the best number where they can be reached, If not get a number which is most convenient_____)

25. Can we please have your first name. This information will help use to correlate the responses you just gave us with your blood test results. _____

****(DO NOT ASK IF THIS IS OBVIOUS & USE DISCRETION****

26. Even though it is fairly obvious by your voice, can you please just confirm whether you are male or female?

1. Male
2. Female
3. Refused

27. What is your age in years? ____

28. What is the highest level of formal education you have completed? *(DO NOT READ, CHECK ONLY ONE OF THE FOLLOWING. FILL IN GRADE IF APPLICABLE).*

____Grade _____
____High School Diploma/ G.E.D.
____Some trade, technical, vocational, or business college
____Some (Community) College, CEGEP
____Some University
____Diploma or certificate from trade, technical, or vocational school or business college
____Diploma or certificate from (Community) College, CEGEP
____University degree
____ REFUSED

29. What is your residential address?

a) No. and street: _____
Town/City/Village: _____
Postal Code: _____

(Rural Residents)

b) Legal land location: _____
Township and range: _____
Distance and direction from nearest town/city: _____ miles / kms
_____ direction (N, E, SW, etc.)

30. Is your mailing address different from where you live?

1. Yes
2. No (*Closing Remarks*)

31. What is your mailing address?

PO Box: _____
No. and street: _____
Town/City/Village: _____
Postal Code: _____

Closing:

THANK YOU FOR YOUR TIME. As I mentioned at the beginning of this survey you will be receiving a call in the next day or two from the Victorian Order of Nurses agency to set up a time when a sample of blood can be taken.
If you have any questions at all you can contact:
Tara Schellenberg or Dr. Mark Vooght at the Public Health department of the Five Hills Health Authority, their phone number is 306-691-6400.
Alexia Campbell at 306-787-8044 or Dr. William Osei at 306-787-1580 at Epidemiology, Research and Evaluation Unit of Saskatchewan Health.
Thanks you very much for your time.

GOODBYE.

Unique patient number _____
Selection Area _____

Date of Interview (DD/MM/YY)_____
Interviewer's Initials_____

ISBN 1-55157-050-5