Seroprevalence of West Nile Virus in Saskatchewan's Five Hills Health Region, 2003

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ABSTRACT

Background: The Five Hills Health Region of Saskatchewan reported the highest West Nile virus (WNV) case rates in the 2003 outbreak. A serologic and telephone survey was undertaken to assess the seroprevalence of the virus and the knowledge, attitudes and behaviours of the residents.

Methods: Respondents had to be at least 18 years of age, and residents of the Five Hills Health Region between July 1st and September 15th, 2003. Blood samples of respondents were tested at the National Microbiology Laboratory for flavivirus immunoglobulin using a WNV IgG ELISA and plaque reduction neutralization test. Descriptive analyses performed related to respondents' demographics, knowledge, attitudes, behaviours, and seropositivity. WNV infection risk was assessed using odds ratio.

Results: There were 619 questionnaire respondents, of whom 501 donated a blood sample. The seroprevalence of WNV in the Five Hills Health Region was 9.98% (95% CI 7.37-12.59%). Seropositivity of rural areas was 16.8% and urban was 3.2%. Most (97%) of participants thought WNV was an important health issue. Forty-eight percent of the participants used insect repellents containing DEET most of the time. There was good knowledge regarding WNV transmission and prevention of the spread of WNV. Rural compared to urban residents were six times more likely to be positive for WNV (OR=6.13, 95% CI 2.82-13.34).

Interpretation: This is the highest seroprevalence rate of West Nile virus recorded in North America thus far. Many factors could have influenced this outbreak, such as eco-region, early prolonged hot weather, level of mosquito control programs, urban and rural community differences, and personal protective behaviours.

MeSH terms: West Nile virus; seroepidemiologic study; Saskatchewan; knowledge, attitude, behavior

La traduction du résumé se trouve à la fin de l'article.

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The most recent outbreaks of West Nile virus (WNV) human manifestation have been in Romania (1996-1997),[1] United States (beginning 1999),[2,3] Israel (2000)[4] and Canada (beginning 2002).[5-9]

A seroprevalence study of WNV was conducted after an outbreak in Romania in 1996. The attack rate was 12.4/100,000 and serological prevalence was 4.1%.[2] Lower post-epidemic serological prevalence was estimated among residents of Queens, New York in 1999 with a weighted seroprevalence of 2.6%.[3] Also, Nassau and Westchester Counties, New York[3,10] and Fairfield and surrounding Counties in Connecticut[11] had seroprevalence rates of 0-1%. A serological survey conducted in Oakville, Ontario found a WNV prevalence of 3.1% and a provincial attack rate of 3.3/100,000.[5,6] In 2003, Saskatchewan had 937 WNV human cases – an attack rate of 93/100,000.[5] The serological survey reported in this paper is the first of its kind in Saskatchewan. Similar to the Elliott et al. study,[6] this study was conducted after the previous season's outbreak. It describes the WNV distribution in the population, as well as risk and protective factors associated with WNV infection. The Five Hills Health Region (FHHR) in Saskatchewan was the chosen study location due to its highest number of cases per capita in the province. Individual health region rates ranged from 0 to 400 per 100,000 population.

The objectives of this study were: to assess the extent of WNV prevalence in the FHHR in 2003; to estimate the extent of knowledge, attitudes, and behaviours in relation to WNV infection in the FHHR in 2003; and to identify WNV risk and protective factors in the FHHR.

METHODS

The survey was a cross-sectional prevalence study conducted in the Five Hills Health Region (FHHR), situated in the south central area of Saskatchewan (Figure 1). This region had the largest number of WNV cases per capita (approximate attack rate of 400/100,000) in Saskatchewan during the summer of 2003, and was chosen in order to estimate what was likely the highest WNV seropositivity in Saskatchewan. Residents were contacted in three study areas chosen based on their

mosquito control programs, the size and effectiveness of which may be related to levels of WNV infection present. These programs emphasized larval development site identification, mosquito source reduction, and larviciding. Gravelbourg (1,286 residents), Mossbank (416), and Willow Bunch (407) were areas with low levels of mosquito control programs; Assiniboia (2,710 residents) had medium levels and Moose Jaw (34,185) had a large and intensive mosquito control program. Low- and medium-range mosquito control programs were similar and were based in rural communities; the only high-level program was run in Moose Jaw. Hence the study areas were regrouped along urban-rural lines. All residents of the FHHR who lived in the area between July 1, 2003 and September 15, 2003, and who were 18 years or older were eligible to participate in the study. A previous diagnosis of WNV, by physician or blood test, did not exclude potential subjects from participating in the study. At a presumed prevalence of 4%, and 2% precision, the estimated minimum sample size was 369. A random selection of 619 survey respondents was made through approximately 3,000 telephone calls. Using the reverse telephone directory for Moose Jaw, Gravelbourg, Mossbank, Willow Bunch, Assiniboia and surrounding areas, names and addresses were removed and numbers were randomly assigned to one of four telephone interviewers. Thus residents without a listed landline phone number were excluded from the survey. Residents were contacted weekday evenings and Saturday morning and afternoon. The first person in a household to meet the eligibility criteria was invited to participate. Before answering the questionnaire, measuring knowledge, attitudes and behaviours, participants were also invited to agree to provide a blood sample at a later date (blood collection rate of 81%).

A pilot study was conducted to assess the questionnaire and interview process. Pilot study subjects were recruited from another community in FHHR; the pilot participants had only an interview, no blood was collected. Figure 1 shows the FHHR and study areas.

Blood samples were tested at the National Microbiology Laboratory (NML) in Winnipeg. All sera were screened for flavivirus IgG using a WNV antibody capture ELISA, as described previously.[12] Reactive samples were confirmed for the presence of WNV specific antibody by plaque reduction neutralization test (PRNT). The standard PRNT was performed essentially as described by Beaty et al.[13]

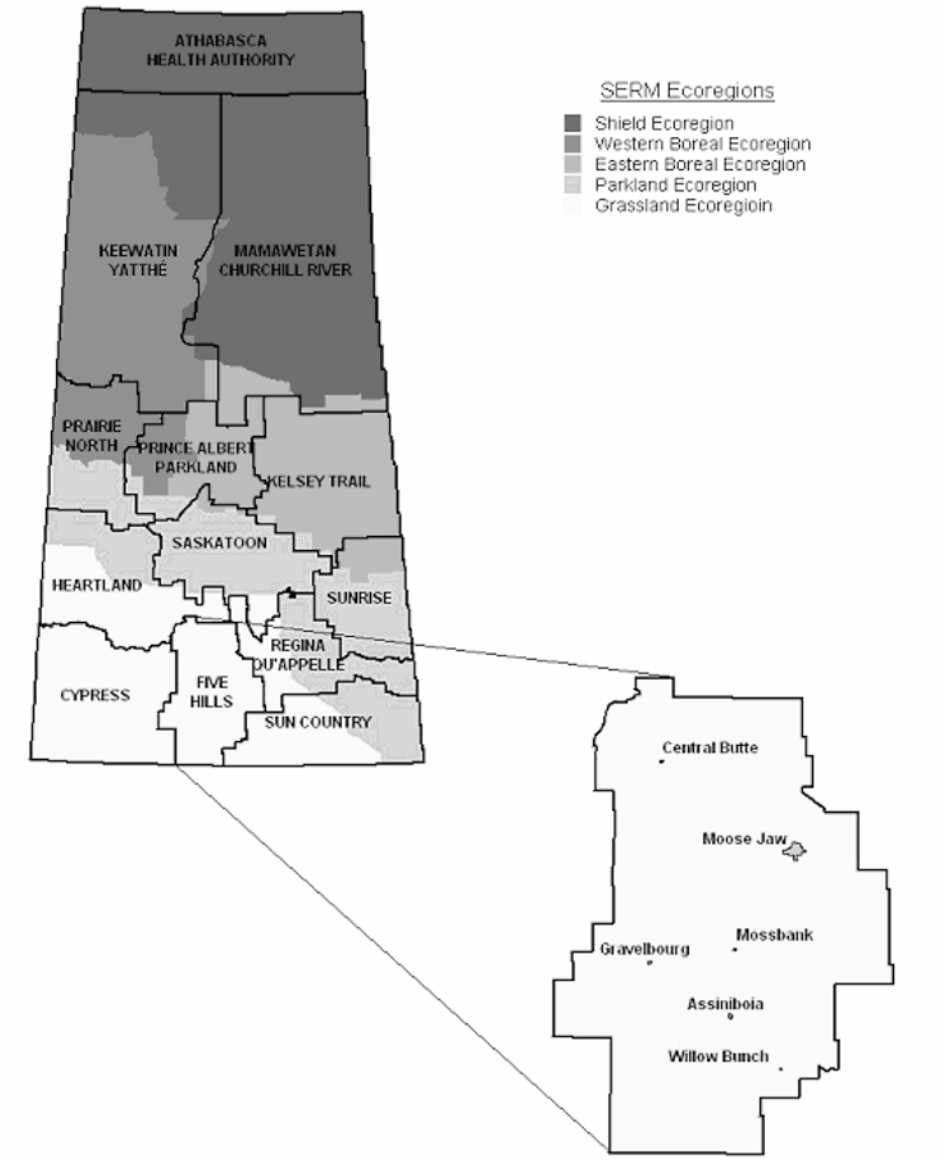

Figure 1. Map of Saskatchewan Eco-Regions, Five Hills Health region and Study Sites 2003

Laboratory results were matched to the questionnaire responses and analyzed using EPI Info software, version 2002 designed by Centers for Disease Control, Atlanta, Georgia and by the World Health Organization in Geneva, and SPSS, Version 11.5, SPSS Inc., Chicago, IL.

Descriptive analysis of variables in the study focussed on demographic and survey responses. Then unadjusted odds ratios for selected characteristics were derived from two by two tables and simple logistic regression models.

Ethics approval was provided by the Bio-medical Ethics Committee at the University of Saskatchewan, as well as by the ethics boards in the FHHR and Health Canada.

RESULTS

Of the 501 sera tested, 50 were found to be positive for WNV specific antibody. The overall WNV seroprevalence was 9.98% (95% CI 7.37-12.59%). Rural residents were approximately six times more likely to be positive for WNV, compared to urban residents (OR=6.13, 95% CI 2.82-13.34) (Table I).

TABLE I

WNV Serology Results vs. Selected Characteristics (N=501)

Variable	Positive n=50 (10%)	Negative n=451 (90%)	OR	95% CI
Age				
0-59	33 (66.0%)	328 (72.7%)		
60+	17 (34.0%)	123 (27.3%)	1.37	0.74-2.56
Sex (				
Male	14 (28.0%)	129 (28.6%)		
Female	36 (72.0%)	322 (71.4%)	1.03	0.54-1.97
Education (n=499)				
≤ high school	29 (58.0%)	205 (45.7%)		
Post secondary	21 (42.0%)	244 (54.3%)	1.64	0.91-2.97
Residence				
Rural	42 (84.0%)	208 (46.1%)		
Urban	243 (53.9%)	243 (53.9%)	6.13*	2.82-13.34
Job Type (n=378)†				
Outdoor	19 (46.3%)	107 (31.8%)		
Indoor	22 (53.7%)	230 (68.2%)	1.86	0.96-3.58
Avoid areas where mosquitoes were likely to be a problem‡				
Yes	14 (28.0%)	171 (37.9%)		
No	36 (72.0%)	280 (62.1%)	1.57	0.82-3.00
Restricting outdoor activity‡				
Yes	2 (4.0%)	46 (10.2%)		
No	48 (96.0%)	405 (89.8%)	2.73	0.64-11.59
Wore long sleeves and long pants when outdoors‡				
Yes	23 (46.0%)	185 (41.0%)		
No	27 (54.0%)	266 (59.0%)	0.82	0.45-1.47
Applied insect repellents containing DEET‡				
Yes	19 (38.0%)	214 (47.5%)		
No	31 (62.0%)	237 (52.5%)	1.47	0.81-2.69
Applied insect repellents containing non-DEET‡				
Yes	3 (6.0%)	28 (6.2%)		
No	47 (94.0%)	423 (93.8%)	1.04	0.30-3.54
Used two or more personal protective measures‡				
Yes	17 (34.0%)	196 (43.5%)		
No	33 (66.0%)	255 (56.5%)	1.49	0.81-2.76
Self reported previously diagnosed with WNV				
Yes	13 (26.0%)	2 (0.44%)		
No	37 (74.0%)	449 (99.6%)	78.89*	17.45-362.81

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

TABLE II

Demographic Characteristics of the Study Sample (N=619)

Variable	Frequency N=619 (%)
Sex	
Male	184 (29.7%)
Female	433 (70.0%)
N/A	2 (0.3%)
Age	
18-29	75 (12.1%)
30-39	87 (14.1%)
40-49	159 (25.7%)
50-59	136 (22.0%)
60-69	72 (11.6%)
70+	88 (14.2%)
N/A	2 (0.3%)
Mean age	49.7
Education*	
Grade School	125 (20.2%)
High School	166 (26.8%)
Post Secondary	323 (52.2%)
N/A	5 (0.8%)
Residence	
Rural	312 (50.4%)
Urban	307 (49.6%)
Indoor/Outdoor Job	
Mainly Indoor	306 (49.4%)
Mainly Outdoor	172 (27.8%)
Unemployed	135 (21.8%)
N/A	6 (1.0%)
Occupation	
Farming	85 (13.7%)
Government	21 (3.4%)
Education	36 (5.8%)
Health Care	52 (8.4%)
Trade	46 (7.4%)
Parks & Recreation	7 (1.1%)
Office, factory, retail & restaurant	129 (20.8%)
Other	9 (1.5%)

* Highest level completed

Respondents were predominantly female (70%). The average age of participants and the FHHR population were similar. The age distribution of the study sample reflected that of the health region, with the exception of the 18-29 year old population, which was undersampled in this study. Half of the study sample (52.2%) had completed at least some post-secondary education. Respondents were about equally divided between rural and urban residences (50.4% and 49.6% respectively), which differs from the FHHR rural and urban population distributions (38% and 62% respectively). The detailed demographic characteristics of the study sample are available in Table II.

Most respondents felt that West Nile virus was an important health issue (96%). Almost half of the respondents used insect repellents containing DEET most of the time (48%). Just over a third (38.8%) of 18-59 year olds were using insect repellent containing DEET most times compared to 9.5% of 60+ year participants. There was overall good knowledge regarding WNV transmission by "mosquitoes" (99%), "blood transfusions" (70%), and "organ transplants" (48%). Two thirds (65%) of respondents thought WNV could be contracted by "contact with dead birds". Some of the erroneous assumptions regarding possible means of contracting WNV included: "shaking hands with a WNV positive patient" (6%); "having sex with a WNV positive patient" (6%); and "drinking infected water" (28%). Knowledge of WNV prevention was high: "wearing protective clothing" (96%), "applying insect repellents containing DEET" (95%), and "avoiding going outside during peak mosquito times" (96%). However, 51% and 72%, respectively, responded that "washing hands regularly" and "using a bug zapper" were effective preventive measures. Table III includes details of the above responses.

DISCUSSION

West Nile virus seroprevalence in the FHHR in Saskatchewan in 2004 was found to be approximately 10%. To date, this is the highest seroprevalence rate for WNV in North America.[2,6,10] Saskatchewan experienced the highest number of WNV cases in Canada during the summer of 2003.[5] Evidence suggests that approximately 80% of WNV infections are asymptomatic,[14] therefore a high WNV survey seroprevalence was to be expected. The FHHR was selected for study as it had the highest number of reported cases in 2003. Limited resources were concentrated in one geographical area of the province, where the highest seropositivity rate was likely to be measured. Other regions of the province had fewer or no reported cases.

Biases and limitations

It is possible that previously diagnosed WNV patients were more likely to volunteer for this serosurvey, introducing selection bias. If the 13 previously diagnosed subjects are removed from the seroprevalence calculation, the rate is 7.4% (as opposed to 10%). These subjects might have artificially increased the seroprevalence to a rate greater than what would be expected in a random sample of FHHR residents.

Females were oversampled in this study, as we did not employ sex-specific selection methods; the first person to answer the phone who met the eligibility criteria was invited to participate. It is unknown how this could have influenced results, as WNV infection rates are similar between the sexes, but personal protective behaviours and knowledge of WNV could be different.

Recall bias could have had an impact on survey responses. Participants were telephoned in March and April 2004 and the questionnaire was designed to recall participants' behaviour which occurred during the summer of 2003.

Selection bias was introduced by excluding from the survey residents without telephones, who use cell phones exclusively or had an unlisted number. According to SaskTel, the main telephone service provider in Saskatchewan, approximately 5% of Saskatchewan phone numbers are unlisted.[15]

No laboratory-confirmed human cases of WNV were reported in Saskatchewan in 2002. WNV was present in 2002, in corvids and horses; it is possible that 2003 was not the first year WNV was introduced in the human population, and that cases went undiagnosed due to the high rate of asymptomatic infection.[14] It is possible the seroprevalence could be a cumulative rate of WNV over two years (or more), instead of a point prevalence for 2003. However, there was still a chance that this study was measuring a point prevalence of WNV due to the lack of reported human cases in 2002.

Bird and mosquito factors related to survey seroprevalence

In birds, corvids are very susceptible to the virus and have a high fatality rate.[16,17] Infected corvids usually die before or early into the onset of human cases,[17] which makes them a good sentinel for human infections. In 2003, 9 documented WNV positive deaths were detected among 34 dead corvids tested in the FHHR.[18]

Several other contributing factors relating to the ecology of the region and presence of the mosquito vector can help explain the high seroprevalence rate. These factors include above normal temperatures in the spring and summer period,[6] an extended frost-free period in the fall and long WNV exposure period.[19] Other factors included a pronounced enzootic amplification in birds and other animals, with the resultant increased transmission of the virus to humans. The primary "bridging" vector – a vector that carries the disease from one animal group (birds) to another group (humans) – driving the outbreak in birds and humans in Saskatchewan was *Culex tarsalis*.

The FHHR is located in a mixed grassland eco-region, and *Culex tarsalis* is widely distributed throughout the area. The region is consistently hotter and drier than other areas in Saskatchewan, and *Culex tarsalis* development usually starts earlier in the season with more generations of adult mosquitoes than in more northerly areas.[20]

TABLE III

Survey Respondents' Knowledge, Attitudes and Behaviours (N=619)

Variable	Frequency N=619 (%)
Has heard of WNV	615 (99.4%)
Believes WNV can be contracted through:	
Mosquito bites	611 (98.7%)
Blood transfusions	434 (70.1%)
Organ transplants	298 (48.1%)
Contact with dead birds	401 (64.8%)
Believes WNV can be contracted through:	
Sexual contact with WNV case	38 (6.1%)
Same room as WNV case	18 (2.9%)
Drinking infected water	170 (27.5%)
Shaking hands with WNV case	38 (6.1%)
Believes WNV to be an important health issue	592 (95.6%)
Believes repellents with DEET are worth using	518 (83.7%)
Supports adulticiding	466 (75.3%)
Believes the following behaviours prevent WNV:	
Wearing long-sleeved/ protective clothing outdoors	597 (96.4%)
Avoiding outdoors during peak mosq. hours of dusk & dawn	593 (95.8%)
Applying DEET	590 (95.3%)
Believes the following behaviours prevent WNV:	
Washing hands	316 (51.1%)
Applying repellent with ingredients other than DEET or citronella	196 (31.7%)
Wearing a mask	90 (14.5%)
Using a bug zapper	445 (71.9%)
Always or most of the time practiced the following behaviours:	
Avoid mosquito areas	217 (35.1%)
Restrict outdoor activity	55 (8.9%)
Wear long sleeves or pants	255 (41.2%)
Apply repellent – DEET	298 (48.1%)
Apply repellent – Non-DEET	38 (6.1%)
Always or most of the time used DEET, by age category	
18-59	240 (38.8%)
60+	58 (9.4%)

WNV knowledge and behaviour

Respondents believed insect repellents containing DEET were worth using, however half did not use them most times while outside. Persons who were over the age of 60 were less likely to use insect repellents containing DEET most times, compared to respondents 18-59 years. The Elliott et al. study also found that younger people were more likely to use insect repellent containing DEET.[6] This finding has potential implications for public messaging regarding WNV risk.

In this study, the odds of being positive for WNV were six times higher for rural compared to urban residents. Larger, urban communities, with mosquito control programs that included intensive larviciding, had significantly lower levels of *Culex tarsalis* mosquitoes.[21] Rural communities, due to their small size and close proximity to abundant *Culex tarsalis* habitat, could not employ intensive larviciding programs over large areas. Their mosquito control programs focussed on source reduction, public education, and personal protection. This coupled with the fact that rural residents may be outdoors more often and for longer periods of time, in addition to the region's eco-system, may help explain differences in the distribution of rural and urban seropositivity in the FHHR.

Individuals who reported a previous WNV diagnosis were about 80 times more likely to be serologically confirmed WNV positive compared to those who did not report a previous WNV diagnosis (OR=78.0, 95% CI 17.45-362.81). Therefore, self-reported diagnosis of WNV infection was an accurate measure for laboratory-confirmed WNV positivity in our study sample. This finding could be useful for subsequent questionnaires that rely on self-reported WNV infection alone.

This high WNV seropositivity (10%; 95% CI 7.37-12.59%) in FHHR was uneven in rural and urban areas. The potential interaction between rural-urban communities and other contributing factors, such as the level of mosquito control programs, is an interesting area for further investigation.

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RÉSUMÉ

Contexte : La région sanitaire de Five Hills, en Saskatchewan, est celle qui a déclaré le plus grand nombre de cas de virus du Nil occidental (VNO) durant la flambée épidémique de 2003. Nous avons mené une enquête sérologique et téléphonique pour évaluer la séroprévalence du virus ainsi que les connaissances, les attitudes et les comportements des résidents.

Méthode : Les répondants devaient avoir au moins 18 ans et avoir habité la région sanitaire de Five Hills entre le 1er juillet et le 15 septembre 2003. Le Laboratoire national de microbiologie a examiné les échantillons de sang des répondants à l'aide de l'ELISA IgG et d'un test de séro-neutralisation par réduction des plaques pour y détecter les immoglobulines dirigées vers le flavivirus. Nous avons effectué des analyses descriptives des données démographiques des répondants, ainsi que de leurs connaissances, de leurs attitudes, de leurs comportements et de leur séropositivité. Le risque d'infection à VNO a été évalué selon un rapport de cotes.

Résultats : Les répondants du questionnaire étaient au nombre de 619, dont 501 ont produit un échantillon de sang. La séroprévalence du VNO dans la région sanitaire de Five Hills était de 9,98 % (IC de 95 % = 7,37–12,59). La séropositivité était de 16,8 % dans les zones rurales et de 3,2 % dans les zones urbaines. La plupart des participants (97 %) considéraient le VNO comme un problème de santé important. Quarante-huit p. cent utilisaient la plupart du temps des insectifuges contenant du diéthyltoluamide (DEET). On connaissait bien le mode de transmission du VNO et les mesures pour prévenir sa propagation. Les résidents des zones rurales étaient six foix plus susceptibles d'être séropositifs pour le VNO que les résidents des milieux urbains (RC=6,13, IC de 95 % = 2,82–13,34).

Interprétation : Il s'agit du plus fort taux de séroprévalence du virus du Nil occidental enregistré en Amérique du Nord jusqu'à maintenant. De nombreux facteurs pourraient avoir influencé cette flambée, dont l'écorégion, la chaleur précoce et prolongée, l'ampleur des programmes de désinsectisation, les différences entre les communautés urbaines et rurales, et les comportements de protection individuels.