Estimating the Infection Rate:
West Nile Virus Seroprevalence in Alberta

Alberta

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Executive Summary

This report presents the results from the second West Nile virus (WNv) Seroprevalence Study, undertaken by Alberta Health and Wellness during March through June 2007.

Alberta experienced its first locally acquired clinical cases of WNv (275 confirmed human cases) in the summer of 2003. Since then, clinical (e.g., symptomatic) infections have been detected every year, though the numbers have fluctuated. From 2004 to 2006, there were very few clinical cases with a combined total of 51. In 2007 there was an increase in the number of infections with 320 cases and the first two deaths associated with WNv in the province. During the 2008 WNv season, only one clinical infection was detected in Alberta and it is thought to be travel-related.

For every clinical infection there are many more undetected infections in humans, since the majority of WNv infections have no symptoms and are never detected. The WNv seroprevalence studies have been conducted to estimate how many Albertans have actually been infected with the virus and how the virus is spreading in the province. The studies also help to increase our understanding of the cycles of WNv to help predict yearly clinical infection rates. A secondary objective is to investigate the knowledge and attitudes of Albertans about WNv and about measures used to protect against infection.

Study participants were recruited by telephone equally from the Palliser Health Region (which has had the highest incidence of clinical WNv cases since 2003) and the rest of the province. The respondents were also sampled differentially according to place of residence to assess differences between rural and urban Albertans.

Potential participants, 18 years of age and older, were asked to complete a telephone survey and donate a blood sample. The telephone interview used questions adapted from previous studies about WNv.

Seroprevalence results

From March to June of 2007, a total of 3,747 Albertans responded to the telephone survey. Of these, 1,960 also donated a blood sample. The blood samples were analyzed to determine the prevalence of WNv antibody among study participants in order to estimate WNv seroprevalence.

Overall in Alberta, the WNv seroprevalence rate was estimated to be 0.95 per cent just prior to the 2007 WNv mosquito season. The estimate of seropositive Albertans includes any exposure to WNv that could have occurred since 2003. This suggests that more than 20,000 Albertans were infected with WNv at some point from 2003 to 2006.

Albertans living in the Palliser Health Region, located in the southeast part of the province, had the highest seroprevalence rates: 4.17 per cent of individuals living in a rural area and

1.87 per cent of individuals living in an urban area were estimated to have antibodies to WNv.

The findings in this current sero-survey appear slightly higher than what was estimated in 2004; however the point estimates are not statistically different.

Survey results

Awareness of WNv was high in both the 2004 and 2007 survey, and the large majority of Albertans could identify how the virus is spread, who is at greater risk of severe complications, and how to protect themselves from infection. However, some misinformation existed. A large percentage believed incorrectly that young children were at a higher risk for severe complications from WNv infection, and that the virus could be passed to humans from dead birds.

The level of worry about WNv infection appears to have declined slightly throughout the province since 2004. Those living in the south east part of the province where the risk of infection is greater had a higher reported level of worry than in the rest of Alberta.

The percentage who reported using mosquito repellent "always" or "most of the time" increased significantly from 2004 to 2007. Participants from the Palliser region were more likely to use repellent containing DEET than those in the rest of Alberta. Women were less likely to use DEET than men. Overall, people were more likely to report engaging in personal protective behaviours if they were female and worried about contracting WNv. Younger adults, especially in the 18 to 24 year age group, and those who spent more time outdoors were less likely to practice personal protective behaviours often. Interestingly, those that scored higher on the WNv knowledge questions were also less likely to engage in personal protection. This finding highlights the discrepancy between awareness and behaviour that has been observed in other studies of WNv (Elliot et al., 2008; McCarthy et al., 2001; Mostashari et al., 2001).

Conclusions

The findings from this survey suggest that the overall seroprevalence of WNv infection did not change in Alberta between 2004 and 2006. A limitation to the point estimates of seroprevalence is the small number of WNv positive blood samples they are based on, resulting in large confidence intervals.

Results from the telephone survey component provide evidence that self-reported personal protective behaviours have increased since 2004, in particular those using mosquito repellent. Certain groups, such as young adults, and those who spend more time outdoors, are less likely to report practicing protective behaviours. The level of worry about WNv appears to have decreased, although it remains higher in higher risk areas of Alberta.

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Background

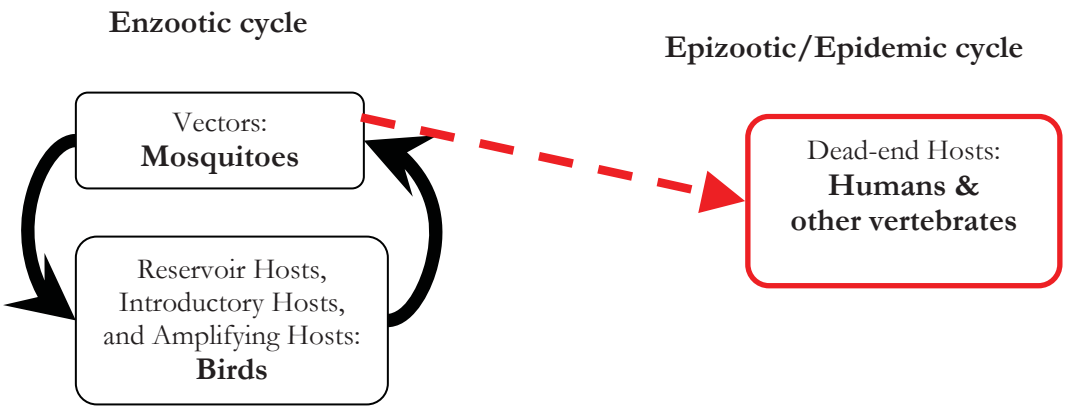
The Virus and its Ecology

WNv is an arbovirus (arthropod transmitted virus) from the *Flaviviridae* family. It is closely related to a group of viruses that cause disease around the globe such as dengue fever, yellow fever, Japanese encephalitis and tick-borne encephalitis. WNv shares a serocomplex with other mosquito-borne viruses that can cause encephalitis namely St. Louis, Murray Valley, the Kunjin subtype, and Japanese encephalitis (Brinton, 2002).

WNv cycles enzootically between mosquitoes and birds, although it can infect and cause illness in a range of animal species including humans in an epidemic/epizootic manner. Infection has been detected in many non-avian species including reptiles (e.g., alligators), rodents (e.g., squirrels, chipmunks), ungulates (e.g., mountain sheep, goats, cattle), equines (e.g., horses, mules, donkeys), skunks, rabbits, bears, dogs and cats (Dauphin et al., 2004). Humans and to a greater degree, equines appear to be most severely affected by WNv, and may develop clinical disease.

Most non-avian species, including humans infected with WNv, are referred to as dead-end hosts and do not generally contribute to viral spread. This is because most develop transient and insufficient viremia to infect mosquitoes and contribute to the virus's cycle in nature (Hayes, Komar & Nasci, 2005). However, recent evidence suggests that some non-avian vertebrates could possibly act as amplifying hosts in the environment. For example, squirrels and alligators have been shown to develop sufficient viremia to infect mosquitoes (Kramer, Styer & Ebel, 2008).

Figure 1. WNv Transmission Cycle



More than 280 species of birds and over 40 species of mosquitoes have been found to be infected with WNv in North America. This wide range of vectors and hosts makes WNv an ecological generalist compared to other arboviruses and has undoubtedly contributed to its geographic spread (Kramer et al., 2008).

Ornitophilic mosquitoes (i.e., those feeding on birds) are the principal vectors of WNv (Rappole, Derrickson & Hubalek, 2000). Among the many mosquito species found carrying the virus, *Culex* types are the most important in transmitting the disease to humans. The *Culex tarsalis* mosquito, which breeds primarily in irrigated farmlands, has been primarily responsible for WNv transmission in western Canada (Hayes et al., 2005; Turell et al., 2001). The ability to over-winter in many species, including *Culex tarsalis*, facilitates virus survival within infected mosquitoes and may play an important role in the persistence of the virus in North America.

Birds generally become infected with WNv after being bitten by an infected mosquito, and can then pass the virus on to other mosquitoes amplifying the prevalence of infection. Direct bird to bird transmission has been observed in laboratory settings where infected corvids have been housed with non-infected corvids, however the importance of this mode of transmission in nature is unknown (Hartemink, Davis, Reiter et al., 2007).

Susceptibility to infection varies among bird species. Those that are susceptible to infection and develop significant blood levels of the virus are important reservoir, introductory, and amplifying hosts. Members of the Corvidae family, including crows, blue jays, magpies and ravens, are very susceptible to the effects of WNv. So too are some species of raptors, robins and house sparrows (Hayes et al., 2005). Competent host birds will develop infectious viremia for about one to four days after exposure, after which they generally develop lifelong immunity (Dauphin & Zientara, 2007).

Infected birds have the ability to spread the virus into new territories, acting as introductory hosts. For the virus to become established in a new territory, several factors are necessary: appropriate climate, abundant presence of mosquito vectors and cross-species transmission to numerous indigene birds, which act as amplifying hosts. Once introduced, the virus has the ability to persist from year to year.

Clinical Characteristics and Pathology in Humans

Mosquitoes are the primary mode of transmission of WNv to humans. The *Culex* species of mosquito have their peak feeding during dusk and dawn, thus the risk for infection in humans is higher during these time periods (Campbell et al., 2002). Direct human to human transmission has not been documented however, other forms of transmission have been observed and include (Hayes et al., 2005; CDC-DVBID, 2004):

- organ transplantation from an infected donor;
- blood transfusion with infected blood;
- mother to child transmission either through trans-placental or breastfeeding; and
- occupational exposure in laboratory workers, turkey-breeders and crocodile farm workers.

Most infected individuals have no symptoms. When symptoms do appear they normally occur two to 15 days after the exposure and include flu-like symptoms (e.g., headache, fever, body aches, rash), and are referred to as WNv non-neurological syndrome. A small proportion of those infected develop a more serious illness referred to as WNv neurological syndrome. Symptoms may include encephalitis, meningitis and acute flaccid paralysis (poliomyelitis-like, or Guillan-Barré-like). These patients often require hospitalization and supportive care. WNv neurological syndrome results in prolonged rehabilitation and permanent neurological damage in about 50 percent of those affected, especially in older individuals (Labowitz et al., 2004). It also has an estimated four to 15 per cent case fatality rate (Gubler, 2007).

The distribution of clinical presentations (syndromes) depends on previous WNv activity in the area and consequent background immunity. It will also be influenced by the age structure of the population and the effectiveness of control efforts. In endemic areas, with a high prevalence of background immunity, WNv infection is associated with early childhood and is mostly a non-fatal febrile disease, rarely associated with encephalitis. In contrast, in areas where little or no prior virus activity (e.g. North America), aging and immunologically naïve populations are more likely to experience neuroinvasive disease along with more clinical cases in general.

Serologic surveys indicate that infection rates are similar in every age group, and both sexes are equally susceptible but the frequency and severity of clinical illness increases with age (Petersen, Marfin & G, 2003; Weinberger, Pitlik & Gandacu, 2001; Tsai et al., 1998). About one in five people infected with WNv will experience mild symptoms (WNv non-neurological syndrome), and about one in every 150 infected individuals have been shown to develop severe neurological disease (Mostashari et al., 2001).

There is currently no specific treatment or cure for WNv, however several antiviral treatments are being tested in clinical trials (Kramer et al., 2008). People with serious illness are treated with supportive therapies to ease symptoms and prevent secondary infections. Vaccines are currently in the development process however none are yet licensed for use in humans. Two vaccines for horses are currently licensed for use in Canada.

Predicting West Nile Virus Epidemics

The distribution of WNv within a region is influenced by complex ecological factors; therefore, making predictions for a potential human epidemic is difficult. In order to assess the potential for an outbreak multiple factors must be considered:

- human population characteristics and living conditions
- vector characteristics (mosquito types, abundance, vertical transmission and over wintering)
- reservoir characteristics (population density of the avian reservoir, presence of susceptible hosts)
- virus presence and characteristics (virulence)
- environmental factors (climate-rainfall and temperature, vegetation, landscape)
- implementation of prevention and control measures (vector control programs , public education)

Ecological factors that have been shown to predict WNv outbreaks in humans include warm temperatures favouring mosquito development and over-wintering, mosquito pools testing positive for WNv, corvid mortality, and equine illness (Nielsen et al., 2008; Patnaik Juliusson & Vogt, 2007). Trapping and testing mosquitoes for WNv has been an effective strategy for monitoring virus activity in an area, however the proportion of WNv positive mosquito pools is often low, even when observed infection rates are high (Dauphin & Zientara, 2007). Mosquito abundance has also been shown to be a poor predictor of WNv outbreaks in humans, as outbreaks have been observed with low mosquito densities (Nielsen et al, 2008).

A study examining feeding behaviour of mosquitoes in North America found that *Culex* species preferred host is the American robin in May and June when the birds are abundant (Kilpatrick, Kramer et al., 2006). In July, robins and many other avian hosts disperse after breeding and feeding patterns of *Culex* mosquitoes shift to other available hosts including humans. Thus in the early part of summer, WNv amplifies in susceptible avian hosts and the mosquito vector, and then as the avian hosts disperse, the risk of human infection increases (Kilpatrick, Kramer et al.). The dispersal of birds in later summer may be particularly important for escalating human infections in areas with low biodiversity as humans may comprise a larger proportion of the remaining host pool (Ruiz et al., 2007).

Epidemiology of West Nile Virus Infection

WNv was first identified in 1937 in the West Nile province of Uganda in the blood of a woman with flu like symptoms during research on Yellow fever (Smithburn et al., 1940). After this initial discovery, it was found to be endemic in many areas of Africa where it settled into epizootic cycles, with epidemic peaks when the local host populations had a low prevalence of antibodies. For approximately five decades it was seen as a relatively unimportant human and animal pathogen involving mild flu like illness (Gubler, 2007). During the past decade however, WNv gained the status of an emerging infectious disease, capturing the attention of public health professionals with its recent spread around the globe (Hayes et al., 2005; Dauphin et al,

2004; Zohrabian et al., 2004). The virus has now been found on all continents except Antarctica (Kramer et al., 2008; Hayes et al.).

Sometime during the 1990s, a more virulent strain of WNv developed that was associated with outbreaks having more neuro-invasive disease and deaths in humans and animals. It is believed that this more virulent strain emerged in North Africa following an outbreak in 1994 with higher numbers of serious illness and deaths than previous outbreaks (Gubler, 2007). A series of outbreaks of the more virulent strain in humans, horses, and birds occurred in the Middle East and parts of Europe during the late 1990s: Romania, 1996; Russia, 1999; and Israel, 1998 and 2000 (Dauphin et al., 2004; Murgue, Zeller & Deubel, 2002).

After the discovery of WNv during the summer of 1999 in New York, USA, WNv spread rapidly to become established in nearly every part of the country. From 1999 to 2007, approximately 27,082 cases of illness and 1045 deaths were reported in humans in the USA (CDC, 2008). The rapid spread of the virus is likely due to the migratory patterns of reservoir birds acting as introductory hosts (Gubler, 2007). The virus caused widespread mortality in some indigenous bird species due to lack of previous exposure and adaptation to the virus (LaDeau, Kilpatrick & Marra, 2007; CDC, 2003).

The modality of virus introduction to the Western hemisphere is not fully understood (Dauphin et al, 2004; Lanciotti et al 1999). However the subsequent spread of the virus after 1999 underscores the ability of arboviruses to become established when introduced to new areas where appropriate climate, efficient vectors and hosts are present (Calisher, 2000). Molecular monitoring of the virus in North America revealed the emergence of a dominant genotype different from the original one introduced in 1999 (Snappin et al., 2007). The dominant genotype was named the North American or WN02 and had greater transmission efficiency making it the only genotype circulating in the US within three years of the original introduction (Snappin et al.; Kramer et al., 2008).

Only small numbers of clinical cases in humans and horses have been found in Central America and the Caribbean (Kramer et al., 2008; Hayes et al., 2005). This may be due to pre-existing immunity to other flaviviruses, and differences in ecological factors (Kramer et al.; Rappole et al., 2000). This region however, serves as wintering ground for migratory birds; therefore, it is likely to contribute to the maintenance of the virus in avian reservoirs (Gould, 2004).

West Nile Virus in Canada and Alberta

Surveillance for the WNv was first introduced in Canada during 2000 in response to the outbreak in New York, USA in 1999. The virus was first detected in Canada in August 2001 in Ontario in birds and mosquitoes. Data is now collected on a weekly basis during the transmission season and is reported for wild birds, veterinary cases, human cases and mosquito pools. In Canada, human infections are normally detected from May to December, with the majority of cases occurring in August.

To date, locally acquired human cases have been reported in 5 of the 10 provinces. The number of cases has fluctuated year to year and has been found almost entirely in the Prairie Provinces. As of 2008, no locally acquired cases were found in BC, the Maritimes (NS, NB, PEI, and NFLD) or the Territories. In 2003, 1391 cases were reported across Canada. Between 2004 and 2006, only 406 clinical cases were detected. The 2007 season resulted in the highest number of infections to date with a total of 2318, followed by only 29 cases in 2008.

The fluctuation in cases from year to year is likely due to weather patterns that either favour or hinder the development of *Culex* mosquitoes along with potential over wintering of infected mosquitoes. The levels of human infection observed in seroprevalence studies, discussed in a later section, are too low in North America to have any substantial impact on the frequency or intensity of epidemics (Hayes et al., 2005).

Table 1. Clinical Human WNv Cases in Canada 2003 - 2008

Province	2003	2004	2005	2006	2007	2008
British Columbia	19 (19)	0	0	0	(18)	1 (1)
Alberta	**275**	**1 (1)**	**10 (3)**	**40 (3)**	**320 (48)**	**1 (1)**
Saskatchewan	848	10	58	19 (3)	1397	12
Manitoba	139	3	54	50	570	13
Ontario	89	13	95 (4)	42 (3)	11 (5)	1
Quebec	17	1	7	0	1	1
Maritimes	3 (3)	0	3 (3)	0	0	0
Territories	1 (1)	0	0	0	0	0
Canada	1391	28	227	151	2318	29

Note: Brackets indicate number of cases thought to be travel-related Source: Public Health Agency of Canada, 2008: Data up to Sept. 13, 2008

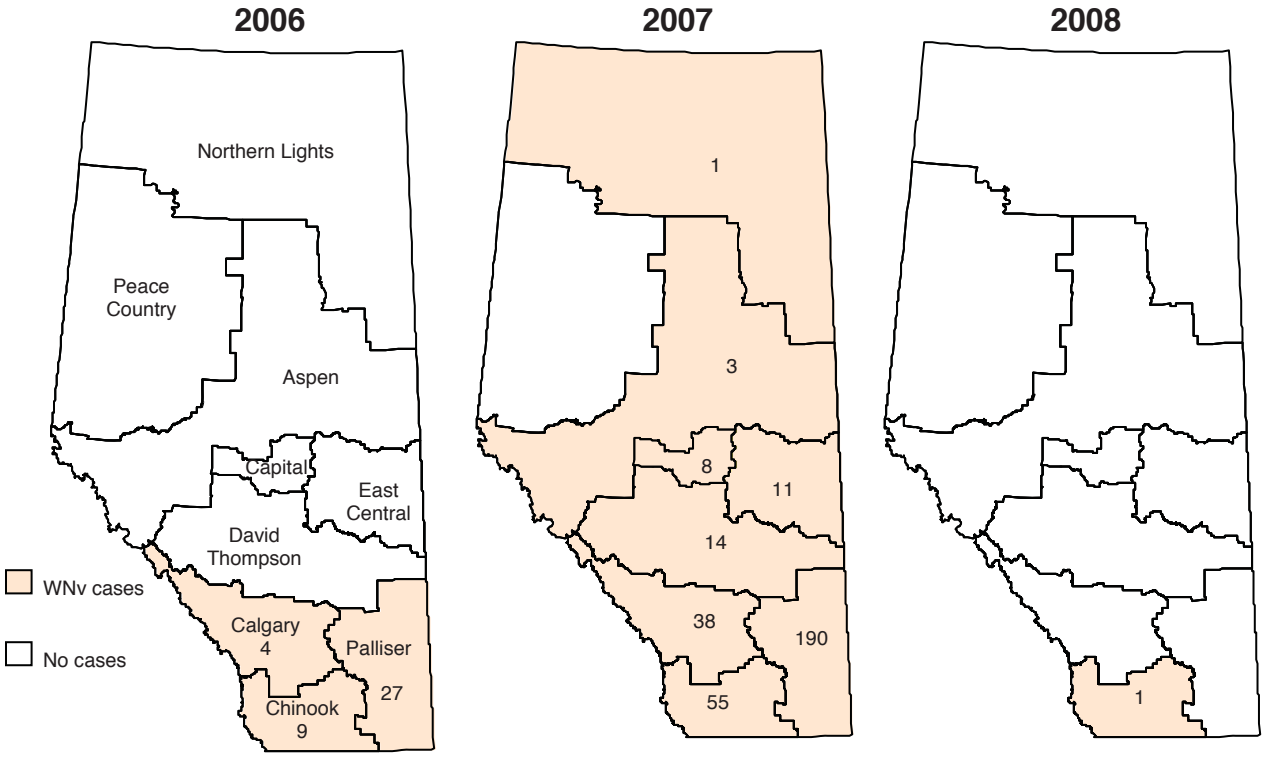
In Alberta, dead bird collection and testing was discontinued following the summer of 2006 as it was determined that further testing would provide no additional information to inform public health. Testing of horses and mosquitoes remains a part of ongoing WNv surveillance (Alberta Health and Wellness, 2008).

The majority of mosquito surveillance occurs in the south east part of the province where the *culex tarsalis* mosquito is more prevalent. The southern part of the province is characterized by higher temperatures and lower precipitation, which are conditions that are known to favor the development of *Culex* mosquitoes. Since 2003, positive mosquito pools have been found in five health regions, Chinook, Palliser, Calgary, David Thompson and East Central. In the same time frame, 232 horses tested positive for WNv infections and approximately 35% of those horses died due to their infection. The horses were not vaccinated and were located in the Chinook, Palliser, Calgary, and David Thompson health regions.

The distribution of laboratory confirmed WNv cases in Alberta is clustered in the south east corner of the province, with the highest number observed in the Palliser Health Region each year to date (Figure 2). Cases found in areas of the province north of the Calgary health region are often thought to be associated with travel to an area of virus activity.

The clinical presentation of human cases in Alberta since 2003 has included both WNv non-neurological syndrome and neuroinvasive disease. The neurological manifestations are in general similar to those previously reported in North America (Sayao et al., 2004). There are no gender differences in WNv infection incidence and no cases have been attributable to blood products, organ transplant or vertical transmission.

Figure 2. Summary of West Nile Human Cases by Health Region, 2006-08



West Nile Virus Seroprevalence

Seroprevalence is a term that refers to the proportion of the population whose blood tests positive for a given pathogen at a given point in time. In the case of WNv, it is the proportion of a population testing positive for WNv antibodies in their blood. It can be difficult to compare the results from different estimates of seroprevalence because methods often differ from study to study. At one extreme, serological surveys revealed endemic areas in Africa, with WNv antibodies reported in more than 60 per cent of the population (Taylor et al., 1956). General background immunity in other regions has been much lower.

In Europe and the Middle East, assessments of seroprevalence in areas with WNv activity have ranged from zero to eight per cent (Batieha, Saliba & Graham, 2000; Hubalek Halouzka & Juricova, 1999; Tsai et al., 1998). In the western hemisphere, a similar range has also been observed, however certain small regions have reported rates as high as 20 per cent following epidemics (Schweitzer et al., 2006). A three per cent seroprevalence rate was estimated in Queens, New York following the virus's first appearance in North America during 1999 (Mostashari et al., 2001).

Of the few seroprevalence studies completed in Canada, rates have ranged from zero to 10 per cent. In 2002, Ontario had it's first epidemic, and seroprevalence was estimated to be 3.1 per cent in the area with the highest number of clinical cases (Elliot et al., 2003). A Saskatchewan study reported the highest seroprevalence in Canada to date with ten per cent of study participants showing evidence of infection following the 2003 season in a region with the most virus activity (Saskatchewan Health, 2004).

Table 2 provides a summary of published seroprevalence studies conducted in the USA, Canada, and Europe. This list may not be exhaustive.

Table 2. Seroprevalence Study Findings

Location	Year	n	Seroprevalence	Citation
USA				
Queens, New York City	1999	677	3%	Mostashari, F., *et al.,* Epidemic West Nile encephalitis, New York, 1999: Results of a household-based seroepidemiological survey. *The Lancet* 2001; 358: 261-264.
Connecticut	2000	731	0%	Hadler, J., *et al.*, West Nile virus surveillance in Connecticut in 2000: An intense epizootic without high risk for severe human disease. *Emerging Infectious Diseases* 2001; 7: 636-642.
Cuyahoga county, Ohio	2002	1209	2 – 3% higher in children than adults	Mandalakas, A.M., et al. West Nile virus epidemic: Northeast Ohio, 2002. *Emerging Infectious Diseases* 2005.

Nebraska state	2003	624	10% range = 4 to 20% by region	Schweitzer, BK, et al. (2006). Geographic factors contributing to a high seroprevalence of West Nile virus-specific antibodies in humans following an epidemic. *Clinical and Vaccine Immunology, Mar.* 314-318.
Wyoming, Goshen County	2003	869	14%	Murphy, TD, et al. (2005). West Nile virus infection among health-fair participants, Wyoming 2003: assessment of symptoms and risk factors. *Vector Borne Zoonotic Dis., 5,* 246-251.
Colorado, Fort Collins community and area	2003	547	4% in chord blood samples taken during birthing	Paisley, JE, et al. (2006). West Nile virus infection among pregnant women in a northern Colorado community, 2003 to 2004. *Pediatrics, 117,* 814-820.
Texas, Houston	2004	397	7% in homeless range = 5 to 16% with longer time homeless being higher	Meyer, TE, et al. (2007). West Nile virus infection among the homeless, Houston, Texas. *Emerging Infectious Diseases, 13,* 1500-03.
CANADA				
Ontario, Oakville	2002	1505	3%	Elliott, S., *et al.*, *Results of a West Nile virus Seroprevalence Survey, South Oakville, Ontario, 2003.September 2003 to Ministry of Health and Long-Term Care (Ontario)*. 2003. p. 21-33.
Manitoba, south western part of the province	2004	1200	3% overall; range = 0.5 - 5%	Manitoba Health. *Sero-survey identifies WNv infection rates in southwestern Manitoba.* [News Release] 2005
Saskatchewan Five Hills region	2004	501	10%	*Saskatchewan Health, 2004. Study shows WNv wide spread in Five Hills Health Region last summer (news release).*
Alberta	2004	2518	0.3% overall; 5% in rural Palliser region	Ivan M, et al. (2005). *Estimating the Infection Rate of West Nile Virus in Alberta.* Edmonton: Alberta Health & Wellness.
EUROPE				
Bucharest, Romania	1996	959	2-4%	Tsai, T.F., *et al.*, West Nile encephalitis epidemic in southeastern Romania. *The Lancet* 1998; 352: 767-771.
Czech Republic, South Moravia	1997	619	2%	Hubalek, Z., J. Halouzka, and Z. Juricova, West Nile Fever in Czech land. *Emerging Infectious Diseases* 1999; 5: 594-595.

Czech Republic, Central Bohemia	2002	497	0%	Hubalek, Z., Zeman, T., et al. Mosquitoborne viruses, Czech Republic. Emerging Infectious Diseases 2005; 11: 116-118.
Spain, southern region	2002	504	0.6% overall; range of 0% urban to 5% rural	Bernabeu-Wittel, M, et al. (2007). West Nile virus past infections in the general population of southern Spain. *Enferm Infecc Microbiol Clin, 25,* 561-5.
Turkey, south eastern region	not stated	181	10% - sera from two state medical hospitals	Ergunay, K. (2007). Seroprevalence of West Nile virus in southeastern Turkey: first evidence for tick-borne encephalitis virus infections. *Vector Borne Zoonotic Disease, 7,* 157-61.

Public Perception and Behaviours in North America

An important component of the public health response to WNv has been educating the public on personal protective behaviours to prevent mosquito bites. Key messages have included promoting the use of effective mosquito repellents, wearing protective clothing, avoiding the outdoors during high risk times, repairing holes in screens, and reducing mosquito larval development sites (i.e., standing water sources). Studies have been completed to evaluate and inform such public health education initiatives by assessing risk perception, behaviours, attitudes and risk factors associated with WNv infection in humans.

A study conducted in New York (Mostashari et al., 2001) during 1999 revealed that although 84 per cent of respondents named one or more personal protective measures against WNv, 39 per cent had not taken any of these measures. Only nine per cent of respondents consistently used repellent, underlining the considerable disparity that can exist between awareness and behaviour.

Other US studies have found that personal protective measures differed between cultural groups, by age, sex, and perceived risk of infection (McCarthy et al., 2001). Practicing personal precautions was associated with a higher perceived risk of infection, being female, being over 50 years of age, and with households speaking English as the first language (McCarthy et al.; CDC, 2003). The belief in the presence of WNv in the vicinity was not an established predictor of engaging in personal protective behaviours (CDC-DVBID, 2004; Blendon et al., 2002).

Studies in Canada have found a high awareness of WNv and that most obtained their information about WNv from news media including television, radio and newspapers (Elliot, Loeb, Harrington & Eyles, 2008; Elliot et al., 2003). A lack of practicing personal protective behaviours, despite public education campaigns has been observed such as rarely or never wearing insect repellant or protective clothing when outdoors (Elliot et al., 2008). A common barrier to wearing repellent is the perception that DEET poses a health risk (Aquino et al., 2004). Being female, and being worried about WNv infection were predictive of practicing personal protective behaviours (Elliot et al., 2008).

The first survey completed in Alberta in 2004 (Ivan, et al., 2005) found that over 90 per cent of Albertans were aware that WNv had been identified in mosquitoes and humans in Alberta. Awareness in the Palliser Health Region was slightly higher than the rest of the province. Almost all respondents recognized mosquito bites as a mode of WNv transmission. The majority of participants also believed incorrectly that contact with dead birds could be responsible for WNv transmission to humans. Television, radio and newspaper were most commonly mentioned as sources of information.

Despite a high awareness of WNv, most respondents rarely or never avoided places or times when mosquitoes were a problem (Ivan, et al,. 2005). The majority of participants indicated sporadic or no use of mosquito repellent. Reasons for not using repellent included: concern about the use of chemicals, allergy, perceived low risk of contracting WNv, and the unpleasant smell of the repellents.

Introduction to the WNv Seroprevalence Survey

WNv is relatively new to North America and there is much to learn about its epidemiology in this new environment. Research has suggested that for every confirmed clinical infection there are many more undetected infections in humans since the majority of WNv cases are believed to be mild or asymptomatic. To determine the actual infection rate, sero-survey methods, in addition to laboratory confirmed clinical cases can greatly enhance surveillance of WNv.

In response to the first human cases of WNv infection detected in Alberta in 2003, an enhanced surveillance system was developed in the form of a sero-survey to complement the information gained through regular clinical surveillance. The enhanced surveillance system was designed to:

1. Add to current knowledge of WNv epidemiology in the broad context of new emerging diseases in Canada and abroad.
2. Enhance baseline surveillance data for WNv infection prevalence in order to evaluate the evolution of this epidemic in Alberta and Canada over time.
3. Increase understanding of the cycles of WNv to help predict yearly clinical infection rates.
4. Assist in evaluating the effectiveness of public communication and awareness campaign components of the WNv response plan in Alberta.
5. Identify potential risk factors of WNv infection and assist in planning preventive strategies and focused control measures.
6. Provide a reference for studies of the ecology and epidemiology of WNv and other arthropod-borne infections in Alberta and Canada.

Methods

Study Design

The study design from the first sero-survey conducted in 2004 (Ivan et al., 2005) was repeated in order to allow for comparison of findings between 2004 and 2007. The cross-sectional surveys were conducted in two phases.

In the first phase, consenting participants answered a telephone survey which assessed knowledge, attitudes and behaviours regarding WNv.

In the second phase, a laboratory requisition was provided and participants donated blood samples at a participating lab in their area of the province.

Target Population

Individuals residing in Alberta since July 2006 who were 18 years and older, as well as linguistically competent in English were eligible to participate in the study.

It should be noted that the sampling strategy excluded certain categories of Alberta residents, specifically those living in a household without a telephone, many of those living in long term care facilities and those residing in correctional facilities.

Sampling Design

A complex sampling design was employed in order to allow for the most accurate estimates across various study strata. The Palliser Health Region was over-sampled because of its typically higher rate of clinical WNv cases in humans. In order to determine whether risks differed between urban and non-urban environments, and since most Albertans live in urban centres, non-urban residents were over sampled. An additional sample of participants who refused to provide a blood sample, but agreed to complete the telephone survey component was also sought to control for differences in those who refused the blood work component of the survey. This additional "survey only" sample was not a part of the sero-survey completed in 2004.

Geographically specific banks of telephone numbers were obtained and assigned to one of two sets: the Palliser Health Region or the rest of Alberta. Within each of these two sets, banks were then allocated to either an urban or rural strata. Within the Palliser Health Region set, the urban stratum included Medicine Hat. For the rest of Alberta set, the urban stratum included Calgary and its suburbs (Okotoks and Strathmore), Edmonton and its suburbs (Fort Saskatchewan, Leduc, Sherwood Park, Spruce Grove, St. Albert and Stony Plain,) and the major regional urban centres of Fort McMurray, Grande Prairie, Lethbridge and Red Deer. All other banks were allocated to the non-urban (rural) strata.

50 per cent of the sample was allocated to the Palliser Health Region and 50 per cent to the rest of Alberta. Based upon estimates of the number of urban and rural residents, 60 per cent were allocated to the urban strata in the Palliser Health Region sample and 53.33 per cent of the rest of Alberta sample to the urban strata.

The sample of 3,747 Albertans comprised of 3243 participants who agreed to the survey and blood work, and an additional 504 who agreed to the survey only was assembled by mid June 2007. Recruitment stopped at that point to avoid overlap with the 2007 WNv mosquito season.

Telephone Survey

The survey questionnaire (Appendix A) was nearly identical to the first sero-survey completed in 2004 (Ivan et al., 2005). However, items that added little to the study findings were removed or revised. The survey questions were adapted from previous studies conducted in Canada and the US, and consisted primarily of close-ended questions that investigated knowledge about WNv, the attitudes toward the virus and the use of measures to protect against infection.

Surveys were conducted by the Prospective Measurement and Evaluation department in the Calgary Health Region who used a computer assisted telephone interview system to facilitate the recording of information. The interviewers followed a strict protocol including a list of standard responses to frequently asked questions (Appendix B). During the survey, interviewers were monitored for adherence to the protocol.

Each selected telephone number was called a minimum of nine times including calls in three different time slots (weekday, evening and weekend) before it was abandoned. Contacted individuals who declined to participate were not called again. Table 3 presents the percentage of participation in 2004 and 2007.

Table 3. Survey Participation

Phase 1 Participation	**Year: 2004**	**Year: 2007**
Agreed to survey and blood work	3,780	3,243
Agreed to survey only	N/A	504
Declined to participate	8,052	9604
Total	11,832	13,351
Participation	**32%**	**28%**

Participation, as described above, is lower than what is typically obtained in telephone surveys that address health concerns. However, the survey involved agreeing to provide a blood sample at a participating laboratory. A study, comparable in this respect, has previously reported a response of 25 per cent (Blendon et al., 2002).

Blood Collection

Survey participants received an information package about the blood collection procedures in the mail. It included an information letter (Appendix C), a requisition form, a list of blood collection sites in their region and a WNv educational brochure. Participants were asked to take the requisition form to a designated participating laboratory to provide a blood sample. Samples were forwarded to the Alberta Provincial Laboratory for Public Health (ProvLab) for analysis.

A reminder telephone call was given to participants who had not provided a blood sample approximately three weeks after the telephone survey date in order to increase participation, and to ensure that the package was received. In addition, packages that were undeliverable were returned to the study coordinator. By the cut-off in mid June, the response for blood work reached 60 per cent. Participation was slightly lower in 2007 compared to 2004. Table 4 presents the blood work participation for both surveys.

Table 4. Blood Work Participation

Phase II Participation	**Year: 2004**	**Year: 2007**
Laboratory requisitions mailed	3780	3,243
Blood samples received at Provincial Lab	2518	1,960
Participation	**67%**	**60%**

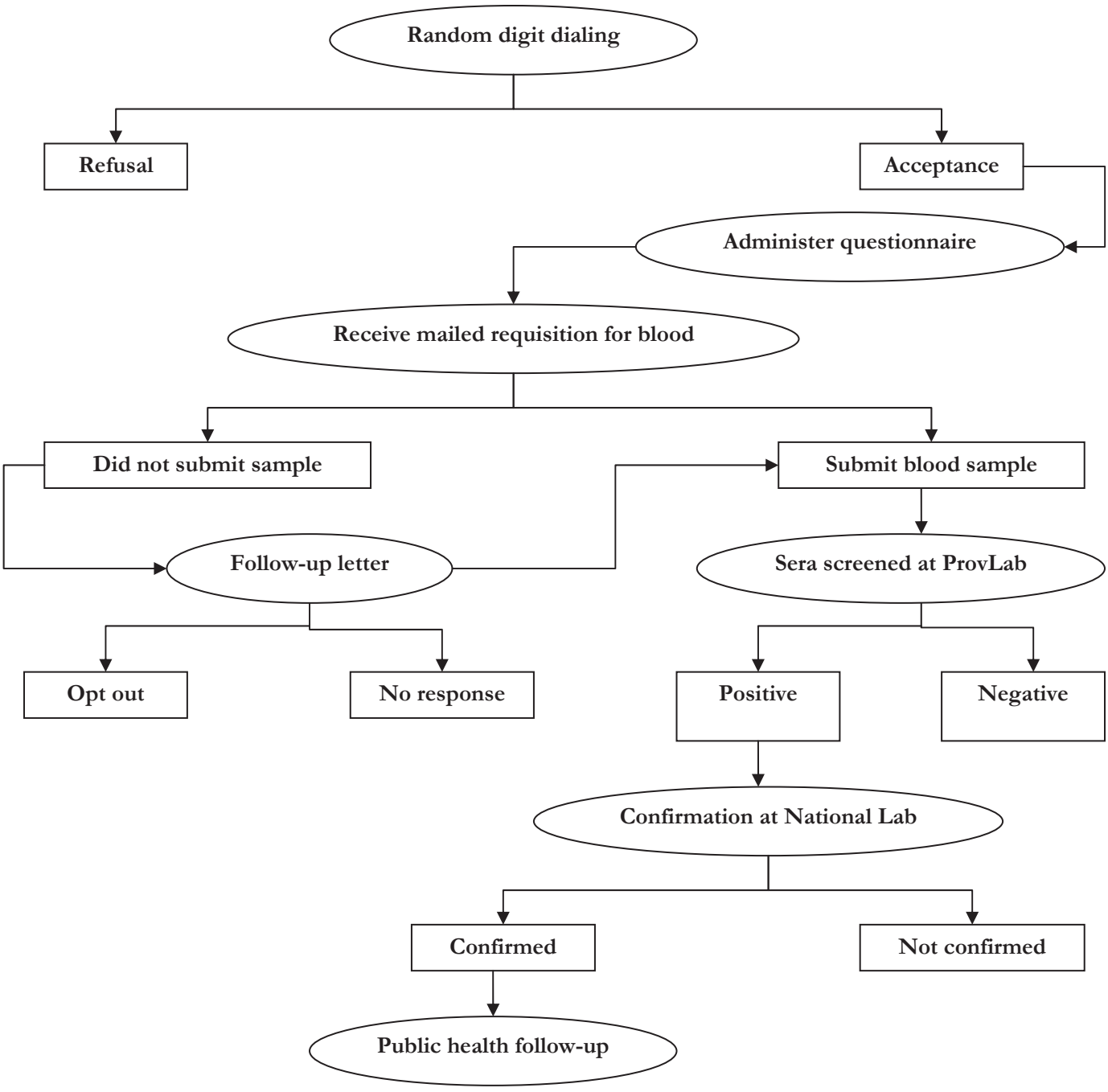
Laboratory Tests

The Alberta Provincial Laboratory of Microbiology screened the blood specimens for IgM and IgG WNv antibodies. Reactive samples were then forwarded to the National Microbiology Laboratory in Winnipeg, Manitoba for confirmation with a plaque reduction neutralization test (PRNT). A detailed description of the serology completed can be found in Appendix D.

The positive results from serological investigation were followed up by regional public health officials after the completion of the project (Appendix E).

Figure 3 presents a visual summary of the study flow.

Figure 3. Study Flowchart



Data Analysis

A unique identifier was assigned to each participant. This identifier was used to link laboratory results and survey data for analysis.

To allow accurate inferences to the provincial population, weights were derived to reflect the differential probability of being selected for participation. In addition to population estimates derived from the Alberta Health Care Insurance Plan Registry for each stratum and each region, the weighting process included corrections for household size and the number of telephone lines to the household. Weights were also post stratified by age and sex.

Data were analyzed using the statistical software SAS version 9.1.

Results

Demographic Characteristics of Survey Participants

From the 3,747 survey respondents, 1,990 were from Palliser Health Region. Among these, 56 per cent were from urban areas and 44 per cent were from rural areas. Among those in the rest of Alberta, 49 per cent of the 1,757 respondents were from urban areas; the remaining 51 per cent were rural residents.

Some demographic categories were under-represented, while others were over-represented when compared with their distribution in Alberta's 2006 population. For example, those aged 18 to 24 were under-represented (6 per cent) compared to the Alberta population (9 per cent) and females were over-represented (62 per cent) compared to the Alberta population (50 per cent). The post stratification weights account for these differences in the analyses.

Survey: Descriptive Results

Descriptive results for variables from the 2004 and 2007 telephone surveys are presented. Point estimates were derived using weighted data and standard errors were calculated from the successive application of bootstrap weights. Provincial estimates are always given. Separate estimates by strata are presented only when differences between strata were statistically significant.

Table 5 presents additional unweighted demographic information about the participants in the 2007 survey.

Table 5. Demographic Characteristics of the Survey Sample, 2007

Parameter	Survey Stratum: PHR-urban n=1123		PHR-rural n=867		RoAB-urban n=853		RoAB-rural n=904		Survey only n=504		AB total n=3747	
	N	%	N	%	N	%	N	%	N	%	N	%
sex												
male	418	37	337	39	333	39	336	37	196	39	1424	38
female	705	63	530	61	520	61	568	63	308	61	2323	62
age group												
18-24	62	6	46	5	47	6	59	7	46	9	214	6
25-34	202	18	138	16	132	16	115	13	110	22	587	16
35-44	209	19	176	20	198	23	192	21	86	17	775	21
45-54	259	23	240	28	227	27	218	24	107	21	944	25
55-64	171	15	156	18	140	16	171	19	68	14	638	17
>= 65	219	20	110	13	107	13	148	16	85	17	584	16
refused	1	0	1	0	2	0	1	0	2	0	5	0
education												
high school or less	436	38	389	44	190	22	351	39	208	42	1366	36
college / technical school	423	38	340	40	319	37	357	40	190	38	1439	38
university - undergrad	182	17	108	13	224	27	142	16	66	14	656	18
university – post graduate	73	7	24	3	117	13	50	5	30	6	264	8
don't know / refused	9	1	6	1	3	0	4	0	10	2	22	1
live within 5 km of urban centre												
yes	1046	93	307	35	782	92	254	28	319	63	2389	64
no	76	7	558	64	69	8	650	72	182	36	1353	36
don't know / refused	1	0	2	0	2	0	0	0	3	1	5	0

NOTE: PHR = Palliser Health Region; RoAB = Rest of Alberta

The demographic characteristics of survey participants in 2007 were comparable to the 2004 sample (Ivan et al., 2005), and similar across stratum. Just over 60 per cent of participants were female, and close to half the sample were in the 35 to 54 year age group. A little over 70 per cent had education up to a college/technical school or less. In the urban stratums over 90 per cent lived within a 5 km radius of an urban centre. In the rural stratum, 35 per cent in the PHR and 28 per cent in RoAB lived within 5 kms of an urban centre.

The main differences between stratum in 2007 were: 1) the "survey only" stratum had a slightly higher percentage in the 25-34 yr age group (22% versus 13-18%); and 2) the urban RoAB

stratum had a higher percentage reporting university or higher education than those in other stratum (40% versus 16-24%).

West Nile Virus Knowledge

Virtually all respondents (~99 per cent) had heard about WNv prior to the survey in 2007. Table 6 presents responses to knowledge questions about transmission of WNv.

Table 6. Knowledge of Modes of WNv Transmission, 2004 and 2007

Can a person become infected with WNv by:		2004		2007	
		%	(CI)	%	(CI)
Mosquito bites	Yes	97.6	(96.6, 98.6)	98.9	(98.5, 99.4)
	No	0.5	(0.1, 0.9)	0.7	(0.3, 1.1)
	Don't know	2.0	(1.2, 2.8)	0.3	(0.1, 0.6)
Being in the same room with an infected person	Yes	1.7	(1.1, 2.3)	3.5	(2.4, 4.7)
	No	83.4	(81.4, 85.4)	92.2	(90.5, 93.8)
	Don't know	15.0	(13.0, 17.0)	4.3	(3.0, 5.6)
Contact with dead birds	Yes	62.4	(59.7, 67.1)	72.8	(70.4, 75.2)
	No	13.6	(11.6, 15.6)	19.9	(17.9, 22.0)
	Don't know	24.0	(21.6, 26.4)	7.2	(5.8, 8.6)
Shaking hands with an infected person	Yes	2.1	(1.3, 2.9)	5.6	(4.3, 6.9)
	No	80.6	(78.6, 82.6)	91.0	(89.3, 92.6)
	Don't know	17.3	(15.3, 19.3)	3.4	(2.3, 4.6)

Overall, knowledge appears to have increased when comparing point estimates between the 2004 and 2007 surveys. The percentage of "don't know" responses decreased, and the correct responses increased with one exception. While almost all respondents correctly recognized mosquito bites as a mode of WNv transmission, the majority also believed incorrectly that contact with dead birds could be responsible for WNv transmission to humans. It is uncertain as to why this misconception exists however, it may be a result of the dead bird collection and testing carried out in areas of Alberta to monitor WNv.

Other findings not presented in a table include:

- Close to half of respondents (48 per cent) recognized that older adults (aged 50 yrs and over) are more likely to develop severe complications when infected with WNv.
- The majority of respondents (54 per cent) incorrectly believed that young children (under five years of age) are also more likely to develop severe complications.

Sources of Information

Table 7 presents the sources of information about WNv identified by participants.

Table 7. Most Mentioned Sources of Information, 2004 and 2007

Where did you hear about WNv?	2004			2007		
	Palliser % (CI)	Rest of AB % (CI)	Alberta % (CI)	Palliser % (CI)	Rest of AB % (CI)	Alberta % (CI)
Television	80.1 (77.9, 82.3)	86.6 (84.6, 88.6)	86.4 (84.8, 88.2)	75.0 (72.6, 77.3)	86.0 (84.1, 87.8)	85.6 (83.8, 87.4)
Newspaper	68.0 (65.5, 70.5)	65.6 (63.1, 68.1)	65.7 (63.2, 68.2)	59.7 (57.0, 62.3)	61.6 (59.0, 64.3)	61.6 (59.0, 64.1)
Radio	48.5 (45.6, 51.4)	46.2 (43.3, 49.1)	46.3 (43.5, 49.1)	51.0 (48.2, 53.8)	42.3 (39.3, 45.2)	42.5 (39.7, 45.4)
Friends/ word of mouth	21.9 (19.5, 24.3)	12.5 (10.7, 14.3)	12.8 (11.0, 14.5)	23.3 (21.0, 25.6)	16.5 (14.4, 18.7)	16.8 (14.7, 18.8)
Pamphlets	4.8 (3.6, 6.0)	4.4 (3.2, 5.6)	4.4 (3.9, 4.9)	11.0 (9.4, 12.6)	6.9 (5.8, 8.1)	7.1 (5.9, 8.2)
Doctors	6.6 (5.0, 8.2)	4.7 (3.5, 5.9)	4.7 (3.5, 5.9)	15.4 (13.5, 17.2)	9.3 (7.7, 11.0)	9.5 (7.9, 11.1)
Internet	3.6 (2.4, 4.8)	5.0 (3.8, 6.2)	5.0 (3.8, 6.2)	4.1 (3.0, 5.2)	5.6 (4.3, 6.9)	5.6 (4.3, 6.8)

The medium by which people reported hearing about WNv was similar in both the 2004 and 2007 surveys. Most participants reported hearing about WNv on television, in newspapers and on the radio. Television was mentioned as a source of information by over 80 per cent of respondents. There was an increase in the percentage of those hearing about WNv from friends/word of mouth, pamphlets and doctors in 2007 compared to 2004. For example, the percentage of those reporting pamphlets and doctors as information sources more than doubled in the Palliser region. People in the Palliser region were more likely to report hearing about WNv via friends or word of mouth compared to those in the rest of the province.

Other less common sources of information mentioned were: the workplace, magazines, and veterinarians.

West Nile Virus Attitudes

Table 8 presents results for the percentage of people who reported being worried about the possibility of being infected by WNv. The response scale differed between the two study periods. In 2004, the scale was a 3-point response option scale ranging from "Very worried" to "Not worried at all". In 2007 the scale was expanded to a 5-point response option scale ranging from "Very worried" to "Not worried at all". Findings from 2004 and 2007 are not completely comparable due to the differing scales employed. In 2004, the responses were dichotomized (i.e., very or a little worried; not worried at all), and in 2007, the responses were categorized into three groups (i.e., worried, middle and not worried).

Table 8. Worried about West Nile Virus, 2004 and 2007

How worried are you about getting WNv?	Year	Palliser Region % (CI)	Rest of province % (CI)	Alberta % (CI)
Very or a little worried (1,2)	2004	67.0 (65.8, 68.2)	48.6 (47.1, 50.1)	49.2 (47.8, 50.6)
Not worried at all (3)		33.0 (31.8, 34.2)	51.4 (49.9, 52.9)	50.8 (49.4, 52.2)
Worried (1,2)	2007	22.1 (20.0, 24.2)	9.8 (8.3, 11.3)	10.2 (8.8, 11.1)
Middle (3)		33.0 (30.5, 35.5)	27.5 (25.0, 30.0)	27.7 (25.2, 30.1)
Not worried (4,5)		44.8 (42.1, 47.6)	62.6 (59.9, 65.3)	62.1 (59.4, 64.7)

Despite the differing scales, overall the level of worry about becoming infected with WNv appears to have decreased in 2007 compared to 2004. In 2007, 62.1 per cent of respondents reported they were not worried, whereas, in 2004, it was 50.8 per cent.

In 2007, the percentage of people who reported that they were worried was twice as high in the Palliser Health region compared to the rest of the province (22.1 per cent in Palliser versus 9.8 per cent in the rest of Alberta). The majority of participants outside of the Palliser region were not worried about becoming infected with WNv. There were no differences between the urban and rural strata.

Behaviours Related to West Nile Virus Prevention

Participants were asked to indicate which personal protective measures they practiced and how often. Responses are presented in Table 9.

Table 9. Personal Protective Measures

Thinking back to last summer, at times when you might be bitten by a mosquito, how often did you do the following things?		2004 % (CI)	2007 % (CI)
Restrict your outdoor activity	Always	1.4 (0.8, 2.0)	1.1 (0.6, 1.5)
	Most of the time	5.1 (3.9, 6.3)	6.4 (5.1, 7.6)
	Sometimes	14.1 (12.3, 15.9)	19.6 (17.5, 21.7)
	Rarely	20.3 (18.1, 22.5)	23.0 (20.8, 25.3)
	Never	59.1 (56.3, 61.8)	49.7 (46.8, 52.5)
Avoid places where mosquitoes were a problem	Always	6.5 (5.1, 7.9)	7.1 (5.7, 8.4)
	Most of the time	20.1 (17.9, 22.3)	27.6 (25.2, 29.9)
	Sometimes	21.6 (19.2, 24.0)	28.1 (25.7, 30.6)
	Rarely	17.0 (14.8, 19.2)	16.0 (13.8, 18.3)
	Never	34.7 (32.2, 37.2)	20.9 (18.7, 23.1)
Wear a long-sleeved shirt and pants	Always	9.7 (8.3, 11.1)	11.3 (9.7, 13.0)
	Most of the time	19.8 (17.4, 22.2)	20.8 (18.6, 23.0)
	Sometimes	38.0 (35.3, 40.7)	41.9 (39.1, 44.7)
	Rarely	16.2 (14.0, 18.4)	14.6 (12.5, 16.6)
	Never	16.2 (14.0, 18.4)	11.3 (9.5, 13.1)
Wear light-colored clothing	Always	7.8 (6.2, 9.4)	7.8 (6.4, 9.3)
	Most of the time	25.6 (22.9, 28.3)	31.2 (28.6, 33.8)
	Sometimes	44.5 (41.8, 47.2)	41.6 (38.9, 44.3)
	Rarely	10.9 (9.1, 12.7)	10.0 (8.3, 11.7)
	Never	11.1 (9.7, 12.9)	8.5 (6.9, 10.0)
Avoid the times when mosquitoes were most active	Always	4.8 (3.8, 5.8)	7.9 (6.6, 9.2)
	Most of the time	21.4 (19.0, 23.8)	27.5 (25.1, 29.9)
	Sometimes	26.0 (23.6, 28.4)	33.5 (30.8, 36.2)
	Rarely	17.1 (15.3, 18.9)	14.3 (12.3, 16.2)
	Never	30.7 (28.2, 33.2)	16.7 (14.6, 18.8)
Wear mosquito repellent	Always	13.7 (11.9, 15.5)	19.6 (17.5, 21.8)
	Most of the time	23.2 (20.7, 25.6)	30.4 (27.8, 32.9)
	Sometimes	29.9 (27.5, 40.3)	28.8 (26.3, 31.1)
	Rarely	17.1 (15.3, 18.9)	10.6 (9.0, 12.2)
	Never	18.0 (16.9, 19.1)	10.2 (8.5, 11.9)

Overall, when comparing the point estimates in 2004 and 2007 there appears to be an increase in those engaging in personal protective behaviours. The largest increase between the two studies is seen in "wearing mosquito repellent".

In 2007, half of participants reported that they wore mosquito repellent "always" or "most of the time" compared to about 37 per cent in 2004. The percentage of people who "rarely" or "never" wore repellent decreased from about 35 per cent in 2004 to 21 per cent in 2007.

Table 10 shows environmental risk reduction activities reported by participants.

Table 10. Environmental Risk Reduction Activities

Last summer did you or anyone in your household do any of the following things to reduce the number of mosquitoes around your home?		2004 % (CI)	2007 % (CI)
Repaired screens that had tears or holes in them	**Yes**	30.9 (28.4, 33.4)	31.0 (28.3, 33.7)
	No	28.5 (26.0, 31.0)	23.4 (21.0, 25.8)
	n/a	40.6 (38.2, 43.0)	45.5 (42.7, 48.2)
Checked and cleaned all rain gutters as required	**Yes**	37.9 (35.0, 40.8)	46.1 (43.4, 48.8)
	No	43.9 (41.0, 46.8)	36.4 (33.7, 39.1)
	n/a	18.3 (15.9, 20.7)	15.5 (13.5, 17.4)
Emptied or replaced pools of standing water regularly	**Yes**	44.2 (41.5, 46.9)	48.5 (45.8, 51.3)
	No	18.6 (16.4, 20.8)	15.5 (13.3, 17.7)
	n/a	37.2 (34.7, 39.7)	35.8 (33.1, 38.6)

Note: n/a refers to "not applicable"

There appears to be little change between 2004 and 2007 in the percentage of people who reported that they engaged in environmental risk reduction activities. There was a slight increase in the percentage reporting that they checked and cleaned all rain gutters as required. The most common environmental reduction activity was "emptying or replacing pools of standing water regularly".

Survey: Predictive Results

Multiple linear and logistic regressions were used to examine predictors of:

- engaging in personal protective measures;
- the use of a mosquito repellent containing DEET; and
- engaging in environmental risk reduction activities.

The dependent measure for engaging in personal protective measures was a composite formed by assigning a number to each category (4 for 'Always' decreasing to 0 for 'Never') and summing across the six activities listed in Table 9. Similarly, a composite for environmental risk reduction activities was formed by summing across the four activities listed in Table 10. Participants were also asked if the repellent they used contained DEET, and this was also used as a dependent measure for some analyses.

Each analysis employed the following independent variables:

- Demographic characteristics: age, gender and education.
- Survey stratum: urban or rural, Palliser Health Region or the rest of the province.
- A composite for knowledge of WNv formed by scoring the WNv knowledge questions listed in Table 6 and the item asking whether the virus had been detected in humans in Alberta and totaling the number answered correctly.
- Time spent outdoors during high and low risk periods for WNv transmission (i.e., AM and PM).
- Whether participants were worried about the possibility of WNv infection or not.

All analyses were conducted on weighted data. Standard errors for regression coefficients were generated by bootstrap, and these were used in tests of significance of the individual coefficients.

Table 11 presents the linear regression results using the personal protective measures composite as the dependent variable.

Table 11. Predictors for Personal Protective Measures, 2007

Estimated Regression Coefficients

Parameter	Point Est.	95% CI		P-value
Intercept	12.07	10.51	13.63	<.0001
Residence – rural vs urban	0.11	-0.34	0.56	0.31
Residence: Palliser vs Rest of AB	-0.06	-0.43	0.31	0.38
Age 18-24 yrs	-1.50	-2.56	-0.45	0.003
Age 25-34 yrs	-1.13	-1.97	-0.28	0.004
Age 35-44 yrs	-0.79	-1.56	-0.03	0.021
Age 45-54 yrs	-1.08	-1.85	-0.31	0.003
Age 55-64 yrs	-0.85	-1.68	-0.01	0.023
Age >= 65 yrs	0	Reference		
Education	<0.01	-0.13	0.13	0.49
Sex – female vs male	1.13	0.60	1.66	<.0001
Knowledge composite	-0.39	-0.77	0.00	0.025
Knowledge: WNv in AB yes vs no	0.05	-0.55	0.66	0.43
Time outside am	-0.27	-0.55	0.00	0.030
Time outside pm	-0.68	-0.94	-0.43	<.0001
Worried about WNv	1.07	0.84	1.30	<.0001

Participants were more likely to engage in personal protective measures if they:

- were female
- were worried about contracting WNv

They were less likely to use protective measures if they:

- were younger than 65 years of age, especially those in the 18 to 24 yrs age group;
- spent more time outdoors during high risk times for mosquito bites
- scored higher on the composite knowledge variable

Table 12 shows the logistic regression results where the use of a mosquito repellant containing DEET is the dependent variable.

Table 12. Logistic Regression Results: Using Repellent Containing DEET, 2007

Odds Ratio Estimates

Parameter	Point Est.	95% CI		P-value
Residence: rural vs urban	1.19	0.68	2.07	0.54
Residence: Palliser vs rest of AB	1.59	1.01	2.50	0.043
Age: 18-24 vs >= 65yrs	0.29	0.07	1.64	0.84
Age: 25-34 vs >= 65yrs	0.35	0.09	1.35	0.13
Age: 35-44 vs >= 65yrs	0.62	0.23	1.71	0.35
Age: 45-54 vs >= 65yrs	0.44	0.18	1.06	0.07
Age: 55-64 vs >= 65yrs	1.02	0.18	5.86	0.98
Education	1.00	0.87	1.15	0.99
Sex: female vs male	0.48	0.25	0.93	0.029
Knowledge composite	1.21	0.79	1.87	0.38
Knowledge: WNv in AB yes vs no	0.59	0.28	1.26	0.17
Time outside am	1.25	0.87	1.78	0.22
Time outside pm	1.00	0.75	1.32	0.98
Worried about WNv	1.32	1.01	1.71	0.039

People were more likely to report using a repellent containing DEET if:

- They lived in the Palliser Health Region compared to the rest of the province.
- They reported being worried about contracting WNv compared to those who were not worried.

People were less likely to report using a repellent containing DEET if:

- They were female compared to those who were male.

Table 13 shows the results of the analysis of the environmental risk reduction composite.

Table 13. Predictors for Engaging in Environmental Risk Reduction Activities, 2007

Estimated Regression Coefficients

Parameter	Point Est.	95% CI		P-value
Intercept	0.82	0.46	1.18	<.0001
Residence: rural vs urban	0.03	-0.09	0.14	0.33
Residence: Palliser vs Rest of AB	-0.01	-0.11	0.08	0.41
Age: 18-24 yrs	-0.29	-0.54	-0.03	0.013
Age: 25-34 yrs	-0.22	-0.43	-0.01	0.019
Age: 35-44 yrs	-0.04	-0.23	0.16	0.35
Age: 45-54 yrs	0.12	-0.06	0.30	0.10
Age: 55-64 yrs	0.19	0.00	0.39	0.025
Age: >= 65 yrs	0	Reference		
Education	-0.003	-0.03	0.03	0.42
Sex: female vs male	0.14	0.01	0.27	0.017
Knowledge composite	0.001	-0.09	0.09	0.49
Knowledge: WNv in AB yes vs no	0.03	-0.12	0.17	0.36
Time outside am	0.07	0.01	0.14	0.018
Time outside pm	0.07	0.01	0.13	0.021
Worried about WNv	0.12	0.07	0.18	<.0001

Respondents were more likely to engage in environmental risk reduction activities if they:

- were female
- were worried about contracting WNv
- spent more time outside at higher risk times for mosquito bites
- were 45 years of age or older

Respondents were less likely to engage in environmental risk reduction activities if they:

- were in the two youngest age groups (i.e., 18 to 24 and 25 to 34 year age groups)

Demographic Characteristics of the Seroprevalence Sample

A total of 1,960 blood samples were received at the Provincial Laboratory of Microbiology. Five samples were not included in the final results as they were not properly labeled, and thus could not be matched conclusively to a survey respondent. A total of 56 per cent of the samples came from the Palliser Health Region and 44 per cent came from the rest of the province. Blood samples were comprised of 51 per cent urban residents and 49 per cent rural residents.

Table 14 presents the unweighted composition of the seroprevalence sample.

Table 14. Demographic Characteristics of the Seroprevalence Sample, 2007

	Survey Stratum									
	PHR-urban n=619		**PHR-rural** n=474		**RoAB-urban** n=385		**RoAB-rural** n=477		**AB total** n=1955	
Parameter	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
gender										
male	228	37	168	35	147	38	171	36	714	37
female	391	63	306	65	238	62	306	64	1241	64
age group in years										
18-24	13	2	9	2	7	2	11	2	40	2
25-34	63	10	44	9	35	9	36	8	178	9
35-44	98	16	82	17	87	23	87	18	354	18
45-54	165	27	141	30	108	28	109	23	523	27
55-64	122	20	115	24	82	21	125	26	444	23
>= 65	158	26	82	17	65	17	108	23	413	21
refused	0	0	1	9	1	0	1	0	3	0
education										
high school or less	234	38	196	42	82	21	174	37	686	35
college / technical school	228	37	185	39	146	38	173	36	732	37
university - undergrad	113	18	74	15	96	25	93	20	376	20
university - graduate studies	44	7	16	3	60	16	35	7	155	8
don't know / refused	0	0	3	0	1	0	2	0	6	0
live within 5 km of urban centre										
yes	586	95	162	34	358	93	121	25	1227	63
no	33	5	312	66	27	7	356	75	728	37

Note: PHR = Palliser Health Region; RoAB = Rest of Alberta

Seroprevalence Estimate

All sera (1,955) were screened at the Provincial Laboratory of Microbiology in Alberta using the WNv IgG test made by Focus Technologies. All positive and borderline results from this screening (n=100) were then sent to the National Microbiology Laboratory (NML) in Winnipeg MB. As a comparison, in the 2004 serosurvey, from 2,518 sera received, 109 were sent to NML for confirmatory testing (Ivan et al., 2005).

NML ran the CDC WNv IgG ELISA and further confirmed samples with plaque reduction neutralization titres (PRNT), which is considered the gold standard method for WNv detection (see appendix D for a description of serology). The confirmatory analysis produced 39 samples considered truly positive from the 2007 sample. In 2004, 35 samples were confirmed to be truly positive.

Table 15 presents the un-weighted descriptive characteristics of the participants representing the positive sera.

Table 15. Unweighted Descriptives of the Positive Sera Samples

Variable	2004 n=35		2007 n=39	
	N	%	N	%
Palliser Region	30	86%	34	87%
Rest of AB	5	14%	5	13%
Urban	6	17%	14	36%
Rural	29	83%	25	64%
Female	18	51%	22	56%
Age 18-24 yrs	1	3%	2	5%
Age 24-34 yrs	6	17%	5	13%
Age 35-44 yrs	6	17%	13	33%
Age 45-54 yrs	12	34%	8	21%
Age 55-64 yrs	6	17%	10	26%
Age 65 yrs and older	4	11%	1	3%

Table 16 presents the weighted seroprevalence estimates and bootstrap generated confidence intervals.

Table 16. Seroprevalence Per cent Point Estimates in 2004 and 2007

Region	Seroprevalence 2004		Seroprevalence 2007	
	Point Est. %	95% CI	Point Est. %	95% CI
Palliser – urban	0.77	0.28, 1.41	1.87	0.74, 2.99
Palliser – rural	4.56	2.79, 6.77	4.17	2.27, 6.06
Rest of the province – urban	0	0.00, 0.01	1.01	0.00. 2.54
Rest of the province – rural	0.84	0.20, 1.82	0.73	0.00, 1.61
Alberta (total)	**0.31**	**0.12, 0.58**	**0.95**	**0.04, 1.85**

WNv activity was first detected in Alberta in 2003, thus it must be noted that the estimate of seroprevalence in the first survey (2004) likely all occurred during the 2003 season. Seroprevalence estimates in the 2007 survey included virus exposure that could have occurred anytime from 2003 up to 2006. The blood screening employed does not indicate how long ago old infections occurred.

The provincial seroprevalence was estimated to be 0.31 per cent in 2004 and 0.95 per cent in 2007. The highest seroprevalence was found in the rural area of the Palliser region in both surveys. There was little difference between the other survey stratums.

The confidence intervals for the estimates in 2004 and 2007 illustrate that there were no statistically significant changes in seroprevalence. The sample size of positive sera were small in both studies and limits the analyses by creating large confidence intervals, thus detecting small changes is not possible.

Extrapolating the 2007 estimates to the 2006 Alberta midyear population 18 years and older (2,511,618), it can be estimated that approximately 23,000 Albertans had been previously exposed to WNv by the end of the 2006 WNv season.

Discussion

Survey Results

In both the 2004 and 2007 surveys, public awareness of WNv was found to be high and was consistent with studies conducted in other parts of North America including other provinces in Canada (Mostashari et al., 1999; Hadler et al., 2001; Elliot et al., 2003; Saskatchewan Health, 2004). Although there was already a high awareness found in the first Alberta survey completed in 2004, the level of public knowledge appears to have increased in the 2007 survey. A higher percentage of respondents were able to identify correct and incorrect modes of WNv transmission.

Across both surveys, information sources about WNv most often cited by participants were the television, radio, and newspapers. There was an increase in the percentage of those hearing about WNv from friends/word of mouth, pamphlets and doctors in 2007 compared to 2004. For example, the percentage of those reporting pamphlets and doctors as information sources more than doubled in the Palliser region in 2007 compared to 2004. People in the Palliser region were more likely to report hearing about WNv via friends or word of mouth compared to those in the rest of the province.

The level of worry about contracting WNv was lower overall in 2007 than in 2004 and was associated with the level of virus activity in the area. The percentage of those who reported being very or a little worried was twice as high in the Palliser region (i.e., 22 per cent) compared to the rest of Alberta (10 per cent). The Palliser region of Alberta has had the most clinical WNv cases and positive mosquito pools detected every year since 2003.

The survey findings suggest the large majority of Albertans have knowledge of personal protective measures to avoid WNv infection; yet, less than half of individuals consistently take precautions. There was, however, an increase in reported protective behaviours in 2007, in particular wearing mosquito repellent. In 2004, about 36 per cent reported wearing mosquito repellent "always" or "most of the time". This increased to about 50 per cent in 2007. The overall discrepancy between knowledge/awareness and behaviour has been found in other similar studies (Elliot et al., 2008; McCarthy et al., 2001; Mostashari et al., 2001).

Respondents were more likely to report engaging in personal protective measures overall, if they were worried about contracting WNv and were female. They were more likely to report using mosquito repellent containing DEET if they were worried about contracting the virus and were from the Palliser region of the province. Those in the 18-24 year old age group, and those who spent more time outside during high risk times (e.g., dawn and dusk), were less likely to consistently practice personal protective measures. These findings were consistent across the two surveys.

Seroprevalence Results

Alberta's overall seroprevalence just prior to the 2007 WNv season was estimated to be 0.95 per cent and was not significantly different than the seroprevalence estimated in 2004 at 0.31 per cent. In addition, seroprevalence estimates varied across the survey strata in a similar pattern in both surveys. The rural area of the Palliser Health Region continues to have the highest point estimate of 4.2 per cent, whereas both the urban and rural areas in the rest of Alberta had the lowest point estimates (1.0% and 0.7% respectively).

The seroprevalence estimates found in the Palliser region of Alberta remain consistent with the Oakville and Manitoba studies as well as those from the northeastern USA and Europe (Elliot et al., 2003; Saskatchewan Health, 2004; Manitoba Health, 2005).

The fact that seroprevalence estimates did not change between the two surveys is aligned with other public health surveillance findings of WNv during the same time frame. There was little WNv activity detected in Alberta between 2004 and 2006. There were only 51 clinical cases in total reported to Alberta Health and Wellness across the three years. In addition, few mosquito pools tested positive for the virus. The 2007 WNv season resulted in favourable conditions for *culex* mosquito development, and subsequently, Alberta had its highest number of clinical cases to date that year. Future seroprevalence assessments following epidemic years, like 2007, would help determine if seroprevalence increases and would help to develop the full picture of WNv evolution in Alberta.

Limitations

As with all survey-based research, there are important limitations that must be considered when interpreting the findings from this survey. Although the sample was randomly selected, respondents volunteered to participate. This has the potential to introduce an element of bias in the sample as those with a greater interest, and/or people who thought they may be infected may be more likely to participate in the study. The response rate of 32 and 28 per cent in 2004 and 2007 respectively, means that the sample may not be representative of the full spectrum of the population. The weighting methodology employed in the analyses attempts to reduce the impact of this limitation but does not eliminate it.

Another important limitation was estimating the provincial seroprevalence using the small number of blood samples testing positive for WNv. The seroprevalence estimates were based on 39 truly positive blood samples across the study stratums. For example, the point estimate for the urban stratum outside of the Palliser region was based on two positive samples. It is possible that those two positive cases were anomalies found by chance. For example, they may represent individuals who had travelled to areas with greater WNv activity where the actual exposure occurred, and not in their assigned study stratum. Patient history was not recorded in this study so this limitation remains an unknown.

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Appendices

Appendix A. Questionnaire

Appendix B. Frequently asked questions

Appendix C. Sample letters

Appendix D. Serology

Appendix E. Follow-up information for those testing positive

Appendix A. Questionnaire

INTRODUCTORY SCREEN
Hello, my Name is <insert own name> and I am calling on behalf of Alberta Health and Wellness. We are conducting a study aimed to increase our understanding of West Nile Virus and to help plan the province's program of West Nile virus prevention. The study involves a 15 minute survey that can be completed over the phone and also requires study participants to give a small blood sample for West Nile virus testing. We need to speak to someone in your household who is - 18 years of age or older - has been living in Alberta since July 1st, 2006 (last summer) or earlier - and has had the most recently past birthday? Is that you, or is there more than one person 18 years of age or older? IF NEEDED: More than 2?

RECRUITMENT AND WAIVER WITH SUBJECT
Participation in this study involves two parts. The first part involves completing a phone survey with Albertans who are also willing to give a blood sample for West Nile Virus testing. If you are interested in participating, I will ask for your name and mailing address. A requisition for blood testing and a list of blood collection sites in the province will be mailed to your house some time this week. The requisition must be taken with you to the blood collection site where a small vial of blood will be drawn and sent to the ProvLab for testing. Because of the size of the study, respondents would only be contacted if their blood sample came back positive for West Nile Virus and this contact would be handled by the medical officer of health for your health region. If you are willing and able to provide the blood sample, then we can proceed right now with a short phone survey about West Nile virus. Do you have any questions about the study as I have described it so far? ANSWER QUESTIONS FROM F.A.Q. AND THEN: Are you willing to participate in both parts of the study?

CONSE: CONSENT
Before I start with the actual survey questions, I want to remind you that your help is voluntary and all your responses are strictly confidential. SAY SLOWLY: You are free to stop the interview at any time. If there are any questions you feel uncomfortable answering, please tell me and I will move on to the next one. May I continue now?

HEAR
The first part of the survey deals with your knowledge about West Nile Virus. Before today, have you ever heard of West Nile Virus?

Yes		
No	**skip to**	**LSUM**
Don't Know	**skip to**	**LSUM**

WHERE
Where did you hear about it?

- Newspaper
- TV
- Radio
- Friends
- Pamphlets

Doctors/health care professionals
Internet
Other: Please specify

MOSQ
To your knowledge, has West Nile Virus been identified in mosquitoes in Alberta?
Yes
No
Don't know

PEOP
To your knowledge, have there been people infected with West Nile Virus identified in Alberta?
Yes
No
Don't know

WORRI
How worried are you about getting West Nile? Are you....
Very worried
A little worried
Not worried at all

LIST
I am going to read you a list of possible ways people can get a disease. As I read each one, please tell me whether or not you think a person can become infected with West Nile virus that way:
Mosquito bites?
Blood transfusions?
Sexual contact with someone who has WNV?
Organ transplants?
Being in the same room with someone who has WNV?
Contact with dead birds?
Shaking hands with someone who has WNV?
Yes
No
Don't know

AGE
To the best of your knowledge, which age group is more likely to develop severe complications when infected with West Nile virus?

OPTIONAL READ: Severe complications may require hospitalization and in rare circumstances can lead to prolonged health problems or can be fatal. Examples include brain or spinal cord membrane inflammation...
Meningitis is an inflammation of the membrane around the brain and spinal cord. Encephalitis is an inflammation of the brain. Meningoencephalitis is an inflammation of the brain and the membrane surrounding it.

YOUNG CHILDREN - Under the age of 5 years

CHILDREN - Between the ages of 6 and 12
TEENAGERS - Between the ages of 13 and 17
ADULTS - Between 18 and 64
SENIORS - 65 years of age or older
DON'T KNOW

LSUM
Thinking back to last summer, at times when you might be bitten by a mosquito, how often did you do the following things?

Restrict your outdoor activities
Avoid the places where the mosquitoes were a problem?
Wear long sleeves and pants?
Wear light colored clothing?
Avoid the times of day when mosquitoes were most active? (If required: Mosquitoes are most active at dusk and dawn)
Wear mosquito repellent?

Always
Most of the time
Sometimes
Rarely
Never
Don't know

NORE
If you did not use repellents, why not?

Concerned about the use of chemicals
Perceived low risk of getting West Nile virus
Did not see mosquitoes
Concern over interaction with sunscreen
Too much trouble
Cost too much
Didn't bother
Other (please specify)
Don't know

DEET
Did the mosquito repellent you use most often contain DEET?

OPTIONAL READ: DEET is the most effective ingredient used to repel pests like mosquitoes or ticks. DEET does not kill mosquitoes; it just makes them unable to locate people and to feed on them.

Yes	**skip to**	REDUC
No	**skip to**	NODE
Don't know	**skip to**	REDUC

NODE
If you did not use DEET repellents, why not?

Concerned about chemical use

Perceived low risk of getting West Nile Virus
Did not see mosquitoes
Concern over interaction with sunscreen
Too much trouble
Cost too much
Didn't bother
Other (please specify)

REDUC

Last summer did you or anyone in your household do any of the following things to reduce the number of mosquitoes around your house?

Put screens on windows or doors that previously had none?
Repaired screens that had tears or holes in them?
Checked and cleaned all rain gutters as required?
Regularly emptied or replaced pools of standing water? Examples of standing water include: birdbaths, tires, children's pools or fountains that don't work.

Yes
No
Does not apply

WORK

Did you work last summer? (Skips work day questions if they did not work at all)
On a typical workday last summer (July to September), how much time did you spend outdoors during the following time periods?

Early morning (4 am to 8 am)
Day time (8 am to 5 pm)
Evening (5 pm to 9 pm)
Nighttime (9 pm to 4 am)

NONW

On a typical day when you were **NOT working** last summer (July to September), how much time did you spend outdoors during the following time periods?

Early morning (4 am to 8 am)
Day time (8 am to 5 pm)
Evening (5 pm to 9 pm)
Nighttime (9 pm to 4 am)

AGREE

In Alberta, mosquito control intervention initiated by communities may include draining standing water and using chemicals to keep mosquitoes from hatching. These chemicals are called larvicides. Would you agree to the use of mosquito control programs in your area to reduce the number of mosquitoes?

Yes	**skip to**	PROG
No	**skip to**	WHYNO
Don't know	**skip to**	WHYNO

WHYNO

What are some of the reasons you would not agree (or don't know if you would agree) to the use of mosquito control programs in your area? (Read brackets if they said they did not know if they would agree to the use)

No insects/pest problems
Child's health
Adult's health
Pet/livestock health
Environmental protection
Unsafe/hazardous
Too expensive
Not enough information about risks
Other: Please specify
Don't Know

PROG
In your opinion, **if** there is a mosquito control program in your area this summer, would it be important to protect yourself from being bitten by mosquitoes by doing things like wearing repellent, light-coloured clothing, pants and long-sleeved shirts or by avoiding times when mosquitoes are most active?

Yes
No
Don't know

LIVE
Do you live within 5 km of a major urban area?
READ IF NECESSARY: Greater Calgary area (Airdrie, Calgary, Chestermere, Cochrane, Okotoks and Strathmore) Greater Edmonton area (Fort Saskatchewan, Leduc, Sherwood Park, Spruce Grove, St Albert and Stony Plain) Fort McMurray, Grande Prairie, Lethbridge, Medicine Hat or Red Deer

Yes
No
Don't know

The following questions are for grouping our results and will not be used to identify you in anyway.

SEXQ
ONLY IF YOU ARE NOT SURE: It may seem obvious, but we have to ask everyone, are you male or female?

Male
Female
Don't know

AGEGR
Which of the following age groups best describes you?

18 - 24 years
25 - 34 years

35 - 44 years
45 - 54 years
55 - 64 years
Over 65

EDUC

What is the highest level of education you have completed?

Elementary, junior high or less
Senior high - incomplete
Senior high - complete
College or technical school - incomplete
College or technical school - complete
University - incomplete
University - complete
Graduate degree
Don't know

HEALT

In general, would you say your health is...?

Excellent
Very good
Good
Fair
Poor
Don't know

Thank you for completing the survey. We will be sending you forms that you will need when you go to the lab to have your blood tested. Do you have any other questions? Thank you very much for your participation. Have a good day/evening. Goodbye.

<End Survey>

Appendix B. Frequently asked questions

What is the purpose of the survey?

The Government of Alberta through Alberta Health and Wellness has the mandate to determine how many Albertans have been infected with West Nile virus since it spread to our province during the spring and summer of 2003. This information will help the province plan its educational prevention programs as well as understand the impact the virus has had thus far. Most people with West Nile virus do not even know they have it because the symptoms are so mild, so the only way to know who has been infected is through testing a small sample of blood from each person. All of the western provinces are now involved in some sort of study like this one to determine who has been exposed to the virus. This study not only asks you to provide a small vial of blood but also asks questions about how you have heard about West Nile virus, what you know about it and things you can do to prevent yourself from getting it. The research team for Alberta Health and Wellness has selected random households in the province to participate in this study so that at the end of the study, a representative view of Alberta will be obtained. The information will be used by the province, and health regions to plan their campaigns and also their medical response to this upcoming season of West Nile virus.

Is the information confidential?

Yes. Throughout the study, your personal information such as your name, address and phone number will be kept strictly confidential and is only used for the purposes of mailing necessary documents to you. At no time will this information be made public and any reports of this data will remove all personal identifiers. Individuals will not be identifiable from the reports.

Will my phone number be kept in a list?

Until the completion of this project, all of the records will be stored on a confidential list on a secured computer. You may be called later in the study to make sure you received the lab requisitions and make sure you were able to get to the lab to give your blood sample. This contact information will also be used by the medical officer of health for your health region if your blood sample does test positive for West Nile virus.

Will I be phoned back in the future?

You may be phoned during the study to ensure that you were able to get to a lab to give your blood sample. This phone call would typically take place a few weeks after the phone survey. You will also be called if your blood sample tests positive for West Nile virus. Because West Nile virus testing can take several weeks or even months to confirm positive cases, this call would probably not come for several months after the phone survey.

In the case of testing positive for West Nile virus, this call would be from the medical officer of health for your health region. Because of the size of this study, it is only possible for us to call the Albertans who have tested positive for West Nile virus.

How will this information be used?

The survey data will be analyzed by research team at Alberta Health and Wellness to help the province plan its educational strategies around preventing West Nile virus. Results from the blood tests will be used to see where West Nile virus has spread in the province. Medical officers for each health region in the province will use this information to plan their medical response to the upcoming mosquito season and also address education and prevention strategies.

How did you get my phone number?

In order to include all residents of Alberta, phone numbers were randomly generated using computer methods. The research team use complex methods to ensure that all parts of the province are included in this study and your household is invited to participate.

Who are the people who are doing this study?

This study is being coordinated by the Health Surveillance Branch of Alberta Health and Wellness from Edmonton. All the regional health authorities are represented by their medical officers of health who are part of a provincial working group to address the issues of West Nile virus in the province. Other experts on West Nile virus have also been invited to participate in this working group and have provided their input into the design, analysis and interpretation of results.

Who is paying for the survey?

The survey is funded by the Government of Alberta and administered by Alberta Health and Wellness.

Can I quit part way through this?

You can refuse to answer any question and you can stop at any time. However, in order to ensure the accuracy of our survey, it is best if all of the questions are answered. If you run out of time, or need to leave the phone, we can call you back at a later time.

Do I get paid for doing this?

You are doing an important service by answering our questions and agreeing to go to a lab and give a small sample of blood for West Nile virus testing. We really appreciate the time you are giving us. However, we are not paying people for answering these questions or giving blood. Your participation will be instrumental in how the province understands and plans for the upcoming West Nile virus season.

Can I participate in the study if I know I have had West Nile virus?

Yes, you can take part in the study. To get a reliable estimate of West Nile virus infection in Alberta, we need to include all Albertans, even those who know they have had it.

Can I participate even if I am on medication?

There are no exclusion criteria for people taking medication. But if you are concerned about the effects it may have on your own health, please consult your physician before agreeing to participate.

Can I participate even if I have another major health issue or disease?

There are no exclusion criteria for people who have health issues or illness. But if you are concerned about the effects if may have on your own health, then please consult your physician before agreeing to participate.

Will I be quarantined if I am found to be positive?

Being positive means that you have been exposed to West Nile virus in the past and your body developed an immune response to the virus. It does not mean that you are sick or that you can transmit the disease to other people. Therefore, no public health measures like quarantine are necessary.

Will I be treated if I find out I have West Nile virus?

Being positive means that you have been exposed to West Nile virus in the past and your body responded to it. It doesn't mean that you are sick. No treatment is needed in this case. If you test positive, you will be contacted by a physician or public health nurse from your health region to discuss your results.

Who can I call if I find a dead animal or bird?

If you find a fresh dead crow, you can drop it off at any Fish and Wildlife office throughout the province. To find the office closest to you, call toll-free 310-0000. When dealing with any 'found dead' wildlife, always wear gloves, pick up the carcass using a bag inverted over your hand, or use a stick to move the dead animal into a container. Do not handle dead wildlife directly with your hands.

What is Alberta Health and Wellness?

Alberta Health and Wellness is the provincial government department responsible for encouraging and supporting healthy living, ensuring quality health services and providing leadership to the health care system.

What will happen to my blood after it is tested?

All the samples will be labeled with a confidential study ID number and stored by the ProvLab for the duration of the study period. This may be for a several months to allow all confirmatory testing to be done.

What are the risks of giving a blood sample for testing?

Having a blood sample drawn does not put you at risk of disease. All needles are sterile, used only once and discarded. Blood is drawn by trained professionals. In some cases, occasional bruising at the needle site may occur.

How much blood will be taken?

Only a very small quantity will be taken - 10cc or 10 ml. This is the equivalent to a standard blood collection tube you may have had drawn as part of a regular physical checkup.

Will my blood be used (or tested) for anything else?

Your blood will not be tested for anything besides West Nile virus and as part of this study.

Appendix C. Sample letters

Dear <respondent's name>

Thank you for agreeing to participate in this important provincial study of West Nile virus. We greatly appreciate the time that you have already spent answering the phone survey. This information will be used to plan further public education campaigns about West Nile virus. As we explained, this study is made up of two parts. The second part involves collecting blood samples to determine how many Albertans have been infected with West Nile virus. We thank you for consenting to provide a blood sample.

We have enclosed a personalized Laboratory Requisition that you MUST TAKE WITH YOU when you go to have your blood sample taken. We have also included a list of laboratories that can take your sample. Please pick the laboratory that is most convenient. There are no other special instructions.

We do request that you provide this sample as soon as possible so that the results of this phase of the study can inform West Nile virus control procedures for the 2004 summer season.

After you have provided your blood sample, we will only contact you again if you have been infected with West Nile virus. It may take several weeks for positive specimens to be identified and confirmed, but if you have been infected you will be contacted by the office of the medical officer of health from your regional health authority.

If you have not provided a blood sample within three weeks, we will contact you as a reminder.

If at any time, you have questions or concerns about this study or your participation in it, please contact:

Thank you once again for your willingness to participate in this study. Your contribution will be invaluable.

Sincerely,

Original signed

Date

Dear <respondent's name>

I would like to thank you for your participation in the Alberta Health and Wellness West Nile virus study. Your contribution will allow us to assess the extent of the infection in Alberta and will be used to guide our public health response to West Nile virus in the future.

Enclosed please find a brochure that explains what West Nile virus is and how to reduce your risk of being infected.

If you have any further questions or concerns about West Nile virus, this study or your participation in it, please contact:

Thank you for contributing your time to help us address this important public health issue.

Sincerely,

Original signed

Appendix D. Serology

Test Name	Test Format	Test Performance and Interpretation
WNv IgM	A high volume enzyme immunoassay test (EIA) which detects WNv-specific IgM in serum	• Only positive in about 50% of cases during the first week of illness. WNv IgM is nearly always positive in cases after the first week of illness. • Little cross-reactivity with other flaviviruses. • WNv IgM antibody persists for >9 months in at least two thirds of cases. A patient with a positive WNv IgM result may have acquired the infection last season.
WNv IgG	EIA for WNv IgG in serum.	• Cross reacts extensively with other flaviviruses, such as St. Louis Encephalitis, Dengue, Japanese Encephalitis and Yellow Fever, including vaccination. • NOT a reliable marker of immunity to WNv. • Useful to show rising IgG levels in acute and convalescent sera, which are strongly suggestive of recent infection
WNv Plaque Reduction Neutralization Titre (PRNT)	Measures ability of patient serum to block live WNv infection in a cell line. Performed in the Containment Level-3 Lab at the National Microbiology Lab in Winnipeg.	• Highly specific for WNv. "Gold standard" serologic test. • Indicates CONFIRMED previous WNv infection. • Not a rapid test. Results take 4-8 weeks. Too slow for acute public health decision making, but performed for laboratory quality

Appendix E. Follow-up information for those testing positive

West Nile Virus Seroprevalence Follow-Up
Key Messages

- Acknowledge study participation
- Communicate that the respondent tested positive
- What does it mean to test positive
- Possible misconceptions about testing positive
- The need to continue using protective measures to avoid mosquitoes bites
- Answer any other related questions from respondent

Thank you very much for participating in the West Nile Virus Seroprevalence Survey last year, in the spring of 2007. The time that you spent answering the telephone survey and submitting a blood sample is greatly appreciated.

We are now able to communicate the results of the study. According to our records, the blood sample that you submitted last spring as part of this study tested positive for West Nile virus.

Testing positive means that you have been exposed to WNV in the past and your body developed an immune response to the virus. Your blood sample was tested for exposure to the virus before or during the 2006 summer season only. These results will not indicate if you were exposed to the virus last summer (2007), or this year.

Testing positive does not mean that you are sick or that you can transmit the disease to other people. No treatment is needed in this case.

Although you have evidence of exposure to WNv, we do not presently know whether this provides protection against illness if you are exposed to WNv in the future, or how long any protection might last. Therefore it is very important that you continue to take action in protecting yourself against mosquito bites in the future.

You can take simple precautions to avoid mosquito bites:

- Use a mosquito repellent containing DEET or other approved ingredients on exposed skin; apply it on clothing as well, as mosquitoes can bite through fabric.
- Avoid outdoor activities at dusk and dawn where possible
- Wear long -sleeved shirts and pants during peak mosquito times as well as light coloured clothing.

It is also important to take measures around your home:

- Make sure that there is no standing water in your yard where mosquitoes can lay their eggs.
- Keep mosquitoes out of house by using window and door screens, etc.

For further information on West Nile virus: www.fightthebite.info