Assessing the Feasibility of Swine Influenza Surveillance in Manitoba

by

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Abstract

This project explored the feasibility of performing swine influenza surveillance in Manitoba using provincial veterinary diagnostic laboratory data and a farm premises identification registry. Diagnoses of swine influenza using polymerase chain reaction (PCR) were obtained from the veterinary laboratory database and linked with registry data on farm location and characteristics. Statistical and space-time analyses, including the Cuzick and Edwards test, Kulldorff Spatiotemporal scan, the Knox test and the modified CuSum method, were used to determine the time and spatial patterns of swine influenza in Manitoba. Analysis showed that swine influenza was endemic but also seasonal and that the frequency of diagnosis was increasing in time. Swine influenza was clustered in several regions across the province, including the southeast, and was clustered in time, particularly during the later time periods of the study. This study demonstrated that the farm premises identification registry is a crucial component of disease surveillance in animals.

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Introduction

The influenza virus is probably the most well studied virus with the exception of the Human Immunodeficiency Virus (1). Despite the effort put into understanding the virus, however, the virus and its patterns of activity remain an enigma. Even one of the world's leading influenza researchers stated that "Influenza is not a tidy or predictable disease, and those who attempt to label it and put it in a box do so at risk" (2).

Influenza viruses are a member of the family *Orthomyxoviridae* and the genera *Influenza*. Influenza virus can infect a variety of species as a zoonotic pathogen and is categorized into subtypes A, B and C. Influenza A occurs in humans, animals and birds. Influenza B and C viruses occur in man only and are nonsignificant types in animals, despite isolations of influenza B from seals (3) and influenza C from pigs (4).

Kilbourne referred to the untidy and unpredictable nature of influenza and 2 components of the virus are responsible for this phenomenon. First, the virus contains 8 segments of linear negative-sense, single-stranded RNA that serve as its genetic code and cause the evolution of the virus through genetic drift and genetic shift. Genetic drift occurs because RNA viruses, including influenza, lack the polymerases to correct errors that occur during replication. These point mutations result in a mismatch between the field and vaccine strains of the virus thereby reducing vaccine efficacy. Genetic shift occurs when the 8 strands of RNA reassort, potentially leading to a substantial change in the subtype

of the virus. Ineffective or nonexistent immunity to this novel virus in a population could result in an epidemic or pandemic.

The second component causing instability of the virus is 2 antigenic glycoproteins situated on the viral membrane. The first glycoprotein, hemagglutinin (H), functions in attaching the virus to sialic acid receptors on host cells. The other glycoprotein, neuraminidase (N), removes sialic acid from the cell surface and facilitates the release of progeny influenza viruses from the host cell. There are 16 hemagglutinin and 9 neuraminidase subtypes and in theory there can be any combination of these hemagglutinin and neuraminidase subtypes, but in reality it appears that the number of feasible combinations is limited. To date, only H1, H2, H3, H5, H7 and H9 have infected humans, while subtypes H1, H2, H3 and N1 and N2 have been involved in pandemics (5, 6). Subtypes H1, H3, N1 and N2 typically circulate in swine (7, 8), although there have been sporadic isolations of unique strains such as H4N6 and H2N2 (9).

The ecology of influenza is extensive and complex. Influenza has been detected in 18 species of mammals as well as birds and has also been reported to be carried by blow flies (5). Influenza is most commonly found in humans, pigs, birds and horses, while infections are sporadic or geographically limited in dogs, mink, seals and whales (10). Each species has several influenza subtypes that are well established within the species, but transmission across species occurs occasionally and the mechanisms of transmission are poorly understood (11). Wild birds are the reservoir for all influenza viruses; in these species the virus does not produce disease but replicates in the intestine and is excreted in

large amounts via the feces (10). Outbreaks in domestic poultry occur primarily through contact with feral birds or by transfer of infected feces by humans (12). Influenza strains found in wild birds are a low pathogenic type, but when the virus is transmitted to domestic poultry it can change to a highly pathogenic form (11).

It is unknown how new strains of influenza become established in human populations (13). It has been proposed that influenza transfers from wild birds to humans via quail, pigs and chickens as intermediate hosts (14); however, this route has not been proven and there is little evidence that pigs are directly involved in the development of pandemic influenza (7, 14). Research into the pathogenicity and transmission factors of the influenza virus is ongoing. Once established in the human population, the transmission of influenza occurs by droplet, direct and indirect contact and airborne spread. In humans, the primary modes of transmission are droplet and direct and indirect contact, while airborne transmission is thought to play a very minor role in the spread of influenza (15).

In pigs, swine influenza virus transmits by droplet and direct and indirect contact (15). While the role of aerosol transmission in the spread of swine influenza is uncertain, it should not be disregarded. There are anecdotal reports that swine influenza virus can spread via an airborne route for up to several kilometres (16–19). In addition, a review of many swine influenza virus outbreaks in turkeys found that the outbreaks were not associated with either animal or human contact between a swine farm and the turkey farm (20). A spatial analysis of the influenza status of turkey farms in Minnesota found that a positive status was associated with the number of swine barns within 3 miles of the

premise, and that a dose-response relationship existed between the likelihood of a positive influenza status of a turkey farm and the number of swine barns within 1, 2 and 3 miles (20).

Regardless, the main mode for the introduction and circulation of swine influenza virus on farms is through contact between infected and susceptible pigs (10, 21). Thus, swine influenza virus will persist on farms with a continuous flow of new pigs, while farms that depopulate and disinfect facilities between batches of pigs should be able to control the spread of influenza (21). A latent or carrier state has been postulated to explain the survival of swine influenza virus between outbreaks; however, there is no scientific evidence for a carrier state and it appears that the introduction of naïve pigs causes the persistence of the virus (10, 21).

There are currently 4 subtypes of influenza circulating in North American swine: H1N1, H3N2, H1N2 and pandemic 2009 (H1N1) influenza. The H1N1 subtype, sometimes referred to as classical H1N1, was first isolated by Richard Shope in 1930 (21) and remained the only strain of swine influenza in North America for over 80 years (22, 23). This virus is characterized by its highly stable nature as its drift rate is very low – even lower than the drift rate of human H1N1 influenza viruses (8, 24). However, since 2001 there has been some reassortment of the H1 hemagglutinin gene resulting in 3 reassortment groups or clusters of H1: classical-like, reassortant-like and H1N2-like (25). The H3N2 swine influenza virus emerged in the southern United States in 1998 with 2 reassortants: a double reassortant consisting of human and avian strains of influenza and

a triple reassortant consisting of human, avian and swine influenza strains (23). In 2004, the H3N2 swine influenza virus was first diagnosed in Manitoba (26). The H1N2 swine influenza virus appeared in 1999 as a result of the reassortment between a classical H1N1 and H3N2 swine influenza virus and became established in the United States swine herd population (24). However, there has only been 1 case of H1N2 diagnosed in Manitoba to date (Tomy Joseph, personal communication). Finally, pandemic 2009 (H1N1) influenza was first reported in humans on April 21, 2009 (27) and the first isolation was from a swine herd in Alberta on May 2, 2009 (28). The virus was first diagnosed in a swine herd in Manitoba on June 30, 2009 and several farrowing, nursery and finishing herds were infected throughout the rest of the year (29). It is believed that the pandemic (H1N1) 2009 influenza virus originated in swine as the virus has significant components of North American and European lineages of swine influenza. The hemagglutinin sequence closely matches a sequence isolated in Indiana in 2000 and the neuraminidase sequence closely matches a sequence isolated in England in 1992 (30). However, its origin in swine has not been proven (14, 31) and the virus also has genes originating from human and avian influenza.

Several studies have tried to ascertain risk factors in the spread of swine influenza virus between swine. It is agreed that swine influenza was once seasonal similar to human influenza but has now become endemic in swine herds (9, 23, 32, 33) and that its epidemiology is becoming more complex (34). This is attributed to the change in swine production from low intensity practices to total confinement systems that introduce susceptible pigs on an on-going basis (21); however, increased surveillance and changes

in viral genetics could also be factors (22). Poljak conducted several studies which found that positive titres for swine influenza were associated with animal and farm density, positive source herds, number of animals and closeness to other herds (35, 36). A study of an outbreak of H3N2 swine influenza in Manitoba (26) found that the outbreak clustered in space and space-time, particularly in the southeastern portion of the province where pig density exceeded 200 pigs per square kilometre. Farms within space-time clusters were more likely to be nursery or finisher sites. A German study reported that swine influenza was prominent in swine dense regions, with multiple strains of swine influenza and a distinct periodic disease pattern in swine dense areas, while low swine density areas tended towards a single strain of swine influenza with an endemic disease pattern (37). A study of swine influenza in the United Kingdom found that farms diagnosed with swine influenza were associated with farm size and biosecurity measures (38). Farms with swine influenza were also more likely to be coinfected with other swine pathogens and had poor respiratory scores on the lungs at slaughter. H1N1 swine influenza also clustered in the northeastern region of the country.

Influenza viruses have been transmitted between pigs and other animal species. There are reports of sporadic and experimental infection of pigs with avian influenza strains including H5N1, H7N7 and several low pathogenic strains; however, avian influenza infects but does not replicate effectively in pigs (8). There are also several documented cases of triple reassortant H3N2 swine influenza infecting and becoming established in turkeys (39–41).

Influenza viruses have also been transmitted between humans and pigs. Reverse zoonosis or anthropozoonosis, defined as the transmission of disease from humans to animals, is a phenomenon that is rarely reported and occurs with influenza. Pigs are easily infected with human strains of influenza (42) and it is believed that these strains adapt to pigs over several years (25). Past pandemic viruses, including the 1918 H1N1 and the 1968 H3N2 influenza viruses, have infected and evolved in swine to become the H1N1 and H3N2 swine influenza viruses currently circulating in North American pigs today (24, 43). Crossover of swine influenza to humans is also reported to have occurred in 1997 and 2003 (44). The human-lineage PB1 gene appears to play an important role in the evolution and establishment of some influenza viruses in the swine population (45). The pandemic (H1N1) 2009 influenza virus was detected in pigs herds after its emergence in the human population (28, 29) and the PB1 gene of the novel pandemic (H1N1) 2009 influenza virus has a close homology with the PB1 gene of the human seasonal H3N2 influenza virus (46).

Swine influenza viruses have infected humans with outcomes ranging from no clinical signs to death. A literature review of human cases of swine influenza found 50 cases published in the literature and calculated a case fatality rate of 14% (47). Other studies have identified antibodies to swine influenza in people with occupational exposure to swine (8, 48, 49). However, serological studies of swine influenza in humans should be interpreted with caution as antibody tests are not reliable in people over 50 years of age or in those vaccinated with any vaccine, particularly the 1976 swine influenza vaccine (8).

There are various surveillance systems that monitor different aspects of the ecology of influenza, including human influenza surveillance systems that operate at the provincial, national and global levels. Surveillance of influenza in Manitoba is performed by the Public Health branch of Manitoba Health (50). This surveillance system tracks cases of laboratory-confirmed influenza, suspected cases of influenza outbreaks reported by a regional health authority or by the provincial diagnostic laboratory, data from sentinel physicians in Canada's FluWatch program, and reports of influenza-related deaths. FluWatch is the national influenza surveillance program started by the Public Health Agency of Canada in 1996 (51). The objectives of FluWatch are early detection of influenza epidemics, monitoring and surveillance of ongoing influenza activity, monitoring of strain genetics and resistance, and provision of information to the World Health Organization for vaccine development. Sources of information for this program include diagnoses from sentinel laboratories, typing and resistance data from the National Microbiology Laboratory, influenza-like illness diagnoses from sentinel physicians, reports from regional representatives, and admission and mortality data from a pediatric immunization monitoring program.

Global activity of influenza is monitored by the World Health Organization's Global Influenza Surveillance Network (52). This network provides data for biannual decisions on vaccine development, as well as alerting for influenza epidemics and pandemics. The data sources for this system include the National Influenza Centres and the WHO Collaborating Centres on Influenza.

Surveillance of human influenza demonstrates that its epidemiology is divided into pandemic and inter-pandemic periods. During inter-pandemic periods, influenza is minimally active during the summer and has a marked seasonal pattern that begins in early winter with influenza A subtype H3N2 and ends in spring with influenza B (53). Regional or community outbreaks climax at 2 to 3 weeks after the start of the outbreak and last anywhere from 5 to 16 weeks, although this is highly variable due to the unpredictable nature of influenza (13, 53, 54). In the northern hemisphere the season begins in late November and ends in June, peaking in January or February (54). Regions near the equator have endemic influenza with 2 peaks that are associated with the rainy season (53). Attack rates of seasonal influenza are generally low at 10 to 20% in the general population, but in susceptible populations such as school age children and the elderly the attack rate may approach 40 to 50% (13). Deaths and complications resulting from influenza tend to occur in people with underlying illnesses. The spread of influenza through human populations demonstrates a pattern of movement from high to low population density termed hierarchical diffusion which reflects mobility and transportation access (54, 55). Influenza spreads from major urban centres to other centres and outlying cities followed by spread along main transportation lines to rural areas.

While inter-pandemic or seasonal influenza is fairly predictable, pandemic influenza is irregular and unpredictable. There is no seasonality to the disease, the virus spreads globally within a period of 6 to 10 months and there usually are waves of infection. The

cause of the waves of infection is unknown but may be related to changes in virulence or effects of season (56). It is thought that the virus may actually take a period of several years to adapt to human hosts (57). Pandemics also mark a shift in predominant strain, as the new influenza subtype replaces its predecessor (13).

Three criteria are required for an influenza pandemic to occur: an influenza virus must undergo an antigenic shift to create a new virus with little immunity in the population, this new virus must cause illness and significantly increased mortality, and the novel virus must readily transmit between people (56). There have been 3 major influenza pandemics during the 20th century. The most famous of these, the 1918 H1N1 pandemic, is believed to have started in China or the midwest United States, featured several waves of infection and had an attack rate of approximately 40% in school-age children (13). The 1957 H2N2 pandemic originated in China and had an attack rate of over 50% in school-age children (13). The H3N2 pandemic of 1968 began in Hong Kong and had an attack rate of 40% in school-age children (13). In 2009, almost 40 years after the last pandemic, the pandemic 2009 (H1N1) influenza emerged in Mexico (58).

There have also been five pandemic "threats" in the last century (59). In 1976, a H1N1 swine-like influenza virus caused an outbreak in a military camp in Fort Dix, New Jersey, while in 1977 another H1N1 strain spread between China and Russia. Highly pathogenic avian influenza strain H5N1 first appeared in 1997 and is still present to this day. In 1999, H9N2 was isolated in 2 children from Hong Kong, while H7N7 was isolated in humans and killed a veterinarian in an avian influenza outbreak in the Netherlands.

While there are extensive surveillance systems for human influenza throughout the world, surveillance systems for influenza in animals are just being established. Two examples of animal influenza surveillance systems include the Canadian Inter-Agency Wild Bird Influenza Survey (60) and the Canadian Notifiable Avian Influenza Surveillance System, also known as CanNAISS (61). The Wild Bird Influenza Survey begin in 2005 in response to the 2004 outbreak of avian influenza in British Columbia and the spread of H5N1 avian influenza across Asia, Europe and Africa. The objectives of this survey are to identify strains of avian influenza present in waterfowl in Canada, to acquire information on the risks that these strains present to commercial poultry biosecurity and to monitor the genes present in these avian influenza strains. The survey uses dead, hunter-killed and live waterfowl. The CanNAISS project is operated by the Canadian Food Inspection Agency and is designed to meet requirements for surveillance set out by the World Organization for Animal Health or OIE. This system performs active surveillance for H5, H7 and highly pathogenic strains of avian influenza in commercial poultry.

Currently, there are no formal surveillance systems to track swine influenza virus in Manitoba or Canada. The lack of swine influenza surveillance has been identified as a gap (62) and is limited in comparison to the surveillance and understanding of human influenza (63). Since the arrival of pandemic (H1N1) 2009 influenza virus in humans and pigs, there have been calls for a formal swine influenza surveillance system (14, 64–66). Surveillance of swine influenza is needed to monitor the unpredictable nature of

influenza and the changes in the virus over time (67) and would also contribute to the monitoring the ecology of influenza in animals (68).

Surveillance of swine influenza is important from both an animal health and public health perspective. Swine influenza surveillance would be an important management tool for swine veterinarians (19). Swine influenza is not devastating as the majority of pigs recover from the disease, but the disease is becoming a major pathogen of swine (69) and is economically important as animals infected with influenza lose weight or have decreased weight gains (10, 69). There are 3 manifestations of swine influenza in pigs (8):

1. Primary pathogen – Clinical signs of classical swine influenza include a sudden onset of fever, lack of appetite, lethargy, coughing, sneezing and nasal discharge. Morbidity is high while mortality is usually low, unless co-infections with other diseases are present (8).
2. Multi-agent respiratory disease complex – Recently, swine influenza has become part of a disease syndrome known as Porcine Respiratory Disease Complex, a combination of respiratory viruses with *Mycoplasma hyopneumoniae* and secondary bacterial pathogens that lead to significant disease and death in swine herds (70).
3. Subclinical – Swine influenza can be isolated year-round from pigs with no clinical signs (8, 42, 68).

A study looking at the control and economics of swine influenza found that outbreaks increased secondary infections and nursery mortality and decreased average daily gain of pigs while increasing costs due to the use of medications (71).

Surveillance of swine influenza is important from a public health perspective because influenza is a zoonotic disease. As indicated earlier, cases of swine influenza in humans have been reported and antibodies to swine influenza have been identified in workers with occupational exposure to swine. A surveillance system for swine influenza would enable veterinarians and public health practitioners to notify producers and farm workers of high levels of swine influenza activity and remind them to take appropriate precautions.

Surveillance of swine influenza is important because of the potential for swine influenza to develop into pandemic strains of influenza. Hemagglutinin binds with sialic acid receptors in the host, which differ between species. Birds have an alpha-2,3 receptor in the intestine while humans have an alpha-2,6 receptor in the trachea. Pigs have both alpha-2,3 and alpha-2,6 receptors in their lungs and this has led to the hypothesis of the pigs as a "mixing vessel" for the next pandemic strain. However, alpha-2,3 receptors have also been found in the lower respiratory tract in humans (8). In addition, H5N1 and H7N7 strains do not replicate easily in pigs (8). A swine influenza surveillance system would signal areas and phases of high influenza activity that could be monitored. It could also identify areas where swine influenza and multiple livestock species overlap – areas where there would be an increased risk of pandemic strains developing because of

influenza virus reassortment. One study found that a spatial analysis of swine influenza could detect overlapping clusters of different swine influenza subtypes and it was postulated that this could be used to identify the development of new subtypes (72).

Swine influenza surveillance is also important because of regulations requiring veterinarians to report cases of influenza. The Reportable Diseases Regulation of *The Animal Diseases Act* came into effect in July 2007 and designates influenza A as reportable by veterinarians to the Chief Veterinary Officer. Under *The Public Health Act*, the Reporting of Diseases and Conditions Regulation came into effect in April 2009, requiring the Chief Veterinary Officer to report cases of influenza to the Chief Public Health Officer. A surveillance system for swine influenza would provide a summary of swine influenza trends that could be provided to the Chief Public Health Officer.

A swine influenza surveillance system would be beneficial in focusing resources in areas of disease activity by identifying areas of high swine influenza activity. Because surveillance and response resources are limited, surveillance systems should be risk-based, using epidemiological studies and geographic information system (GIS) analysis to determine risk-based sampling strata (73).

A swine influenza surveillance system would also help identify areas of high swine influenza activity for further study, particularly for the study of diseases at the interface of human and animal health. During the pandemic (H1N1) 2009 influenza outbreak in humans and pigs, a joint study between Manitoba Health and Manitoba Agriculture, Food

and Rural Initiatives was initiated to study outbreaks of pandemic (H1N1) 2009 influenza in swine herds in Manitoba. In 60% of the known cases of pandemic (H1N1) 2009 influenza outbreaks in swine herds, it was unknown if the outbreaks were due to transmission of the virus from humans or from pigs (Susan Roberecki, personal communication).

Finally, a system for surveillance of swine influenza would implement the principles of "One World, One Health", developed by the Wildlife Conservation Society (74). This paradigm acknowledges that human and animal health are closely connected and states that human and animal health information networks should be developed to assist in early warnings of emerging and resurging disease and to coordinate outbreak responses. Others have also called for greater integration of animal and human health surveillance (63, 64, 75, 76) including the collaboration of both public and private sectors to this end (76).

The purpose of this project is to assess the feasibility of swine influenza surveillance in Manitoba, using existing databases to pilot methods of swine influenza surveillance. This project will answer the following research questions:

- What are the time and spatial patterns of swine influenza in Manitoba?
- What methods are the most effective for swine influenza surveillance?

This project will examine the hypothesis that density of swine and swine farms is a factor in the clustering of swine influenza virus.

The objectives of this project are to:

- Link swine influenza polymerase chain reaction (PCR) diagnostic data to information on swine farm characteristics.
- Apply spatial and temporal statistics to the linked dataset.
- Evaluate the ability of these statistics to detect outbreaks of swine influenza.
- Assess the feasibility, strengths and limitations of this system of swine influenza surveillance.

Methods

Influenza in swine can be diagnosed by polymerase chain reaction (PCR) and by enzyme-linked immunosorbent assay (ELISA). A PCR test is performed on individual lung tissue or nasal swab, while the ELISA test is performed on individual serum samples and is usually interpreted at the herd level. Swine influenza diagnoses by PCR were used in this project.

Two data sources were used in this research project. The first data source was samples tested for swine influenza at Veterinary Diagnostic Services Laboratory, the provincial veterinary diagnostic laboratory operated by the Livestock Knowledge Centre at Manitoba Agriculture, Food and Rural Initiatives. This laboratory serves all veterinary clinics throughout the province of Manitoba and provides diagnostic and surveillance programs for all species of animals. Veterinarians submit tissues, serum and whole carcasses for diagnostic workup. Case histories and test results are entered into an in-house veterinary lab information system. This veterinary laboratory information system, also known as Vet Lab, has been in operation since 2003. Information on all PCR tests for swine influenza as well as available farm information and diagnosis date were downloaded from Vet Lab into a spreadsheet (Excel; Microsoft Corporation, Redmond, Washington, USA) for analysis.

The second data source was a registry of all swine farms located in Manitoba entered in the premises identification database maintained by the CVO/Food Safety Knowledge

Centre at Manitoba Agriculture, Food and Rural Initiatives. This database is operated under the authority of the Animal Premises Identification Regulation under *The Animal Diseases Act*, which requires all swine farms in Manitoba to be registered in the database. This registry was formerly maintained by Manitoba Pork Council through the Hog Producer Registration Order under *The Farm Products Marketing Act*, but was forwarded to the CVO/Food Safety Knowledge Centre to comply with the premises identification requirement. This registry contains data on the location and herd characteristics for registered swine premises located in Manitoba. The location information was verified by CVO/Food Safety Knowledge staff before the premise was entered in the premises identification database.

Herd characteristic information included size (maximum capacity) and barn type (backyard, biotech, conventional). A backyard barn type is defined as an operation raising pigs outdoors, while a biotech is an open air facility with a covered shelter and straw or shavings as bedding material. A conventional barn is a closed structure with controlled ventilation and feed as well as restricted entry of people and animals. Herd characteristic information also included presence of other livestock species at the premises (avian, beef cattle, bison, dairy cattle, goats, horses and sheep) and production type (farrow-to-wean, farrow-to-nursery, farrow-to-finish, nursery, grow-finish, artificial insemination unit). The production types were defined as:

- artificial insemination unit: a facility containing adult males from 6 months of age for the purposes of semen collection
- nursery: a facility containing rooms to raise pigs from 3 to 8 weeks of age

- grow-finish: a facility raising pigs from 8 to 24 weeks of age
- farrow-to-wean: a facility with adult females plus their offspring up to 3 weeks of age
- farrow-to-nursery: a facility with adult females and offspring to 3 weeks of age with an attached nursery facility
- farrow-to-finish: a facility with adult females and offspring to 3 weeks of age with an attached nursery and grow-finish facility.

The farm information was downloaded into a spreadsheet (Excel; Microsoft Corporation) for processing. In cases where farm information was not available from the premises identification registry, the submitting veterinarian was contacted to provide the information.

Both spreadsheets with the swine influenza case information and the premises information were imported into a database (Access; Microsoft Corporation) for processing. The farm name from the Vet Lab case information was manually linked to the farm name in the premises identification registry. A spreadsheet (Excel; Microsoft Corporation) with the case information linked to farm information was generated for analysis. The spreadsheet was imported into mapping software (ArcMap 10; ESRI Incorporated, Redlands, California, USA), into statistical software (IBM SPSS Statistics 19; IBM Corporation, Armonk, New York, USA) and spatial analysis software (ClusterSeer 2; TerraSeer Incorporated, Ann Arbor, Michigan, USA) for analysis.

Analysis of the dataset was performed using the steps of data visualization and data description and exploration as outlined by Gatrell and Bailey (77). Data visualization involves projecting data on a map or graph and subjectively observing for any meaningful patterns. In this project, an epidemic curve of positive diagnoses was drawn using a time interval of 5 days to describe the data in time and to visualize any temporal patterns. Diagnoses of swine influenza were mapped and examined for patterns by a map sequence constructed to visualize spatial patterns over time.

Data description and exploration offsets the subjectivity of data visualization by using spatial analysis statistics to determine if detected patterns are significant or insignificant. A regression equation was used to determine if farm size or other farm characteristics were significantly associated with the diagnosis of influenza. The unit of analysis was the farm site. Predictors were selected based on their availability in the premises identification registry and their potential as risk factors for a positive diagnosis of swine influenza. Farm size and production type were selected based on their association with swine influenza in previous studies (26, 35, 37, 38). The presence of other livestock species was included as influenza is a zoonotic pathogen capable of transmitting to other species and because it is believed that livestock species, particularly the pig, play a role in the reassortment of the virus (8). Barn type was selected to determine if the system of raising pigs influenced influenza diagnosis. In addition, barn type is also important because the backyard and biotech systems have lower biosecurity measures and could permit other species to mingle with pigs, allowing for the transmission of influenza across species. Together, livestock species and barn type could indicate risk of zoonotic

transmission and reassortment potential if various species or non-conventional barn types are found in areas where swine influenza clusters.

Size and production factors were analyzed as factors in influenza diagnosis by comparing farms with positive diagnoses of influenza to farms with negative diagnoses of influenza. A logistic regression model was established with influenza diagnosis as the outcome variable (negative=0, positive=1). Independent variables included farm size as a continuous variable and categorical variables (no=0, yes=1) were established to classify the production type of the farm (farrow-wean, farrow-nursery, farrow-finish, nursery, grow-finish, artificial insemination unit). Independent categorical variables were also set up for barn type (backyard, biotech, conventional) and presence of other livestock species (avian, beef cattle, bison, dairy cattle, goats, horses and sheep).

Large farms could be a frequent submitter of samples to the veterinary diagnostic laboratory compared to smaller farms. In order to control for submission rate as a potential confounder for diagnoses of influenza, a histogram of frequency versus number of submissions was generated and a value to divide farms into low versus high submitters was selected visually from the graph. A categorical independent variable was included in the model to categorize farms as low submitters or high submitters (low=0, high=1). If the variable was significant, separate analyses on farms categorized as low and high submitters were performed.

Models were developed by introducing variables by groups (farm size, production type, barn type, presence of other livestock species, high submission frequency). Variables were introduced by group because some of the variables were mutually exclusive, i.e. a premise could only be 1 production type or 1 barn type. Significant variables by group were then placed into a new model; significant variables from this iteration were used in the final model.

Spatial analysis tests were selected based on their previous applications in veterinary public health, relevance to swine influenza and availability in the ClusterSeer software. The Cuzick and Edwards' test (78) was used to analyze outbreaks of anthrax (79), swine influenza (26, 80), bovine tuberculosis (81) and bovine spongiform encephalopathy (82). This test is used to analyze how farms with positive influenza diagnoses were distributed relative to farms with negative influenza diagnoses. This statistic was run to the 5^{th} nearest neighbour using a Monte Carlo simulation with 999 random outcomes and the Statistical Distance Test was calculated to determine the significance. A large test statistic suggests that positive influenza diagnoses are clustered in space and that farms with positive influenza diagnoses tend to have positive diagnoses on neighbouring farms. A small test statistic indicates non significance and that farms with positive influenza diagnoses generally have negative influenza diagnoses on neighbouring farms.

The Kulldorff Spatiotemporal scan statistic has been used extensively to analyze zoonotic diseases in animals, including anthrax (79), campylobacteriosis (83, 84), West Nile virus (85–88), leptospirosis (89), avian influenza (90–92), bovine spongiform encephalopathy

(93), *Echinococcus multilocularis* (94), swine influenza (26, 80), cryptosporidium (95), crimean-congo hemorrhagic fever (96) and bovine tuberculosis (97). The spatiotemporal scan statistic has also been used concurrently with human and animal data to study West Nile virus (98), leishmaniasis (99) and campylobacteriosis (84). In this project, the spatiotemporal scan was used as a Poisson model to detect clustering of influenza cases (100). The unit of analysis for the scan was the Rural Municipality (RM) by month. The incidence of disease was calculated by dividing the number of farms diagnosed with swine influenza into the number of farms in the RM. The incidence of disease inside the scan window was compared to the incidence of randomly distributed cases outside the scan window. The significance of clusters were determined using a Monte Carlo simulation with 999 permutations. Characteristics of herds diagnosed with influenza and located inside the cluster were compared to herds diagnosed with influenza and located outside the cluster to determine if there were any characteristics that were associated with the clustering of swine influenza. This was performed by using a logistic regression model with clustering as the outcome (no = 0, yes = 1). Independent variables were used for farm size, production type, barn type and presence of other livestock species similar to those used for the analysis of factors in influenza diagnosis described previously.

The Knox test was used as a space-time interaction test to determine if cases were near in both time and space. The Knox test categorizes points into near and far in time versus near and far in space, based on space and time distance cut-offs set by the user prior to the test (101). The space cut-off used was 3.2 kilometres (2 miles), based on the theory that *Mycoplasma hyopneumoniae*, a significant respiratory disease in swine, can travel

this distance. The time cut-off used was 4 days, equivalent to the latency period for swine influenza (21, 102).

A modified CuSum was used to measure significant outbreaks in time. The CuSum is commonly used in industrial applications to detect changes in a process and was modified to fit epidemiological data (103). The CuSum technique adds differences between observed cases and a reference baseline over time. The variation is added over time and compared to a reference value calculated from the population size, the average incidence of disease and a pre-determined relative risk threshold. For this study the relative risk was set at 1.1 with 95% significance and a series of 999 Monte Carlo simulations was run to determine the significance of the test statistic.

A historical outbreak of swine influenza was used to analyze the sensitivity of the surveillance system for monitoring swine influenza. An outbreak of H3N2 swine influenza occurred from September 2004 to May 2005, and it was determined if the statistical techniques could detect this particular outbreak.

This project ensured the privacy and confidentiality of the datasets. Data collected by Veterinary Diagnostic Services Laboratory is collected under authority of *The Animal Diseases Act* and can be used for purposes intended under that legislation, including issues of animal and public health. This data is protected by *The Freedom of Information and Protection of Privacy Act.* Maps generated by this project did not identify the specific locations of farms. Both paper and electronic data were protected. Paper datasets

and other materials related to this project were kept in a locked filing cabinet. Electronic data was kept on the Government of Manitoba's secure network in a password-protected folder. The network has regular back-up procedures.

Upon completion of this project, paper copies of data and other materials will be boxed, sealed and archived as outlined in the Government's archive policy under *The Archives and Recordkeeping Act.* After 5 years, the project materials will be destroyed. The electronic data will also be stored in an electronic archive folder and deleted after 5 years.

The project was submitted to the Research Ethics Board at the University of Manitoba for ethics approval. As this board does not have jurisdiction over research in animals, the project was then submitted to the Animal Care Committee of the University of Manitoba. The project was approved as a protocol with minimal animal involvement, as the samples had already been collected and submitted to the veterinary diagnostic laboratory.

Results

A total of 4893 records were downloaded from the Vet Lab database which included the period of time from January 1, 2003 to December 31, 2009. The Vet Lab database records were linked with the farm names in the Premises Identification database, resulting in a total of 2852 (58.3%) linked records available for analysis. In the Vet Lab dataset, 11.8%, 15.1% and 58.6% of records were positive for H1N1, H3N2 and pandemic (H1N1) 2009 swine influenza virus respectively, while 12.1%, 13.5% and 55.1% of records were positive for the H1N1, H3N2 and pandemic (H1N1) 2009 virus respectively in the linked dataset (Table 1). The percentage of positive diagnoses for each subtype was similar between the Vet Lab dataset and the linked dataset, suggesting that the linked dataset was not skewed by the loss of records that could not be linked.

A total of 2041 (41.7%) records were not linked because the farm name in the veterinary laboratory database could not be found in the premises identification registry. This could occur for several reasons, including: the farm was located outside of Manitoba, the farm was no longer in operation, the farm had not registered with the Premises Identification program, the farm submitted samples to the laboratory under a name different from that listed in the premises identification registry, or the veterinarian could not accurately recall information on farm characteristics. In addition, 2 veterinarians who were asked to provide farm characteristic data for farms not listed in the registry did not provide data. These 2 veterinarians had submitted samples for 68 farms representing 159 records or 3.2% of the Vet Lab dataset.

An epidemic curve of positive diagnoses was drawn for each strain of swine influenza showing the number of positive diagnoses over time (Figures 1 to 4). A time interval of 5 days was used to describe the data in time and to visualize any temporal patterns. All the epidemic curves of influenza show that swine influenza is endemic because isolations occur throughout the year. However, the curves also generally show a seasonal pattern with an increased frequency and number of diagnoses beginning in the fall and extending through the winter into early spring. The isolations of H1N1 swine influenza appear to be sporadic from 2003 to 2005 and the isolations of pandemic (H1N1) 2009 influenza beginning in July of 2009 also appear to be sporadic compared to the other strains. An epidemic curve for all influenza subtypes combined also shows the same seasonal pattern and an increased frequency of diagnosis over the time period from 2003 to 2009.

A map sequence was constructed by dividing each year into seasons (winter – December 21 to March 20, spring – March 21 to June 20, summer – June 21 to September 20, fall – September 21 to December 20) and mapping cases occurring during these time periods. In viewing, the maps showed that diagnoses of H1N1 swine influenza (Figure 5) were sporadic and limited to the southeast and southcentral areas of the province during 2003. During 2004, H1N1 swine influenza was diagnosed on a farm north of Winnipeg and in western Manitoba. Diagnoses were intermittent throughout Manitoba until 2005 when the number of diagnoses substantially increased throughout the province until 2009. H3N2 swine influenza (Figure 6) began in the southeast and southcentral Manitoba in the winter of 2005. It appeared to spread more quickly than the H1N1 subtype as it emerged north

of Winnipeg and in western Manitoba by the spring of 2005. During 2006 it appears that H3N2 swine influenza activity decreased, increased again during the years 2007 to 2009, and declined during the summer and fall of 2009. In general, diagnoses of H1N1 and H3N2 swine influenza occur sporadically throughout the province although the majority of maps showed cases of swine influenza clustering in areas southeast of Winnipeg. Diagnoses of pandemic (H1N1) 2009 swine influenza, on the other hand, occur sporadically throughout the province without any noticeable clustering (Figure 7).

The frequency of the number of submissions in the dataset is shown (Figure 8). Any farm with greater than 7 submissions in the dataset was classified as a high submission frequency, thus a total of 585 (20.5%) records were identified as belonging to farms with a high submission frequency. Logistic regression models compared swine influenza diagnosis by subtype and for all subtypes combined with variables representing farm size, production type, barn type, presence of other livestock and high submission frequency. Significant variables for each subtype are listed in Tables 2 through 4. For the H1N1 subtype, the grow-finish production type was significant in the preliminary model of all production types (P=0.013) and in the final model (P=0.001). Similarly for the H3N2 subtype, the grow-finish production type was significant in the preliminary model of all production types (P=0.000) and in the final model (P=0.000). The odds ratio indicates that farms diagnosed with the H1N1 subtype were 1.625 times more likely to have a grow-finish production type than farms not diagnosed with H1N1 swine influenza. In contrast, the odds ratio of 0.496 for the H3N2 subtype indicates that farms diagnosed with H3N2 swine influenza were less likely to be a grow-finish production type. There

were no significant factors for the pandemic (H1N1) 2009 subtype. When all subtypes were combined (Table 5), high submission frequency was a significant factor in the preliminary model (P=0.041) and in the final model (P=0.041), but the odds ratio of 0.748 indicates that farms with frequent submissions for swine influenza virus testing were less likely to be diagnosed with swine influenza. Separate regression equations were modeled for low versus high submission frequency across all subtypes (Tables 6 and 7). The model indicated that the presence of beef cattle was the only significant variable for the high submission frequency (P=0.037), but this variable was not significant in the final model (P=0.854).

The results from the Cuzick and Edwards analysis are shown in Table 8. This analysis demonstrated that the H1N1 (P=0.002), H3N2 (P=0.001) and all swine influenza subtypes combined (P=0.001) were clustered, indicating that farms diagnosed with the respective subtype of swine influenza were more likely to have neighbouring farms with a positive diagnosis. In contrast, the pandemic (H1N1) 2009 subtype was not clustered in space (P=1.000), indicating that neighbouring farms were not likely to be diagnosed with the pandemic (H1N1) 2009 subtype. Maps generated by the software (Figure 9) show that farms diagnosed with H1N1, H3N2 and all swine influenza subtypes are located closed to each other, while farms diagnosed with pandemic (H1N1) 2009 swine influenza are randomly scattered amongst control farms.

A total of 92 RMs contained farms that submitted samples for swine influenza virus testing. Several significant space-time clusters were detected for the H1N1, H3N2 and

pandemic (H1N1) 2009 influenza, indicating that the incidence of disease within the cluster was significant compared to cases distributed at random outside the cluster. The results and significance of the spatiotemporal scan are shown in Table 8. For the H1N1 subtype, there was a significant primary cluster (*P*=0.001) from October 2006 to November 2009 in the southeastern portion of the province including the RMs of Stuartburn, LaBroquerie, Franklin, Piney, Hanover and DeSalaberry. A second significant cluster (*P*=0.001) was detected in the RM of Turtle Mountain from September to December of 2009. The scan indicated a third cluster in the RMs of Portage la Prairie, Grey and Cartier from October 2003 to April 2007, but this cluster was not significant (*P*=0.161). The H3N2 subtype was significantly clustered (*P*=0.001) in the RM of La Broquerie from October 2005 to April 2008, and secondarily clustered (*P*=0.025) in the RM of Morris from October 2008 to October 2009. A third significant cluster (*P*=0.036) was present in the RMs of Hamiota, Blanshard and Miniota from June to September of 2005. The pandemic (H1N1) 2009 swine influenza was significantly clustered (*P*=0.001) in the RM of Turtle Mountain from September to November of 2009 and in the RMs of Stuartburn, La Broquerie and Franklin from August to October of 2009 (*P*=0.033). A third nonsignificant cluster (*P*=0.495) was located in the RM of Macdonald during August 2009. Maps showing the results of the space-time scans are shown in Figures 10 to 12.

Characteristics of herds inside the cluster were compared to herds outside the cluster to determine if there were any characteristics that were associated with the clustering of swine influenza. Results of the analysis are compiled by swine influenza subtype in

Tables 9 to 11. A total of 88 H1N1 swine influenza diagnoses were located inside the clusters and farm size (P=0.022), conventional barn type (P=0.026) and avian species (P=0.030) were significant in the preliminary models, while farrow-to-finish production type was marginally significant (P=0.053) and was put into the final model. The final model indicated that farrow-to-finish production type was a significant factor (P=0.000) in the clustering of H1N1 swine influenza and the odds ratio of 0.213 indicating that farms within the H1N1 cluster were less likely to be farrow-to-finish production types compared to farms diagnosed with H1N1 swine influenza outside the cluster. The presence of avian species was an important but nonsignificant (P=0.063) factor inside the H1N1 subtype cluster in the final model. A total of 38 H3N2 subtype diagnoses were located inside clusters and the conventional barn type was a significant factor in the preliminary model examining barn types (P=0.012). In the final model, conventional barn type was a significant factor (P=0.024) and the odds ratio indicated that farms within the cluster were 2.3 times more likely to be conventional barns compared to farms diagnosed with H3N2 swine influenza outside the cluster. Finally, there were no significant variables for the 11 premises diagnosed within the pandemic (H1N1) 2009 cluster.

The results of the Knox statistic (Table 8) were significant for the H1N1 (P=0.001) and the H3N2 (P=0.001) swine influenza subtype but insignificant (P=0.307) for the pandemic (H1N1) 2009 subtype. This statistic indicates that cases of H1N1 and H3N2 swine influenza are close in both space and time and cases of pandemic (H1N1) 2009 swine influenza are not related in space or time. The visual output of this statistic generated by the software is shown in Figure 13.

The modified CuSum detected several clusters of swine influenza (Table 8), indicating that the incidence of disease was elevated over specific periods of time compared to the baseline incidence of disease over time. The H1N1 subtype significantly clustered in November (P=0.002) and December (P=0.033) of 2009 and in January of 2007 (P=0.011). The H3N2 subtype significantly clustered in April (P=0.020) and May (P=0.005) of 2005 and was nonsignificantly clustered (P=0.081) in January of 2009. There were no significant outbreaks detected for the pandemic (H1N1) 2009 strain.

Historical outbreaks can be used to evaluate the ability of a surveillance system to detect outbreaks. An outbreak of H3N2 occurred in Manitoba between September 2004 and May 2005. The outbreak was detected by the modified CuSum near the end of the outbreak in April and May of 2005.

Discussion

Surveillance of swine influenza in Manitoba demonstrates the temporal patterns of the virus. All the epidemic curves in this study show that the disease is seasonal, with an increase in the number of positive diagnoses starting in the fall, peaking in the winter and declining in the early spring. The epidemic curves also reveal that the disease is endemic, as the virus, regardless of subtype, is isolated year-round. This agrees with several observations that the virus appears to be endemic with less seasonal activity (9, 19, 23, 33). Seasonal peaks of swine influenza have been credited to sudden changes in weather (42), while the endemicity of swine influenza is attributed to changes in swine production systems where naïve animals are introduced from various sources and young animals with waning maternal immunity are present in large populations of animals (7, 9, 21, 104). However, a German study found that swine influenza was strongly seasonal in high swine-dense areas while endemic in low swine density areas (37).

The epidemic curves also show that the frequency of diagnosis of swine influenza is increasing with time and this was also illustrated by the map sequences. An increase in diagnosis over time is also suggested by the CuSum technique and the Kulldorff Spatiotemporal Scan. The CuSum technique identified significant clustering of the H1N1 and H3N2 subtypes during later time periods of the study, especially in the year 2009, and the spatiotemporal scan indicated clustering of H1N1 and H3N2 swine influenza during later time periods of this study. Increased numbers of diagnoses of swine influenza can be attributed to increased activity of the virus, although increased surveillance,

alterations in viral genetics or changes in production systems could also be factors (22). In contrast, there was no significant clustering of the pandemic (H1N1) 2009 subtype, however, the study period for this virus was less than 1 year. Further data will be required to determined the temporal trends of this subtype.

Selection of the time interval for an epidemic curve is crucial, as one must balance short time intervals that exaggerate random patterns with long time intervals that combine too many cases and hide the true pattern of the disease. It is suggested that the time interval for an epidemic curve be between one-fourth and one-half of the incubation/latency period (105). The incubation period for swine influenza is 1 to 3 days (21). A human influenza study suggested a chain length of between 4 to 5 days, based on the midpoint of the average latency period plus the midpoint of the average infectious period (102). A household transmission study calculated 3.6 days as the mean serial interval of human influenza, although it reported that this figure was longer than other similar studies (106). Thus, it seems that a serial interval of 4 days for an epidemic curve of swine influenza is appropriate.

This study also demonstrates that surveillance of swine influenza in Manitoba identifies the spatial patterns of the disease through mapping and spatial analysis. Mapping is a powerful tool for visualizing the locations of diagnoses of swine influenza. The map sequences show that diagnoses of pandemic (H1N1) 2009 swine influenza occur randomly throughout the province while diagnoses of H1N1 and H3N2 swine influenza are prominent in the southeastern region of the province.

While data visualization is an important first step in examining spatial patterns of disease, data description and exploration are essential to determine significant versus insignificant patterns (77). The Cuzick and Edwards statistic indicated that farms with swine influenza are more likely to have neighbouring farms with swine influenza. This agrees with a study of an H3N2 swine influenza outbreak that calculated a significant Cuzick and Edwards statistic (26). Other studies have found that closeness to other farms and high density of pig farms were associated with a positive influenza diagnosis (35, 37). Simultaneous outbreaks over widespread geographical areas with high densities of swine farms have also been reported (17, 18, 42). In comparison, the Cuzick and Edwards statistic was nonsignificant for the pandemic (H1N1) 2009 swine influenza subtype and supports the conclusion that the disease is spread primarily by human-animal contact rather than by animal or farm contact. However, the number of records for pandemic (H1N1) 2009 swine influenza was significantly smaller compared to the other subtypes and this may be a factor in the outcome of this statistic.

The Kulldorff Spatiotemporal scan indicates that swine influenza subtypes H1N1 and H3N2 cluster primarily in the southeastern region of the province, although second and third significant clusters of H1N1 and H3N2 swine influenza were found in RMs in western Manitoba. The southeastern region of the province is densely populated with pigs and pig farms, as the RM of Hanover contains 388,905 pigs compared to the RM of Elton in the west part of the province which contains 18,356 pigs (107). In contrast, the pandemic (H1N1) 2009 swine influenza was clustered in the RM of Turtle Mountain,

located in the southwestern region of the province and a second cluster was located in the southeastern region of the province. However, more cases of pandemic (H1N1) 2009 swine influenza should be analyzed to determine the spatial patterns of this subtype.

The Knox test indicated that the H1N1 and H3N2 subtypes are significantly clustered in space and time. In contrast, the pandemic (H1N1) 2009 subtype is not clustered in space-time, possibly indicating the role of human-animal contact in transmitting the virus, although the sample size is considerably smaller than the other subtypes.

Each spatial and temporal statistic has advantages and limitations. The Cuzick and Edwards statistic is very relevant to veterinary medicine, as the nearest neighbour order can be changed and it can be used with non-uniform populations which is characteristic of animal populations (108). The Kulldorff Spatiotemporal scan is a robust method, as it is able to test for the significance of a cluster and identify its location, can correct for multiple testing and avoids bias as it does not require the user to determine the size and location of clusters (100, 109). However, the spatiotemporal scan requires data on, or a stable estimate of, the distribution of the population in order to avoid false clusters, assumes that the shape of the disease is circular, and can misidentify exposure location (109). The CuSum method is a powerful test as it is highly sensitive to small changes because it adds variation over time (110) and can detect significant events for a predetermined false-positive rate (111) and perform multiple sequential tests without increasing the rate of false positives or Type I error (112). CuSum works well with small numbers (110) and was found to be superior to other temporal-based tests including the

sets method (113, 114) and the Early Aberration Reporting System (EARS) (115). However, one study reported that CuSum performs better with less variable data compared to highly variable data (116). CuSum also assumes that data is normally distributed and that there is no autocorrelation (117). This study found that the H1N1 and H3N2 swine influenza subtypes were clustered in space and time, therefore the data would be autocorrelated. A transformation could be used on non-normal data but this technique does not work well on small numbers of cases (117). In addition, the CuSum technique detects abrupt changes in a process and therefore would be limited in detecting gradual increases of influenza (118).

Logistic regression was used to identify farm characteristics that may be associated with a positive swine influenza diagnosis and with the time and space patterns of the disease. Regression analysis of characteristics associated with farms diagnosed with swine influenza showed that diagnoses of H1N1 swine influenza were associated with grow-finish premises. In addition, the comparison of farm characteristics inside versus outside the H1N1 subtype cluster shows that a farrow-to-finish type of farm was significantly less likely to be located within the cluster. This contrasts with the studies of Poljak who found that finisher herds were more likely to be positive for H1N1 swine influenza if they were a farrow-to-finish type of farm (35). Diagnoses of H3N2 swine influenza were significant for the grow-finish production type, but there was a protective effect, because a diagnosis was less likely on a grow-finish premise. This difference of risk factor versus protective effect could be attributed to differences in population immune status between the 2 viruses. In addition, age also affects expression of clinical signs as younger animals

show clinical signs while older animal have subclinical disease (19); therefore, differing animal ages between the 2 subtypes could affect the presentation of clinical signs and the likelihood for samples to be submitted.

The regression analysis also indicated that farms with a high submission frequency were less likely to be positive. This could represent farms that have ongoing respiratory disease problems and perform diagnostic workups that include testing for swine influenza. It also suggests that this analysis is not biased by farms submitting multiple samples for swine influenza testing.

The regression analysis identified factors that were associated with the clustering of the H1N1 and H3N2 subtypes. Clustering of H1N1 swine influenza on premises with avian species present was marginally nonsignificant but is of public health concern because of the possibility of viral reassortment resulting from transmission across different species. However, the odds ratio of 0.231 suggests that farms within the cluster were less likely to have avian species present. Farms diagnosed with H3N2 swine influenza within a cluster were 2.3 times more likely to be a conventional farm compared to farms diagnosed with H3N2 swine influenza outside of the cluster. Conventional farms are larger than backyard and biotech farms and this could indicate that farm size plays a role in the clustering of the disease.

This study demonstrated that a surveillance system for swine influenza in Manitoba is possible through the use of epidemic curves and maps to effectively visualize the patterns

of the disease. In addition, several statistical methods were successful in detecting clusters of H1N1, H3N2 and pandemic (2009) H1N1 swine influenza, including the Cuzick and Edwards test, Kulldorff's Spatiotemporal scan, the Knox test, and the modified CuSum method. Historical outbreaks can be used to provide wholly authentic data for evaluating surveillance systems (109). The advantages of authentic data include that it is "real" data and can truly test the ability of the system and it simulates the experience of using real surveillance data (109). The disadvantages of using wholly authentic data include defining the outbreak and the limited supply of data with "true" outbreaks (109). This project used knowledge of an initial outbreak of H3N2 swine influenza as criteria to determine the ability of the surveillance system. The modified CuSum detected this retrospective outbreak; however, this statistic detected the outbreak near the end of the epidemic and suggest that this statistic may not be able to detect the initial start of an outbreak. However, wholly authentic data requires a sufficient number of outbreaks to adequately evaluate the system (109) and this project only used 1 outbreak as an assessment.

This project did not directly support the hypothesis that density of swine and swine farms is a factor in the clustering of the swine influenza virus; however, the H1N1 and H3N2 subtypes primarily clustered in the southeastern region of the province, a region highly populated with swine and swine farms.

This project demonstrates that surveillance of swine influenza and other zoonotic diseases in Manitoba is feasible when it is linked with a farm premises identification

registry. The farm premises identification registry is a crucial component for surveillance because it contains farm characteristic and location data that is complete and verified and that can be matched with the diagnostic data in the veterinary laboratory database for analysis. This is in contrast to the National Animal Identification System, the premises identification program in the United States, that may not succeed due to a lack of support from industry and little progress in collecting farm location data (119, 120). Researchers have warned that the failure of this program will make it difficult to locate farms and operate disease surveillance and control programs, which is ironic given the advances in GIS technology and analysis (32, 119). Premises identification registries should also include all species and specialty farms such as backyard farms (120) and this information is included in Manitoba's premises identification registry.

Verification of data before it is entered in a farm premises identification registry is important; in this study farm location was verified before it was entered in the premises identification registry by confirming it with municipal property ownership maps. Knowledge of how data in a premises identification registry was collected and verified is also important, as a study identified that GIS data held by public agencies was biased or inaccurate compared to field verified data, in part due to differences in data collection and geocoding (120). This study also found that 10% of farm locations were inaccurate by more than 900 metres, a distance large enough to include adjacent farm sites. This inaccuracy would lead to erroneous and possibly disastrous decisions when operating disease surveillance and control programs. In addition, it may be difficult to maintain the quality of registry data in a highly evolving industry such as the swine industry (120).

This study also shows that a swine influenza surveillance system can achieve the objectives of the "One World, One Health" initiative by using animal health information to monitor for trends of disease and disease outbreaks. This information can be linked to human health information systems for the purpose of joint surveillance of zoonotic diseases. Surveillance systems have traditionally tracked disease movement from animals to people. However, the experience with pandemic (H1N1) 2009 swine influenza has emphasized that diseases can be transferred from people to animals. This experience highlights the need to build and implement surveillance systems that can capture the bilateral movement of disease between people and animals.

The advantage of this surveillance system for swine influenza is that it is a passive surveillance system. A passive surveillance system is defined as a system that includes diseases reported by practitioners at their discretion, instead of an active surveillance system that incorporates systematic recording of a designated disease (121). Passive surveillance is especially proficient at identifying changes and trends that need further workup (121). Passive surveillance is more cost and time efficient compared to an active surveillance system, especially when the disease is rare (68, 121). This surveillance system used existing datasets that were compiled for other purposes, therefore eliminating the requirement to collect data for a stand-alone surveillance projects. This project also demonstrated that this surveillance could easily be performed using commercially available software for data management and spatial and statistical analysis.

There are several limitations to this swine influenza surveillance system. This surveillance system may be limited to monitoring patterns and trends, as none of the methods detected the new H3N2 swine influenza until well into the course of the outbreak. This system would need to be augmented by other methods of reporting emerging disease, such as personal interaction with veterinarians and especially front-line practitioners. In fact, many sources of data are required to accurately assess the activity and prevalence of disease (121). This study also highlighted the large number of submissions required for analysis, as only 60% of the submissions to the veterinary laboratory were usable. Another disadvantage of this system is the workload required to link the premises registry data with the laboratory diagnostic data and to operate the software for analyzing the data. Therefore, it is recommended that the veterinary laboratory database be equipped with a data field to enter the premises registration number and that veterinarians and producers be required to submit this registration number with their samples. It is also recommended that these methods be incorporated into an automated system that enables a user to easily perform surveillance of swine influenza, as well as other diseases of veterinary and public health interest.

Another limitation of this swine influenza surveillance system is its subjectivity. While active surveillance is costly, it is highly proficient at systematically collecting disease information and monitors for disease regardless of clinical signs or lab diagnosis (68). In comparison, a passive surveillance system would be highly likely to miss cases of subclinical influenza which are common in pigs (38, 68, 122). A passive surveillance system can also be influenced by awareness of a disease (121) or by producer concerns

with the expense of testing and trade implications, which are relevant to swine influenza (63). In addition, this study obtained data from a veterinary laboratory and there is the possibility of submission bias in this study. With a large presence of swine farms in the southeastern region of the province, veterinarians and producers in this area may be more likely to submit samples to the laboratory compared to those elsewhere in the province. This would cause swine influenza to appear more prevalent in this region of the province. However, the regression analysis of all swine influenza diagnoses indicated that although high submission rate was a factor, it was more related to a negative diagnosis than a positive diagnosis, suggesting that submission bias is not a factor. In addition, further analysis of low versus high submission premises did indicate any significant risk factors for swine influenza.

Finally, this surveillance system for swine influenza identifies several risk factors for a positive diagnosis of swine influenza but it does not identify factors related to the spread of the disease. The next step in disease risk assessment is to incorporate GIS with network analysis of pig transportation to assist in determining the effects of local versus regional spread of swine influenza (34).

In conclusion, this project demonstrates that surveillance of swine influenza in Manitoba is feasible using a farm premises identification registry and that it can identify trends of the disease. This project suggests that a risk-based surveillance strategy for swine influenza in Manitoba should include farms located in the southeastern region of the province, farms with a low number of submissions to the veterinary diagnostic laboratory

and facilities with grow-finishing pigs. Further research should include a network analysis to better understand the ecology of swine influenza of pigs as well as incorporation of swine influenza surveillance information with human influenza surveillance data to better understand the ecology and interactions of influenza between pigs and humans.

Tables

Table 1. Number and percentage of positive diagnoses for H1N1, H3N2 and pandemic (H1N1) 2009 influenza

Influenza strain	Vet Lab dataset 4893 records	Linked dataset 2852 records
H1N1		
positive samples	346	221
total samples	2921	1819
percent positive	11.8%	12.1%
H3N2		
positive samples	342	193
total samples	2258	1432
percent positive	15.1%	13.5%
Pandemic (H1N1) 2009		
positive samples	65	27
total samples	111	49
percent positive	58.6%	55.1%

Table 2. Summary of significant variables by logistic regression, H1N1 swine influenza

Subtype	Variable	Regression coefficient	*P*-value	Odds ratio
H1N1	Farm size	0.000	0.937	1.000
	Farrow-to-wean	-0.283	0.384	0.754
	Farrow-to-nursery	-1.121	0.162	0.326
	Farrow-to-finish	0.214	0.573	1.239
	Nursery	0.248	0.287	1.281
	Grow-finish	0.532	0.013	1.702
	Artificial insemination	-0.042	0.895	0.959
	Backyard	-19.205	0.999	0.000
	Biotech	0.493	0.531	1.638
	Conventional	0.071	0.642	1.074
	Avian	0.254	0.371	1.289
	Bison	-	-	-
	Beef cattle	-0.381	0.233	0.683
	Caprine	-	-	-
	Dairy cattle	-1.188	0.265	0.305
	Equine	-1.052	0.316	0.349
	Ovine	-0.184	0.807	0.832
	High frequency	-0.003	0.986	0.997
H1N1 (final model)	Grow-finish	0.486	0.001	1.625

Table 3. Summary of significant variables by logistic regression, H3N2 swine influenza

Subtype	Variable	Regression coefficient	*P*-value	Odds ratio
H3N2	Farm size	0.000	0.477	1.000
	Farrow-to-wean	0.247	0.347	1.281
	Farrow-to-nursery	-0.369	0.493	0.691
	Farrow-to-finish	-0.653	0.080	0.520
	Nursery	0.216	0.355	1.242
	Grow-finish	-0.847	0.000	0.429
	Artificial insemination	0.152	0.607	1.164
	Backyard	0.279	0.721	1.322
	Biotech	-19.315	0.999	0.000
	Conventional	0.102	0.536	1.107
	Avian	-0.280	0.383	0.756
	Bison	-	-	-
	Beef cattle	0.197	0.521	1.218
	Caprine	-	-	-
	Dairy cattle	-19.074	0.998	0.000
	Equine	0.683	0.411	1.979
	Ovine	0.382	0.554	1.466
	High frequency	0.113	0.604	1.120
H3N2 (final model)	Grow-finish	-0.700	0.000	0.496

Table 4. Summary of significant variables by logistic regression, pH1N1 swine influenza

Subtype	Variable	Regression coefficient	*P*-value	Odds ratio
pH1N1	Farm size	0.000	0.443	1.000
	Farrow-to-wean	-	-	-
	Farrow-to-nursery	-	-	-
	Farrow-to-finish	20.661	1.000	9.396 E -8
	Nursery	0.999	0.261	2.716
	Grow-finish	-0.417	0.656	0.659
	Artificial insemination	-42.406	0.999	0.000
	Backyard	-	-	-
	Biotech	-	-	-
	Conventional	-0.719	0.286	0.487
	Avian	-	-	-
	Bison	-	-	-
	Beef cattle	-	-	-
	Caprine	-	-	-
	Dairy cattle	-	-	-
	Equine	-	-	-
	Ovine	-	-	-
	High frequency	21.116	0.999	1.481 E-9

Table 5. Summary of significant variables by logistic regression, all swine influenza subtypes

Subtype	Variable	Regression coefficient	*P*-value	Odds ratio
SIV	Farm size	0.000	0.967	1.000
	Farrow-to-wean	-0.108	0.604	0.898
	Farrow-to-nursery	-0.466	0.288	0.627
	Farrow-to-finish	-0.094	0.710	0.910
	Nursery	-0.009	0.954	0.991
	Grow-finish	-0.057	0.714	0.945
	Artificial insemination	-0.067	0.744	0.936
	Backyard	-0.188	0.804	0.828
	Biotech	0.053	0.945	1.054
	Conventional	-0.101	0.375	0.904
	Avian	0.159	0.477	1.173
	Bison	-	-	-
	Beef cattle	-0.125	0.586	0.882
	Caprine	-	-	-
	Dairy cattle	-1.502	0.151	0.223
	Equine	-0.107	0.868	0.899
	Ovine	0.458	0.365	1.582
	High frequency	-0.290	0.041	0.748
SIV (final model)	High frequency	-0.290	0.041	0.748

Table 6. Summary of significant variables by logistic regression, all swine influenza subtypes, low frequency submission

Subtype	Variable	Regression coefficient	*P*-value	Odds ratio
SIV, low frequency submission	Farm size	0.000	0.291	1.000
	Farrow-to-wean	0.062	0.780	1.064
	Farrow-to-nursery	-0.432	0.356	0.649
	Farrow-to-finish	-0.024	0.929	0.976
	Nursery	0.146	0.412	1.158
	Grow-finish	-0.028	0.871	0.973
	Artificial insemination	-0.162	0.453	0.850
	Backyard	-0.921	0.375	0.398
	Biotech	0.013	0.986	1.013
	Conventional	-0.046	0.710	0.955
	Avian	0.086	0.719	1.089
	Bison	-	-	-
	Beef cattle	-0.384	0.132	0.681
	Caprine	-	-	-
	Dairy cattle	-19.578	0.999	0.000
	Equine	-0.139	0.829	0.870
	Ovine	0.612	0.361	1.843

Table 7. Summary of significant variables by logistic regression, all swine influenza subtypes, high frequency submission

Subtype	Variable	Regression coefficient	*P*-value	Odds ratio
SIV, high frequency submission	Farm size	0.000	0.142	1.000
	Farrow-to-wean	-1.361	0.054	0.257
	Farrow-to-nursery	-1.154	0.377	0.316
	Farrow-to-finish	-0.680	0.413	0.507
	Nursery	-0.396	0.297	0.673
	Grow-finish	-0.014	0.971	0.986
	Artificial insemination	0.708	0.309	2.030
	Backyard	23.125	1.000	1.104 E-10
	Biotech	-	-	-
	Conventional	-0.375	0.189	0.687
	Avian	-0.509	0.551	0.601
	Bison	-	-	-
	Beef cattle	1.645	0.037	5.179
	Caprine	-	-	-
	Dairy cattle	0.353	0.795	1.423
	Equine	-	-	-
	Ovine	0.537	0.495	1.711
SIV, high frequency submission (final model)	Beef cattle	-0.036	0.854	0.965

Table 8. Summary of spatial data analyses

Test	Statistic	*P*-value	Clustering
Cuzick and Edwards			
H1N1	4.54	0.002	S
H3N2	7.74	0.001	S
pH1N1	0.72	1.000	NS
SIV	9.24	0.001	S
Kulldorff's SpatioTemporal Scan			
H1N1			
1st: Stuartburn, La Broquerie, Franklin, Piney, Hanover, De Salaberry (2006-10 to 2009-11)		0.001	S
2nd: Turtle Mountain (2009-09 to 2009-12)		0.001	S
3rd: Portage la Prairie, Grey, Cartier (2003-10 to 2007-04)		0.161	NS
H3N2			
1st : La Broquerie (2005-10 to 2008-04)		0.001	S
2nd : Morris (2008-10 to 2009-10)		0.025	S
3rd: Hamiota, Blanshard, Miniota (2005-06 to 2005-09)		0.036	S
pH1N1			
1st: Turtle Mountain (2009-09 to 2009-11)		0.001	S
2nd: Stuartburn, La Broquerie, Franklin (2009-07 to 2009-10)		0.033	S
3rd: Macdonald (2009-08)		0.495	NS
Knox			
H1N1	10.0	0.001	S
H3N2	9.0	0.001	S
pH1N1	3.0	0.307	NS
Modified CuSum			
H1N1			
November 2009	5.287	0.002	S
January 2007	4.644	0.011	S
December 2009	2.932	0.033	S
H3N2			
May 2005	3.713	0.005	S
April 2005	3.356	0.020	S

Test	Statistic	*P*-value	Clustering
January 2009 pH1N1 No time periods were associated with a statistic value greater than zero	2.356	0.081	NS

Table 9. Comparison of farm characteristics inside versus outside the cluster, H1N1 swine influenza

Subtype	Variable	Regression coefficient	*P*-value	Odds ratio
H1N1	Farm size	0.000	0.022	1.000
n = 88	Farrow-to-wean	-0.717	0.279	0.488
	Farrow-to-nursery	-21.367	0.999	0.000
	Farrow-to-finish	-1.724	0.053	0.178
	Nursery	0.217	0.604	1.242
	Grow-finish	-0.215	0.589	0.807
	Artificial insemination	-0.264	0.745	0.768
	Backyard	-	-	-
	Biotech	-20.581	0.999	0.000
	Conventional	0.649	0.026	1.914
	Avian	-1.754	0.030	0.173
	Bison	-	-	-
	Beef cattle	-0.783	0.360	0.457
	Caprine	-	-	-
	Dairy cattle	-19.230	1.000	0.000
	Equine	-20.984	1.000	0.000
	Ovine	-20.984	0.999	0.000
H1N1 (final model)	Farm size	0.000	0.631	1.000
	Farrow-to-finish	-1.547	0.000	0.213
	Conventional	0.519	0.111	1.680
	Avian	-1.465	0.063	0.231

Table 10. Comparison of farm characteristics inside versus outside the cluster, H3N2 swine influenza

Subtype	Variable	Regression coefficient	*P*-value	Odds ratio
H3N2	Farm size	0.000	0.189	1.000
n = 38	Farrow-to-wean	0.044	0.937	1.045
	Farrow-to-nursery	-18.626	0.999	0.000
	Farrow-to-finish	0.304	0.719	1.355
	Nursery	-0.713	0.209	0.490
	Grow-finish	-0.943	0.093	0.390
	Artificial insemination	-1.278	0.072	0.279
	Backyard	23.052	0.999	1.026 E-10
	Biotech	-	-	-
	Conventional	0.943	0.012	0.157
	Avian	-20.255	0.998	0.000
	Bison	-	-	-
	Beef cattle	0.842	0.200	2.321
	Caprine	-	-	-
	Dairy cattle	-	-	-
	Equine	42.860	0.999	4.109 E-18
	Ovine	-19.801	0.999	0.000
H3N2 (final model)	Conventional	0.832	0.024	2.298

Table 11. Comparison of farm characteristics inside versus outside the cluster, pH1N1 swine influenza

Subtype	Variable	Regression coefficient	*P*-value	Odds ratio
pH1N1	Farm size	0.000	0.093	1.000
n = 11	Farrow-to-wean	-	-	-
	Farrow-to-nursery	-	-	-
	Farrow-to-finish	-1.190	1.000	0.304
	Nursery	-20.106	0.999	0.000
	Grow-finish	19.656	0.999	3.441 E-8
	Artificial insemination	-	-	-
	Backyard	-	-	-
	Biotech	-	-	-
	Conventional	-20.441	0.999	0.000
	Avian	-	-	-
	Bison	-	-	-
	Beef cattle	-	-	-
	Caprine	-	-	-
	Dairy cattle	-	-	-
	Equine	-	-	-
	Ovine	-	-	-

Figures

Figure 1. Epidemic curve, H1N1 swine influenza in Manitoba, 2003 to 2009

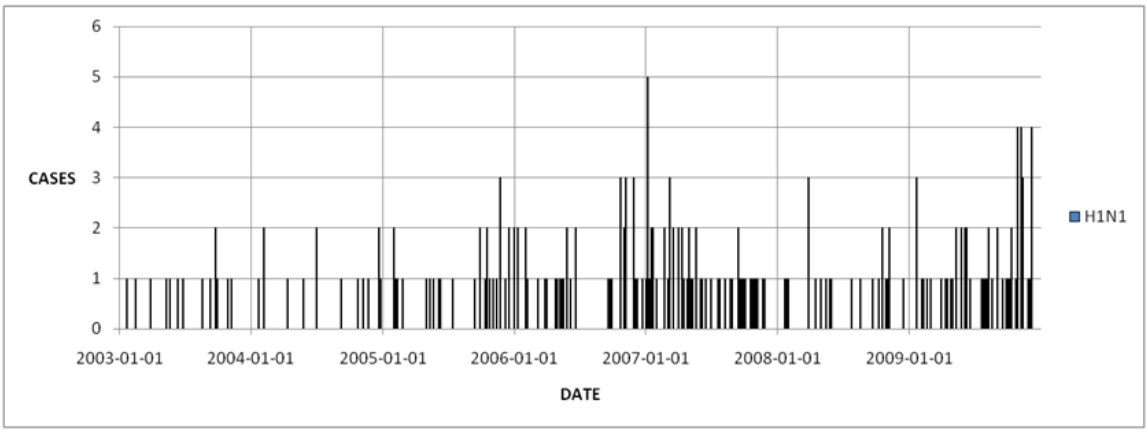


Figure 2. Epidemic curve, H3N2 swine influenza in Manitoba, 2005 to 2009

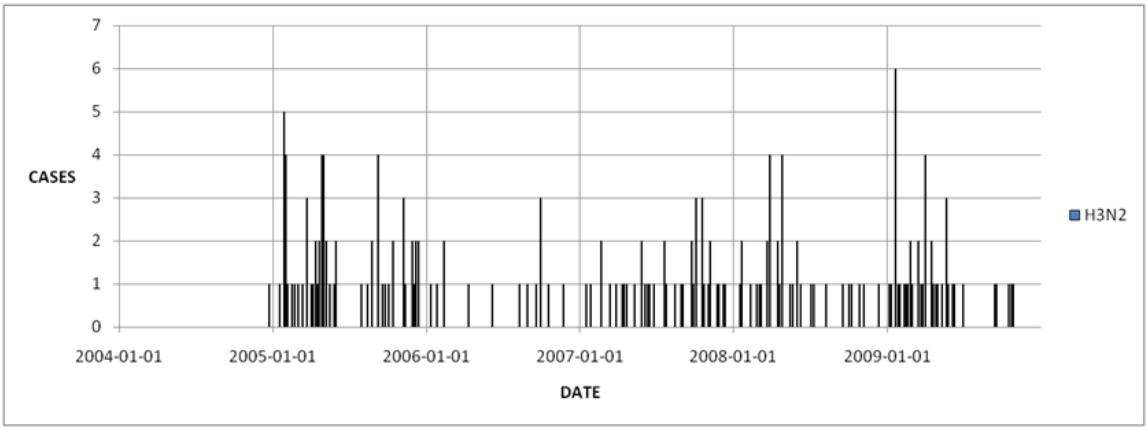


Figure 3. Epidemic curve, pH1N1 swine influenza in Manitoba, 2009

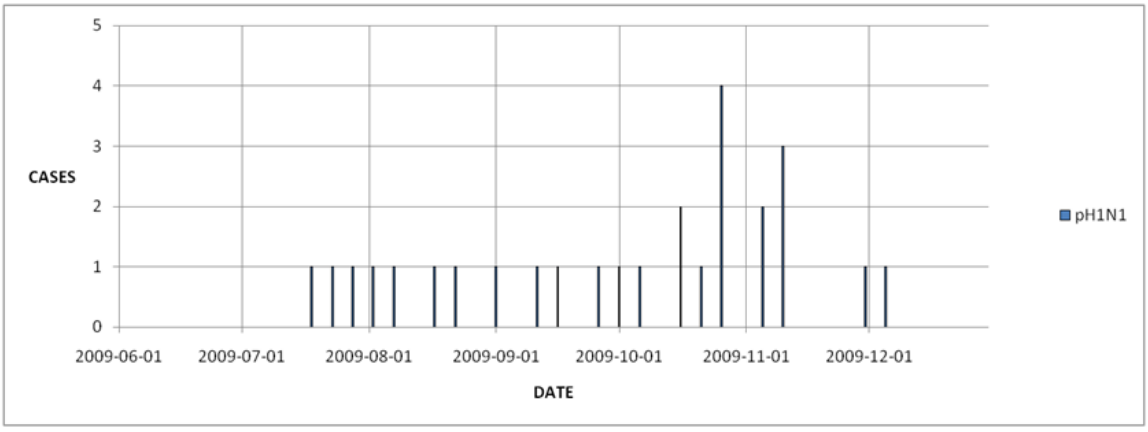


Figure 4. Epidemic curve, swine influenza in Manitoba, all subtypes, 2003 to 2009

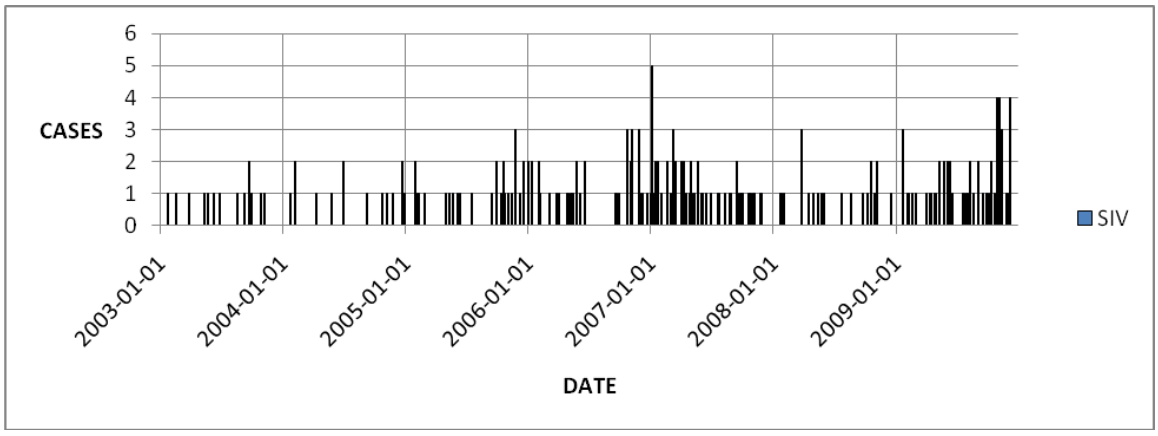
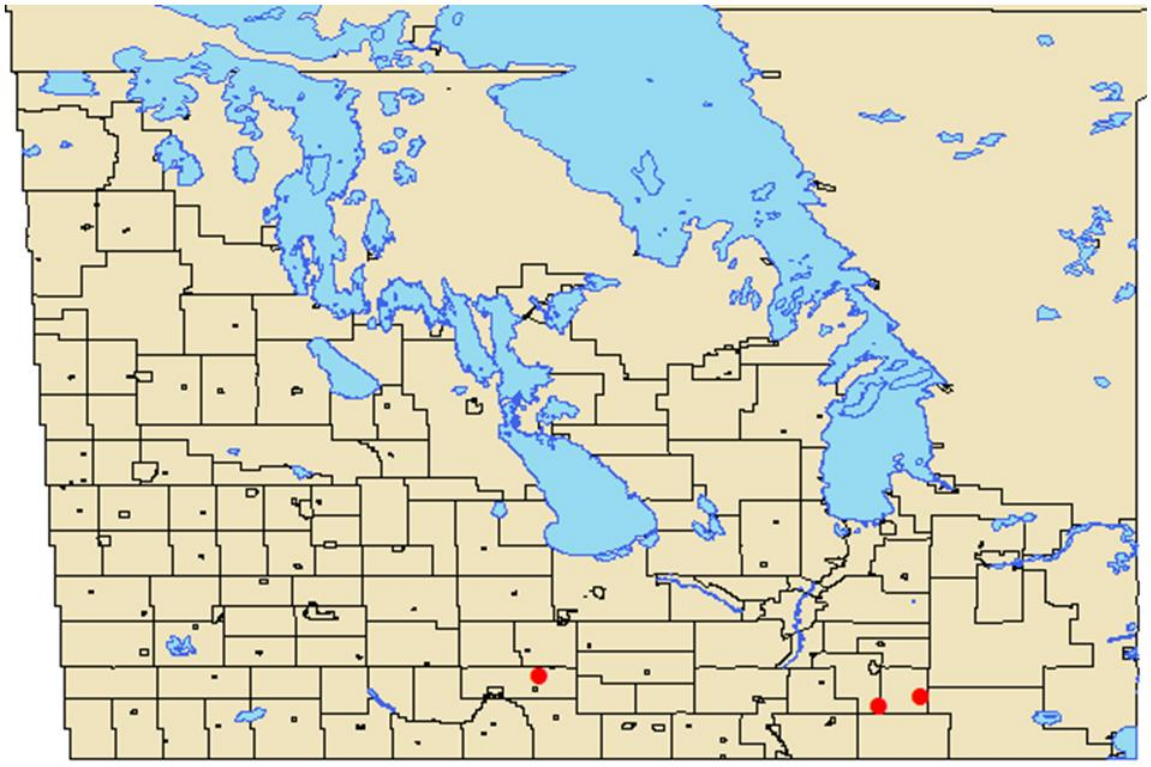


Figure 5. Map sequence, H1N1 swine influenza in Manitoba, 2003 to 2009

H1N1 SIV – Spring 2003



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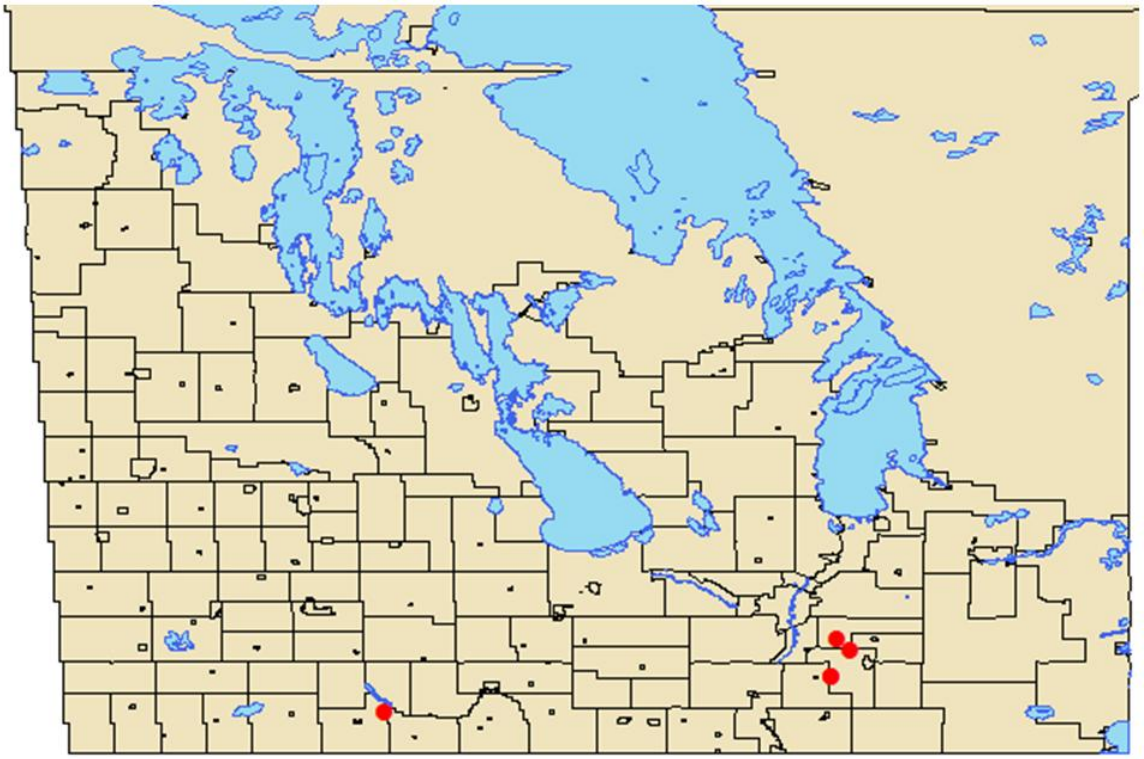

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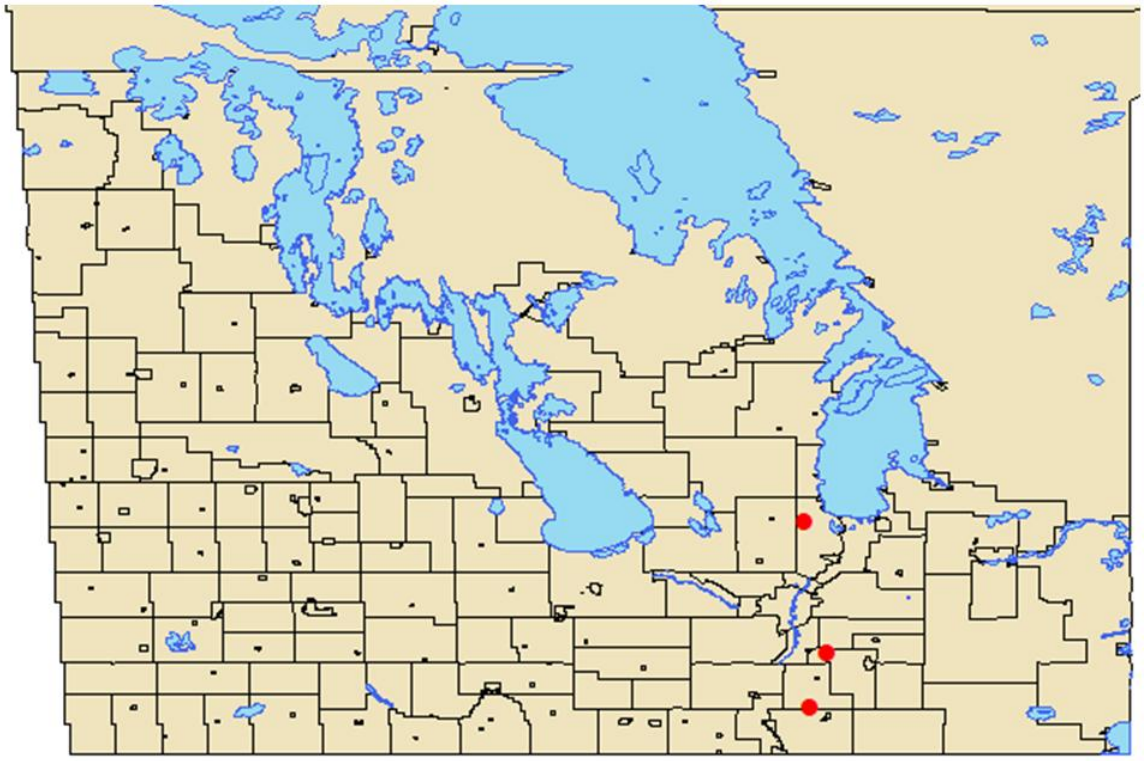

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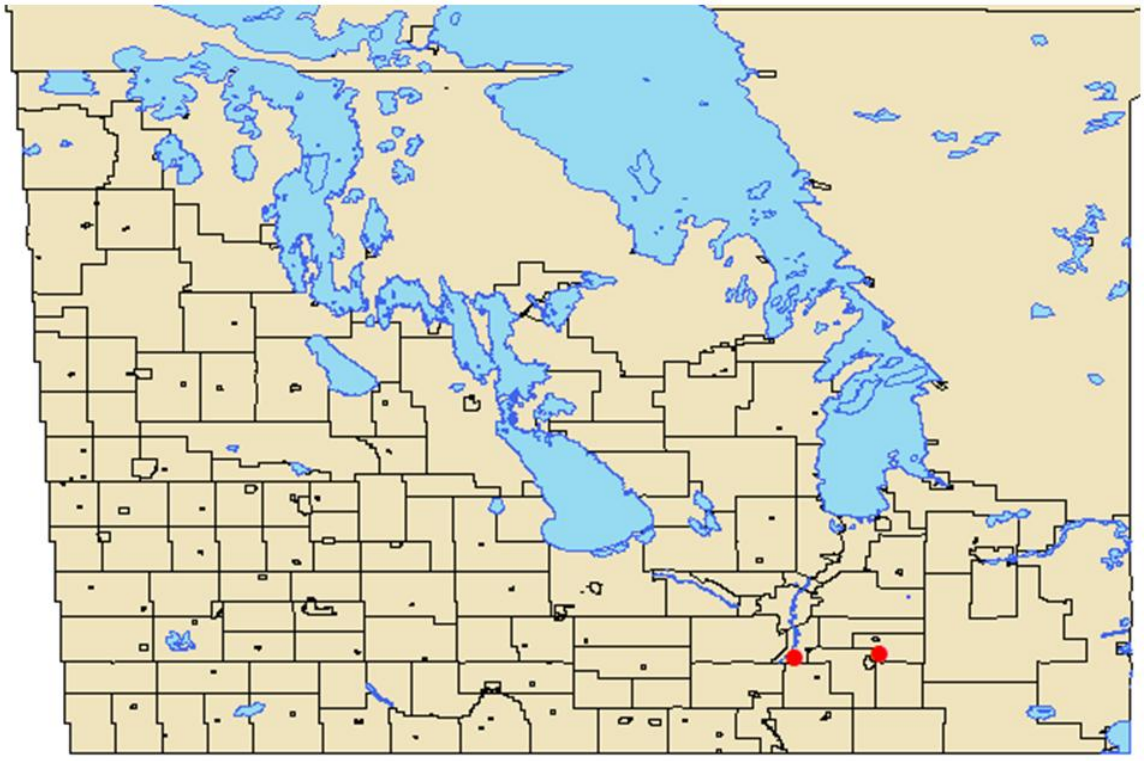
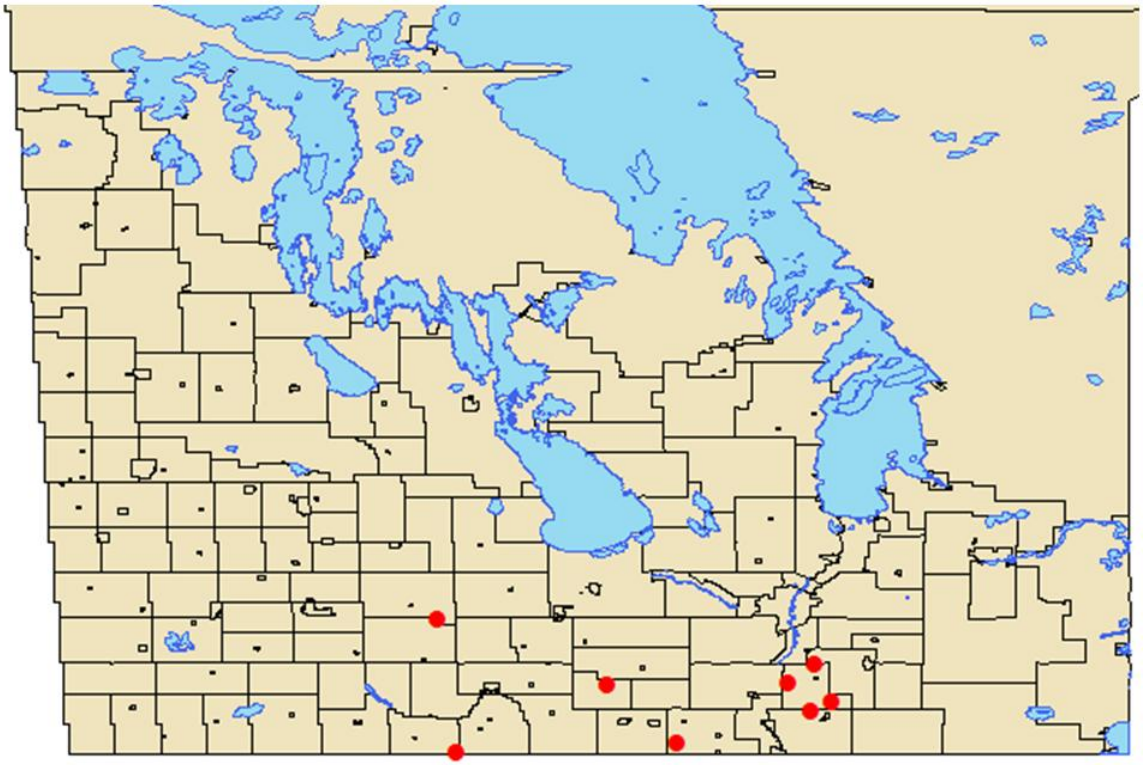


H1N1 SIV – Spring 2004

H1N1 SIV – Summer 2004

H1N1 SIV – Fall 2004



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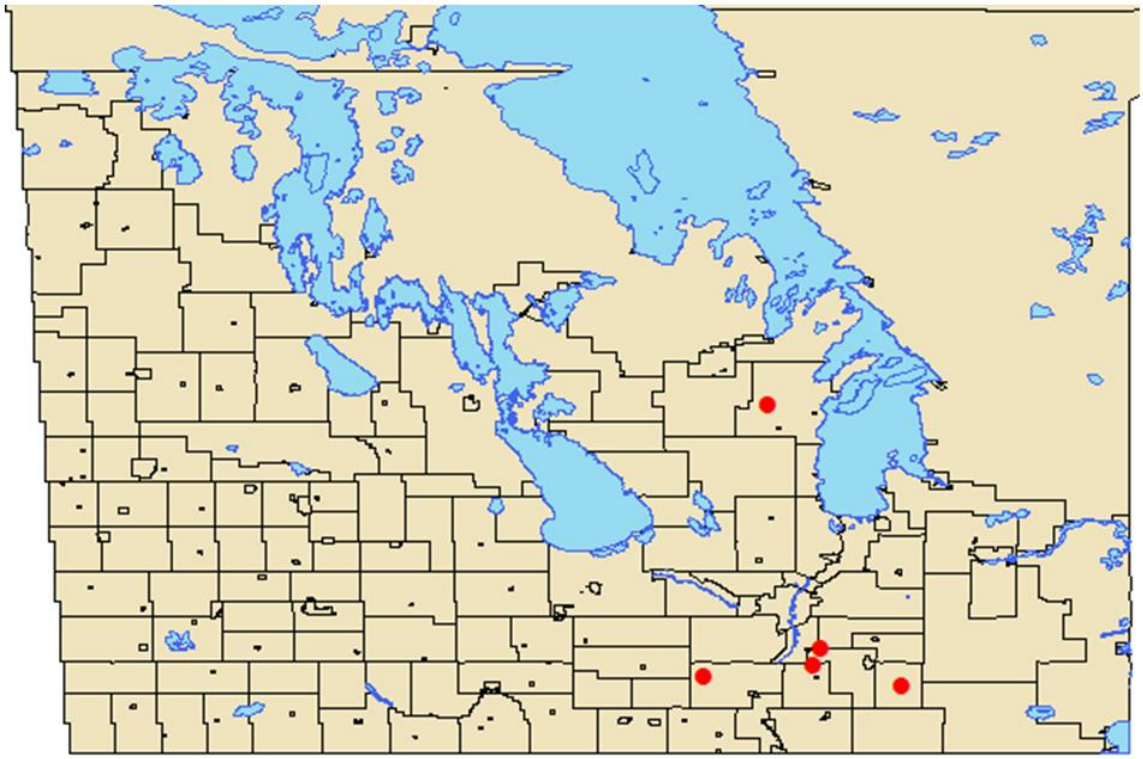
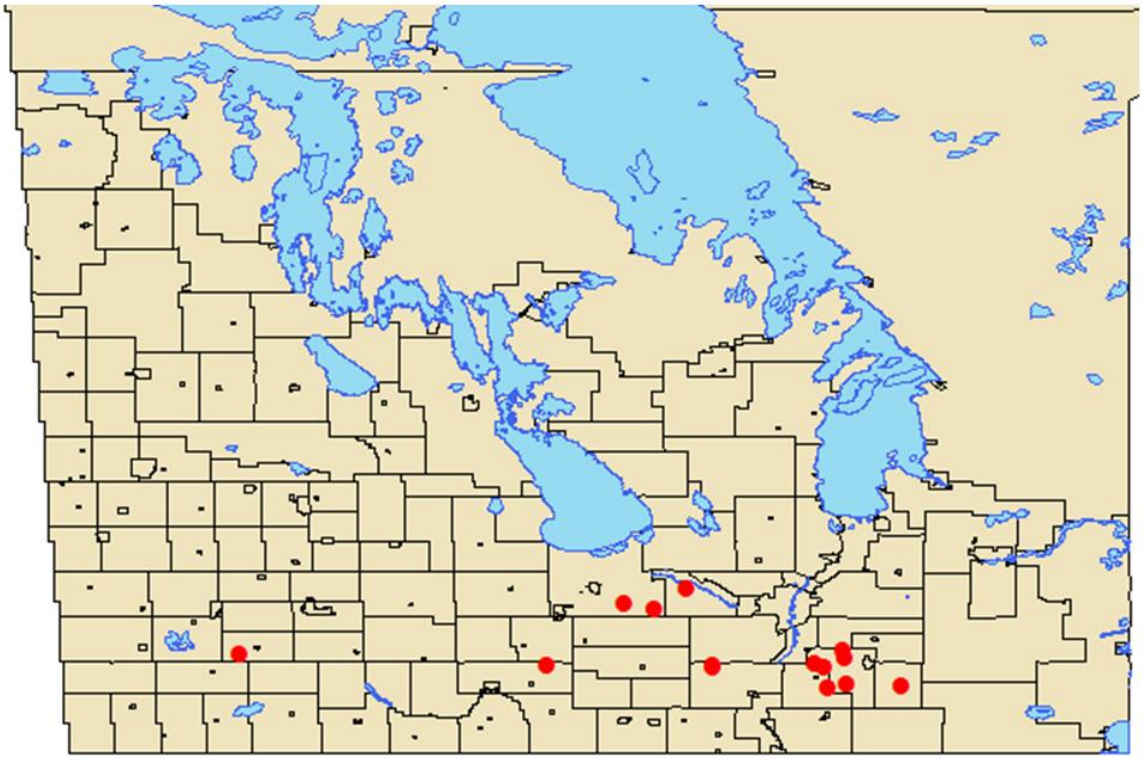


H1N1 SIV – Spring 2005

H1N1 SIV – Summer 2005



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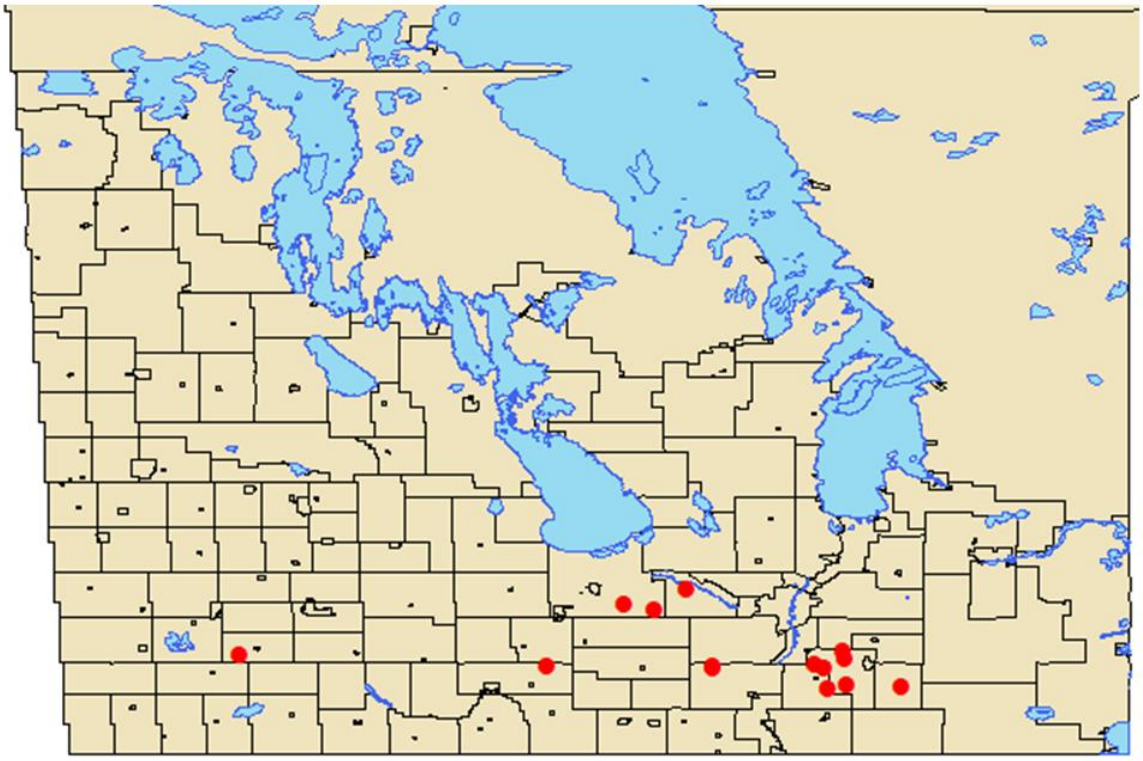

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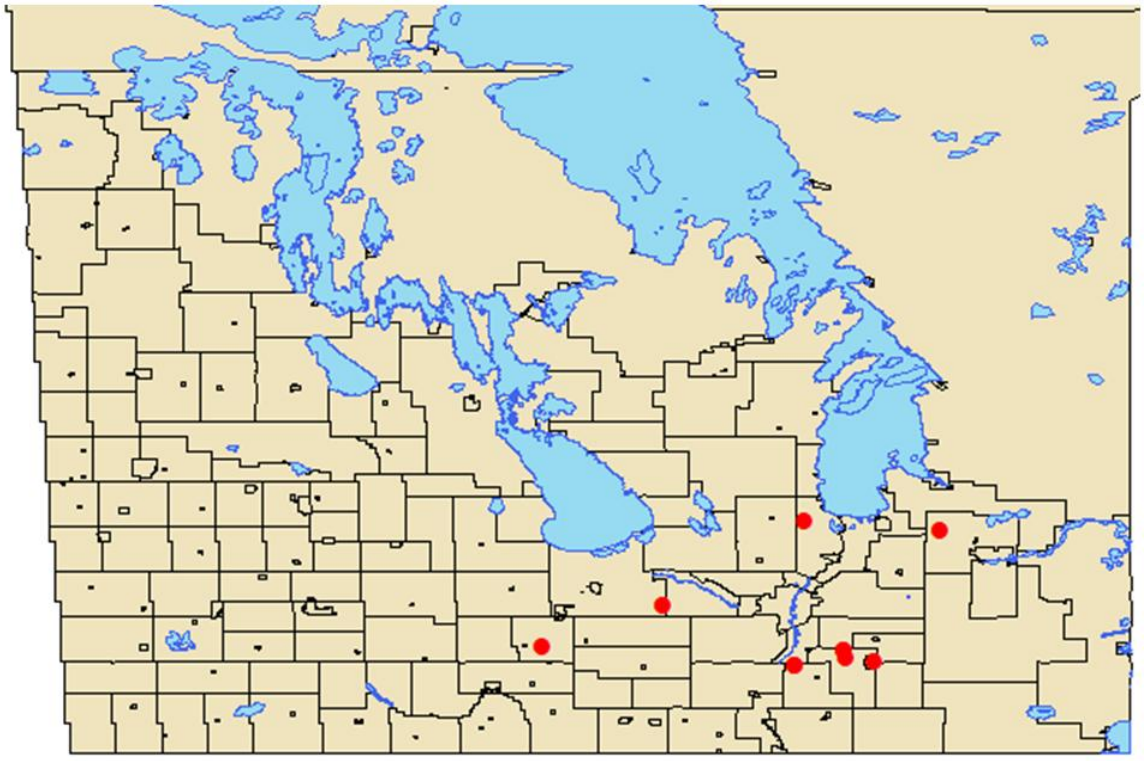

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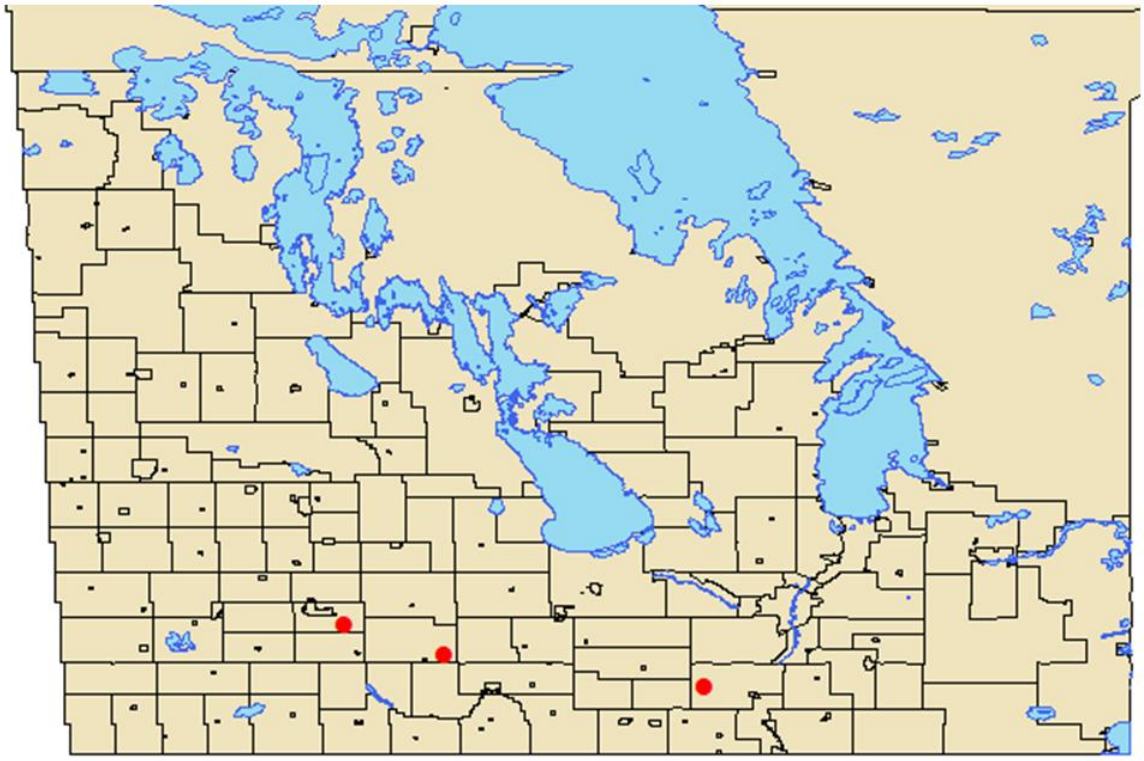

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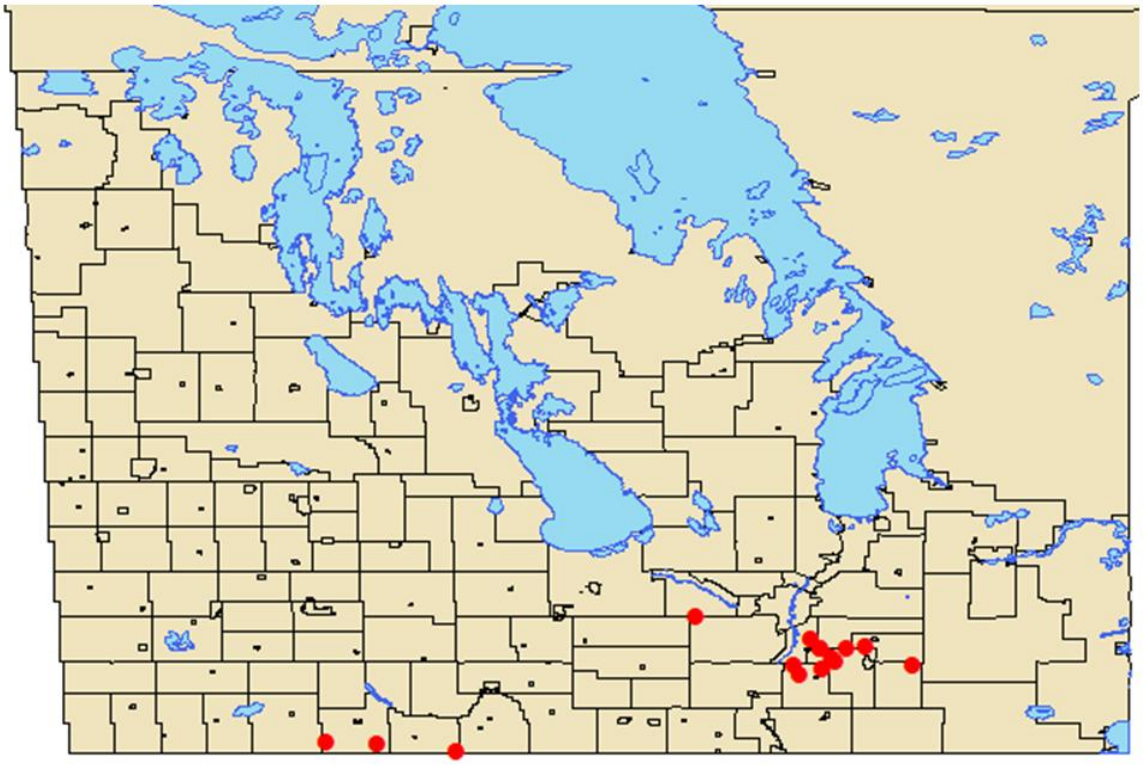

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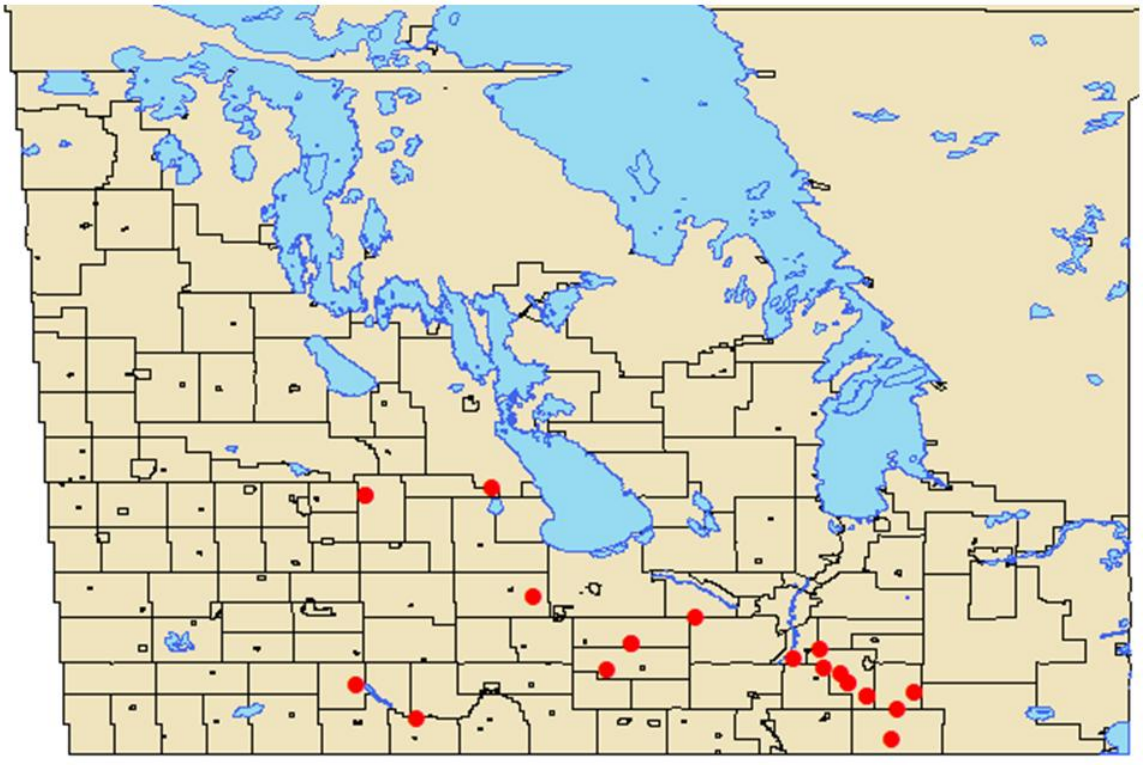

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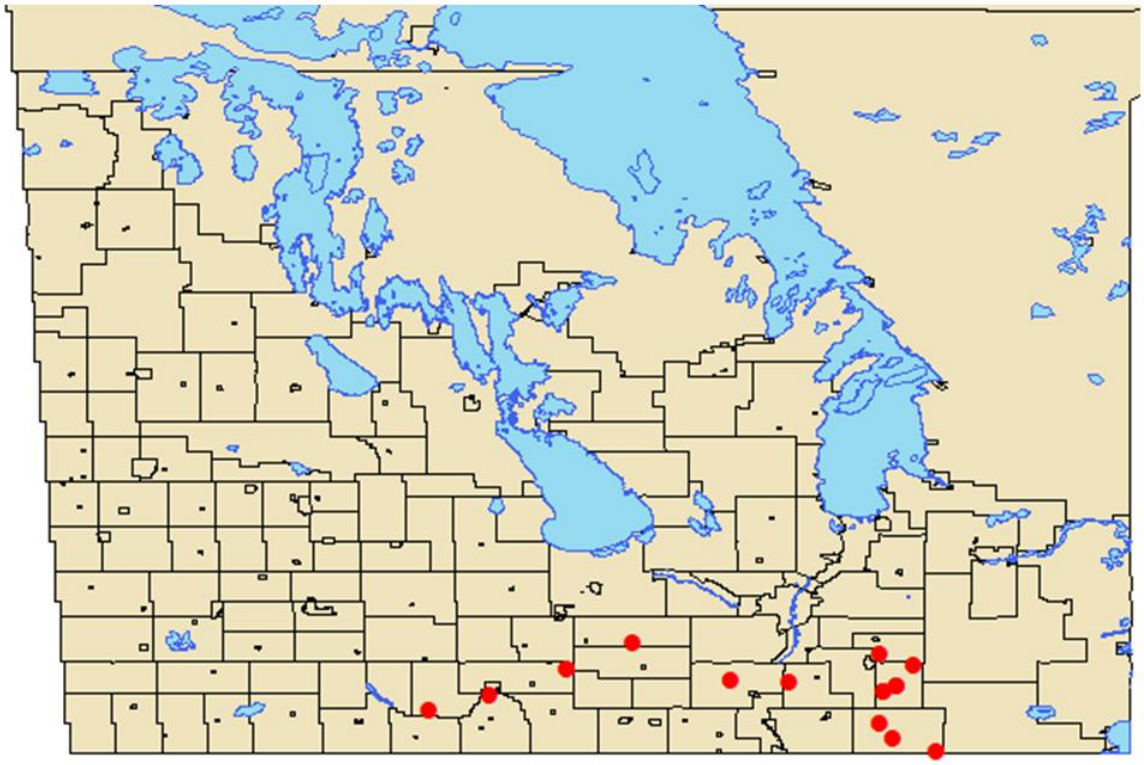

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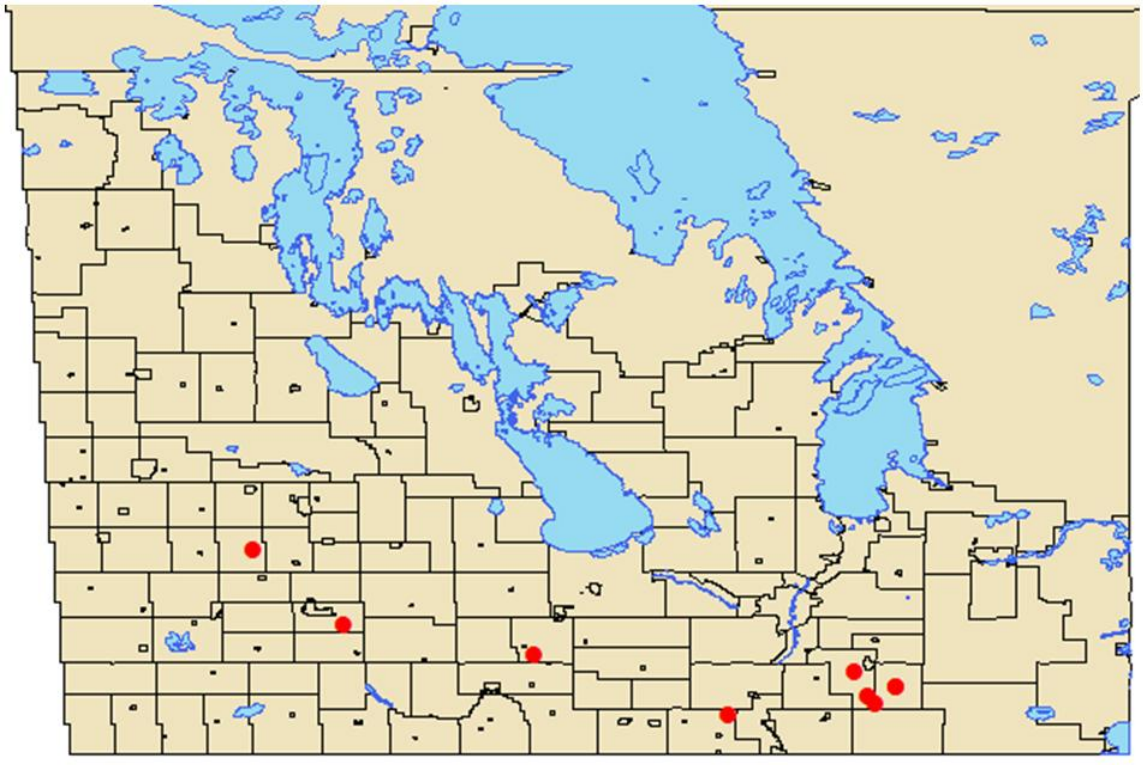

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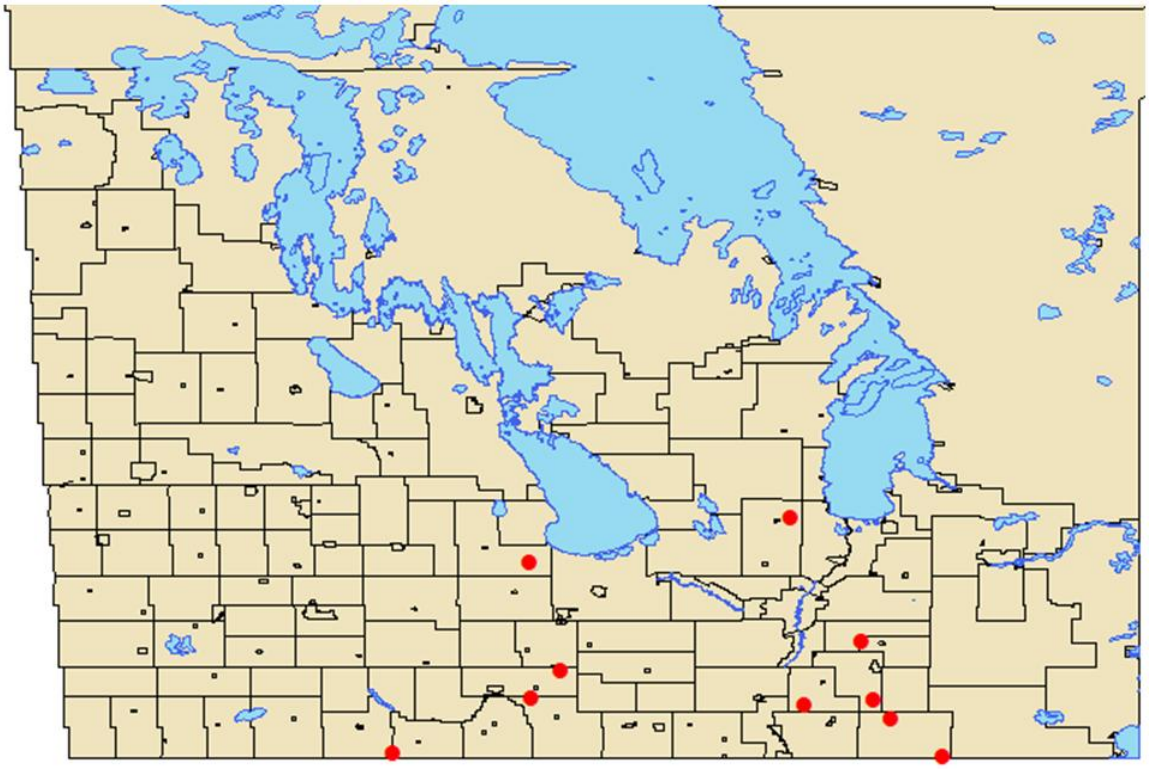

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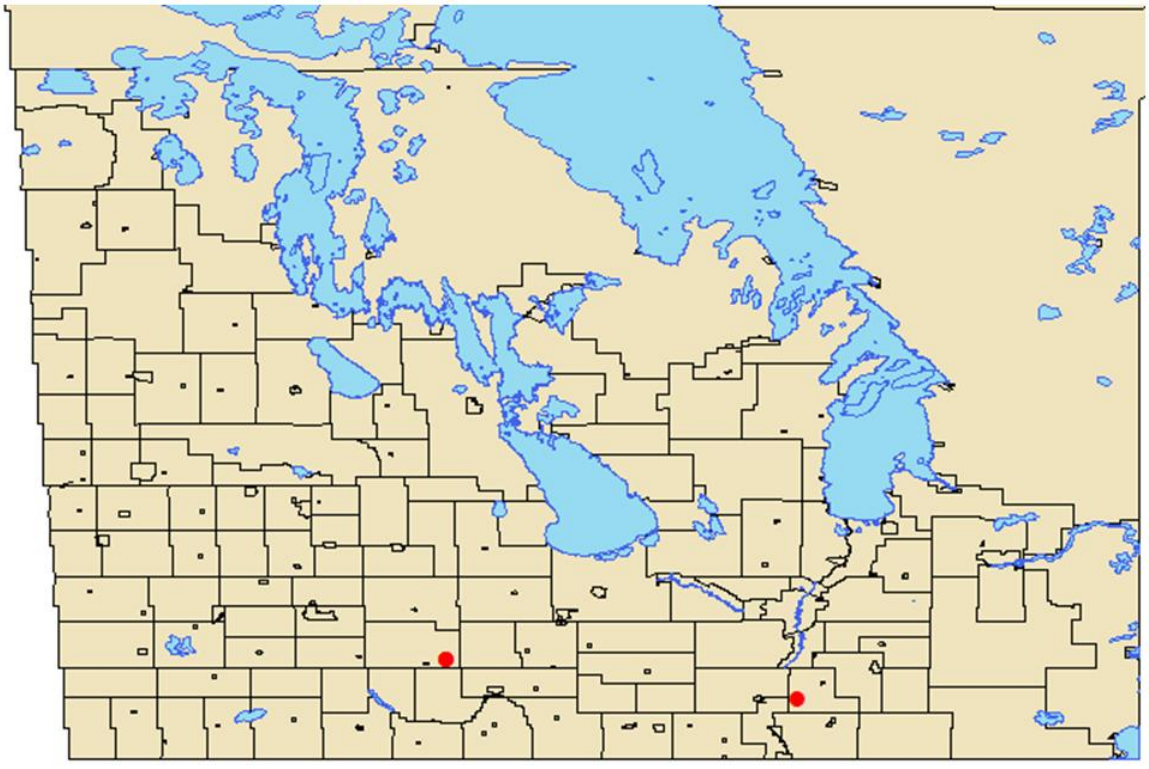
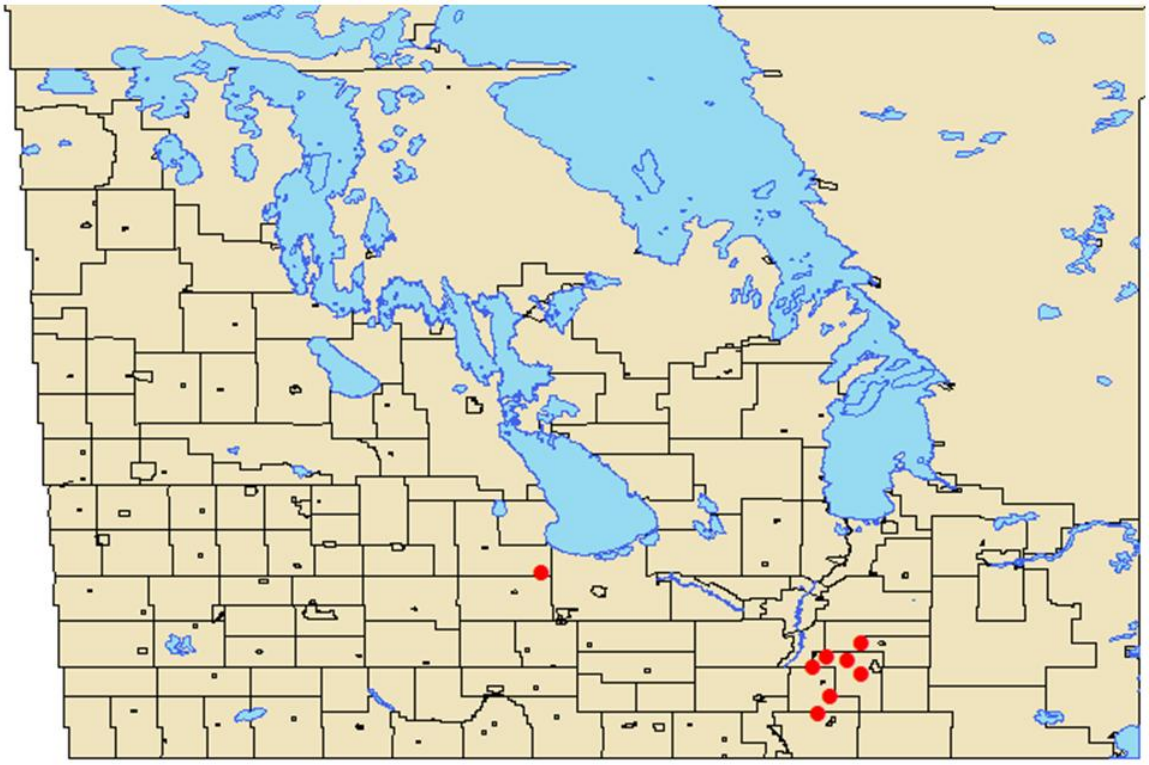
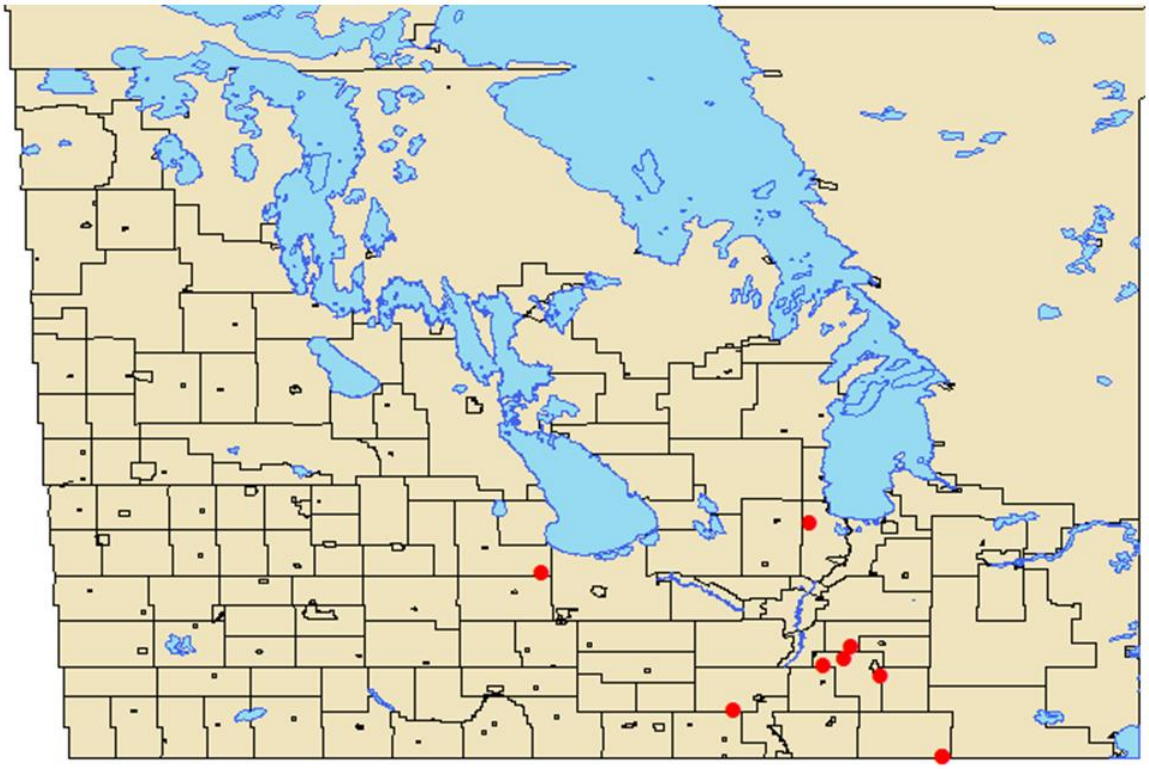


H1N1 SIV – Winter 2008

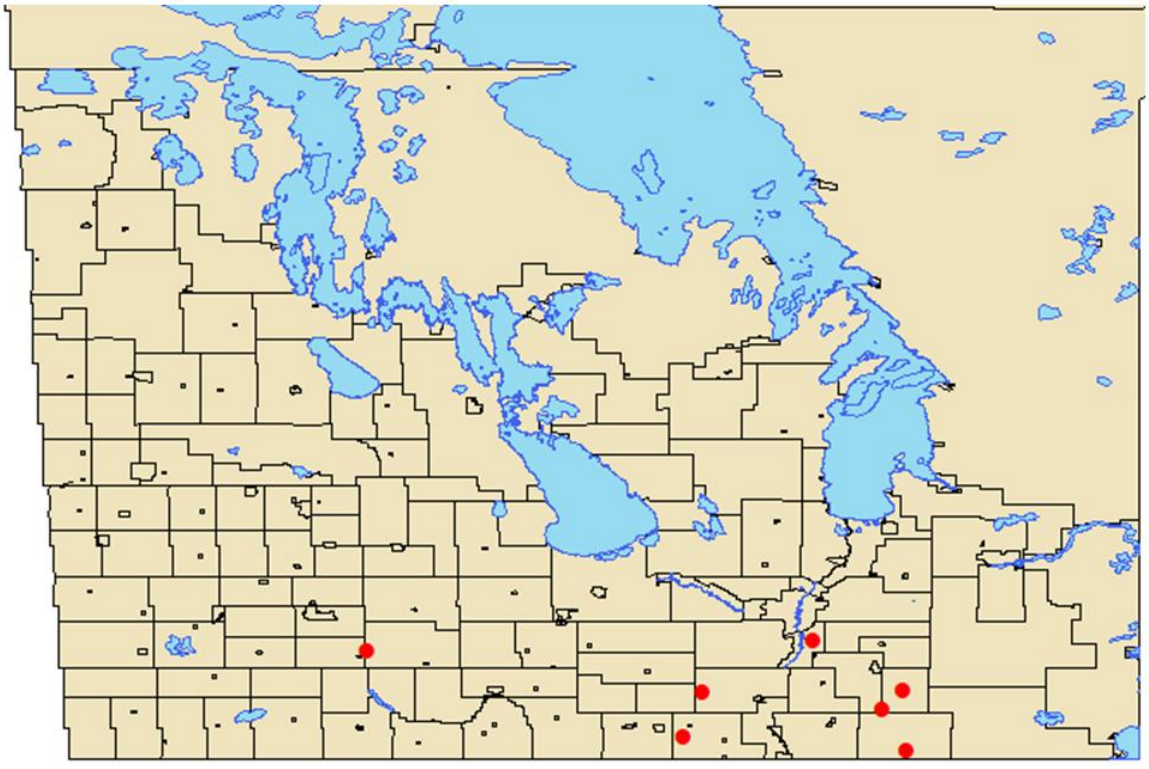


H1N1 SIV – Spring 2008

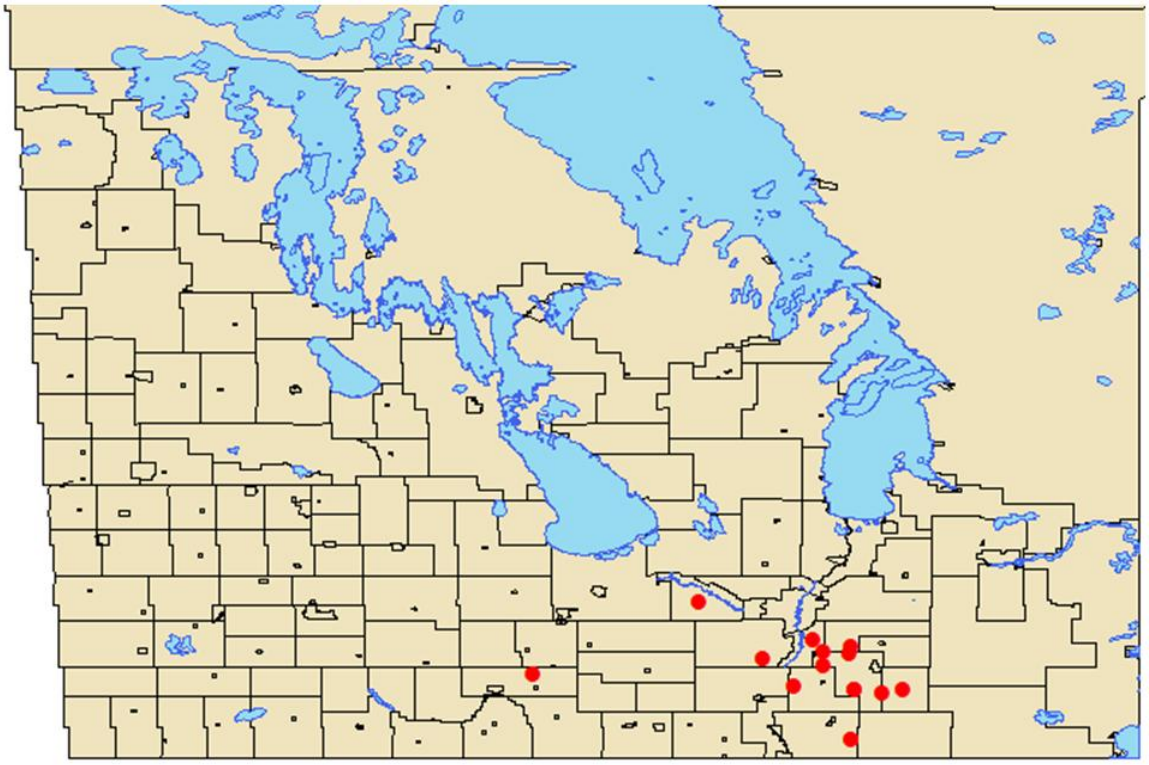
H1N1 SIV – Summer 2008



H1N1 SIV – Fall 2008



H1N1 SIV – Winter 2009



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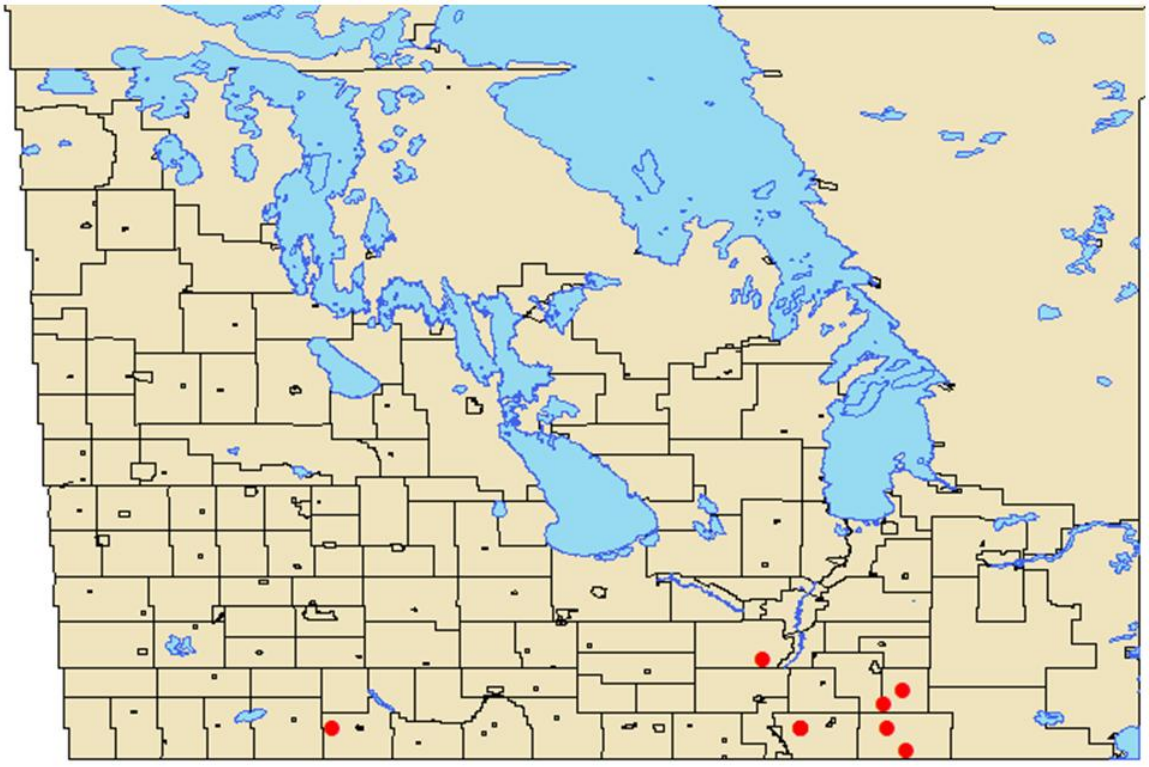

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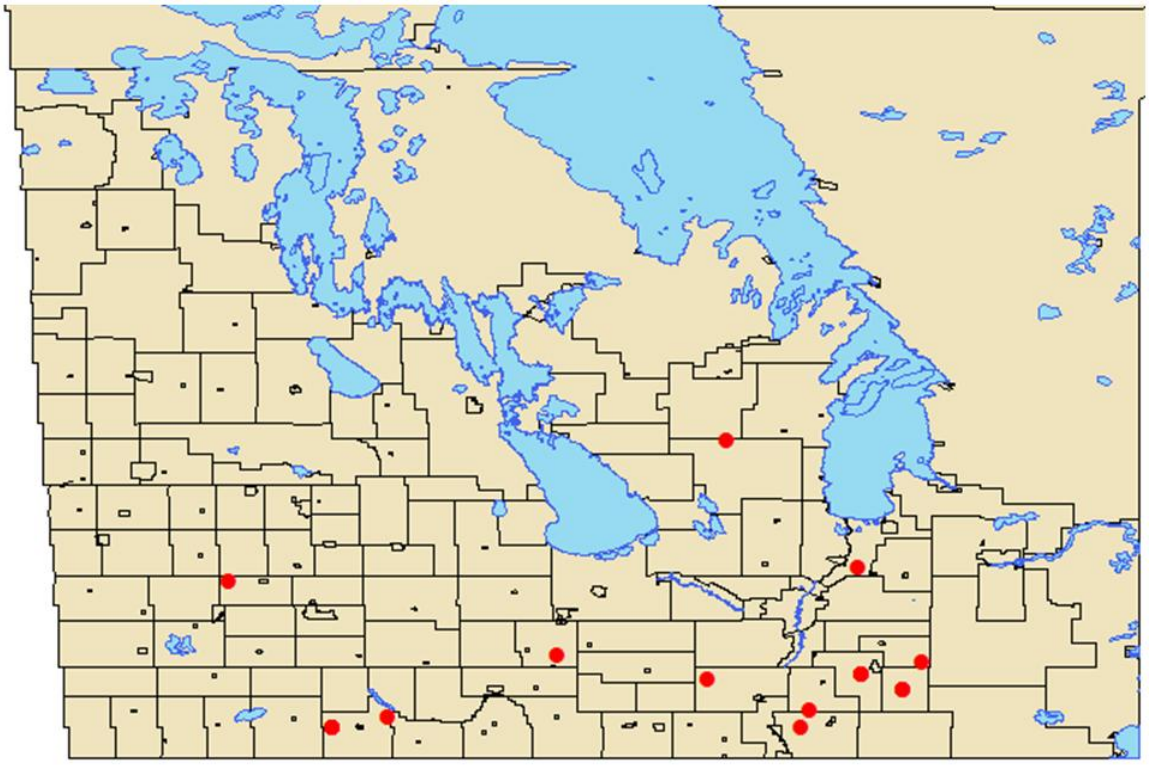
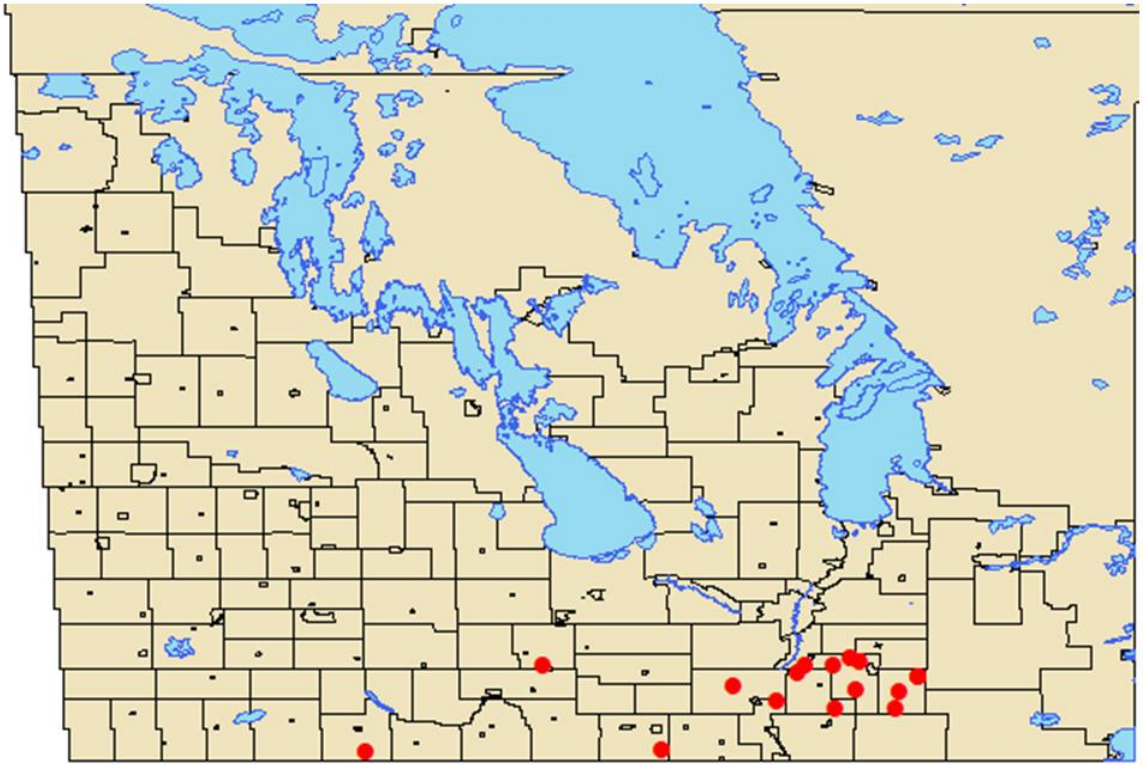
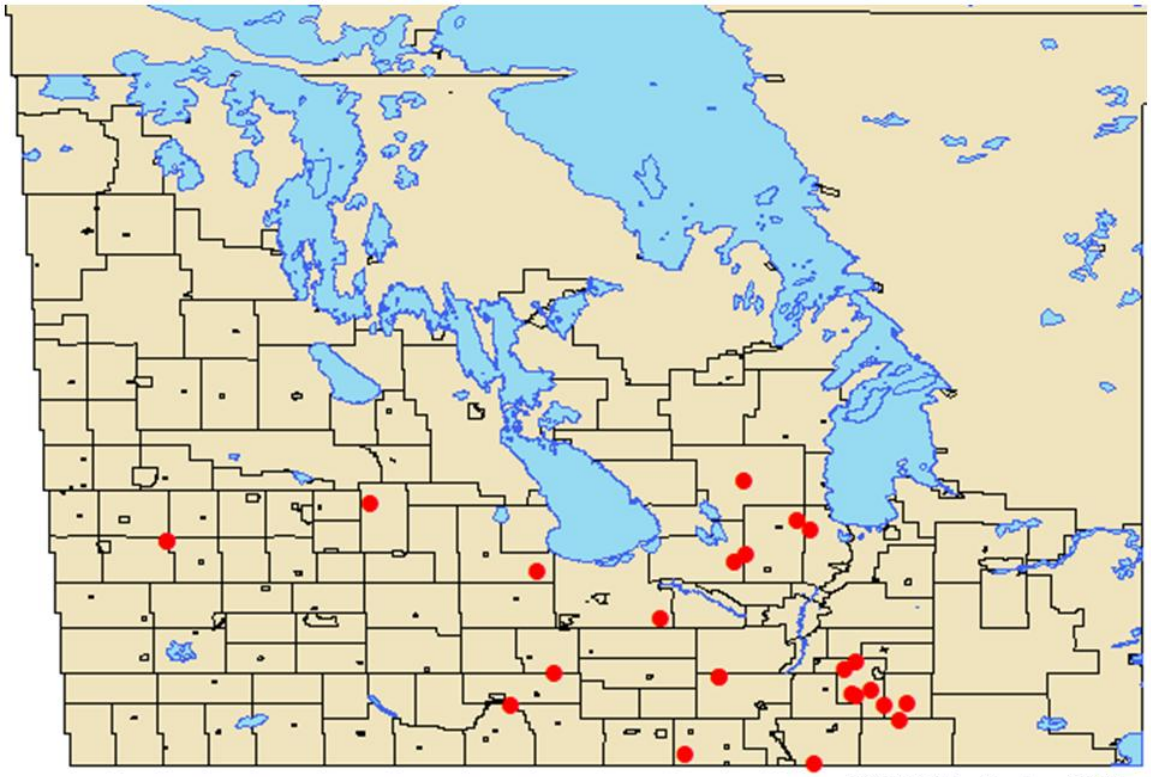


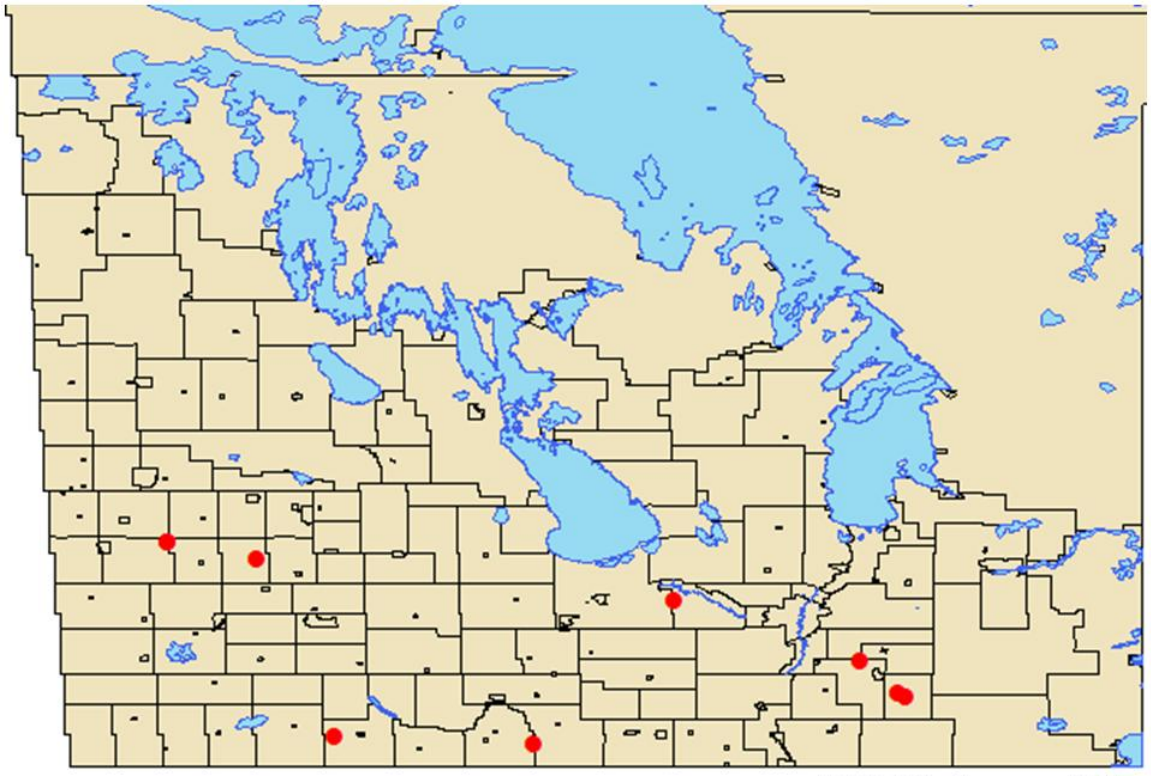
H1N1 SIV – Fall 2009

Figure 6. Map sequence, H3N2 swine influenza in Manitoba, 2005 to 2009





H3N2 SIV – Spring 2005



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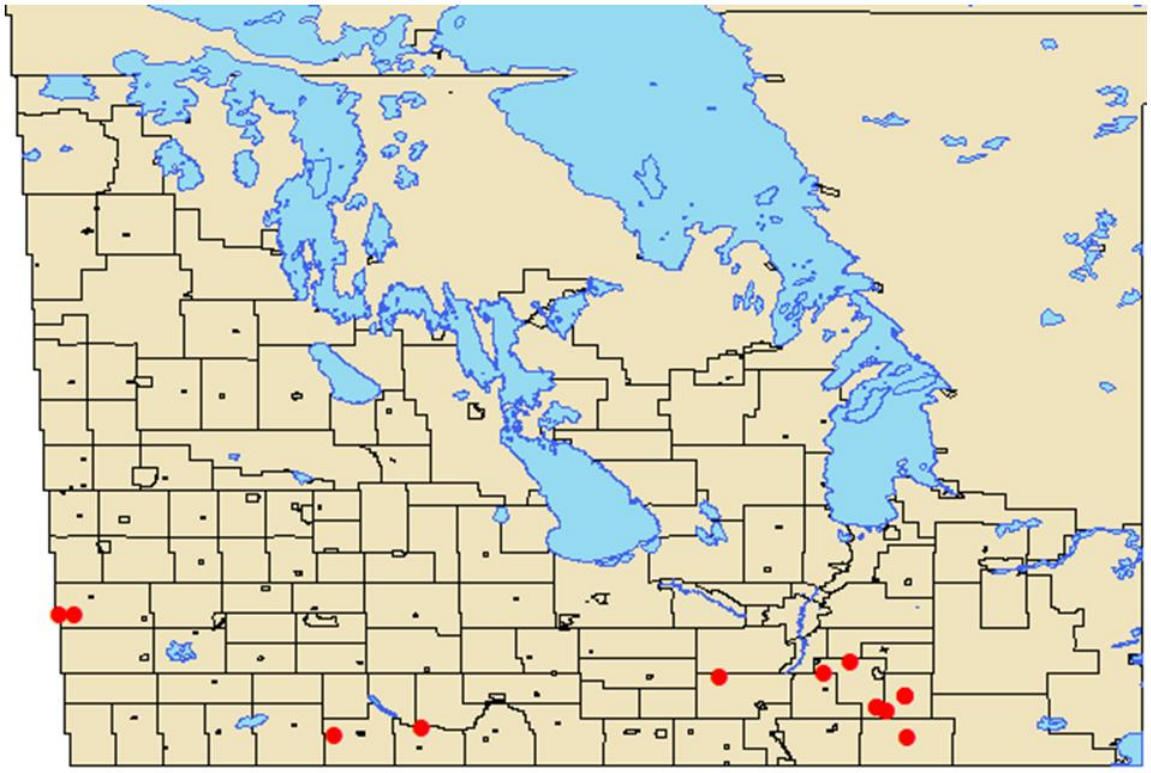

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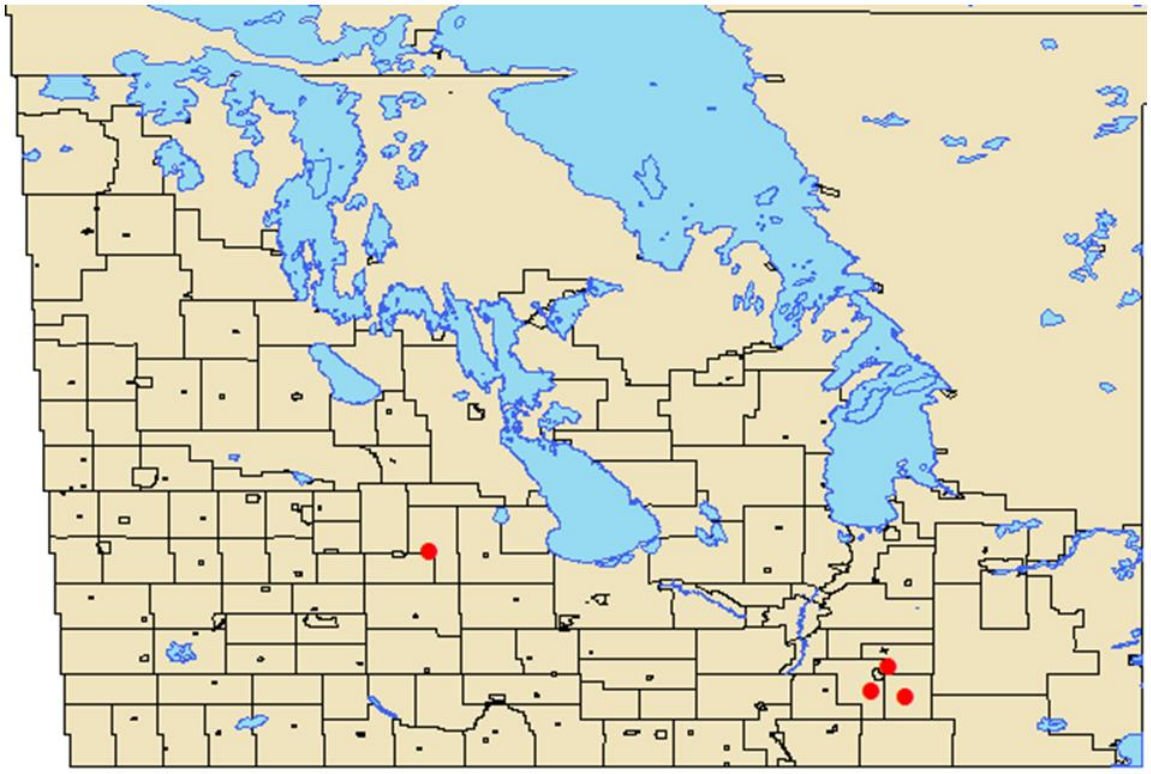

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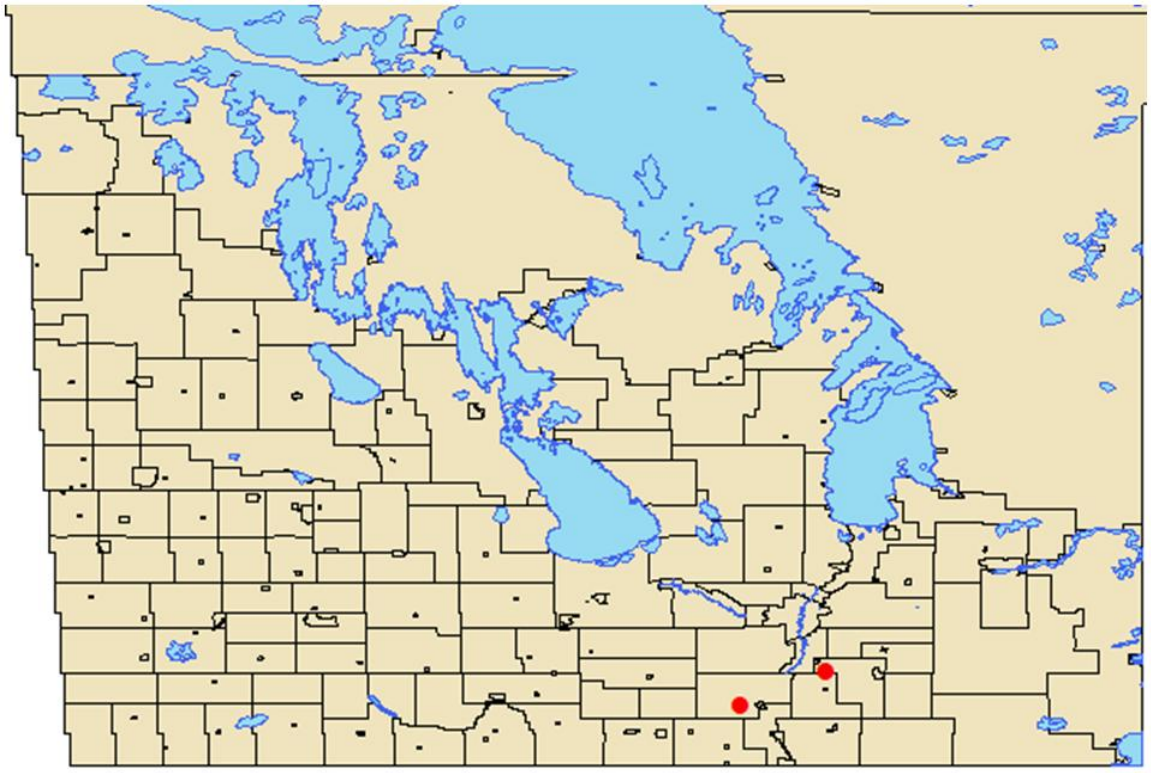

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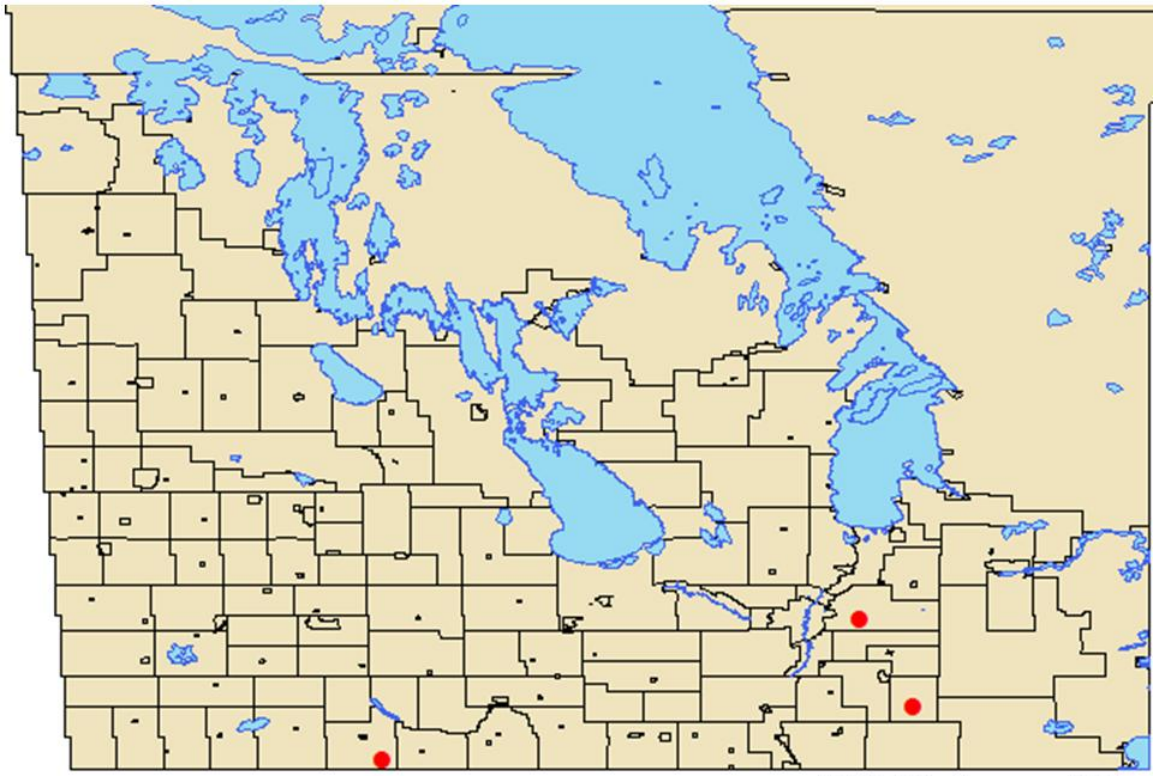

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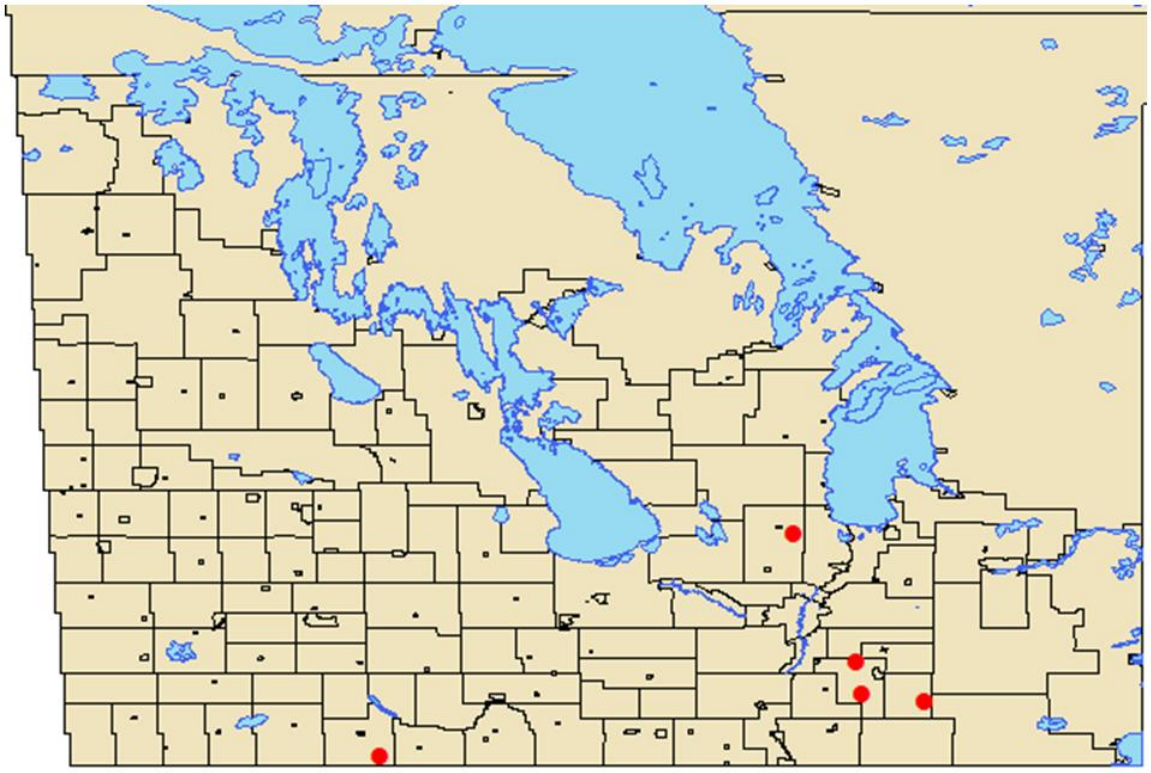

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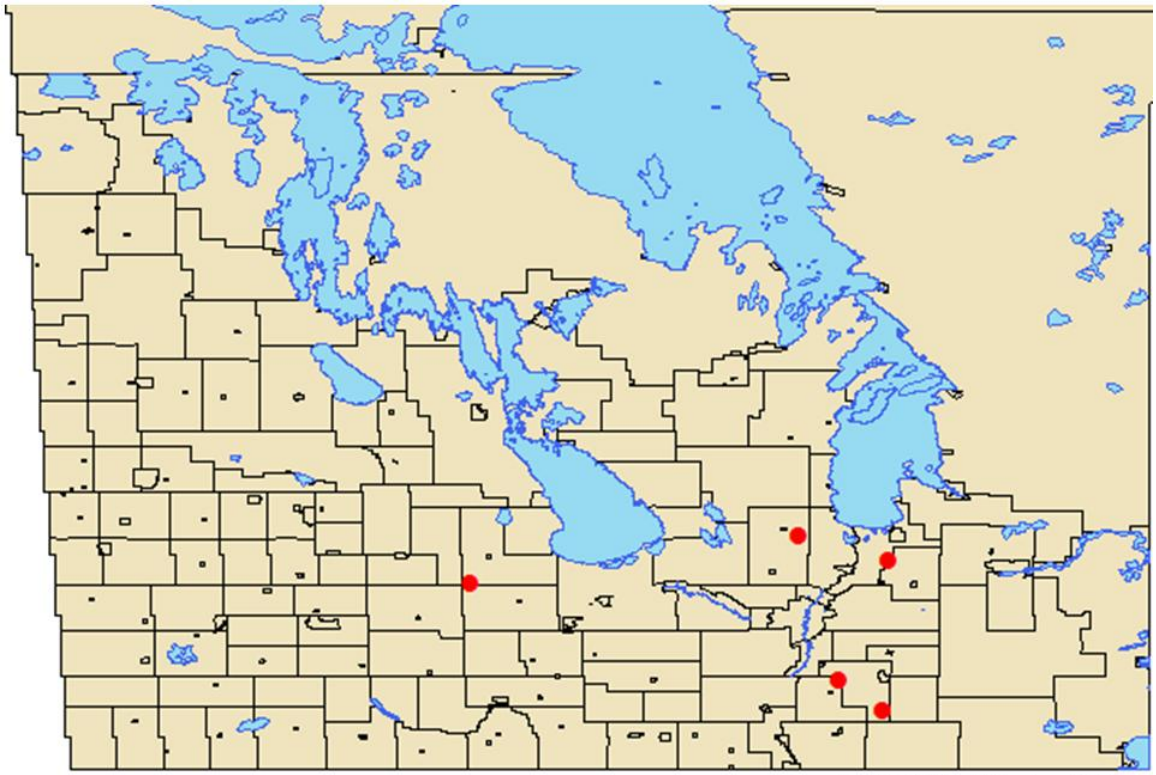
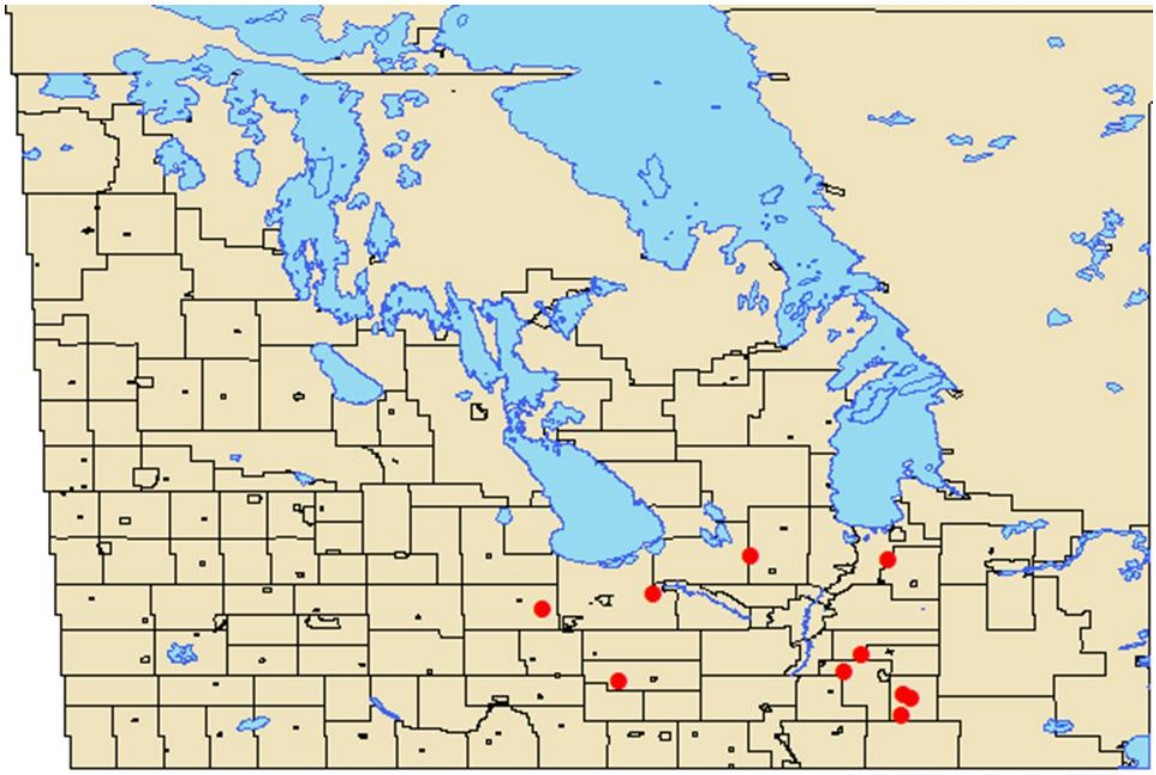


H3N2 SIV – Winter 2007



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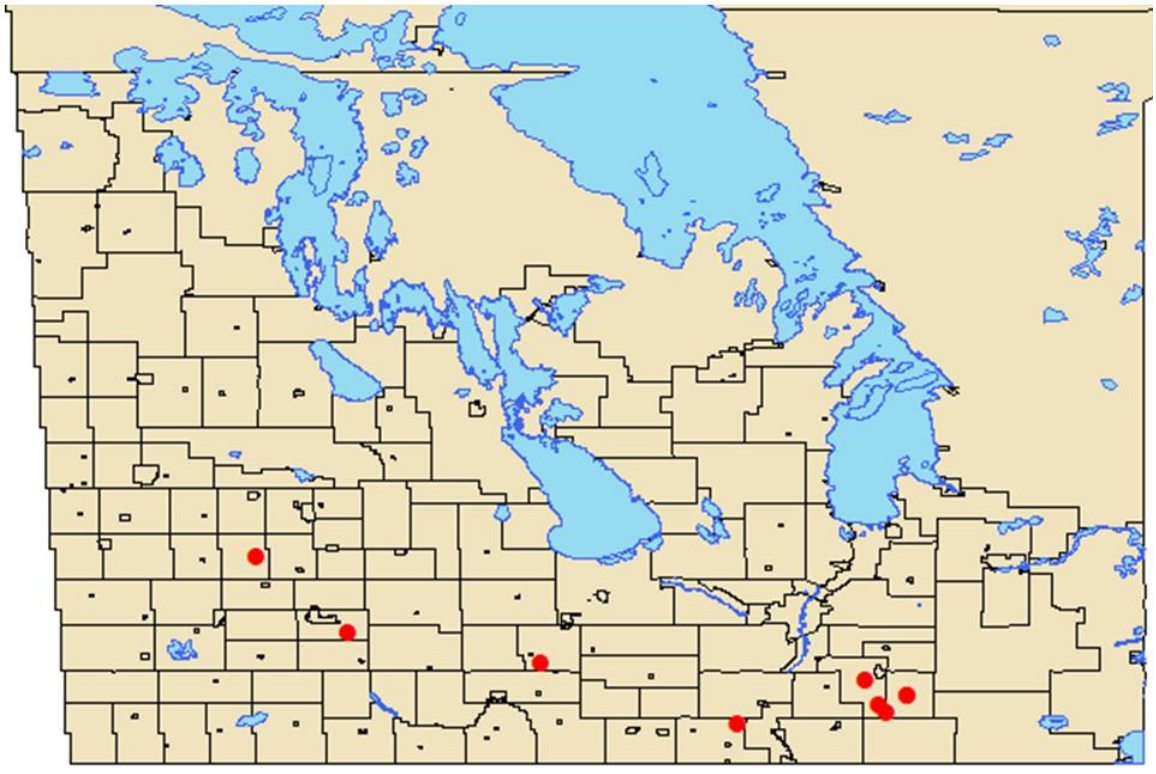

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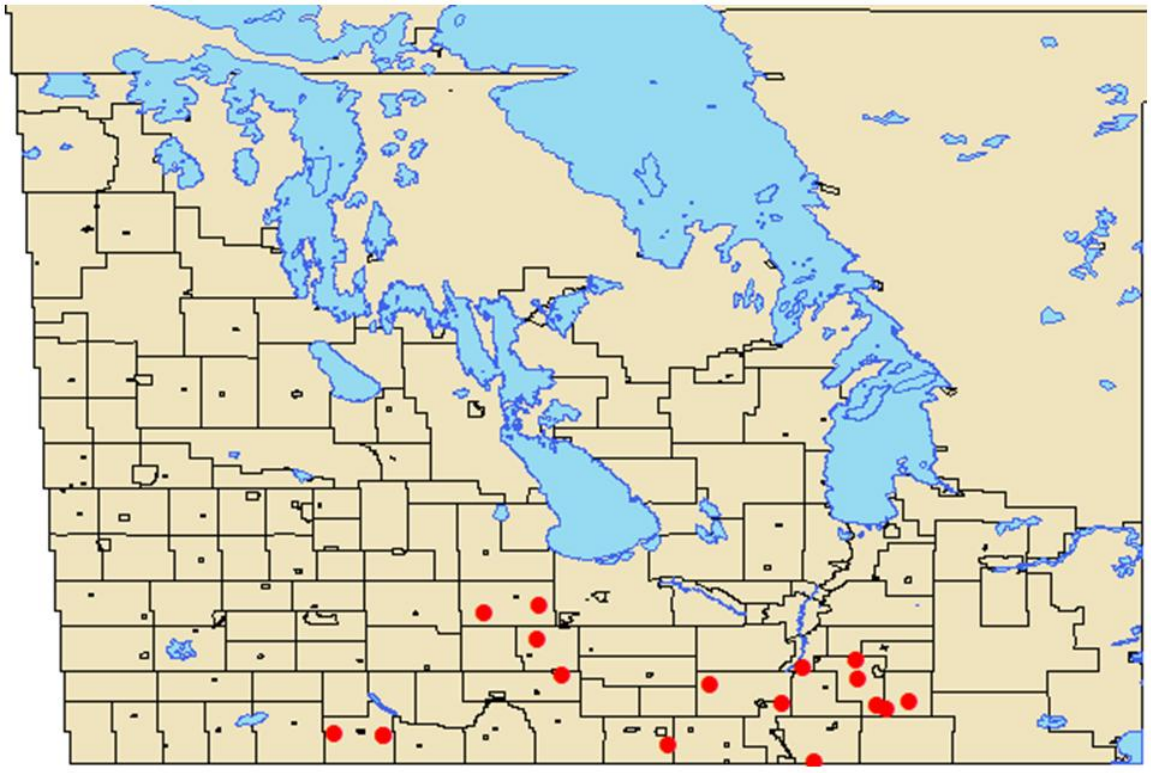
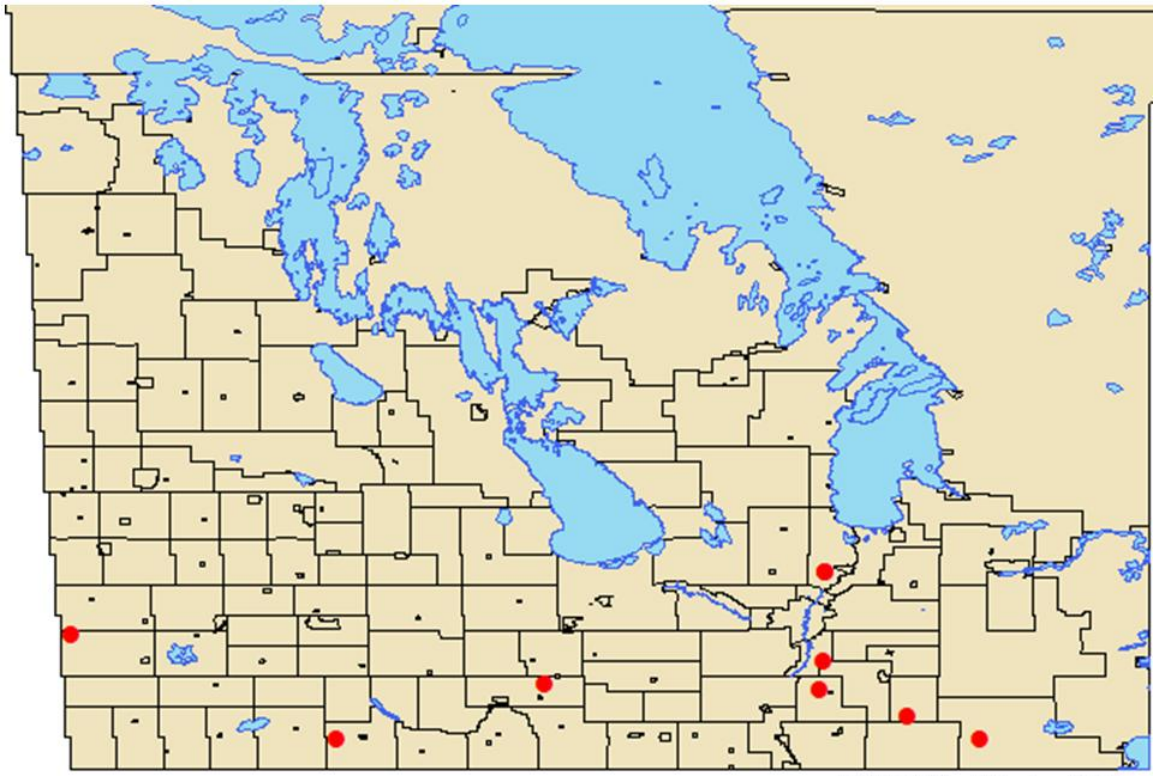


H3N2 SIV – Fall 2007



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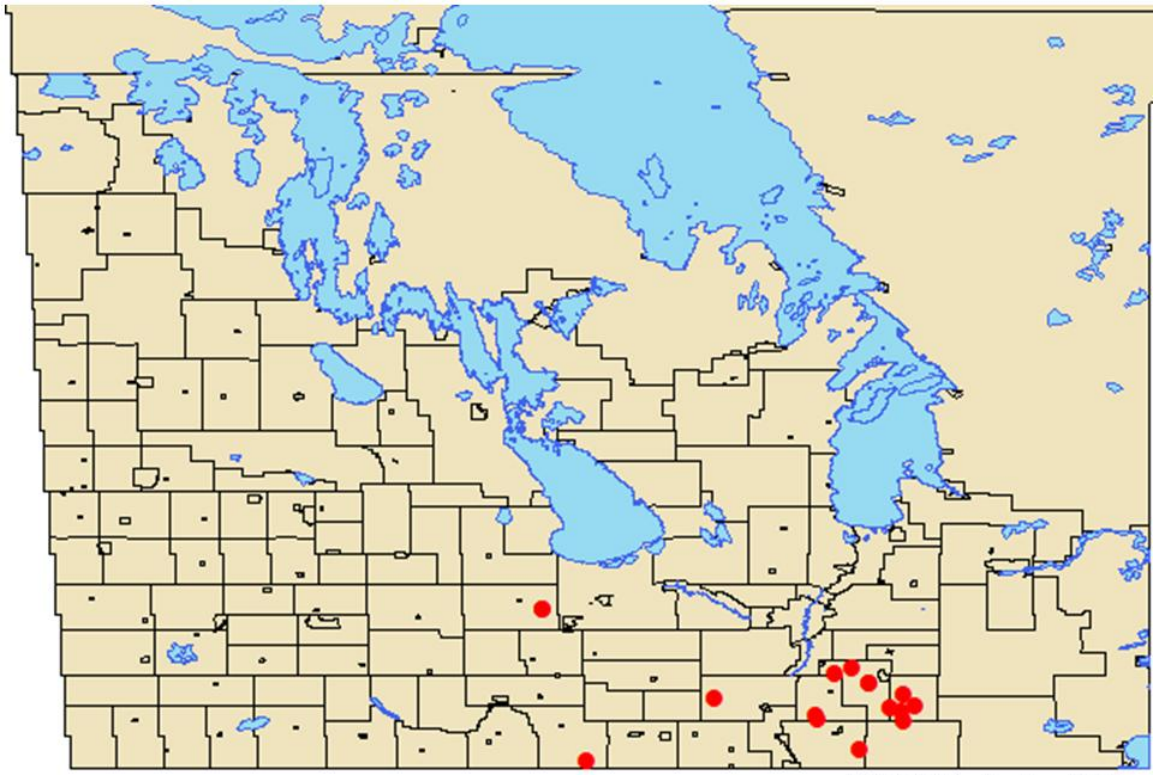

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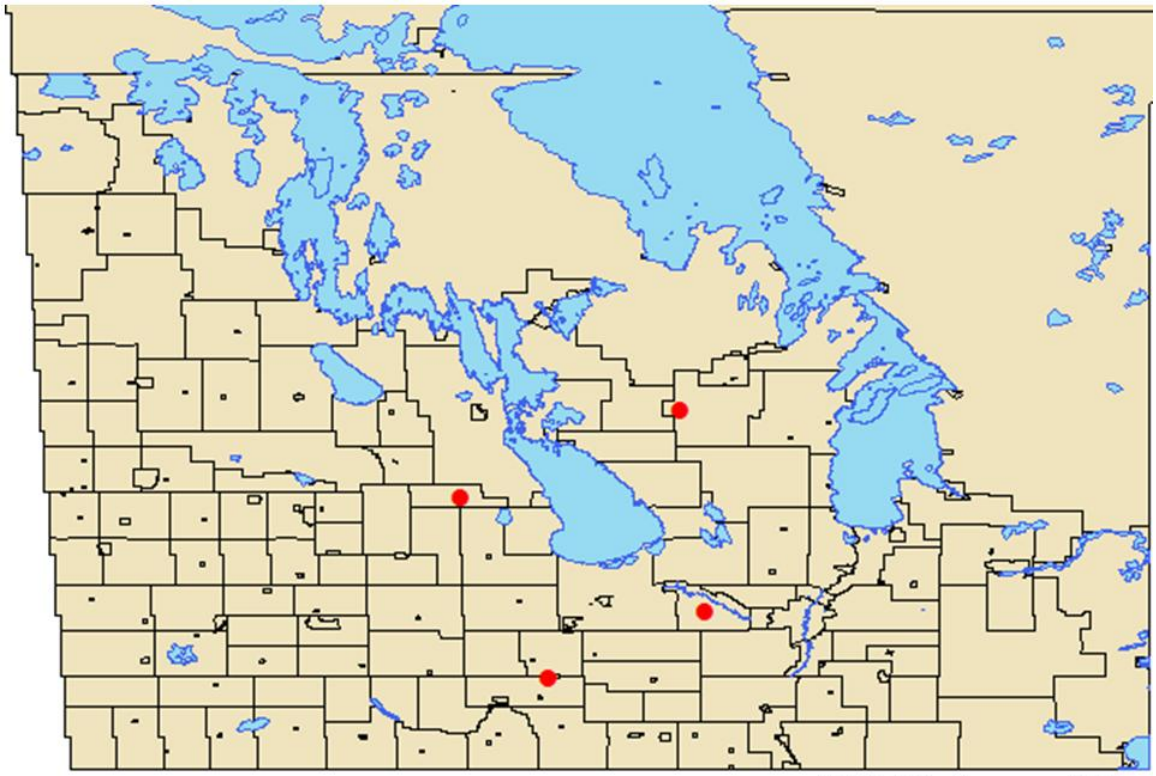

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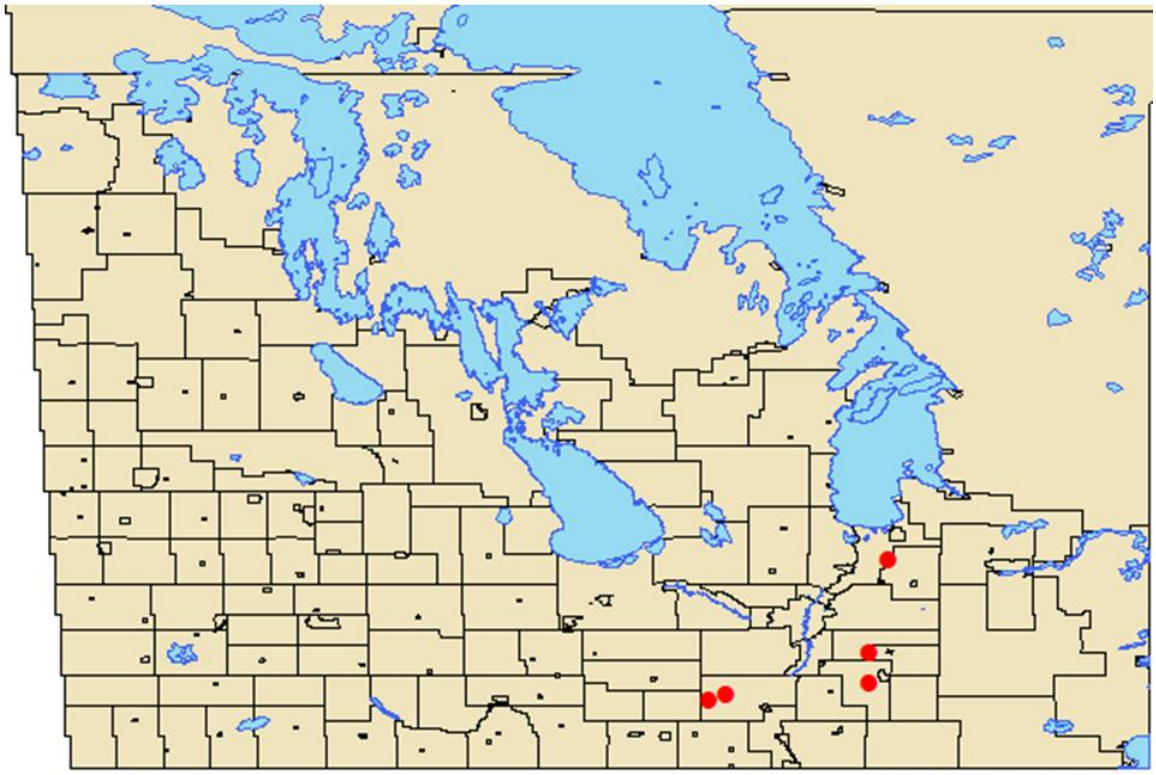

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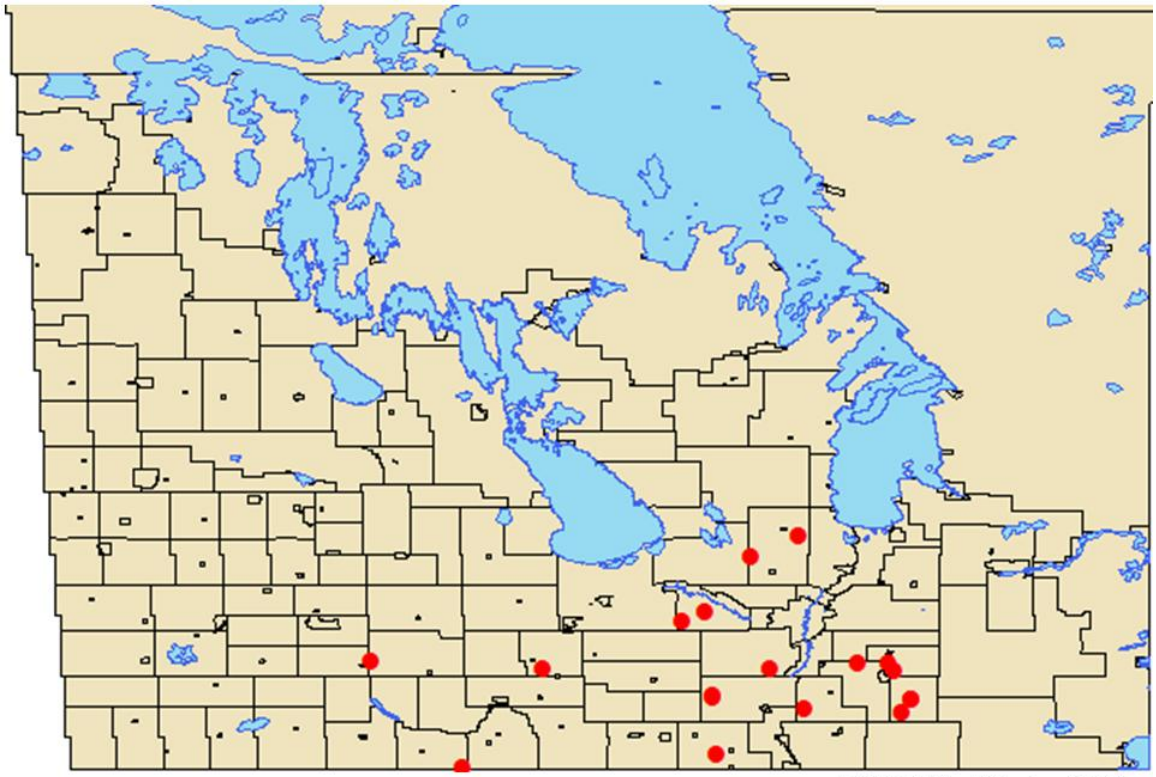

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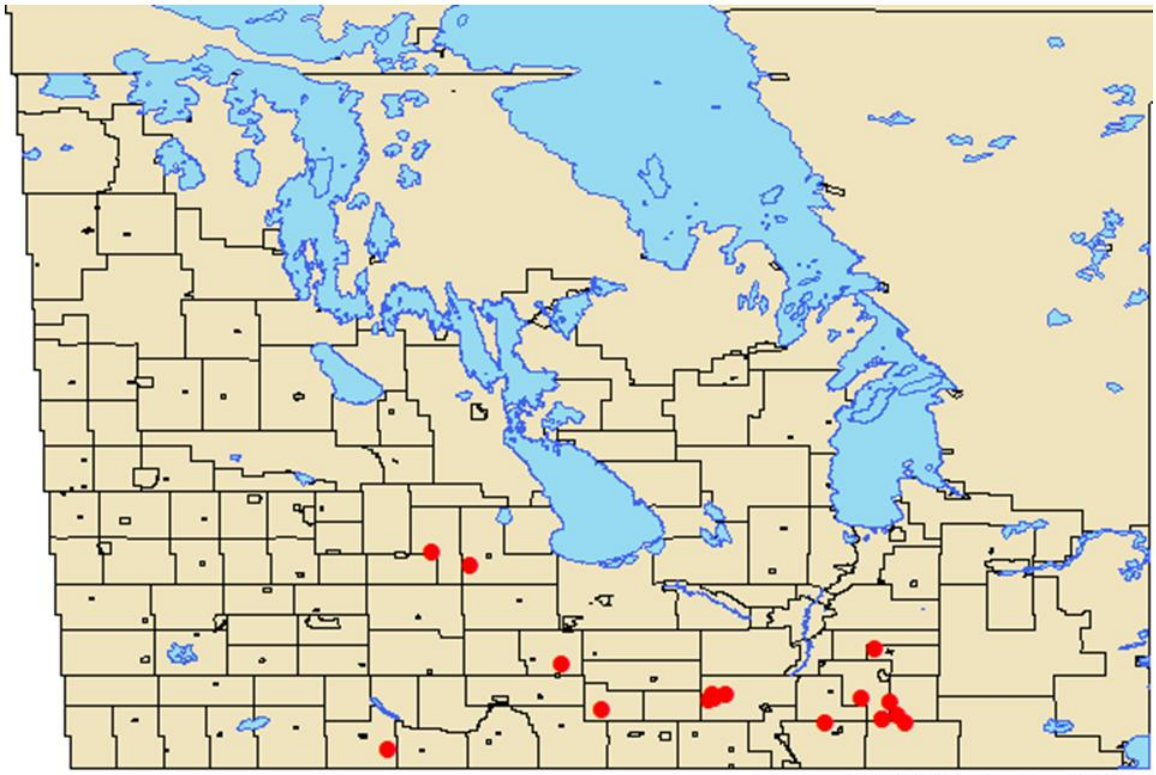

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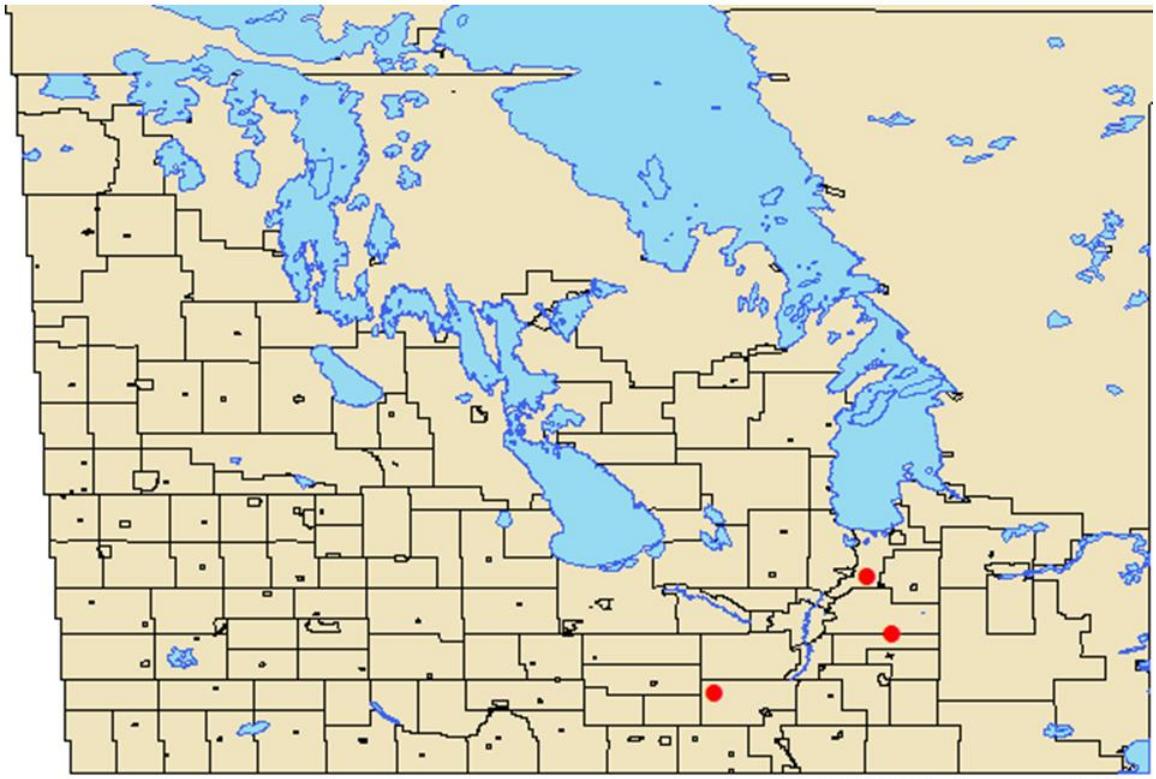

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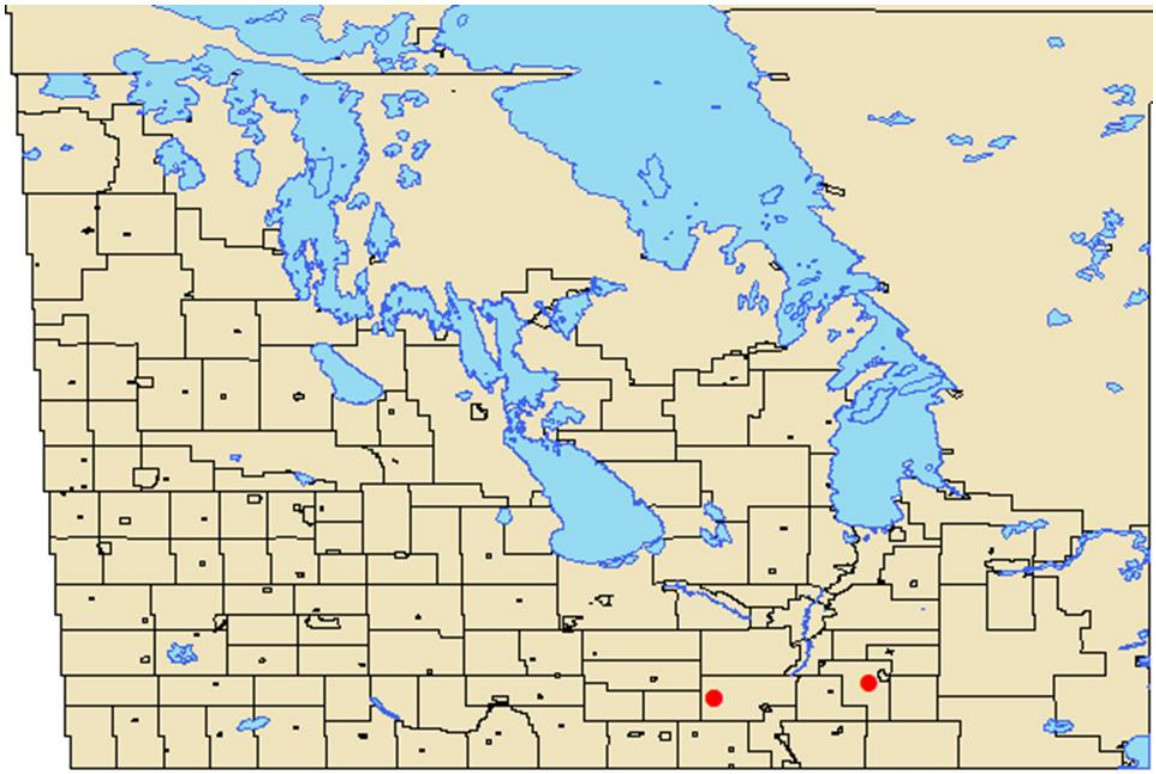
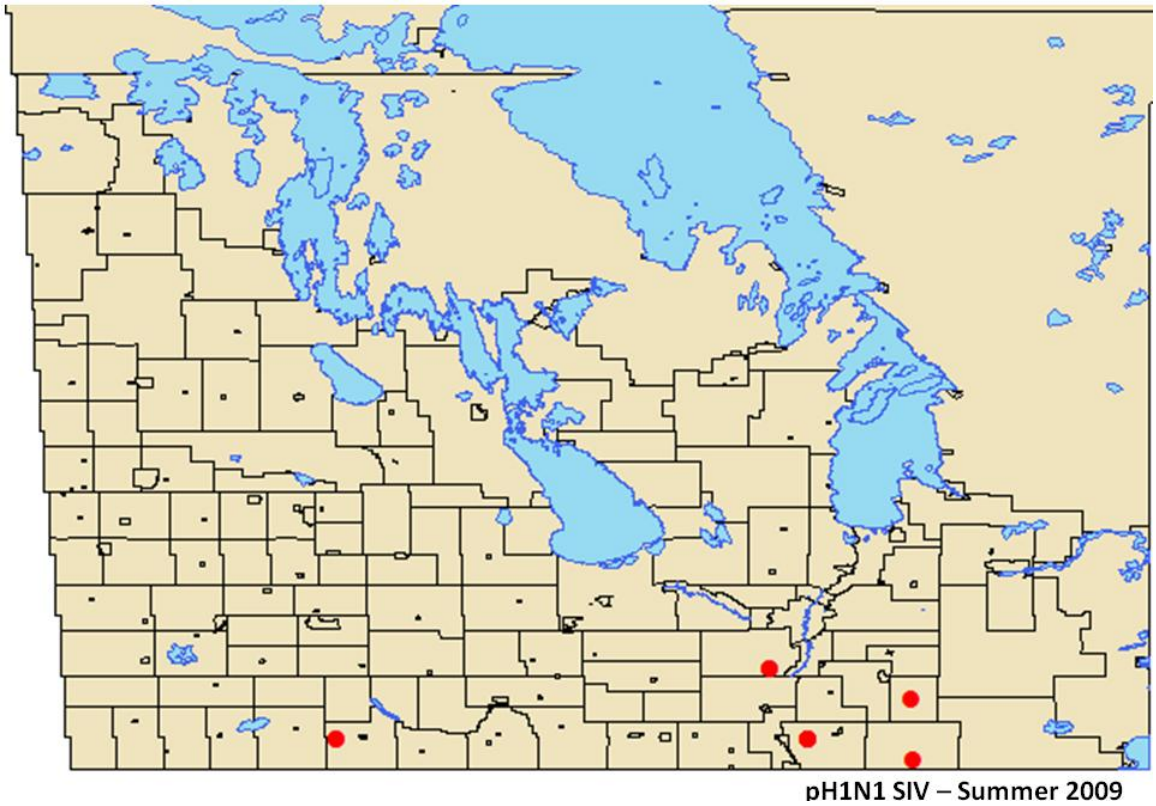
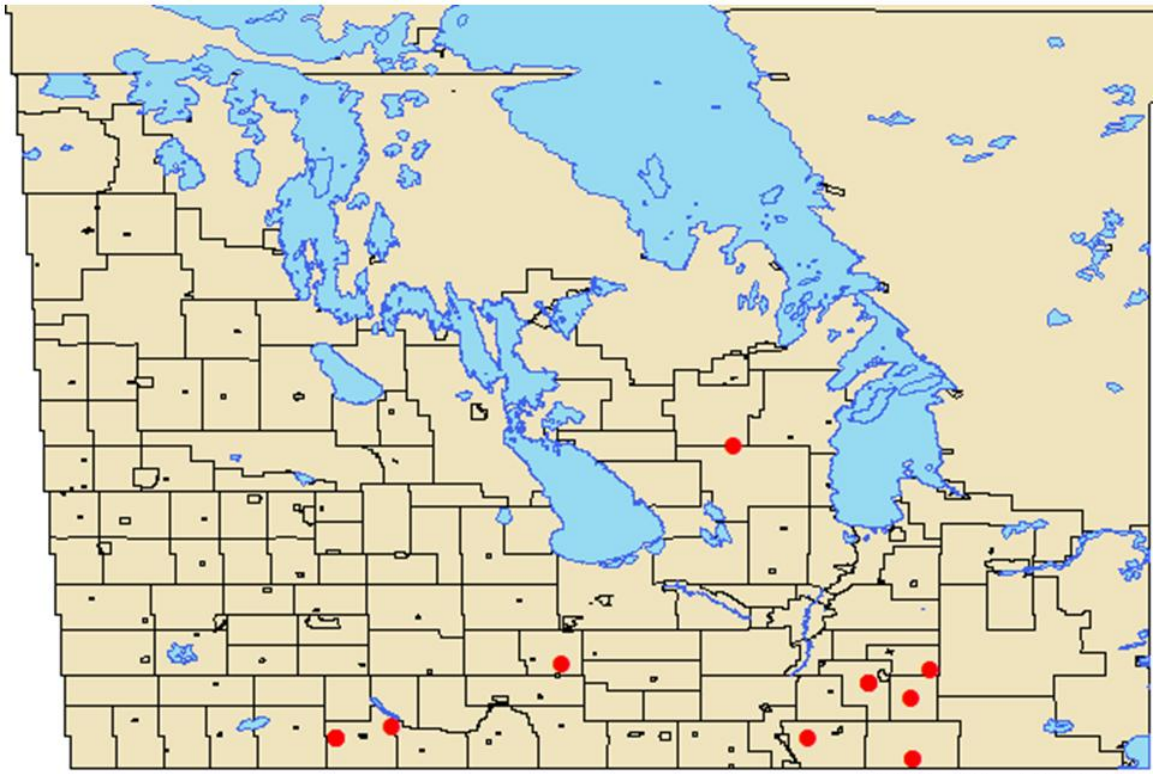


H3N2 SIV – Fall 2009

Figure 7. Map sequence, pH1N1 swine influenza in Manitoba, 2009





pH1N1 SIV – Fall 2009

Figure 8. Histogram, swine influenza virus submission frequency

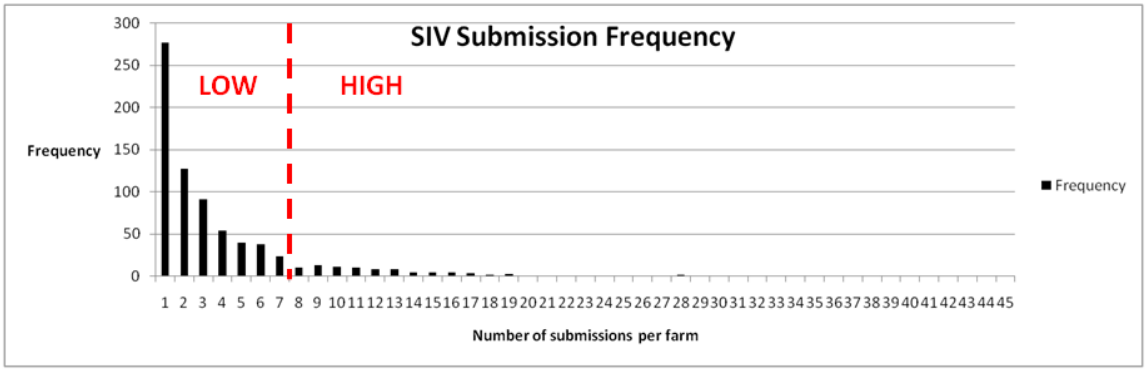
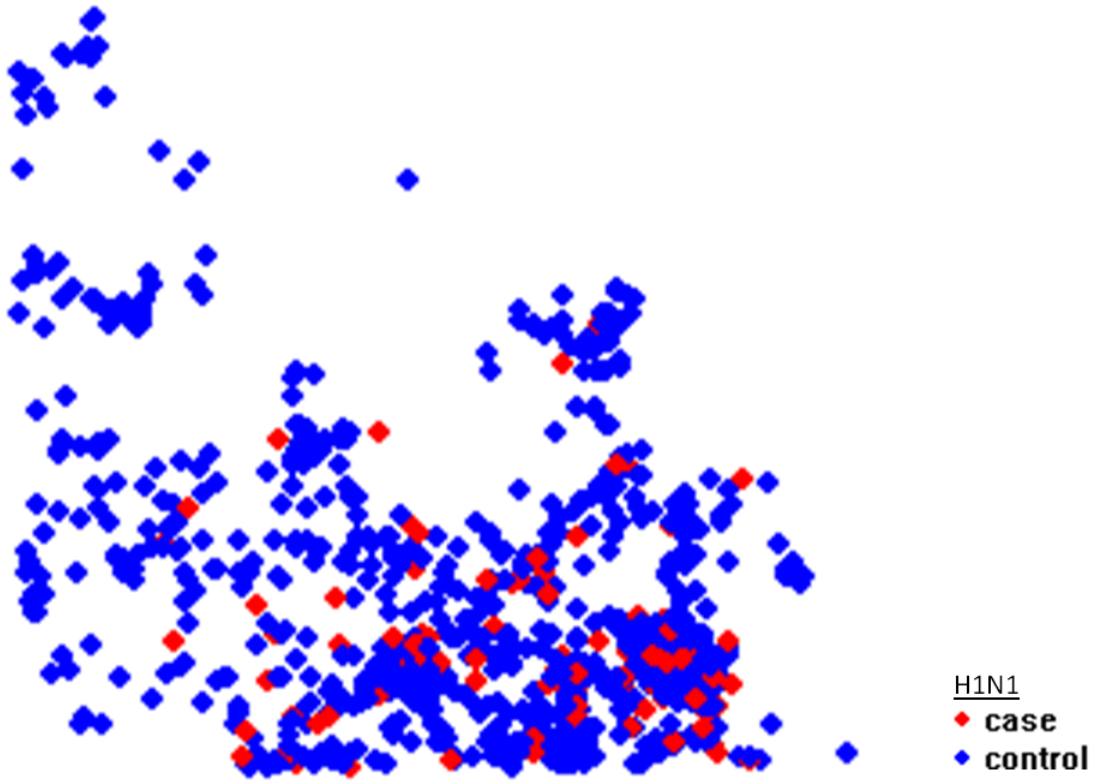
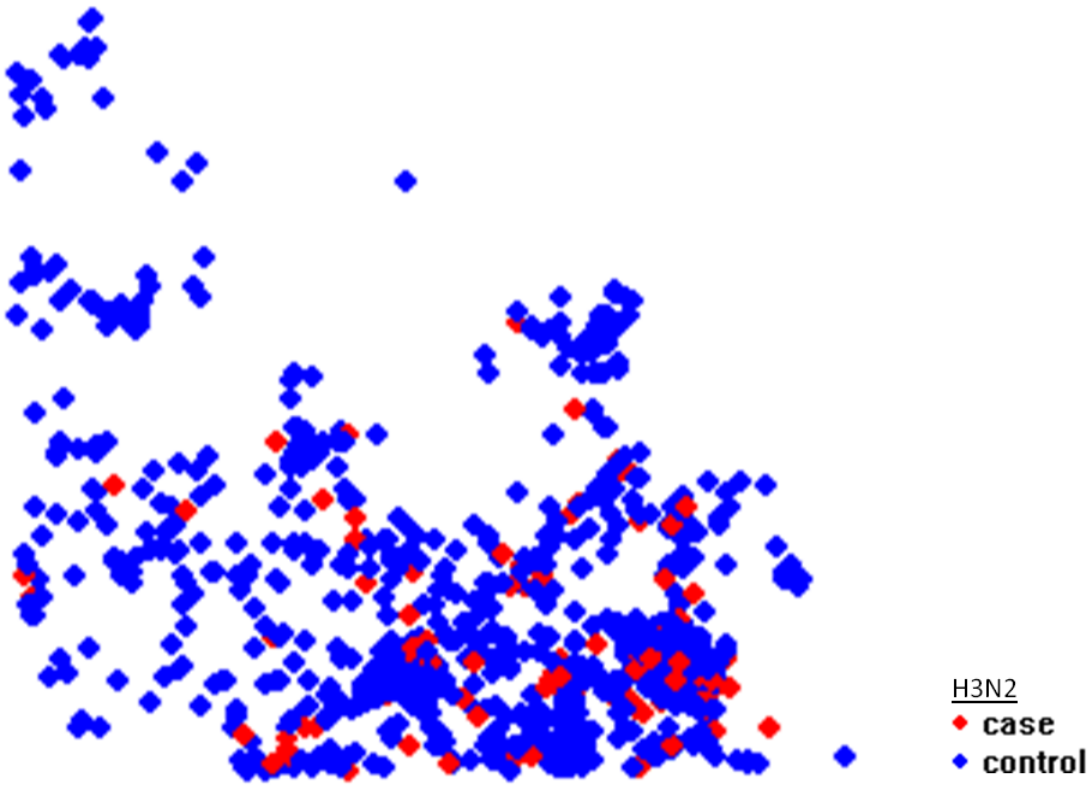


Figure 9. Cuzick and Edwards' maps, swine influenza in Manitoba, 2003-2009




H3N2
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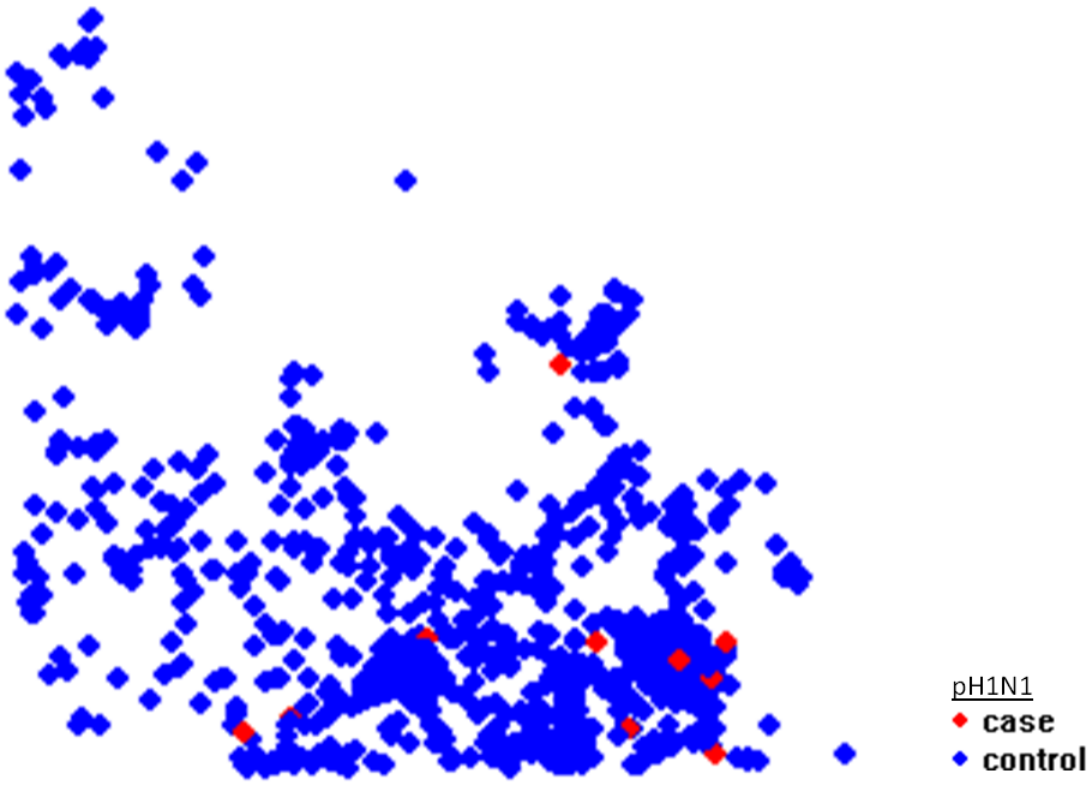
pH1N1
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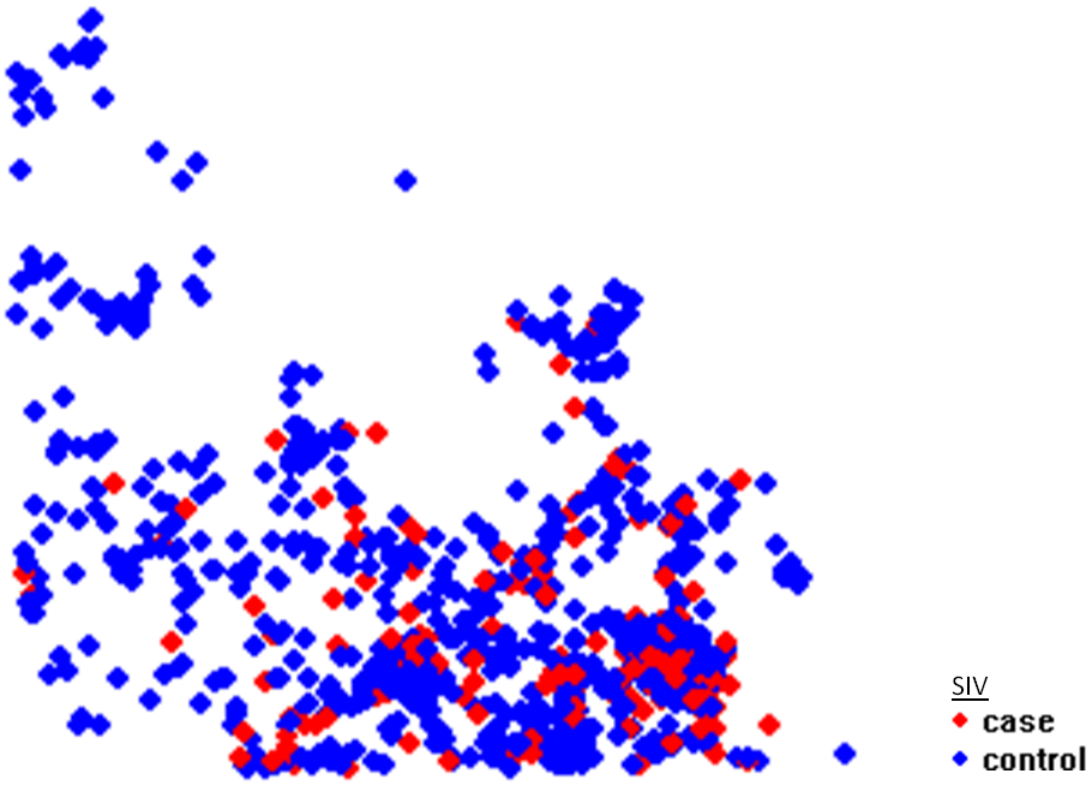
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Figure 10. Kulldorff Spatiotemporal Scan, H1N1 swine influenza in Manitoba, 2003 to 2009

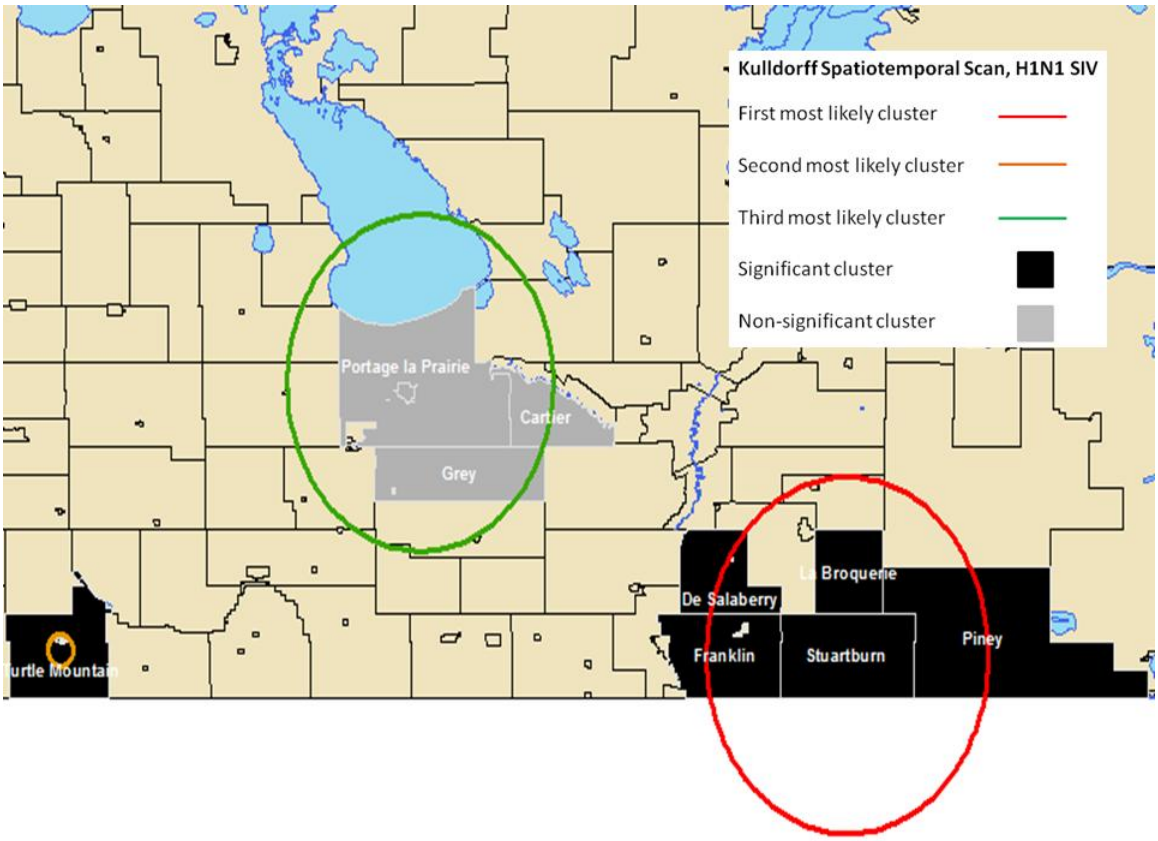


Figure 11. Kulldorff Spatiotemporal Scan, H3N2 swine influenza in Manitoba, 2005 to 2009

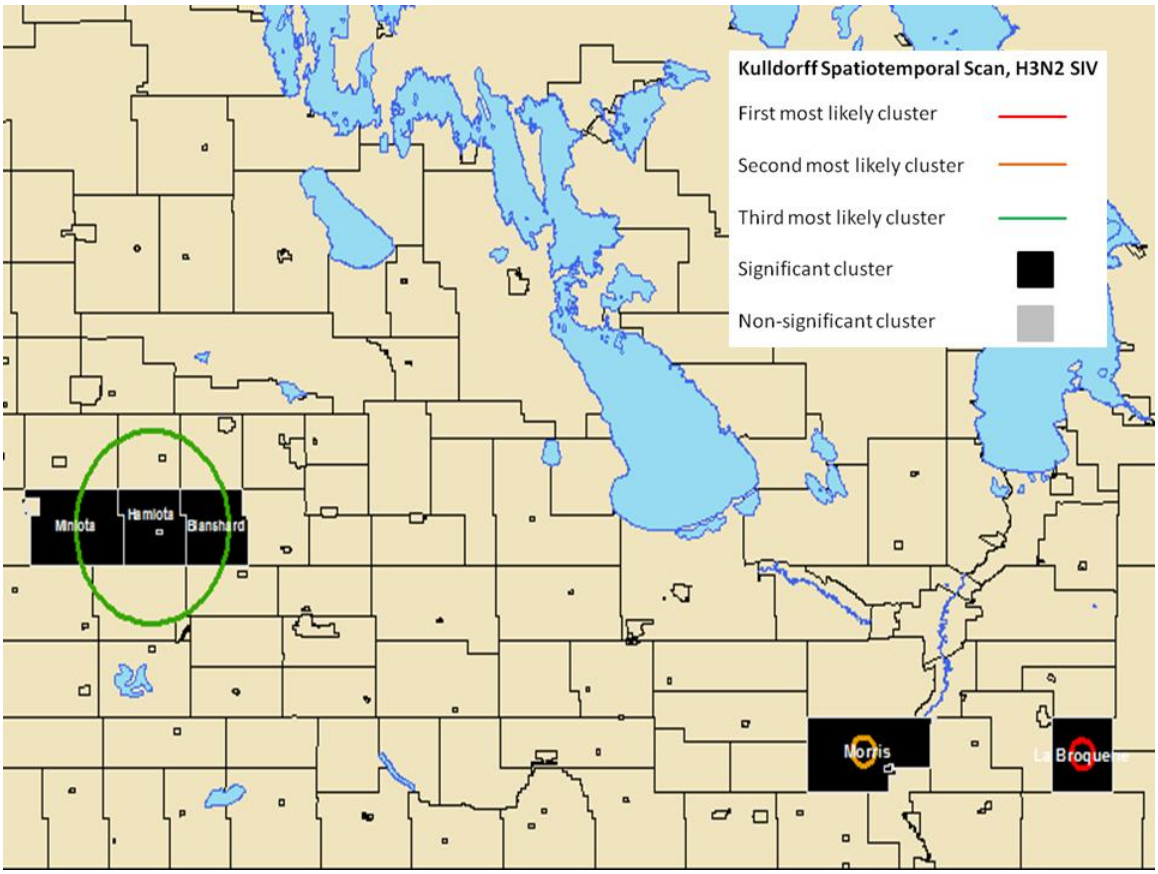


Figure 12. Kulldorff Spatiotemporal Scan, pH1N1 swine influenza in Manitoba, 2009

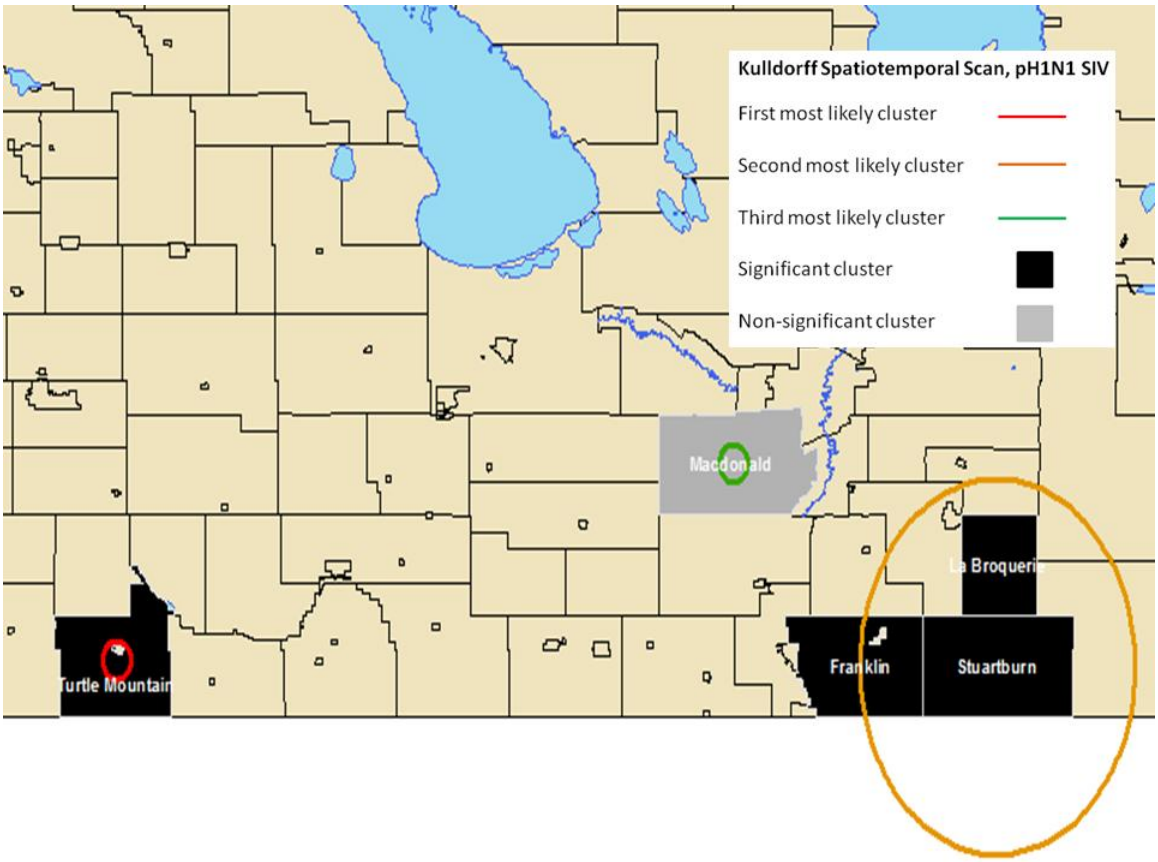
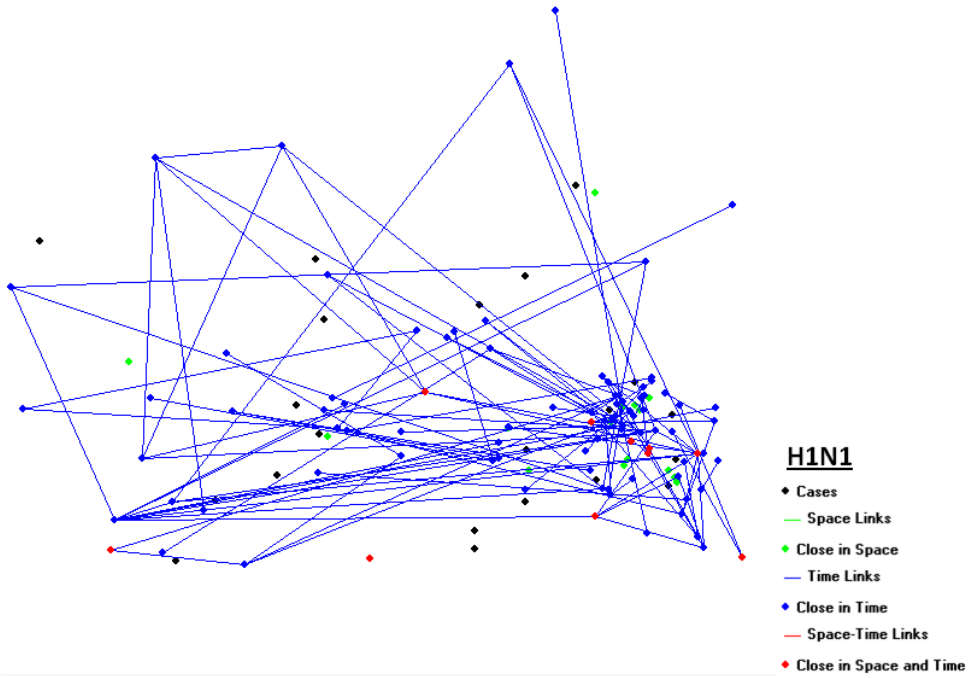
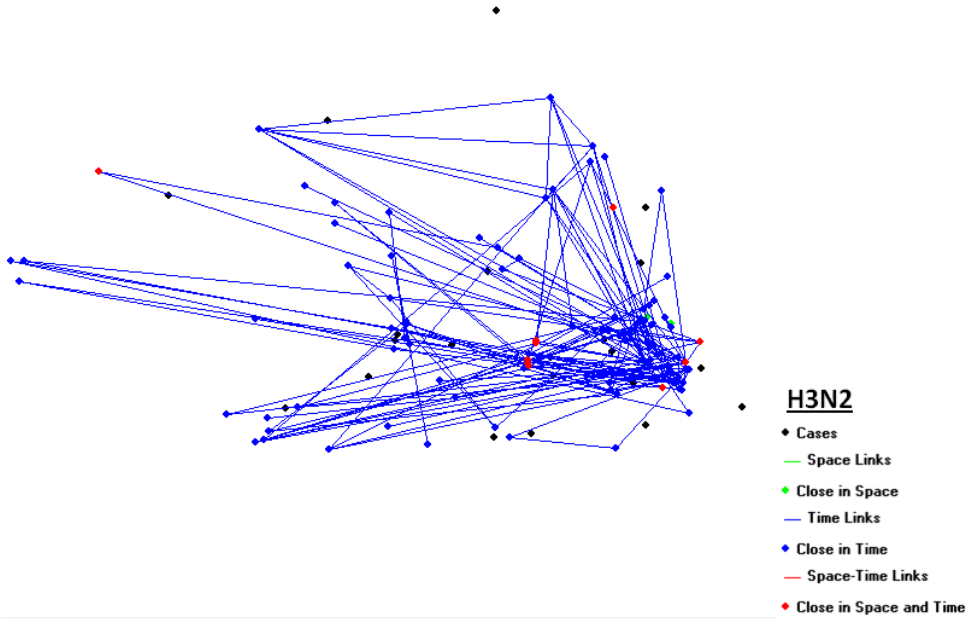
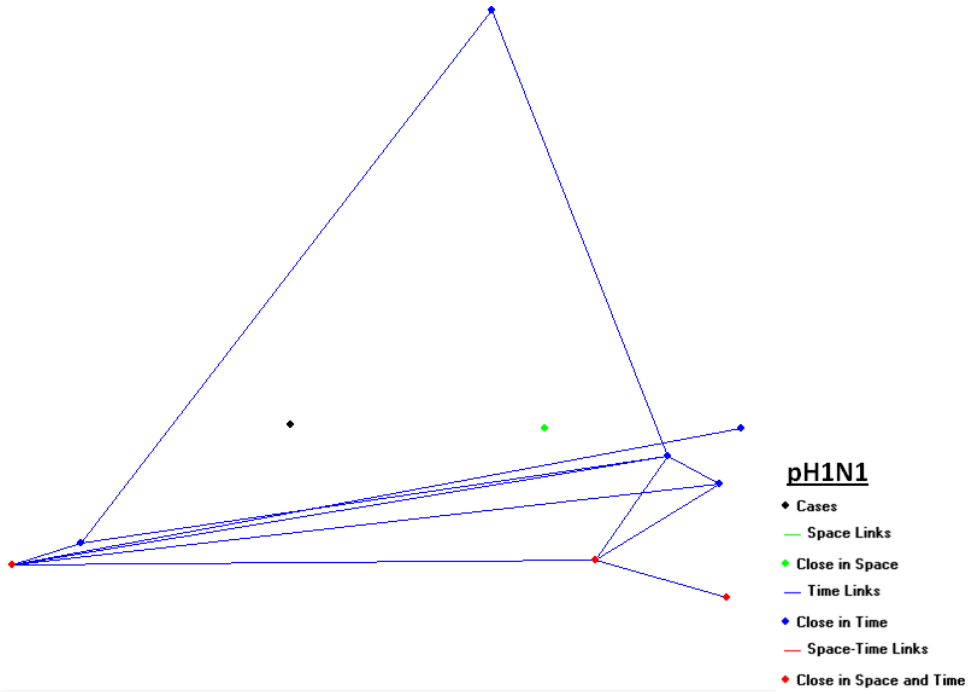


Figure 13. Knox test maps, swine influenza in Manitoba, 2003 to 2009






pH1N1
Cases
Space Links
Close in Space
Time Links
Close in Time
Space-Time Links
Close in Space and Time

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