QUANTITATIVE SUPPORT FOR A THEORY OF WEST NILE VIRUS TRANSMISSION USING SPATIO-TEMPORAL MODELING

By

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A dissertation submitted to the Graduate Faculty in Earth and Environmental Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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APPROVAL PAGE

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ABSTRACT

QUANTITATIVE SUPPORT FOR A THEORY OF WEST NILE VIRUS TRANSMISSION USING SPATIO-TEMPORAL MODELING

by

Constandinos Theophilides

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The West Nile virus is a mosquito borne agent that has infected hundreds of thousands of birds and mammals and thousands of humans since its arrival in the United States in 1999. While there have been numerous hypotheses concerning the transmission cycle of the West Nile Virus, to this day, there has been no comprehensive theory of transmission of the virus to humans nor any quantitative evidence to support it.

In this research a conceptual model of transmission is introduced and evidence is offered which shows that viral amplification peaks 15-16 days prior to onset of symptoms in humans and begins to subside significantly 8 days later. These results are consistent with extrinsic incubation in mosquitoes and intrinsic incubation in humans.

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The quantitative analysis which modeled this transmission involved the design and implementation of a system that is based on a geographic model that uses a Knox test to capture the nonrandom space-time interaction of dead birds, as an indicator of an intense West Nile virus amplification cycle, within a 1.5-mile (2.41-km) buffer area and 21- day moving window. The Knox analysis is implemented as an interpolation function to create a surface of probabilities over a grid of 1,400 cells overlaying New York City. The model's parameters are calibrated using year 2000 data and information on the vector-host transmission cycle. The system is implemented in a geographic information system and used operationally in New York City and, the City of Chicago where it is modified with an unconditional extension of the Knox to address weaknesses inherent in the Knox methodology and significance testing. The results from the traditional Knox method and the unconditional extension of the Knox method were evaluated using a novel space-time implementation of the kappa index of agreement for the Chicago data. They kappa results were used to quantify the success of each method and offer evidence for the role of dead birds in human West Nile virus transmission. It is shown that the discriminatory power of the unconditionally extended Knox is greater and that the two tests produce significantly different results.

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CHAPTER I

INTRODUCTION

A widespread view among Epidemiologists is that using Geographic Information Systems is not more than a way of visually presenting results of statistical analysis or assisting the analysis with simple spatial and/or attribute queries. This approach limits the utility of the spatial approach to a set of tools offered by a software vendor. In contrast to this view, Geographic Information Science provides us with the underlying geographic principles to model some aspect of the real world. These principles are related to the uniqueness of the spatiality of geographic data and its dynamics in time (Goodchild 1992).

One problem that can benefit from the integration of Geographic Information Science with Public Health is West Nile virus, a newly introduced pathogen in the United States. The epidemics caused by West Nile virus in the United States are dynamic and predominately spatial in nature. As such, they can be studied, analyzed and modeled from their geographic perspective as a special case of environmental modeling with Geographic Information Science.

Environmental models, which are wide ranging, and can encompass any aspect of the earth's atmosphere, hydrosphere and biosphere, have a need for both the spatial and temporal dimension (Couclelis 2002, 306). Geographic Information Science provides a

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unique set of tools for building real world models that can accommodate both the spatial and temporal dimensions (Egenhofer and Golledge 1998) and have proven effective at integrating a variety of sub-models, sometimes with differing theoretical bases, into a single framework (Couclelis, 2002). Environmental models tend to be particularly complex, are dynamic, cross-disciplinary and data intensive (Couclelis, 2002), characteristics which are not an impediment to Geographic Information Systems implementation.

Geographic Information Science enables the spatial investigation of environmental systems in all steps of the geographic problem solving process from cartographic visualization through object and statistical implementation of conceptual models. Geographic Information Systems implementations of models of human-environmental systems have focused on a variety of issues that are urban or rural in nature, and relate to epidemiology, and anthropogenic environmental change (Grove et al. 2002). Dynamic models for human-environment interactions have relied on the incorporation of ecological variables in statistical models (Theophilides et al, 2003), cellular automata (Clarke et al. 1998), and agent-based systems (Ahearn et al. 2001, Batty 2003).

In this dissertation, a Geographic Information Science approach is taken to model the transmission process operating behind West Nile virus epidemics. During model construction, several novel concepts and methods of spatio-temporal analysis are introduced.

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CHAPTER II

BACKGROUND

Origin.

The West Nile virus is a mosquito born agent that infects a wide variety of animals and occasionally can cause illness in humans (Hayes 2001). The virus was first discovered in the West Nile district of Uganda in 1937 by Smithburn et al. (1940) in the blood of a woman suffering from fever, but has probably caused more epidemics throughout history and may have even influenced historical events (e.g. death of Alexander the Great, Marr et al, 2003). It is genetically related to the *flavivaridae* family, which includes the viruses that cause Yellow fever, St. Louis encephalitis, Eastern and Western equine encephalitis, Lacrosse encephalitis, Japanese encephalitis and Kunjin encephalitis.

Geographical Distribution of Human West Nile Virus Illnesses in the World and the United States.

Since the 1950's epidemiologists surveying for West Nile virus sera have identified the virus in Africa, Europe, the Middle East, west and central Asia, and Oceania. Encephalitis outbreak epidemics of West Nile virus in humans and animals have been reported in Egypt (Melnick et al. 1951, Taylor et al. 1956), Turkey (Radda 1973), Greece (Papapanagiotou et al. 1974), South Africa (McIntosh et al. 1976), the Middle East (Katz et al. 1989), Romania (Campbell et al. 2001), Russia (Platonov 2001), France (Durand et al. 2002), Italy (Autorino et al. 2002), and many African countries (Burt et al. 2002). In

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1999, the virus appeared for the first time in the United States (Lanciotti et al. 1999) and became the only mosquito borne encephalitis-causing virus that exists in both the Old and New Worlds (Gould et al. 2004). In 2000 the virus spread to Connecticut, New Jersey, Maryland, Vermont, New Hampshire, Rhode Island, Massachusetts, Pennsylvania, Delaware, Virginia and North Carolina. By 2001, the virus had caused illness in humans in all states east of the Mississippi river but South Carolina and West Virginia. In 2002, the all states east of the Rocky Mountains, except New Mexico, had reported human West Nile virus illnesses. In 2003 the virus had caused human illnesses in all states except Washington and Oregon (Centers for Disease Control and Prevention, 2004) and in all indications it has become established in North America.

Human Disease Counts.

Between 1999 to 2001 the virus had caused confirmed encephalitis or other related disease in 155 people in the United States, including approximately 48 deaths (the exact number of illnesses and deaths is unknown as Health Departments constantly update and reclassify cases due to uncertainty and new information often appears months after an illness). In 2002, there were 4156 illnesses in humans 284 of which were fatal and in 2003, there were 9858 human illnesses including 262 deaths (Table 1).

Despite its dramatic introduction in 1999 in New York with 68 human illnesses and 9 deaths (New York City Department of Health and Mental Hygiene 2001, 2002) the virus was relatively quiescence for humans with only 18 cases in 2000 and 66 in 2001. The 2002 and 2003 years proved different from the past with the virus crossing the Mississippi river and causing a remarkable 14,014 illnesses in humans.

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Table 1. Human West Nile virus related illness and deaths for selected years in the US (Data Source, CDC and USGS). Human West Nile virus related illness and deaths for selected years in the US (Data Source, CDC and USGS).

Years	Human West Nile virus Illness (approximate)	Deaths (approximate)
1999	68	9
2000	21	2 (uncertain)
2001	66	30 (uncertain)
2002	4156	284
2003	9858	262

USGS maps that summarize the West Nile virus activity in the United States show an extraordinary geographic pattern (Figure 1) that seems to follow geographic barriers. In 2000, the barrier was the Adirondacks and the Delaware river. In 2001 the barrier was the Mississippi river and in 2002 the barrier was the Rocky Mountains (Centers for Disease Control and Prevention 2004). This pattern is remarkable although the possibility that the apparent "stops" of the virus are due to seasonal cessation of the activity exists.

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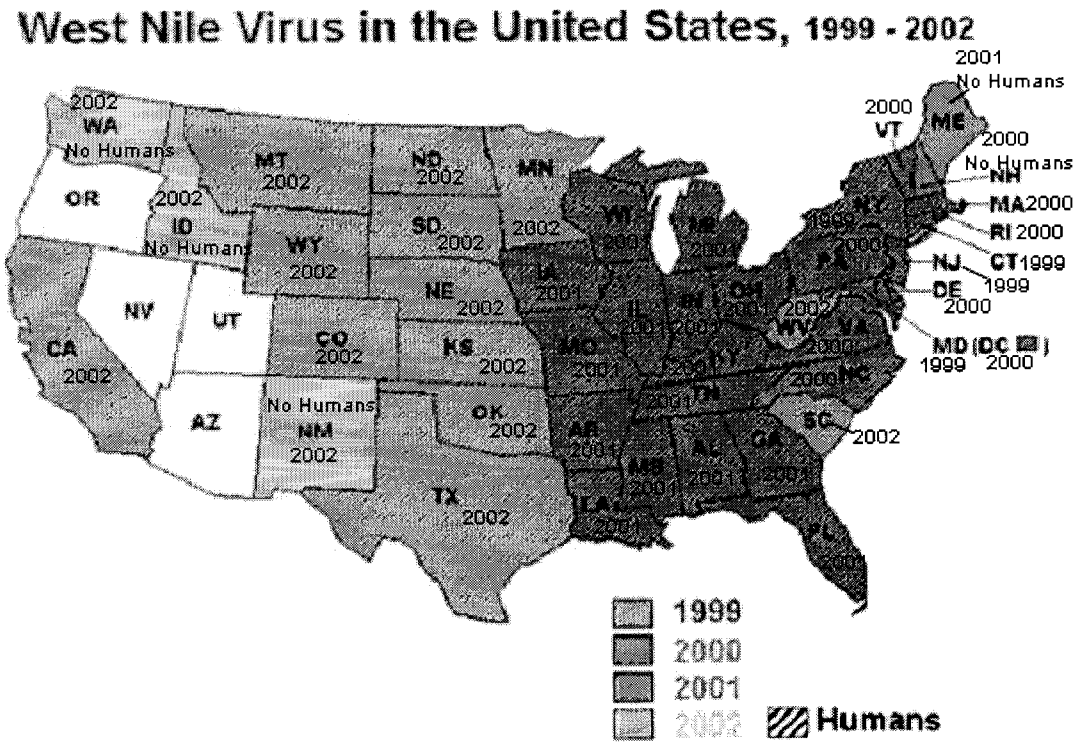

Figure 1. Summary of West Nile virus activity in the United States, 1999-2002. Source, CDC, (available from: http://www.cdc.gov/ncidod/dvbid/westnile/surv&control03Maps99_02.htm, June 29, 2002)

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Human Pathology

Source of Infection in Humans.

Human West Nile Virus infections are most frequently the result of an infected mosquito bite (Campbell et al. 2002). Other identified methods of human West Nile virus infection include blood transfusions (Pealer et al. 2003), direct mother to fetus transmission (Alpert et al. 2003), organ transplantations (Iwamoto et al. 2003) and possible transmission through breast milk (Centers for Disease Control and Prevention 2002) all of which require at least one infected human. For this reason they are thought of as secondary modes of transmission. Once infected a human may develop West Nile virus disease in 3-15 days (Olejnik 1952) although most humans develop symptoms in 2-6 days (Goldblum et al. 1954). Petersen et al. (2002) report that intrinsic incubation period in humans is still unknown and Theophilides et al. (2004) have shown evidence that in most cases it does not exceed 7 days.

Symptoms

Most West Nile virus human infections are asymptomatic and only one to 150-200 humans infected with West Nile virus will develop West Nile virus related disease (Tsai et al. 1998, Mostashari et al. 2001). Whether or not West Nile virus disease will develop depends on complex biological factors. However, most people that do develop the disease following infection, are the elderly and the immune weak (Petersen et al., 2002). If developed, symptoms are usually mild and include fever, headache, body aches, sometimes skin rash and swollen lymph glands. Severe infection is marked by headache,

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high fever, neck stiffness, stupor, disorientation, with coma, tremors, convulsions, paralysis and occasionally death. The clinical characterization of West Nile virus related disease includes encephalitis and/or meningitis with the possibility of neurological complications from these diseases (Petersen et al., 2002).

In the recent outbreaks of West Nile virus in the United States, approximately 60% of diseases in humans were classified as encephalitis while the rest were classified as meningitis (Campbell et al., 2002). Because in most infected humans the viral levels do not reach high enough concentrations, humans are considered dead end hosts and do not contribute to the cycle of the disease in nature.

Clinical Treatment

There is no cure or vaccine for West Nile virus yet. Treatment for West Nile virus infection in humans remains supportive while vaccines are under research and currently not available (Gould and Fikrig 2004).

West Nile virus in Wild Animal populations

Birds

Since the discovery of West Nile virus, researchers have implicated wild animal avian species for its spread and maintenance. Birds were considered the major reservoir hosts in the Old World (Work et al. 1955, Hannoun et al. 1972) and the same appears to hold for the New World (Rappole et al. 2000). Although the virus has been isolated from other animals, there is no proof or indication that those animals play a significant role in

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the West Nile transmission cycle. In the United States, West Nile virus has for the first time infected a naïve bird population. The net results are fatal infections among resident and native birds. Among its first victims were thousands of crows and, native and exotic birds of the Bronx Zoo (Eidson et al. 2001, Steele et al. 2000).

Of wild birds, corvids (crows, magpies and blue jays) are especially vulnerable to West Nile virus and mortality in laboratory experiments has been shown to be as high as 100% in crows, 75% in Blue Jays (Komar et al. 2003, Table 2). Viremia in birds that die peaks 4-5 days post infection and is generally maintained until death (Komar, personal communication). This interval is enough for blood sucking mosquitoes to feed on them and acquire the disease. The high mortality rates of corvids by West Nile virus in laboratory experiments has been corroborated by bird population studies that showed population reduction in crows of up to 80% (Yaremych et al. 2004, Watson et al. 2004).

Table 2. Bird mortality and viremia data (Source: Komar et al. 2003)

Species	Mortality Rate	Average days to death	Mean day of highest viremia	Highest viremia (log(10) titer per Ml serum)	Number of samples
American Crow	100%	5.1	4	10.2	8
Fish Crow	55%	9.6	4	8.9	9
Blue Jay	75%	4.7	3	12.1	4
House Sparrow	50%	4.7	3, 4	10.3	6
Ring-Billed Gull	100%	9	3	8	2
Ring-Billed Magpie	100%	6	3	8.8	3
Common Grackle	33%	4.5	3, 4	11.8	6
Note: >10 viremia titters are sufficient for infecting Culex Pipiens					

Other passerine bird species also have high mortality rates. House sparrows have 25% mortality rates from West Nile virus (Komar et al. 2003, Table 2). These birds are

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known to occur in abundance near anthropogenic environments, and their role in the transmission cycle is perhaps of some importance although one could argue that their smaller biomass (and the much lower than crows mortality rate) could relegate them to a secondary role.

Since, during the winter months, West Nile virus activity subsides in both vector and hosts the seasonal reintroduction of the virus in the bird populations is a question that requires further investigation. Corvids (especially the American crow *Corvus brachyrynchos*), which are thought to be the primary amplification hosts in the urban environments of cities like New York and Chicago (Eidson et al. 2001a, Eidson et al. 2001b, Theophilides et al. 2003, Watson et al, 2004), are among the first birds that are reported as positive by Health Departments in the Spring. They may play an important role in the reintroduction of the virus in the wild that follows the gap of the winter season during which mosquito biology is quiescent. Apart from transmission by infected mosquitoes, direct oral transmission of the virus between crows has also been reported (McLean et al. 2001) and some think that a latent infection in crows in the winter months is responsible for the reintroduction. Unpublished data from New York City and Chicago indicate a peak in crow deaths in May, prior to the emergence of the *Culex* mosquitoes from hibernation.

Other animals

In the United States West Nile virus has been isolated from wolfs and dogs (Lichtensteiger et al. 2003), monkeys (Ratterree et al. 2003), geese (Swayne et al. 2001),

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squirrels (Kiupel et al. 2003) and recent more events show that it is increasingly becoming a threat for the food supply, infecting pigs (Newswise, 2004).

West Nile virus causes disease in horses and it has significantly affected the horse industry, especially in the mid-west states. Significant press coverage on the issue indicates that it is becoming a growing concern (Google News 2004). Though horses never develop high enough viremias to play any role in the amplification cycle (Bunning et al. 2002), there is valid concern that it may be causing fatal illness in some (Trock et al. 2001) resulting in significant financial losses to the industry. A horse vaccine against West Nile virus is available.

West Nile virus in Mosquitoes

Perhaps the most important player in the maintenance and outbreak of West Nile virus is the vector, in this case mosquitoes. In the United States, West Nile virus has been isolated from 43 species of mosquitoes since 1999 (Centers for Disease Control and Prevention 2003). Members of the *Culex* genus (e.g., *Culex. pipiens*, *Culex tarsalis*, *Culex molestus* , and *Culex Univittatus)* are the main vectors of the virus in birds (Turell et al. 2002) in the United States. Of these, the *Culex pipiens* species is the one that has been mostly associated with West Nile virus outbreaks in New York and New Jersey (Nasci et al. 2001, Turell et al. 2000). This species prefers to breed on standing, polluted with organic matter water and is one of the world's most adapted urban mosquitoes (Savage and Miller 1995). Back yard concentrations of water left unclean as long as four days can become the perfect breeding ground for these mosquitoes.

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The Culex pipiens species mosquitoes are considered the primary maintenance vector of West Nile virus in the avian population as they are very susceptible to infection (Ilkal et al. 1997). Its role as a bridge species for transmission of the virus to humans has been disputed by some (Gould and Fikrig 2004, Kulasekera et al. 2001); however, recent studies have shown the North American *Culex pipiens* mosquito to be a hybrid that is capable of feeding on both birds and humans, perhaps reluctantly (Fonseca, 2004). The latter explains limited outbreaks in the North East and Mid West where the *Culex pipiens* is the primary enzootic/epizootic amplifying vector (Kulasekera et al., 2001).

The *Culex salinarius* species is suspected to act as a bridge species for transmitting the virus to humans (Kulasekera et al., 2001). *Culex salinarius* prefers a different habitat than the *Culex* pipiens, namely permanent bodies of water and is more likely to feed on humans (Spielman and D'Antonio 2001 p.199).

The large outbreaks of West Nile virus that occurred in the Western United States are attributed to the *Culex tarsalis*, mosquito species that will indiscriminately feed on birds and humans (Gould and Fikrig 2004).

The initiation of West Nile virus activity in the avian population is attributed to the *Culex restuans* mosquitoes as they become abundant earlier in the season, however their competence for the virus is still under question (Andreadis et al. 2001)

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Mechanisms of transmission.

There are two major recognized modes for the transmission of West Nile virus by mosquitoes. The first mode of transmission is mechanical transmission. In mechanical transmission, a mosquito transmits the infection by contaminated mouthparts after feeding on a viremic bird. Since this requires very high viremias and sequential feedings, it is unlikely that it happens in nature (Chamberlain and Sudia 1961). The second and perhaps more important mode is biological transmission. In this process, the mosquito becomes infected after ingesting blood from an infected host (bird). An incubation period follows during which the infection spreads from the mosquito's mid-gut to the salivary glands from where it will be transmitted to another host (Chamberlain and Sudia 1961). Mosquitoes are cold-blooded animals and incubation periods tend to vary with environmental temperatures. The *Culex pipiens* were shown to be infectious at 100% rates in laboratory settings as early as 6 days at 30 degrees Celsius (Dohm et al. 2002b). Vertical transmission (from an infected mosquito to its eggs) is also a possible for West Nile virus infection in the vector population, however the rate of virus recovery from the offspring population was shown to be approximately 1 to 1600 (Turell et al. 2001) and 1.8 to 1000 (Dohm et al. 2002a). Furthermore, in the same studies (Turell et al. 2001, Dohm et al. 2002a) the mosquitoes were inoculated intrathoracically with the virus. This process results in faster and more efficient infection and development of viremias by approximately 7 days (maybe less with higher temperatures) as it by passes the gut. The mosquito gut is considered an infection barrier. Hence, high concentrations of the virus in the ingested blood are required for infection (Chamberlain and Sudia 1961). The rate of vertical transmission of the virus is far less than the 8-14 per 1000 minimum field infection rates found in New York City (Kulasekera et al., 2001) and its significance

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probably lies with the over-wintering of the virus in mosquitoes and its re-introduction into the wild the following season (Dohm et al. 2002a). However, the exact mechanism of transmission of an infection to another host remains unclear, as only about 1 to 100 mosquitoes will take a second blood meal. Once a mosquito has acquired the infection, it will retain it throughout its life span, usually no more than 2-3 weeks (Chamberlain and Sudia 1961).

West Nile virus Ecology

The foundations of the study of the ecology of West Nile virus were laid out in researches conducted principally in Egypt and Israel. Extensive serological tests showed correlation of West Nile virus human infections with infections in various avian species and the *Culex pipiens* mosquitoes (Goldblum et al. 1957, Taylor et al., 1956). These tests have shown that high viremias only appear in birds following inoculation with the virus. This high viremia allows dissemination of the disease to the arthropod vectors (mosquitoes). In addition, the relative abundance of birds and their high rates of reproduction aid the maintenance of sufficient supply of the virus in the environment and, maintain the cycle of infection (Work et al., 1955, Schmidt 1965). In areas where the virus is endemic, birds typically survive infections and maintain antibodies in their blood (Taylor et al., 1956).

The outbreak of West Nile virus related disease in Romania in 1996 (Tsai et al., 1998) signaled the emergence of West Nile virus as a disease of the urban/suburban environment. The study of the Romanian epidemic indicated that human infections were closely linked to flooded basements that served as a breeding ground for the *Culex pipiens* mosquitoes (Han et al. 1999). The first outbreak of West Nile virus in the United

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States occurred exclusively in an urban environment, New York City (Lanciotti et al. 1999). Campbell et al., (2002) argue that it is a reminder that even the affluent cities are vulnerable to arboviral disease. It should then come as no surprise that Ringia et al. (2004) in a serosurvey of birds conducted in 2002 in Illinois found that captive and urban birds had higher antibody prevalence for West Nile virus than the ones collected from natural areas.

The maintenance of West Nile virus in nature, requires cycling between birds and mosquitoes. Birds, act as the primary reservoir hosts that once infected develop enough viremias to allow transmission of the virus to other mosquitoes (Figure 2).

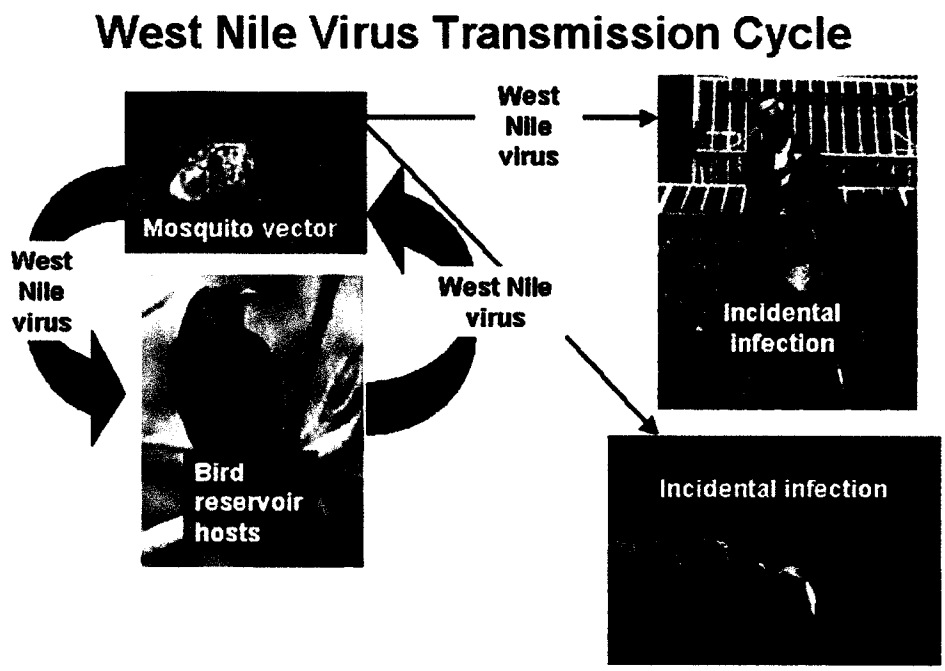

Figure 2. West Nile Virus Transmission Cycle. (Source: Centers for Disease Control and prevention, available from:

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http://www.cdc.gov/ncidod/dvbid/westnile/images/WNVcycle4_18_big.jpg, May 31st, 2004)

Under favorable environmental conditions, the cycle intensifies and an excess of the virus is likely to "spill-over" to humans. In the United States, there seems to be a unique process behind human epidemics as West Nile virus has found an abundance of naïve hosts (birds) that develop high viremias and have high mortality rates (Komar et al., 2003. When birds die in large numbers or leave the area, the infected mosquitoes turn to mammals and humans for blood meals, resulting in human infections (Despommier 2001).

Seasonality of West Nile virus.

Human West Nile epidemics in the United States are clustered in the months of July, August and September with the numbers peaking in August and September and generally following the mosquito season. In the Southern states the virus seems to be constant with slight winter pauses.

West Nile virus and Weather patterns.

West Nile virus follows the biology of primarily the vector and to a lesser extent the host. The evidence indicates that the majority of human cases and infected birds in the North East coincide with the peak of the mosquito period. The *Culex pipiens* mosquitoes thrive in polluted rich organic matter that can result from extended periods of heat and drought in the months leading up to the mosquito season (Monath et al. 1987, Spielman 1967). Epstein et al. (2001) found that West Nile virus and St. Louis encephalitis epidemics

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were preceded by prolonged dry spells. Research also suggests that when a rain event follows these hot dry weather patterns, infection can spread more easily to humans. For example, the 1974 epidemic in South Africa that resulted in over 3000 cases of West Nile virus occurred right after a rain event that broke a sustained dry hot weather (McIntosh et al. 1976). Prior to the 1999 epidemic, New York City had experienced the worst drought in 100 years. When rain broke out in August the infection rates among humans rapidly accelerated. Despommier (2001) states that in addition to increased mosquito concentrations, drought spells can cause the hosts (birds) to concentrate around the dwindling water resources, providing a "concentrated" host target population for the mosquitoes. Although further research still needs to be conducted for establishing a link between weather patterns and West Nile virus epidemics, preliminary observations and prior experience allows us to hypothesize that dry hot weather followed by rain events can favor the mosquito reproduction and cause West Nile epidemics.

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CHAPTER III

MOSQUITO CONTROL AND WEST NILE SURVEILLANCE

Mosquito control in New York City dates as far back as 1901 when the New York City Board of Health ordered the ditching of wetlands and the elimination of standing water for potential breeding grounds (Miller 2001). The primary concern back then was Malaria which, is transmitted and maintained by the *Anopheles quadrimaculatus* mosquitoes that prefer to breed on permanent bodies of water. For fifty years, from the 1940's to the 1990's there was no recognizable threat from mosquito borne diseases in New York City and control efforts were scaled back (Miller, 2001).

The appearance of West Nile virus in New York City in 1999 caught the authorities by surprise. The newly introduced public health threat followed an entirely different biological cycle than malaria. The transformed urban environment that has resulted from increased affluence over the years (i.e. outdoor pools) accompanied by sloppy water use habits, now served as the perfect breeding ground for peridomestic mosquito vectors (Despommier 2001). Thus, the breeding grounds were now on private property. In addition, wetland ditching may have eliminated natural predators of mosquitoes and caused mosquitoes to concentrate elsewhere. The initial response was a widespread use of mosquito adulticides that covered the city, at least twice (McCally et al. 2001). Mosquito adulticides pose acute direct risks to humans that include, allergic and respiratory risks, and indirect risks which are not fully understandable (Thier 2001), not

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properly monitored (O'Hara 2001) and, have potentially legal consequences (Lopez et al. 2002). In addition, widespread use of adulticides can create mosquito resistance and can end up becoming ineffective in the long run (Brogdon and McAllister 1998). Widespread spraying of pesticides is costly and the spread of the virus in the whole country resulted in "rationing" Federal resources for surveillance, remediation and control.

The West Nile virus surveillance problem.

Following a review of the 1999 outbreak, the New York City Health Department, assisted by CDC guidelines, started to understand the biological dynamics of West Nile virus better. The Department hired a mosquito entomologist and in 2000, mosquito remediation was based on targeted mosquito surveillance rather than widespread pesticide spraying. At the same time, it became evident that the disease outbreak was geographically and temporally specific. Human West Nile virus disease appeared to cluster around specific locations and at specific times (Mostashari et al. 2001). In the late summer of 2000, the New York City Health Department contacted the Center for Advanced Research of Spatial Information (CARSI) at Hunter College and sought assistance for becoming better enabled with geographic information technologies in order to address problems that existing surveillance methodologies could not address because they were neither spatially specific nor temporally sensitive.

Surveillance measures and limitations.

The two major surveillance measures employed were mosquito trapping and collection of dead bird reports at the street level and/or testing for positives. Mosquito surveillance is not very efficient for several reasons. The number of traps dictates the spatial resolution

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of the mosquito surveillance and the frequency of testing of collected mosquito pools affects the temporal sensitivity. In order to identify one positive mosquito from a collected mosquito pool from one trap at least 10 samples need to be tested. This requires a very fine grid of trap locations and daily testing for accurate and sensitive identification of the risk areas. Such a devotion of resources is unfeasible. Mosquito infection rates at the peak of the season are less than 10 percent (Kulasekera et al. 2001) and at the early stages of the amplification cycle they are probably less. As a result, mosquito surveillance needs to be guided and resources need to concentrate in hot spots of West Nile virus activity. Dead bird testing for positives suffers from similar limitations although early season positives have been suggested as predictors of the intensity of West Nile virus activity (Guptill et al. 2003).

Surveillance of dead birds is less expensive as it relies on passive public participation. At the same time, in order to be effective, the analysis of the reports has to take into account the limitations and biases that can result from different levels of sensitivity of the public to report dead birds, the spatial configuration of the analysis units and, the ecology of the disease.

Eidson et al. (2001b) first proposed the use of dead crows as an early warning system for West Nile virus. The proposed measure was the density of dead crows (or crows per unit area). The density of dead crows is limited for several reasons; (1) the results of this analysis can differ depending on the selection of configuration of the spatial units used

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and the scale of analysis, (2) it is not possible to statistically test the significance of each density, and, (3) underreporting and edge effects in certain areas can bias the results.

The proposed units of analysis of Eidson et al. (2001b) are census tracts. Census tracts are political subdivisions of variable size that are not related to the ecology of the disease. The use of variable size units has been studied extensively by Geographers and is part of the Modifiable Aerial Unit Problem (MAUP), which is the spatial equivalent of the Ecological Inference problem (Klein and Friedman 1993). The MAUP occurs because the results of two identical analyses based on the same data aggregated on two different spatial configurations schemes and/or scales can differ. Openshaw and Taylor, (1979) demonstrated this problem by creating different spatial aggregations of the same 99 counties in Iowa into larger units and measuring the correlation between Republican voters and elderly voters for each aggregation. They reported correlations that ranged from -0.97 to +0.99, yet no relationship existed between the two variables. The density of dead crows suffers from the same problem. Different ways of aggregating dead crow reports result in different densities. Figure 3 shows an example of a hypothetical cluster of dead birds in Staten Island that can be missed with aggregation of the data into census tracts (upper portion). The lower portion of Figure 3 shows the actual cluster encircled.

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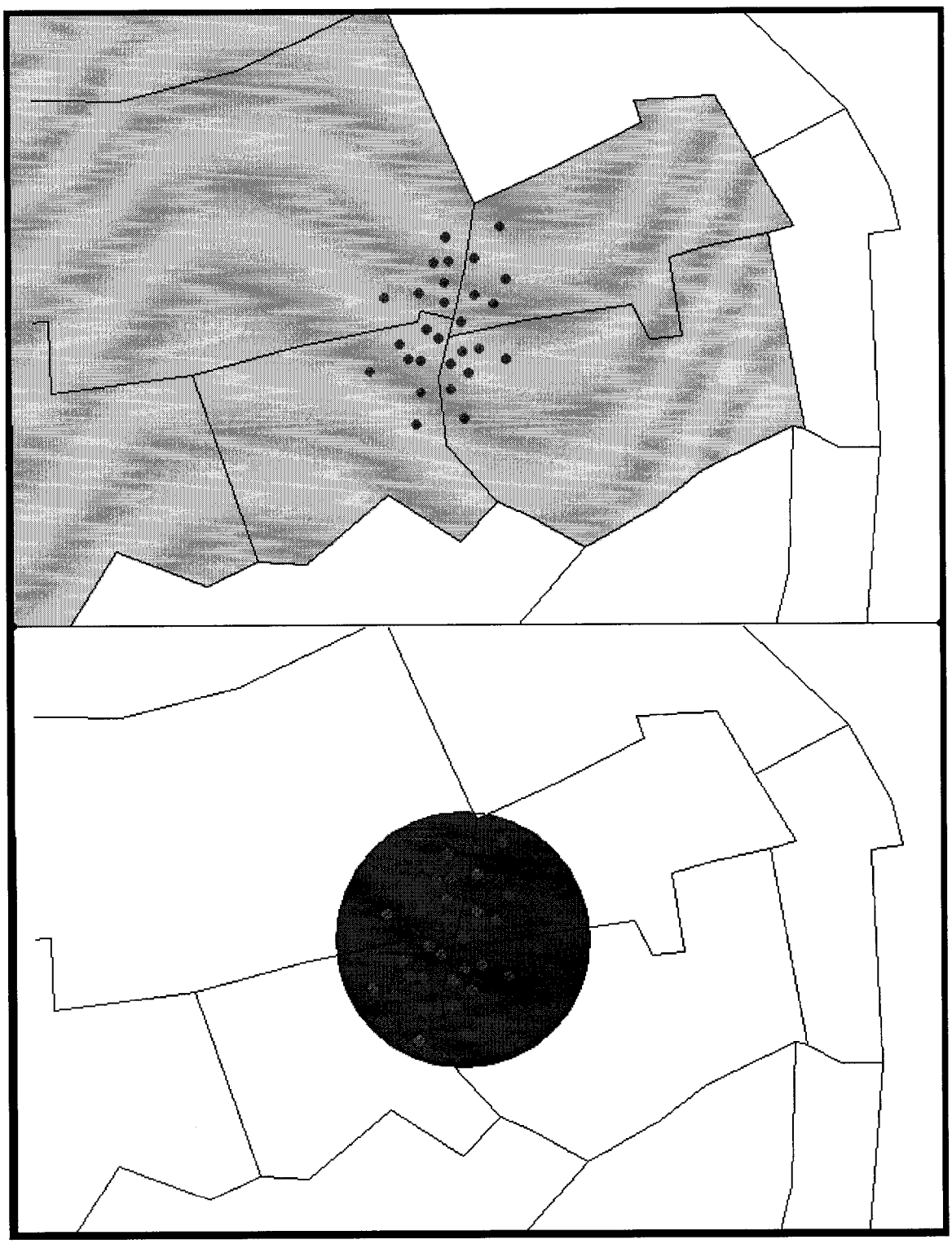

Figure 3. Missed cluster (upper portion) due to aggregation artifacts. Cluster is seen clearly in the lower portion.

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In addition, aggregation of point data in large spatial units is changes the *data support* (Arbia 1989) and reduces the resolution of analysis. The outcome of this is an inference made for a larger unit than the data actually support. This is also known as the ecological fallacy problem outside Geography. As a solution to the *change of data support* problem (Tobler 1979) suggested interpolating a smoothed surface from the point data. This idea is widely utilized by Remote Sensing scientists that combine it with Shannon's (1949) principle of reconstruction of a signal without information loss.

The loss of information that occurs when the *data support* base is changed is unnecessary in the West Nile virus case because the scale in which the disease propagates is approximately known. It will generally follow the vector's travel distance (1.1km for the *Culex pipiens*) and the vector and hosts incubation periods. In dead bird analysis ecological information could help adjust the scale of analysis without much information loss.

To cap everything, though unusual numbers of bird deaths (primarily corvids) are suspected to be dying of West Nile virus, no quantitative evidence has ever been presented to support this and relate it to human outbreaks. To this day, it remains a hypothesis.

Lack of conceptual model design.

In addition to the MAUP problem, the Eidson et al. (2001b) weekly densities ignore the spatial and temporal parameters over which the disease operates. This approach is not new in spatial-epidemiological statistics. In most similar studies, the research design and

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the results do not provide a full understanding of the ecological characteristics of the disease spread or outbreak as they lack a *conceptual model design.* Other approaches that are widely used for investigating the clustering of epidemics remain purely statistical (i.e. Kulldorff 1997, Knox 1963) and ignore the ecological context of the phenomenon being studied. Thus, the statistical procedures are designed to look for spatial patterns, and do not model the underlying processes of the phenomenon (i.e. amplification of West Nile Virus). In this sense, geo-statistical methods for investigating epidemics have been for most cases unconnected from the conceptual understanding of the disease transmission process. To be effective, an early warning system for West Nile virus risk identification based on bird deaths needs to have a conceptual basis and to be parameterized based on data regarding the ecology and pathology of mosquitoes, birds and humans. Above all, it must be able to answer the question of WHY it worked, if it was successful, and validate the conceptual model; in this case that birds die in large numbers by West Nile virus and their deaths are an integral part of the West Nile virus amplification cycle.

The CARSI conceptual approach.

An exploratory investigation of the dead bird data provided by the New York City Health Department revealed that bird deaths appeared to concentrate in specific locations and peak at those locations at specific times. The Center for Advanced Research of Spatial Information (CARSI) determined that in order to prospectively and dynamically track the potential for West Nile virus human outbreaks successfully, the problem needs to be approached and modeled as a space-time phenomenon. A review of current epidemiological methodologies for epidemic modeling revealed that epidemiological modeling was for its most part a-spatial (Lawson 2001).

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Conceptual model.

The occurrence of human outbreaks depends primarily on the availability of large amounts of virus in the host population (the birds) and the mosquitoes that maintain this cycle. West Nile virus amplification in the United States propagates in a naïve host population. This causes great harm to the ecosystems by potentially causing a massive die-off of birds (Komar et al. 2003, Yaremych et al. 2004). From the surveillance perspective, this is an advantage. Unusual bird deaths could mark the presence of an intense amplification cycle (Figure 2) between the vector and host population and that can help dynamically track the spread of the virus in space. When the amplification cycle in corvids is intense, a big pool of the virus is available in the hosts, and with substantial die-offs of birds the mosquitoes will likely turn to humans therefore causing epidemics.

Amplification of West Nile virus in the host population is a phenomenon that occurs in space and time. Despite what some ecologists argue, amplification does not occur universally in an urban area for the sole reason that the host population will not exist everywhere at the same time. Corvids are highly intelligent social animals that tend to assemble in big flocks during the night and in small groups during the day (Savage 1995). Since the mosquitoes that bite them and maintain the amplification cycle feed during the night hours (Spielman and D'Antonio 2001), this is the most probable the time that West Nile virus transmission takes place. Once a crow becomes infected and develops West Nile virus the disease progresses fast, until it dies (Komar et al. 2003). It has been shown that at or near the time of death, infected corvids maintain their highest viremias (Komar, personal communication). As they are limitedly mobile (or immobile) from neurological complications they are sitting targets for the mosquitoes to bite and acquire the infection.

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Hence, it is highly possible that while the corvids become infected with West Nile virus at their roosting places, they transmit the disease to mosquitoes at the place where they die. This location of death is where the mosquitoes that potentially transmit the disease to humans acquire the infection. Because this vulnerability of corvids to become sick and succumb to West Nile virus infection and their social nature, unusual numbers of crows dying close in space and time signify the presence of an intense amplification cycle and human infection potential. This intense amplification cycle and the resulting human risk, manifested in corvid deaths, needs to be modeled as a phenomenon that occurs when the interaction of close space deaths and close time deaths exceeds an expected value. A statistical test must judge the excess over this expected value in order to avoid arbitrary decisions. In addition, statistical testing reduces problems associated with differential propensities of the public to report bird deaths as the decision is based only on available data. The only assumption made is that any underreporting is equally distributed within the local space-time domains used as study areas.

The interaction of space and time in disease incidents has traditionally been diagnosed using Knox's (1963) space-time interaction measure. The Knox test (Knox, 1963, Knox 1964a) is a statistical procedure that identifies space-time interactions of point data that are labeled with specific times of disease occurrence. It consists of pairing all possible data points and examining the pairs for the interaction of their proximity in space and time. Existing parametric or Monte Carlo statistical procedures are then used for testing the significance of this interaction at a given probability level, usually the 95% interval.

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In this research, the Knox test for space-time interaction of bird deaths parameterized using ecologically relevant information, is combined with geographic principles that allow the reliable dynamic tracking of the amplification of West Nile virus at the local level. The discriminatory power of the Knox test is compared with a new test based on the Knox that uses a novel Monte Carlo method and does not suffer from its current significance test limitations. Furthermore, a space-time version of the Cohen (1960) *kappa* non-chance agreement is proposed, in order to measure to model the peak and retraction of the amplification cycle therefore, providing evidence for the hypothesis presented by Despommier (2001); that the role of dead birds is integral for the spill-over of the disease to humans, in places where the primary vector is the *Culex pipiens.*

The null hypothesis tested in this research is that dead birds play no role in the occurrence of human West Nile virus epidemics. The dismissal of this hypothesis occurs in two stages. In the next chapter (Chapter IV) a novel technique is introduced to dynamically measure and model unusual close space-time bird deaths. The technique is calibrated, applied and, *qualitatively* evaluated with New York City data from the 2000 and 2001 season. In Chapter V, the technique is modified with a new unconditional Monte Carlo extension of test to address a weakness of the Knox procedure, and is applied with Chicago 2002 data. Using a novel *kappa* space-time technique the first *quantitative* evidence ever for the role of dead birds in human epidemics is provided. Chapter VI provides a comparison between the Knox chi-square significance measure and its unconditional Monte Carlo extension. Their discriminatory power is examined directly and indirectly. Each chapter serves as a standalone paper.

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CHAPTER IV

MEASURING AMPLIFICATION: THE DYNAMIC CONTINUOUS-AREA SPACE-TIME SYSTEM (DYCAST)

Introduction.

In 1999, West Nile virus made its first appearance in the Western Hemisphere and New York, New York (New York City). By 2001, 68 New York City area residents were diagnosed with laboratory-confirmed West Nile virus infection, and there were nine deaths (New York City Department of Health, 2001, New York City Department of Health, 2002). Remediation and control measures were implemented to reduce the numbers of mosquitoes, identified as the vectors of West Nile virus (Miller 2001). West Nile virus is a mosquito-borne flavivirus belonging to the Japanese encephalitis virus serocomplex. In New York City, the *Culex pipiens* species is the primary vector of West Nile virus as evidenced by its high competency to transmit West Nile virus, its abundance, and its strong ornithophilic habits (Despommier 2001, Turell et al., 2000, Means 1968).

The primary hosts are native bird species that lack West Nile virus immunity, particularly the American crow and blue jay. Pigeons, although abundant in New York City, have very low infection rates. Humans are dead-end hosts, and their infections are most likely incidental and the result of a spillover effect. Spillover effects occur when the transmission cycle between mosquitoes and birds intensifies (a process termed the

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amplification cycle), or when the avian pool is reduced and species of mosquitoes that are opportunistic feeders act as a bridge vector for transmitting West Nile virus to humans (Despommier, 2001, Turell et al. 2001b).

In New York City, during the 1999 season, remediation and control efforts were based on positive West Nile virus human infections, and in the 2000 season, efforts were based on laboratory confirmation of West Nile virus in mosquitoes and birds, which had a delay time of up to 2 weeks. Recently, the New York City Department of Health implemented an in-house laboratory for faster positive mosquito identification. Although these approaches provide a definite confirmation of West Nile virus, they lack timeliness, because positive results in mosquitoes may not appear until West Nile virus activity has substantially intensified. In addition, they identify West Nile virus activity only at discrete point locations, where mosquito traps were positioned and dead birds were found.

An important issue for the New York City Department of Health was how to use data points to identify the areal extent of West Nile virus activity in a timely fashion. To address this issue, the New York City Department of Health in cooperation with the Center for Advanced Research of Spatial Information (CARSI) Laboratory of Hunter College, City University of New York, embarked on an effort in January 2001 to develop an area-based system to identify areas of West Nile virus activity that could lead to human infection, for targeting remediation and control efforts. The system needed to be

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both prospective and dynamic, while providing the New York City Department of Health with the geographic extent of West Nile virus activity.

A widely used methodology for monitoring West Nile virus activity relies on dead crow densities (Eidson et al., 2001a, Eidson et al., 2001b) or the number of dead crows per unit area. This approach has a number of limitations: 1) It does not ascribe statistical meaning to the results, leading to the selection of an arbitrary cutoff (critical) density for judging high-risk areas for West Nile virus; 2) density is highly susceptible to reporting bias; 3) density calculations for areas that vary in size, shape, and scale rely on a false assumption of uniformity of crow densities throughout the region, resulting in the aggregation problem of the modifiable areal unit problem (MAUP) (Arbia, 1989); 4) densities calculated using kernel functions are subject to edge effects (Baily and Gatrell 1995), although corrections for edges are available when the process is assumed to be stationary and isotropic (Lawson, 2001); and 5) density measures ignore the pathology and ecology of the West Nile virus transmission cycle.

Other methodologies used to model infectious diseases generally factor only the temporal and not the spatial component of epidemics (i.e. Daley and Gani, 2001). Those methods that do account for space often use single un-partitioned areas or non-overlapping spatial units specifically designed for unrelated purposes (i.e., census tracts). The latter can lead to an artificial split of clustered data. In addition, these studies are often retrospective and require the use of controls, the selection of which may be biased when knowledge of the ecology of an infectious disease is incomplete (Kulldorff et al. 1999). Research on

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prospective methods that account for both space and time localities is recent and limited (Kulldorff 2001, Rogerson 1997, Rogerson 2001).

Knox (Knox 1963, Knox 1964a) proposed a method that allows for statistical testing of the interaction of incidents of infectious disease in space and time that does not suffer from the limitation of density measures that use an arbitrary critical value of density for determining risk localities. The Knox statistic is calculated by pairing all possible data points (e.g., location in space and time of the death of birds) within a clearly defined geographic area and temporal window and testing them against assigned values of what is "close" in space and time. The number of close space-time data pairs is compared with what would be expected if there were no interaction of space and time, and a probability of nonrandom space-time interaction is determined. When the probability is less than 0.05, the likelihood of space-time interaction is significant.

The Knox test is widely used in epidemiologic studies (Knox 1964b, Andersson et al. 1995, Samuelsson et al. 1994, Gilman et al. 1995, Williams 1984). However, many of these studies are retrospective with the intent of evaluating infectious etiology from disease incidence in single unpartitioned areas. Only recently has the Knox test been implemented prospectively for local regions (Kulldorff 2001, Rogerson 1997, Rogerson 2001). Rogerson (2001) used the Knox test on data updated prospectively with a cumulative sum method; however, this method did not account for dynamic spatial phenomena that exhibit spatial movement in time (Lawson, 2001). In the case of West Nile virus in New York City, prospective monitoring is necessary for targeting

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remediation and control efforts, and dynamic monitoring is essential for tracking the changing spatiality of viral activity. Dynamic monitoring reduces false positive-risk areas where viral activity has subsided, it keeps remediation and control efforts focused on the current viral hot spots, and it can be used to monitor the efficacy of remediation and control initiatives.

The Dynamic Continuous-Area Space-Time (DYCAST) system was developed to identify and prospectively monitor high-risk areas for West Nile virus, and it was used to assist in guiding the remediation and control efforts of the New York City Department of Health. The DYCAST model is prospective and dynamic, and it relies on the Knox test to statistically assess the significance of space-time interaction and, hence, risk. Reporting bias is decreased using this approach, because the decision to identify a high-risk area is not dependent on a cumulative density measure but on a statistical assessment of the significance of space-time interaction. In fact, significant space-time interaction can occur in low-density situations, and non-significant interaction can occur in high-density situations. The Knox analysis is implemented as an interpolation function to create a surface of probabilities over a grid of 1,400 cells overlaying New York City. Each grid cell is assigned a probability based on a Knox analysis of dead birds within a 1.5-mile (2.41-km) radius of its centroid (spatial domain) and a 21-day moving window (temporal domain) preceding each day's daily run. The DYCAST model was calibrated using year 2000 data on dead bird and human West Nile virus incidence reports and was implemented and tested operationally with year 2001 surveillance data.

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Materials and Methods.

Data.

Data on human cases and dead bird reports were provided by the New York City Department of Health for the years 2000 and 2001. Dead bird reports were collected by passive surveillance, relying on public reporting through telephone and Internet entry systems. All dead bird reports were geocoded (assigned a coordinate to the street address of where the dead bird was found) and entered into the geographic information system, GE Smallworld technology (GE Smallworld core 2002). Attribute information, such as species and found date, was attached to each record. The complete DYCAST system, including the Knox methodology for space-time interaction of dead bird reports, was modeled and written in Magik programming language (GE Smallworld core 2002).

Methodology.

The major assumptions of the model used in this study are as follows: 1) West Nile virus is a continuous phenomenon across space; 2) humans are infected at their place of residence; 3) nonrandom space-time interaction of bird deaths is attributed to West Nile virus infection; and 4) each dead bird has an equal opportunity of being reported.

Knox test.

The Knox method is used to test for no interaction of incidents in space and time within a clearly defined spatial and temporal domain. Closeness in space and time is based on a

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set of criteria (e.g., ecology), and pairs of data points are tested as to which of four categories they fall into: close in space only; close in time only; close in space and time; or close in neither space nor time. Knox (1964b) suggests the construction of a 2x2 contingency table as shown in Table 3.

Table 3. Knox contingency matrix.

Knox matrix	Space		
Time		Close	Not Close
	Close	o_{11}	o_{12}
	Not Close	o_{21}	o_{22}

$T(o11)$ is the test statistic or the actual number of pairs found close in space and time, and it is calculated as:

$$T(o_{11}) = \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} s_{ij} t_{ij} \qquad \text{(Equation 1)}$$

where s_{ij}, t_{ij} is 1 when the ith-jth pair is close in space and/or time, respectively, and 0 otherwise. The Knox statistic compares the observed number of pairs close in space and time with the expected number of pairs close in space and time under a random process, given that $s = o11 + o21$ pairs were found close in space and that $t = o11 + o12$ pairs were found close in time (Knox, 1964a). The expected number of pairs is calculated as

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$$E(T) = \left(\sum_{i=1}^{n-1} \sum_{i=j+1}^{n} t_{ij} \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} s_{ij} \right) / N \qquad \text{(Equation 2)}$$

The variance of the Knox statistic was developed by Barton and David (1966).

Model calibration.

Residential location and the presumed date of West Nile virus infection in humans were used as the basis for model calibration, because they were considered the most reliable indicators of amplified West Nile virus activity. A Knox test was performed on all dead birds, except pigeons and unknown species, found within a 1.5-mile (2.41 km) spatial domain of the residential location of each human case and within a 21-day temporal domain prior to the case's presumed date of West Nile virus infection. The presumed date of human infection was estimated as 7 days prior to the reported date of onset of symptoms, which is above the mean incubation period of hospitalized patients, 5.3 days (Nash et al. 2001), and within the range of human infection, 3–15 days (Olejnik 1952). The 1.5-mile (2.41 km) buffer represented local areas of relatively high risk based on twice the feeding distance of *Culex pipiens*, 0.68 miles (1.09 km) (Savage and Miller 1995). The 21-day window accounted for two infectious cycles in birds (e.g., infected birds die within 7 days) and the possibility of a spillover effect. Statistical significance was evaluated at different combinations of critical space-time parameters within the spatial and temporal domains.

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Selection of critical parameters.

A challenging task in the Knox methodology is the selection of critical parameters. Because of the uncertainty in their statistical significance, studies will usually set a "range" and systematically perform the Knox test over the span of the range (Gilman et al. 1995, Williams 1984.). However, the inference of space-time interaction, based on these ranges over the same data set, results in multiple testing (Kulldorff et al 1999, Williams 1984). Additionally, a purely statistical decision for space-time parameter selection disregards factors inherent to the nature of the phenomena that are being studied. In the DYCAST model, the selection of values for the critical parameters happens in the calibration phase and is based in part on ecologic considerations. Critical distance (or measure of space) does not exceed 0.75 miles (1.2 km), reflecting the limited mobility of ill birds and avoiding a distance close to the spatial domain that would reduce the test to one of temporal clustering. Critical time lies between 2 and 7 days, reflecting the period within which infected birds experience limited mobility and die. The Knox test was therefore run for the distances of 0.25 miles (0.4 km), 0.5 miles (0.8 km), and 0.75 miles (1.2 km) and the times of 3 and 6 days, producing six combinations of critical parameters. The actual number of close pairs of dead birds was counted, and the expected number of close pairs and variance (Barton and David 1966) were calculated. The probability of significance was assessed using 1) Poisson (if one or more of the Knox contingency table cells had less than five pairs) or chi-square (if all the Knox contingency table cells had five or more pairs) distribution (Knox 1964a, Knox 1964b, (Barton et al. 1967), 2) a normal approximation (Barton et al. 1967), and 3) 1,000 Monte Carlo random permutations as adapted by Mantel (1967) at the $p = 0.05$ level. The Monte

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Carlo method is explained graphically in Figure 4 (the problem mentioned is examined in more detail in Chapters V and VI).

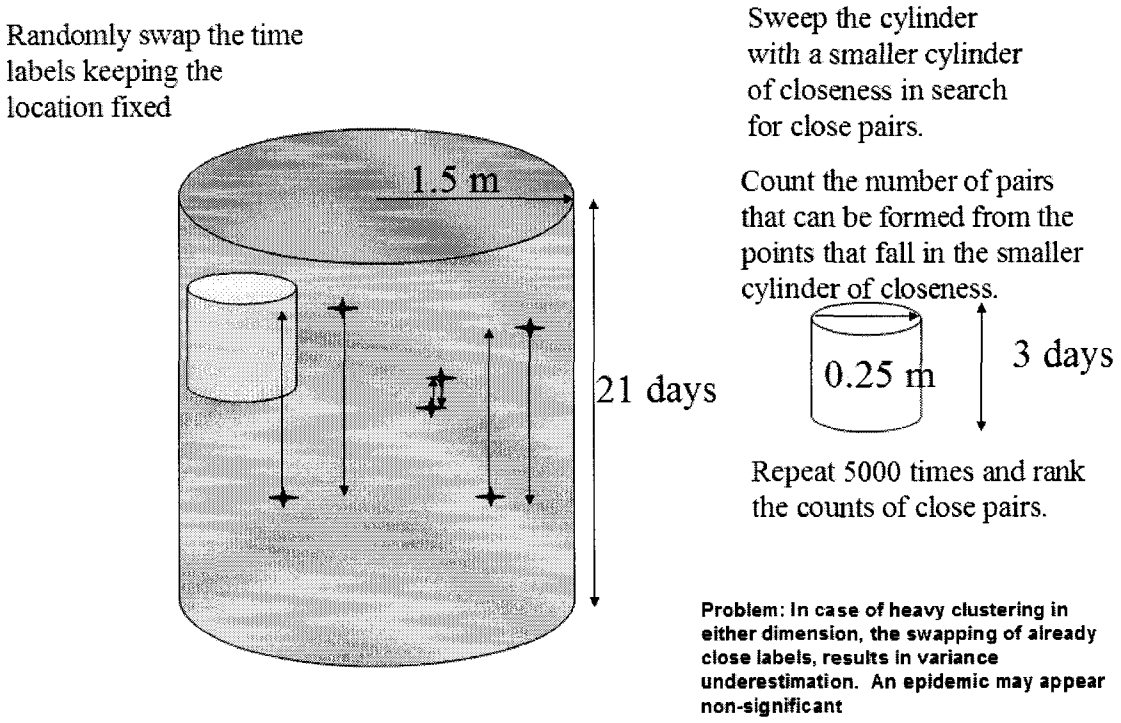

Figure 4. Monte Carlo time label switching significance test.

The critical parameter combination that resulted in significance of space-time interaction of bird deaths within the spatial and temporal domains (1.5-mile buffer, 21 days prior to presumed infection date) for the greatest number of the year 2000 human cases was chosen as the optimal critical parameter combination and set for all future city-wide analyses.

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Spatial design.

The citywide spatial design of the model was used for both the retrospective calibration phase in the year 2000 and for prospective implementation in the year 2001. The spatial design consists of laying a 0.5-mile grid across New York City, running the Knox test on each cell centroid (n = 1,400) as if it were the center of a high-risk area, and assigning to the cell the resulting probability (Figure 5).

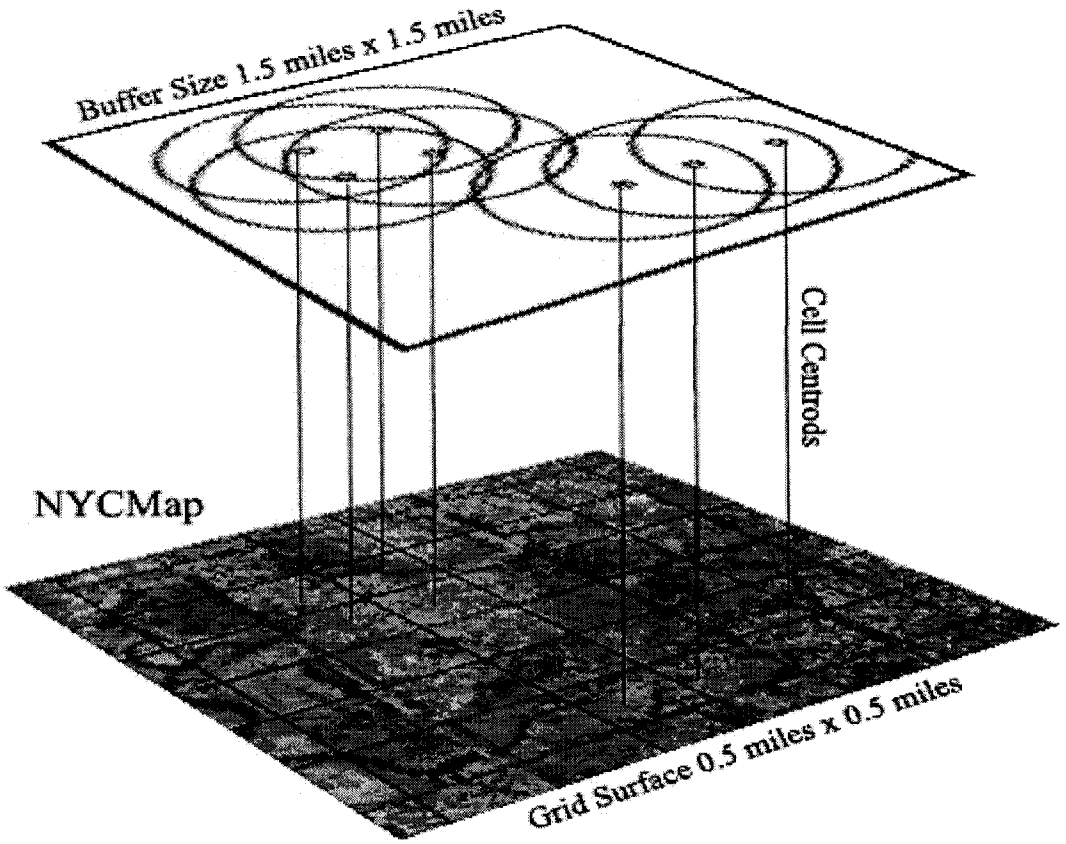

Figure 5. Interpolation methodology.

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The localized Knox test uses a 1.5-mile radius as its spatial domain and a 21-day window as its temporal domain. The rationale for using a 1.5-mile-radius domain to evaluate the probability for a 0.5-mile grid cell is that West Nile virus activity is a continuous phenomenon and, therefore, should be modeled as a continuous surface rather than as a collection of discrete adjoining regions. The creation of surfaces has traditionally been accomplished using a function that interpolates the value of a cell based on its neighborhood (Tobler 1979). The grid's centroid spacing of 0.5 miles (0.8 km) (0.707 miles diagonally) was selected to be less than half the feature of interest (e.g., the 1.5-mile range of *C. pipiens*) to avoid information loss during the interpolation process (Shannon 1949). Similar grid size selection procedures are widely used in remote sensing (Sonka et al. 1998). Treating West Nile virus activity as a surface also avoids the MAUP associated with the arbitrary partitioning of space.

Results.

Model calibration.

Critical parameter calibration.

The critical parameters of 0.25 miles (0.4 km) and 3 days were selected as the best space-time combination for use in this model's Knox test. These parameters demonstrated agreement in significance of the Poisson or chi-square assessment with the Monte Carlo trial in nine (64 percent) of the 14 human cases (although this comparison is not adjusted for chance agreement). Furthermore, although the normal approximation showed only

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four significant cases of space-time interaction, they were all found at the combination of 0.25 miles (0.4 km) and 3 days (Table 4).

When the critical time was extended to 6 days, agreement between the Poisson or chi-square test and the Monte Carlo trial was observed in only seven (50 percent) human cases. As the critical distance was extended to 0.5 miles (0.8 km) and the critical time was kept at 3 days, agreement in significance was observed in only four (29 percent) human cases. At 0.5 miles (0.8 km) and 6 days, there was agreement in only one human case. When critical distance was increased to 0.75 miles (1.2 km) and critical time was kept at 3 days, there was agreement in four (29 percent) human cases, and at 0.75 miles (1.2 km) and 6 days, agreement increased to six (43 percent) human cases. This decrease and then increase in the numbers of significant space-time interaction areas suggest that, as the critical distance approaches the 1.5-mile buffer size, most pairs are close in space, and the Knox test is reduced to a test of pairs close in time. Hence, the probability results refer to a related but different test quantity. In summary, the critical parameter combination of 0.25 miles (0.4 km) and 3 days proved to be the most appropriate for this study because of its strong likelihood of avoiding false negatives.

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Table 4. Results of significance tests for combinations of critical distances of 0.25-0.75 miles and critical time of 3-6 days.

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Significance testing.

The Poisson and chi-square tests for significance assume that the data used for testing are independent of each other. Data pairs that share points in the Knox procedure violate this assumption of independence. In this study, the violation proved particularly acute when the number of data points used (birds) was small. This problem may explain the disagreement shown between the Poisson or chi-square test and the Monte Carlo trial for small numbers of birds. For example, with case 1,002, 22 birds were found within the 1.5-mile buffer and 21-day window. Significance was shown with the Monte Carlo trial at 0.5 miles (0.8 km) and 6 days ($p = 0.05$), but the Poisson or chi-square test showed no significance ($p = 0.128$). An examination of the seven close pairs formed from these 22 birds showed that six pairs shared at least one data point, indicating statistical instability due to a violation in the pair independence assumption.

The results of the retrospective citywide analysis on year 2000 data demonstrated additional problems associated with the diverging results of the Poisson or chi-square test and the Monte Carlo trial and the dependencies at smaller bird numbers. Therefore, a threshold was set at 25 birds and was based on 1) the convergence between the Poisson or chi-square and the Monte Carlo probabilities as the numbers of birds increased, 2) the attempt to reduce pair dependencies that could bias the results in both the Poisson or chi-square and the Monte Carlo methods, and 3) the attempt to minimize false positives at lower bird numbers. For example, a statistically significant result of one close pair with only two data points would not raise the risk (and should not raise the risk alarm) in that area. Because the results of the Poisson or chi-square test with the Monte Carlo trial

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were similar after the threshold was implemented, all year 2001 analyses were run using only the Poisson or chi-square significance test.

Model implementation

The results of this study demonstrate that the DYCAST model was successful in identifying areas of high risk for West Nile virus at least 13 days prior to the onset of illness in five of the seven human cases in the year 2001. One of the missed cases appeared in an area that was indicated by the model 3 days after the person's onset of illness; however, this case occurred after dead bird surveillance was virtually halted on September 11, 2001, because of the terrorist attack on the World Trade Center buildings in New York City. A high-risk area for West Nile virus was identified on July 2nd in northern Staten Island, 25 days prior to the first human case of West Nile virus with onset of illness on July 26th (Figure 6). South of this location, another area of significant space-time interaction of dead birds appeared 17 days prior to the second human case with onset of illness on August 5th (Figure 7). In northeastern Queens, an area of high risk for West Nile virus appeared on July 9th, 38 days prior to the third human case with onset of illness on August 16th (Figure 8). Subsequently, significant space-time interaction of dead birds was observed in southwestern Brooklyn on August 20th, 13 days prior to the fourth human case with onset of illness on September 2nd (Figure 9).

This area of high risk for West Nile virus in Brooklyn was an expansion of an area first observed on August 4th, which persisted and expanded until September 20th. In northern Queens, significant space-time interaction of dead birds was seen from August 3rd through August 28th, 35 days prior to the fifth human case with onset of illness

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September 7th. Two days later, on September 9th, a sixth human case of West Nile virus was diagnosed in central Brooklyn, where the DYCAST system failed to capture significant space-time interaction at any time. In the aftermath of the terrorist attacks on the World Trade Center buildings in New York City, the model failed to capture significant interaction, reflecting decreased surveillance of dead birds. During this time, all areas showing significant citywide interaction gradually receded and disappeared completely 9 days later on September 22nd. These findings demonstrate both the temporal sensitivity and the dynamics of the model. The 9-day interval was not necessarily a disadvantage of the DYCAST system, as there was interest in both current and prior areas of significant interaction for targeting remediation and control efforts. Furthermore, a decrease in birds during this time could potentially have caused mosquitoes to switch feeding habits to mammals, increasing the risk for humans. When bird surveillance did resume in early October, the model demonstrated significant space-time interaction on October 8th in lower Manhattan, just 2 days after the seventh and last human case of West Nile virus with onset of illness on October 6th (Figure 10). This same area of high risk expanded and remained significant until October 31st, the last date for which the analysis was conducted. A review of these analyses also demonstrates significant space-time interaction of dead birds at the edges of all the boroughs, although a potential edge effect could have existed when the edge caused the reporting numbers in a buffer to drop below the threshold (n = 25). However, this was not a problem in this study. Edge effect concerns were also viewed in the perspective of the amplification cycle, which suggests that a reduced number of birds at the edges (i.e., birds will not exist as mosquito targets in the water) will depress the amplification cycle in the buffer.

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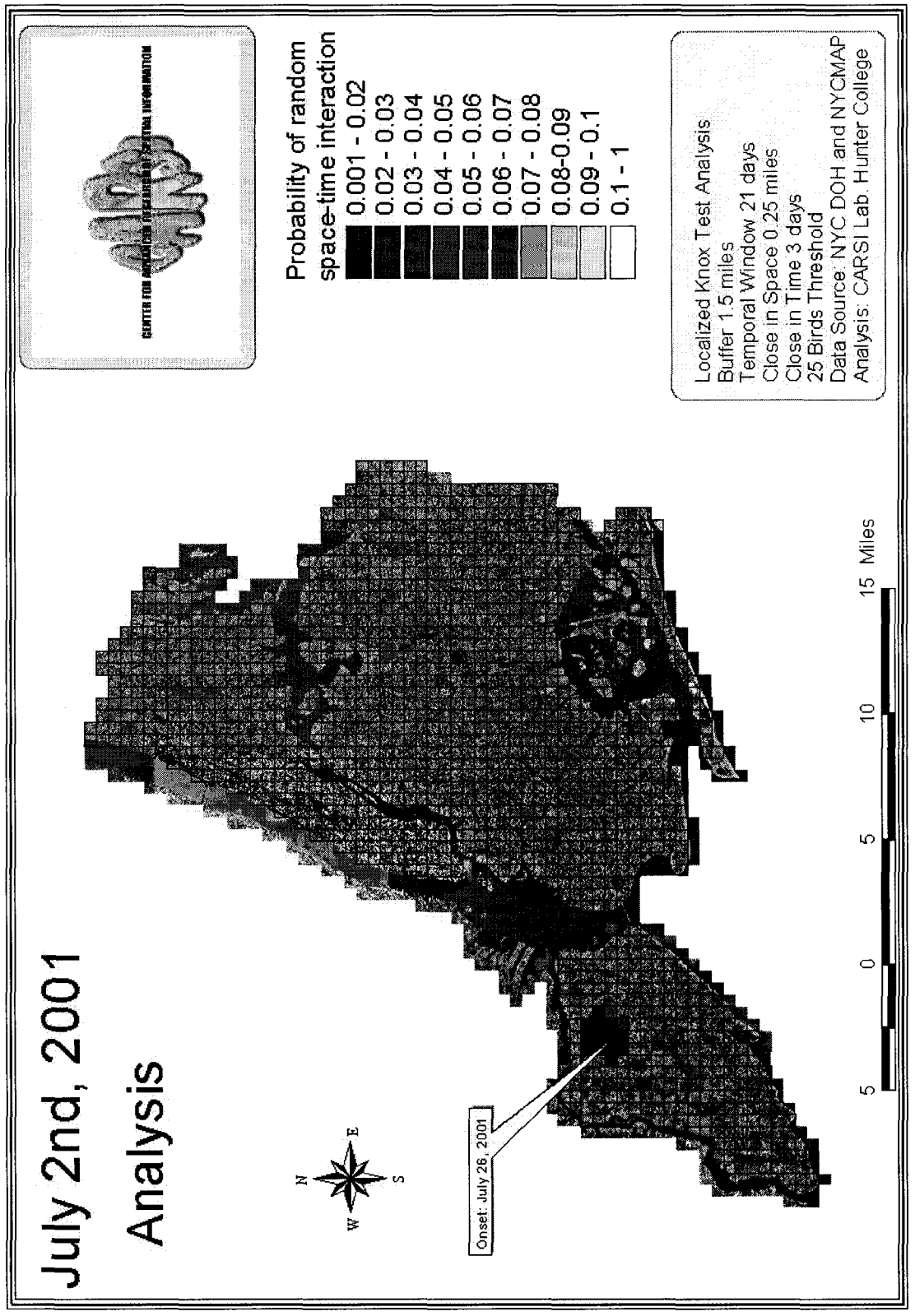

Figure 6. Map of June 2nd, 2001 New York City analysis.

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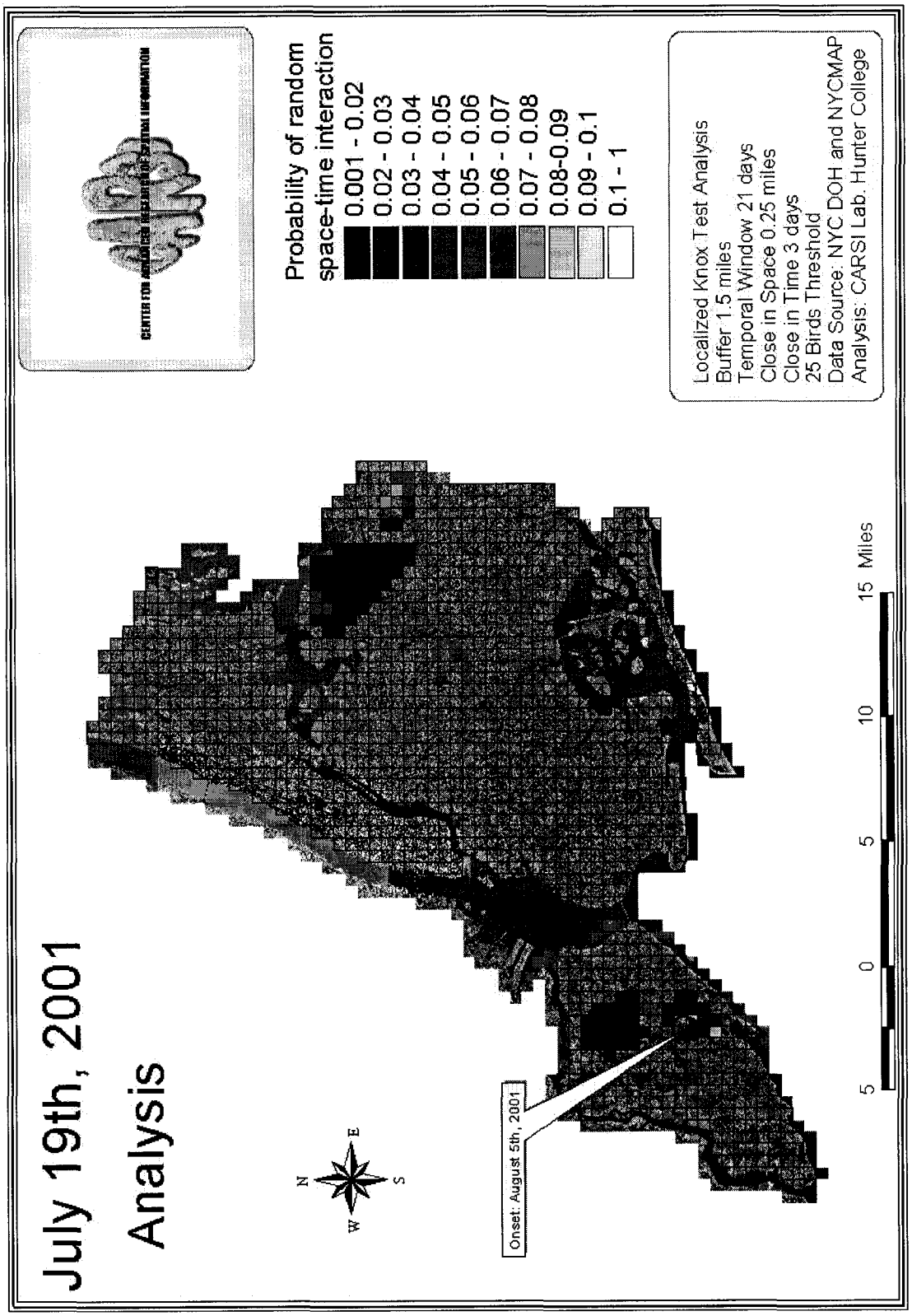

Figure 7. Map of July 19th, 2001 New York City analysis.

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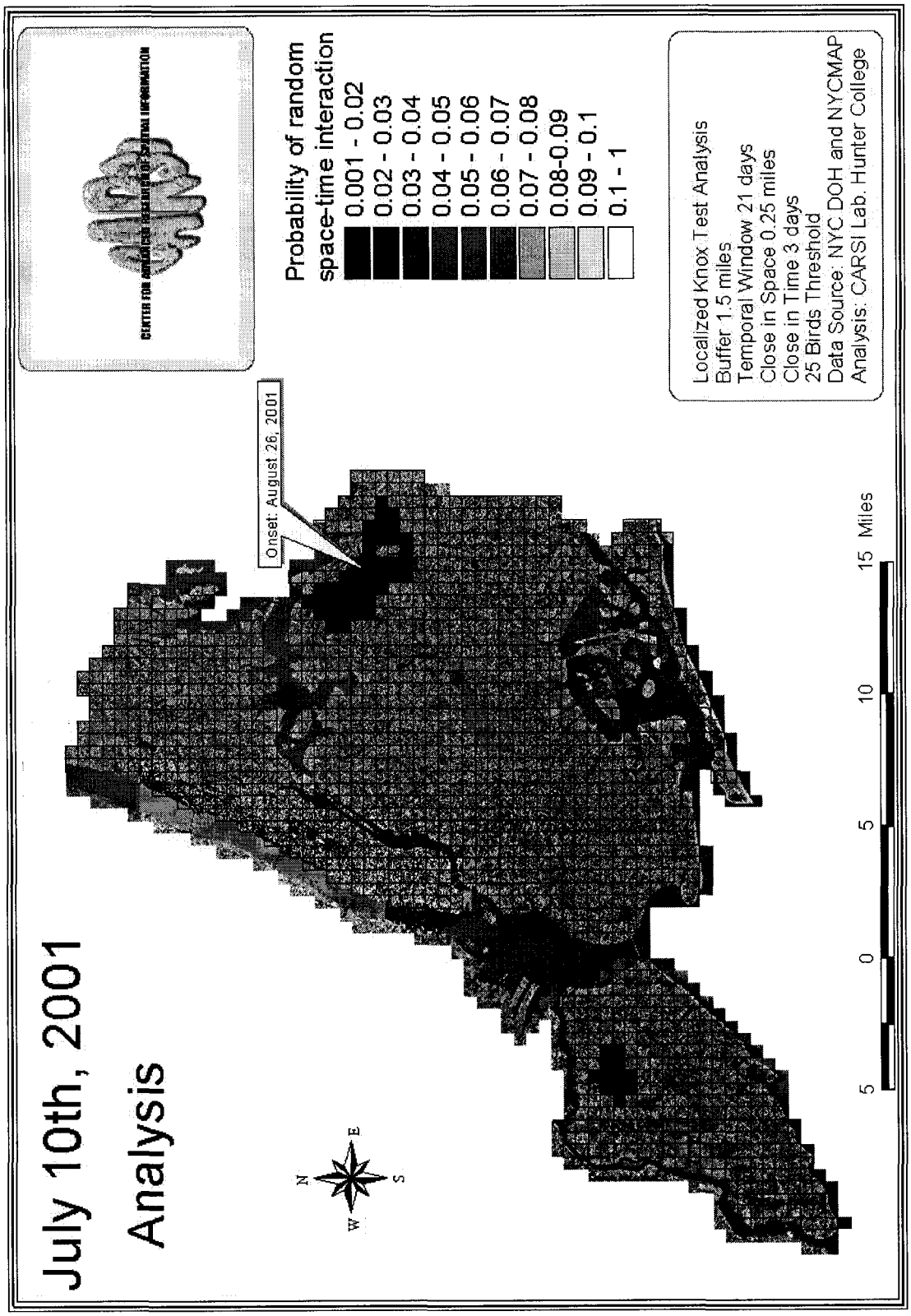

Figure 8. Map of July 10th, 2001 New York City analysis.

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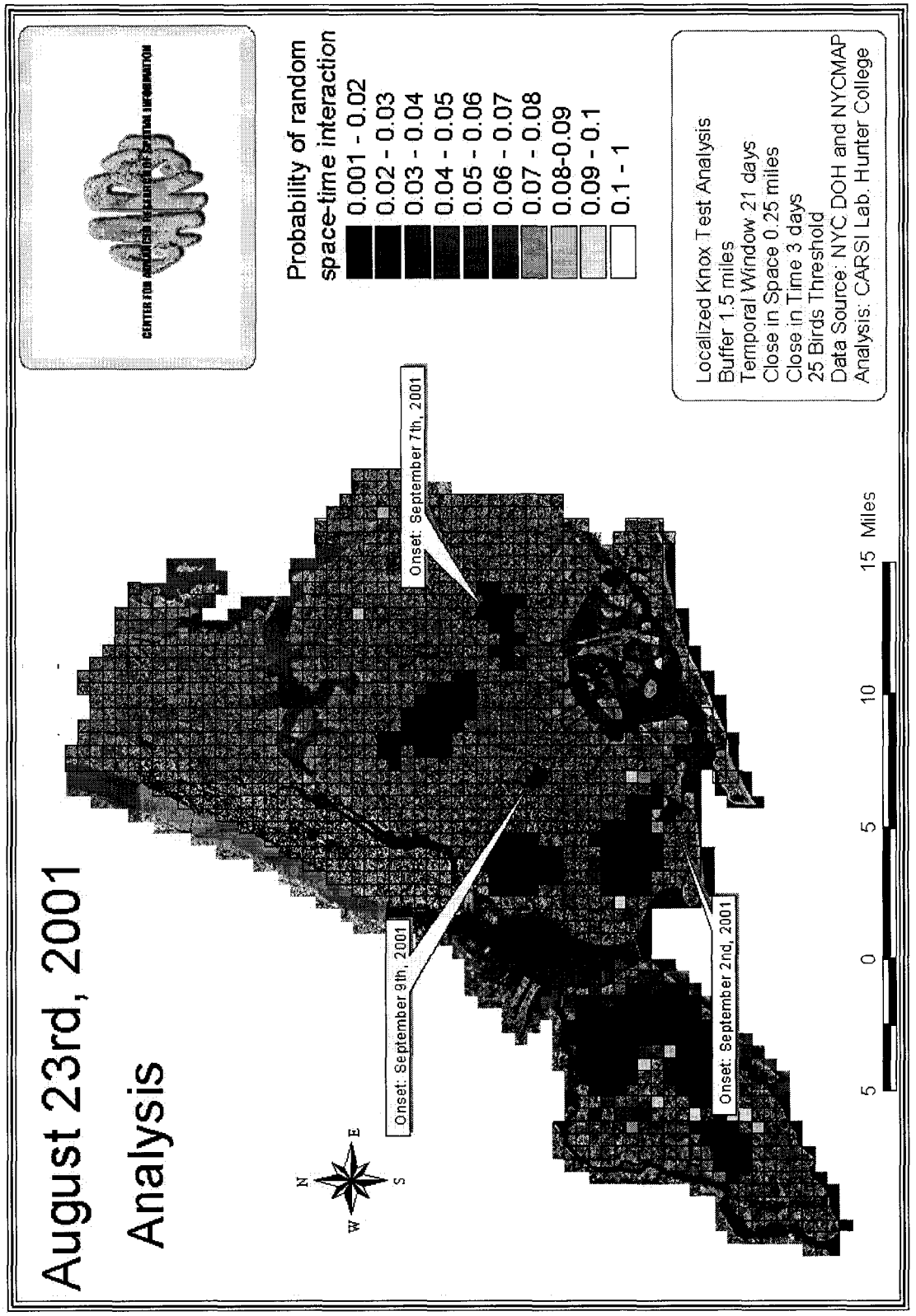

Figure 9. Map of August 23rd, 2001 New York City analysis.

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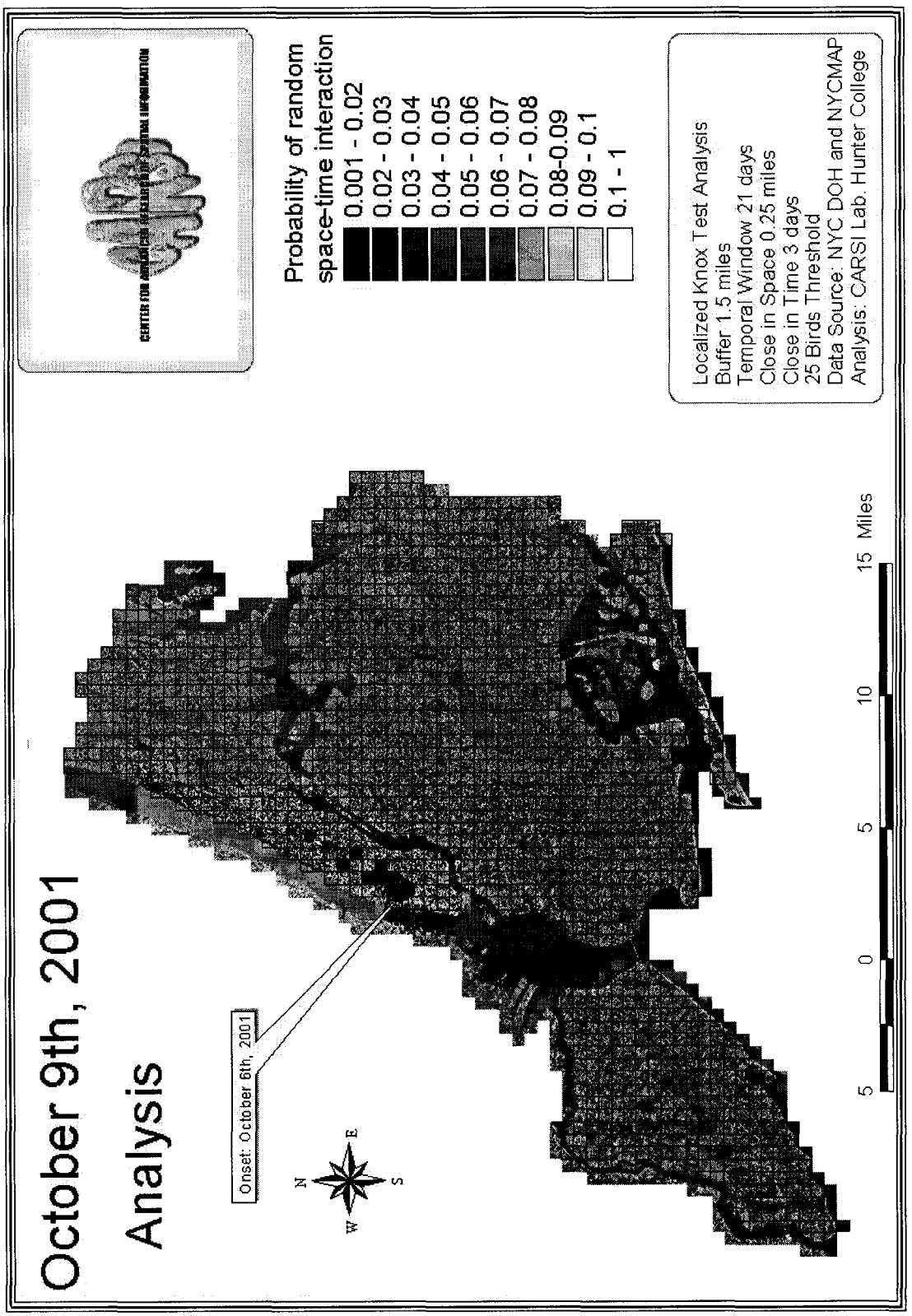

Figure 10. Map of October 9th, 2001 New York City analysis.

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Sensitivity analysis.

A sensitivity analysis was performed on data for September 1, 2001, because this date reflected the peak of the season's West Nile virus activity. The results of the analysis using the critical parameter combinations of 0.25 miles (0.4 km), 0.5 miles (0.8 km), and 0.75 miles (1.2 km) with 3 and 6 days are summarized in Table 5. This table shows the number of cells that were identified as being high-risk areas on the basis of the dead bird interaction effects with the respective parameters, at the $p = 0.05$ and the $p = 0.1$ significance levels.

The more constrained critical parameters (0.25 miles (0.4 km) and 3 days) produced the maximum number of cells in the citywide grid that were identified as high risk. Furthermore, the temporal critical parameter appears to be more important than the spatial critical parameter, because the combination of 0.25 miles (0.4 km) and 6 days produced fewer high-risk cells than did the combination of 0.5 miles (0.8 km) and 3 days. There is also a decrease in the number of high-risk cells with increasing space distance. Critical parameters with the least number of significant buffers appear at the 0.75-mile (1.2-km) and 6-day critical parameter combination. This sensitivity analysis validates the use of the most constrained critical parameters to minimize false negatives. Despite differences in the number of high-risk cells, all critical combinations resulted in high-risk cells in the same approximate areas with minor differences in extent. This finding demonstrates that the model is highly robust with respect to its critical parameters. In summary, the DYCAST system was stable over the range of ecologically constrained parameters, and the most constrained parameters (0.25 miles (0.4 km) and 3 days) were

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the best for initiating remediation and control activities, because they minimized false negatives.

Table 5. Results of sensitivity analysis.

	Time		Time	
Distance and p values	3	days	6	Days
0.025 miles	No.	(%)	No.	(%)
p value < 0.05	254	18.79	156	11.53
p value 0.05 to 0.1	26	1.92	34	2.51
non-significant	1072	79.29	1162	85.94
Total	1352	100	1352	100
0.05 miles				
p value < 0.05	168	12.42	115	8.5
p value 0.05 to 0.1	26	1.92	28	2.07
non-significant	1158	85.65	1209	89.34
Total	1352	100	1352	100
0.75 miles				
p value < 0.05	121	8.87	59	4.36
p value 0.05 to 0.1	40	2.95	48	3.55
non-significant	1191	88.16	1245	92.08
Total	1352	100	1352	100

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Discussion

In conclusion, in the year 2001, the DYCAST system successfully identified areas of high risk for West Nile virus in humans at least 13 days prior to their onset of illness in five of the seven human cases. The reason for this success is the unique combination of geographic principles, ecologic knowledge of the West Nile virus vector-host transmission cycle, and statistical analyses to overcome the limitations observed in previous studies to model West Nile virus.

The innovations of the DYCAST system include the use of a statistical measure to assess areas of high risk for West Nile virus rather than the selection of an arbitrary "critical" density and the use of data regarding the ecology of the host and vectors in the parameter calibration phase that attunes the model to the real world phenomenon. In addition, the DYCAST system did not suffer from MAUP inconsistencies, because it was designed as a continuous area system that used a neighborhood interpolation technique in accordance with centroid spacing selection principles. Moreover, the identification of areas of high risk for West Nile virus in locations of low population density (i.e., Staten Island) demonstrates no visible evidence of reporting bias when a minimum bird threshold was calculated and implemented. Edge effects were reduced to cases where an edge spuriously caused the number of dead bird data points not to reach the appropriate 25-bird threshold. Finally, the dynamic nature of the system provided timely identification and incorporation of temporal effects.

Despite the success of the DYCAST system, certain issues require further research. Two points arising from the calibration results include the issue of threshold and the validity of

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significance tests. Another important issue that should be addressed is reporting bias in areas of varied socioeconomic characteristics. Finally, the model could be optimized further by incorporating additional data, such as the location of mosquito breeding grounds, environmental conditions, high-risk populations, and feedback mechanisms for further calibration on positive bird and mosquito results and control activities.

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CHAPTER V

FIRST EVIDENCE OF WEST NILE VIRUS AMPLIFICATION AND RELATIONSHIP TO HUMAN INFECTIONS

Introduction.

The West Nile virus is a mosquito borne disease-causing infectious agent that affects wildlife and domestic animals. It can occasionally cause fever and encephalitis in humans and in rare cases lead to death. First isolated in the West Nile district of Uganda (Smithburn et al. 1940) it was considered enzootic to the Middle East, Africa and Eurasia (Hayes et al. 2001) until 1999. In 1999, it was identified for the first time in the Western Hemisphere (Lanciotti et al. 1999, Nash et al. 2001), and since then it has been causing seasonal epidemics infecting thousands of people and infecting a wide variety of species in great numbers, mostly birds.

Since its introduction to the Americas, the disease has received renewed attention by researchers. A significant part of this research, dealt with the identification of proxies for West Nile Virus presence in specific areas so that public health officials could intervene with control measures (Eidson et al. 2001b) and reduce the risk of infection in humans. One of the proxies used is the death of birds in close spatial and temporal proximity (Theophilides et al. 2003). The DYCAST system, which identifies local West Nile virus risk areas, is based on the hypothesis that West Nile virus propagation occurs in a cycle between birds (hosts) and mosquitoes (vectors). The cycle eventually amplifies resulting

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in an increased pool of the virus among birds. Mosquitoes that feed on both birds and humans are then more likely to carry and transmit the virus to humans (Campbell et al. 2002). The most competent reservoir hosts are resident passerines and mortality is higher in corvids (McLean et al. 2001, Komar et al. 2003). According to Komar et al. (2003), American crows develop the highest viremia in 4 to 5 days post infection and on average die on day 5. The same study showed their mortality rate was found to be 100% and a study by Yaremych et al. (2004) has verified this high mortality of crows due to West Nile Virus in the wild. Blue Jays develop highest viremia levels on days 1, 2, 3, and 4 post infection and on average die 4.7 days post infection at a 75% rate (Komar et al. 2003). Our hypothesis is that unusually high numbers of crow and Blue Jay deaths occurring close in space and time signal the presence of an intense amplification cycle and increased risk to humans.

The Dynamic Continuous Area Space-Time (DYCAST) (Theophilides et al. 2003) models the amplification process as a continuum in space and time. It has demonstrated that West Nile virus risk in New York City in 2001 could be identified in a timely and specific way, prior to the onset of human infections. Others have shown that local high dead crow densities precede the occurrence of human West Nile virus infections (Eidson et al. 2001a, Watson et al. 2004). Despite the consensus of observations that bird deaths precede human infections in specific areas, to date, no quantitative evidence has ever been presented that links bird deaths to the spatial and temporal parameters of the amplification process, and the spillover of the virus from avian to human hosts.

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Here, a retrospective analysis of dead birds and human infections collected by the Chicago Department of Health from June 30th through October 5th of 2002 is presented. The analysis uses DYCAST (Theophilides et al. 2003) modified with an unconditional Monte Carlo extension of the Knox test. The results are analyzed for predictability beyond random chance using a weighted Kappa analysis for a range of temporal windows selected prior to the date of each human case.

Materials and Methods.

Data description.

The data were provided by the Chicago Department of Health and includes the location and date of dead bird reports (primarily crows and blue jays, n = 3837), as called in by the public, and the location and date of the onset of human West Nile virus infection (n = 215). The infection of humans was assumed to take place at the place of residence. The analyses were performed over a 0.8km (half-mile) grid (with 1,189 cells) overlaid across the City of Chicago.

Analysis.

DYCAST Procedure

The data were imported into the modified DYCAST (Theophilides et al. 2003) system and processed for every day between June 30th, 2002 and October 5thth, 2002, (the period of Chicago Health Department, dead bird surveillance) across the entire grid. The procedure was run using the same parameters as set by Theophilides et al. (2003) for

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New York City. New York City is a similar urban environment with the same mosquito vector, the *Culex pipiens.* In a departure from Theophilides et al. (2003) significance testing was accomplished by an unconditional Monte Carlo extension of the Knox test method Figure 11.

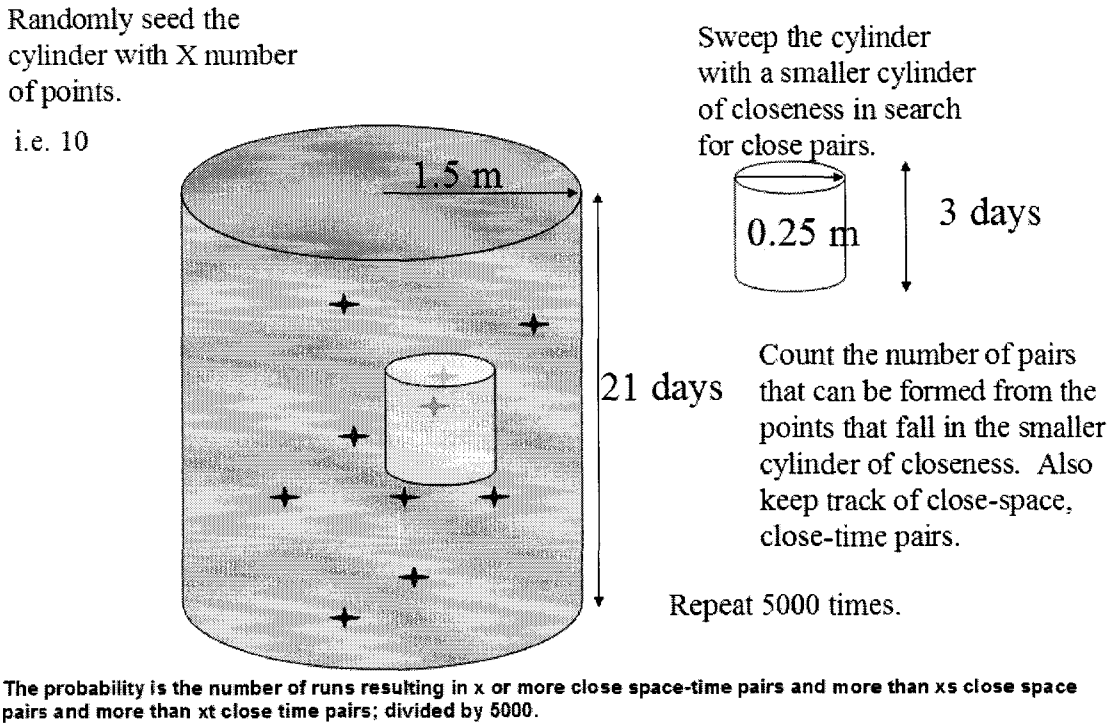

Figure 11. Unconditional Monte Carlo extension of the Knox test.

For a number of points *n* found within the Spatial Domain and Temporal Domain, random sets of *n* points were generated within the same domain. For each set, the number of close space-time pairs (st), the number close space pairs (s) and, the number of close time pairs (t) were counted. Based on the distribution of these random runs the probability level was assessed as:

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$$p = P\{(st \geq ST) \cap (s \geq S) \cap (t \geq T)\} \quad \text{(Equation 3)}$$

where S, T, and ST are the actual number of points found close in space, time, and both respectively. This unconditional formulation should be contrasted with that of the traditional Knox test where:

$$p = P\{(st \geq ST) \mid [(s \geq S) \cap (t \geq T)]\} \quad \text{(Equation 4)}$$

Here 5000 random distributions were generated for each possible count of birds found within the spatial and temporal domain of a cell centroid. These were defined as the area covered by a 2.41km (1.5 mile) radius circle and the 21 preceding days respectively. The actual count of dead birds found within the domain of each cell centroid, were used to select the appropriate distribution and the actual number of close space-time dead bird pairs (given the individual close in space and close in time pairs) was then ranked with respect that random distribution. This resulted in the assignment of each of the 1,189 cells with a daily probability of random space-time interaction of dead bird pairs. A cell was identified as "at-risk" for human West Nile virus infection when its probability was less than or equal to 0.1.

Result Evaluation

Evaluation of the results was conducted in two ways. First, the percentage of human West Nile virus infections that occurred in cells that were shown "at-risk" in the days prior to the onset of the human illness was calculated. Second, the evaluation and testing of the significance of the overlap of at-risk cells with human cases that occurred beyond

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chance was achieved. In these evaluations were done using the results from modified DYCAST and the location and date of the onset of human cases.

Percent of success

In order to calculate the percentage of success, the location of each human case was overlaid on the grid. Each cell containing a human case was queried to determine if it was at-risk. This query was performed for each day starting with 21 days prior and leading up to the date of onset of illness. If the query returned true, risk identification was considered successful for that day for that cell. Two histograms were constructed. One shows the cumulative percentage of successful risk identification x-days (range 21 to 0) prior to onset (Figure 16). The other shows the cumulative percentage of successful risk identification appearing for x-days (Figure 17).

The results of percent success indicate the potential association of dead birds and human risk. However, that association cannot be statistically tested and does not account for successful identification occurring by chance (henceforth-called chance agreement). Stated differently, the high sensitivity of the results may have a reciprocal low specificity. In such a case many more risk cells would have been identified increasing the potential that the high sensitivity is the result of the high probability of chance intersection of the human cases with the numerous risk cells. To control for this and statistically test the association, the kappa index of agreement (or kappa statistic, Cohen 1960) was used. The kappa statistic is used to quantify and test the non-chance agreement between a set of data independently assigned by two raters or methods into nominal classes.

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The general form of the kappa statistic is:

$$\hat{\kappa} = \frac{N\sum_{i=1}^{r} x_{ii} - \sum_{i=1}^{r}(x_{i+} * x_{+i})}{N^2 - \sum_{i=1}^{r}(x_{i+} * x_{+i})}, \qquad \text{(Equation 5)}$$

where:

N is the total number of areas considered,

and x_{ii}, x_{i+}, x_{+i} are the elements of the following matrix (Table 6):

Table 6. Kappa rater table.

	Rater 1		
Rater 2		Class 1	Class 2
	Class 1	x_{11}	x_{12}
	Class 2	x_{21}	x_{22}

The sum of which amounts to *N*.

In this case the "methods" consist of the modified DYCAST procedure and the actual human infections. Each method can classify each grid cell as at West Nile virus risk (or actual West Nile virus sickness) or no risk (no West Nile virus sickness) independently from the other. A chi-square statistical test for the significance of the agreement can be performed.

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Kappa

The parameters for calculating the kappa statistic consist of total possible agreement, expected chance agreement, and observed agreement. The total possible agreement is calculated as the product of the number of human illnesses times the temporal window. Expected chance agreement is the ratio of at-risk cells to total cells (over the windows) multiplied by total possible agreement. Observed agreement is the sum over all human cases of the number of at risk cells contained within the temporal window overlapping the location of a human case. The agreement for no-risk/no-human-illness cells was calculated in a similar fashion. Kappa values for both classes of agreement were calculated and were combined together based on the ratio of total possible agreement in absence of West Nile virus cells divided by the total possible agreement in presence of West Nile virus cells (the former was far higher than the latter). The results are shown in Figure 18 and Table 7. The significance of the resulting weighted kappa values was tested using a chi-square statistic (CI: 95%).

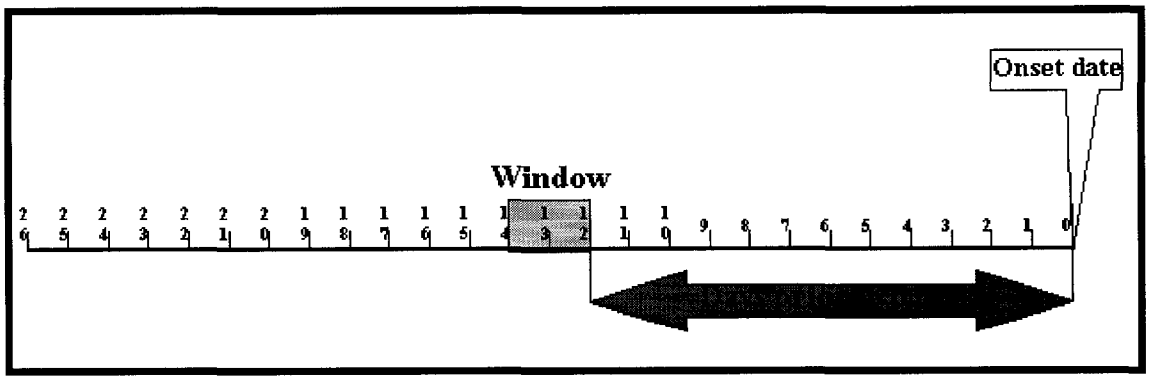

Figure 12. Illustration of combination of window size of two days, 12 days prior.

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Calculation and scaling of kappa values

The spatial version of the kappa statistic was introduced by Congalton and Mead (1983), Congalton (1991) and is widely used in Remote Sensing; however it was never implemented for a space-time database. Here is presented for the first time a methodology for implementation of kappa over space and time. In this methodology kappa values are calculated for unique combinations of differently sized temporal windows of consecutive days, and lags of given number of days prior to the onset of each human infection. The temporal windows ranged from 1 to 21 days, and the lags range from 0 to 17 days prior to onset. An example of a combination of 12 days prior with a 2-day window is shown in Figure 12.

Results

Figure 13, Figure 14 and Figure 15 show the mapped results of the Monte Carlo DYCAST analysis results for July 26th, July 31st and August 4th 2002. The figures show clearly that close space-time dead bird activity commences from specific foci (center north for July 26th and south west for July 31st) and gradually diffuses from those foci and increasingly covers more areas. This provides further evidence that West Nile activity is not widespread but spatially and temporally specific. The significance of this early identification of spots of activity is significant for two reasons; (1) Health authorities can closely monitor and control those areas before the activity spreads and, (2) retrospective studies can later on investigate the environmental reasons that facilitate the initiation of this activity and its spread.

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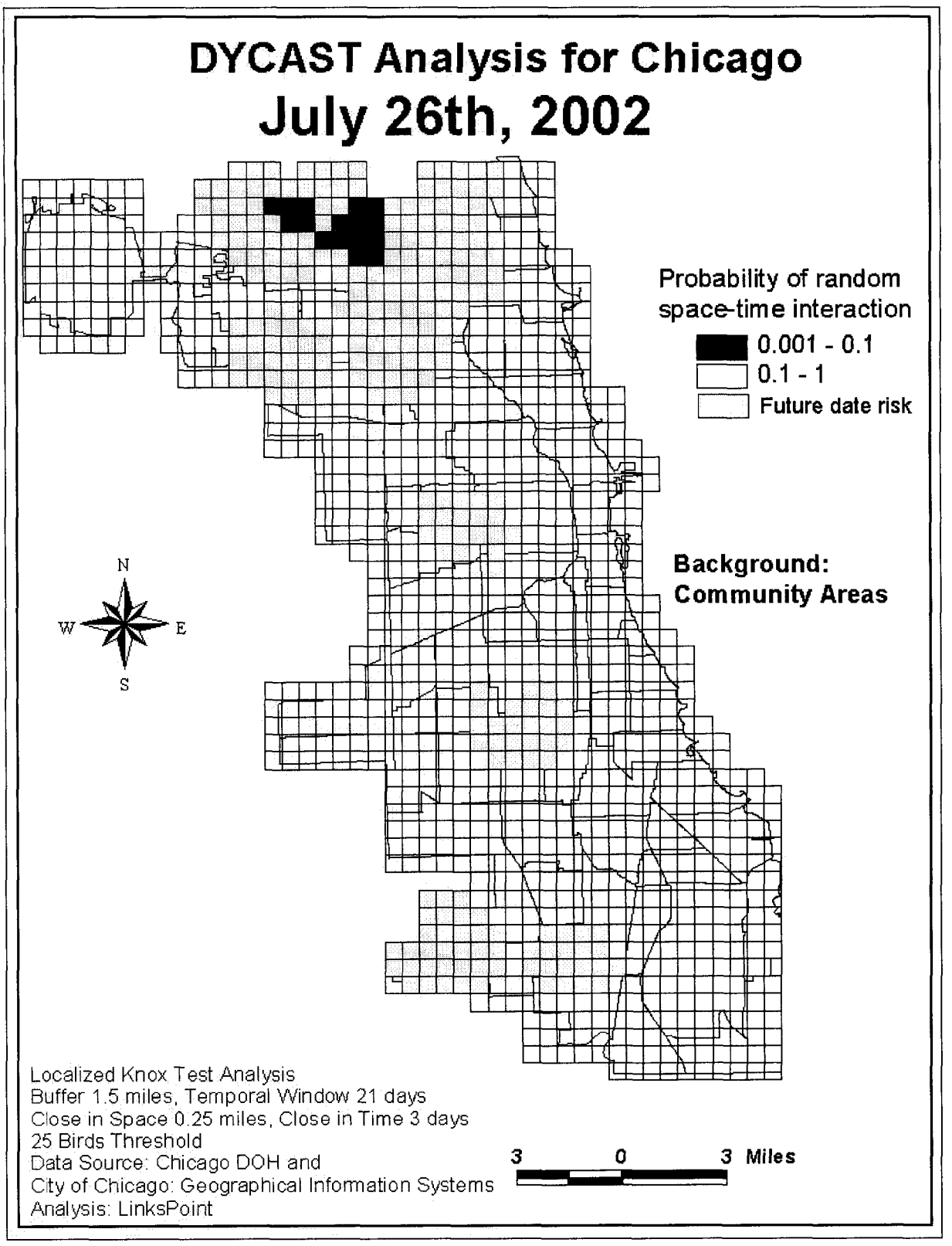

Figure 13. Map of July 26th, 2002 Chicago Analysis.

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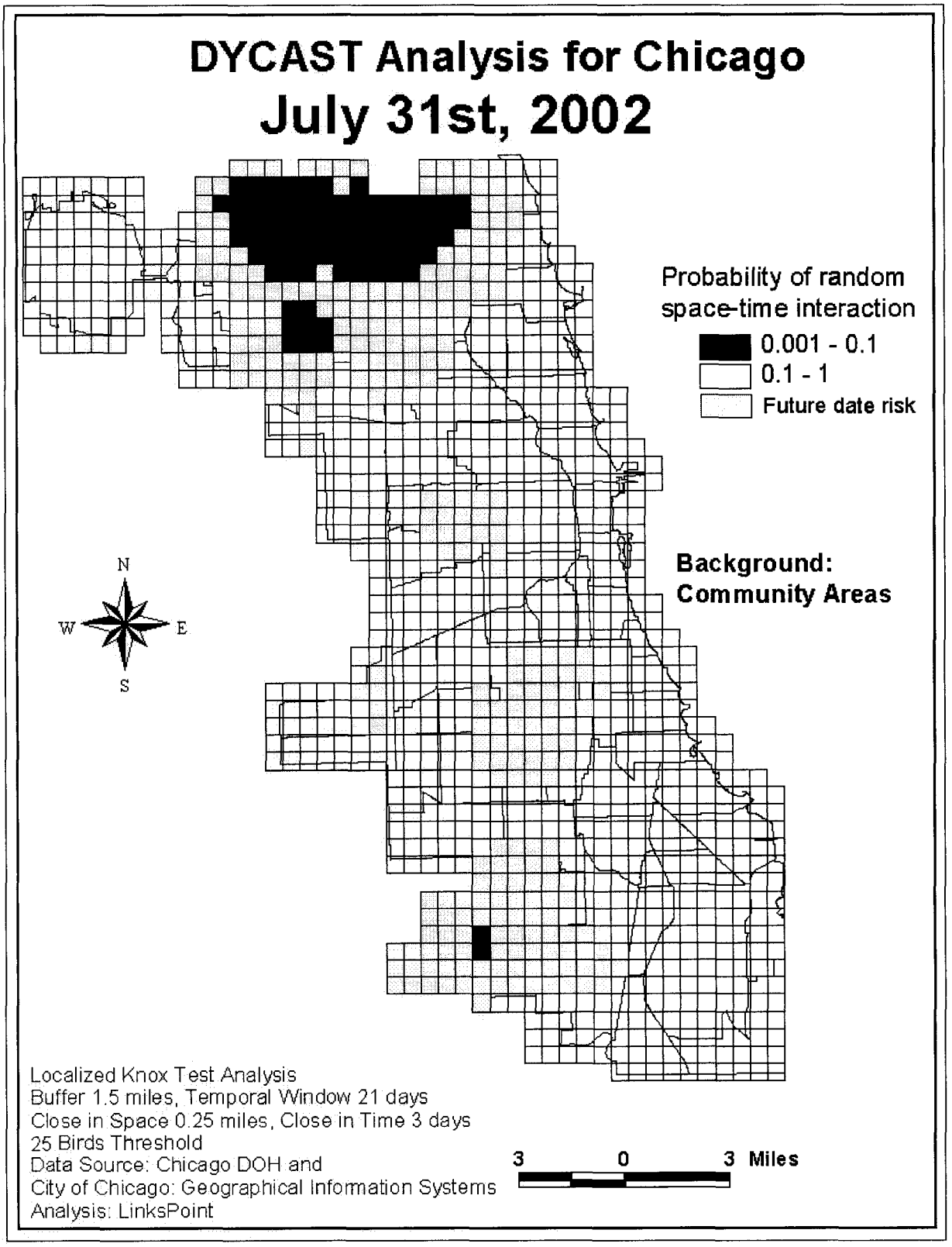

Figure 14. Map of July 31st 2002, Chicago analysis.

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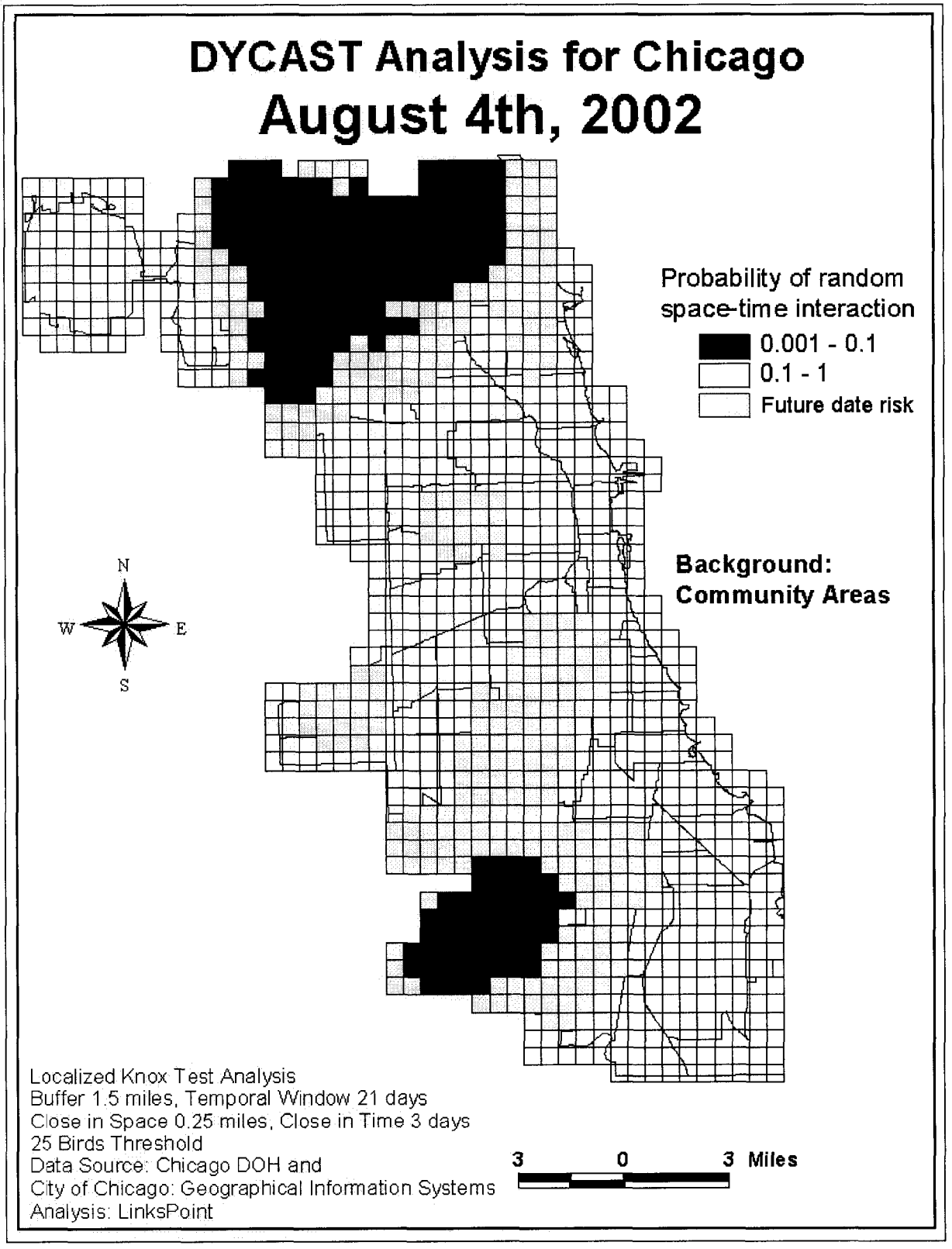

Figure 15. Map of August 4th 2002, Chicago analysis.

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Percent Success.

The results show that 14 days or more, prior to the onset of illness, risk was identified in 79.14% of 0.162sq.km (0.25 square mile) cells in which human cases appeared Figure 16). In 84.19% of cells containing human infections, the risk was consistently shown to appear for at least 10 days (Figure 17). This means that risk was identified prior to the onset of human cases, and existed for many days. In fact, for 76.18% of the cells in which human cases occurred, risk was shown at least 15 days before onset, possibly before the humans were bitten by mosquitoes, assuming the widest range of incubation is 3-15 days (Olejnik 1952).

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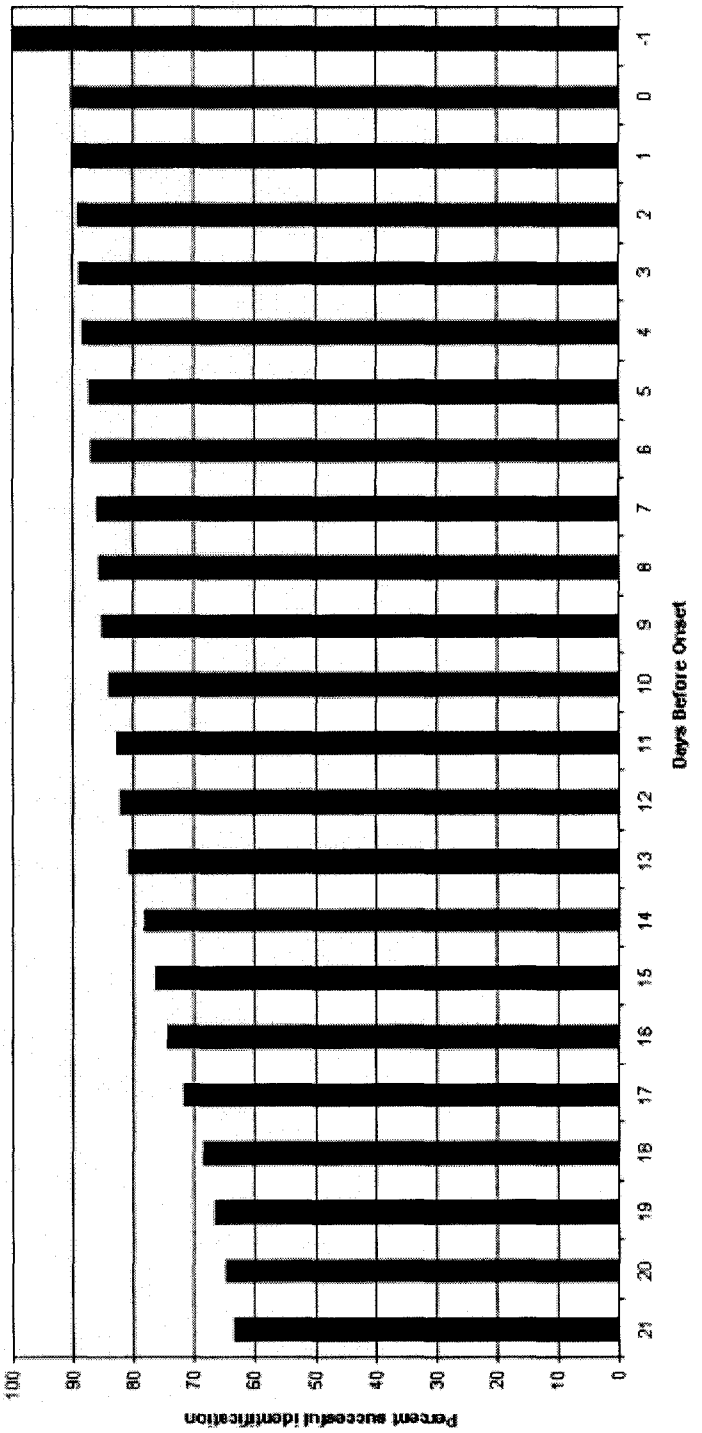

Figure 16. Cumulative histogram of number of days prior to onset of human illness, risk was identified in those areas.

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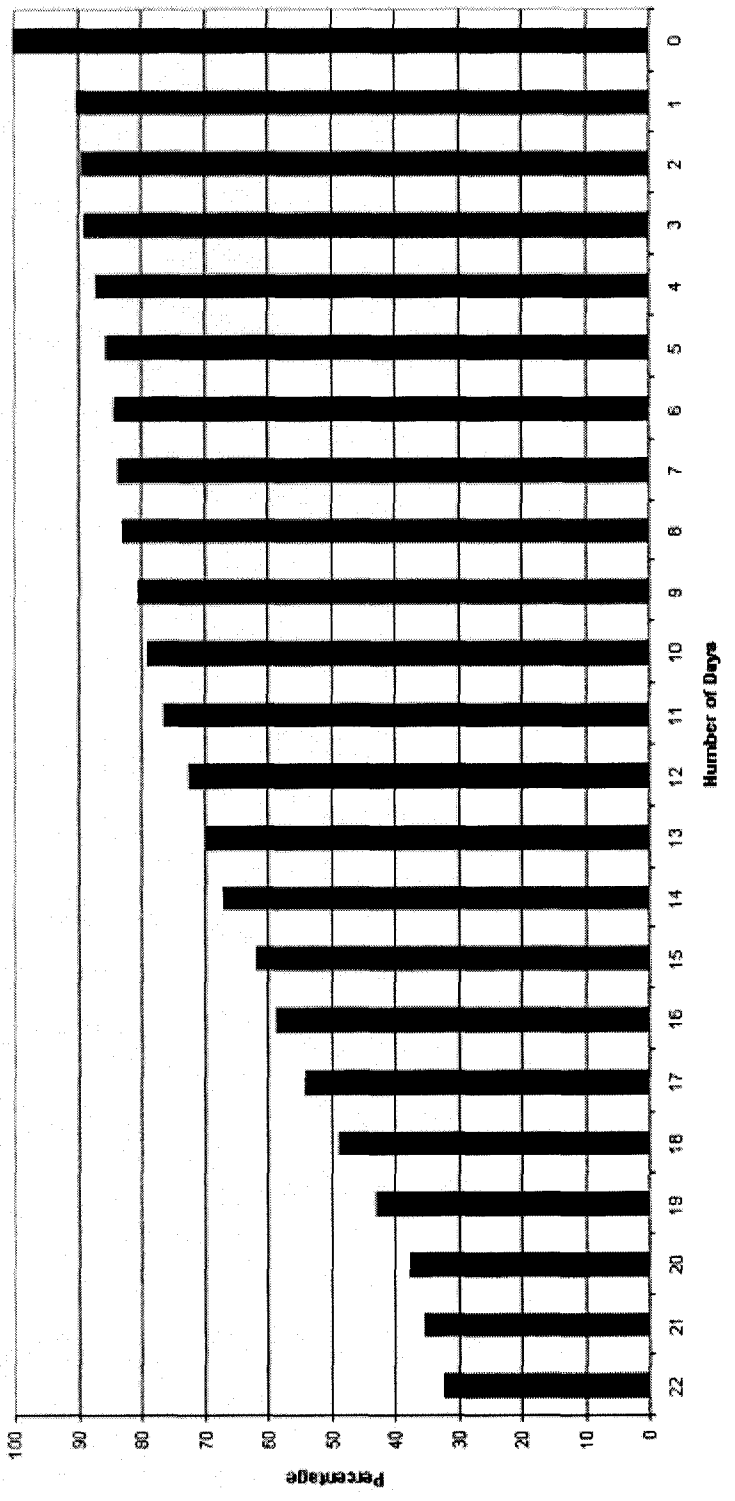

Figure 17. Cumulative histogram of number of days risk identification was sustained prior to human illness.

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Kappa results.

Figure 18 shows a surface created out of kappa values, which indicate non-chance agreement between human cases and close space-time bird deaths, for a range of different combinations of number of days prior to onset of human illness (henceforth called days prior) and window sizes (Figure 12). All kappa values of more than 0.025 were statistically significant. The results clearly show that there is a statistically significant spatio-temporal relationship between non-random space-time interaction of bird deaths and human illnesses for a limited range of combinations of days prior to the onset of illness and window sizes. The strength of this relationship varies with days prior to onset and window size.

The variation in the strength of this relationship is more clearly seen if one considers the combinations of window size and days prior that have non-chance agreement of over 50% (kappa >= 0.5, Table 7). These combinations are bound by an upper and lower limit of days prior to onset and are characterized by an increase in the kappa value to a maximum followed by drop-off as the day of onset of human illness approaches (Figure 18, Table 7). The highest kappa values in descending order occur for windows of 2, 3, 1, and 4 days and this is consistent with the fact that viremia-sufficient to infect mosquitoes-in crows and blue jays lasts for 1-4 days (Komar et al. 2003). The maximum kappa value of 0.59 occurs at 12 days prior with a two-day window (Figure 18, Table 7). Adjusting for a one-day reporting lag (by the public), the maximum non-chance agreement occurs during days 13 and 14 prior to onset of illness. Based on this and the fact that peak viremia occurs 1-2 days prior to bird deaths (Komar et al. 2003) it can be deduced that the maximum pool of virus in the avian hosts that die, occurs at approximately 15 to 16

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days prior to the onset of human illness. Assuming that mosquitoes constantly feed on all available birds, this peak die-off also represents the peak of the amplification cycle and the time the maximum pool of the virus circulates in the entire avian population. Hence, it is the most likely time that mosquitoes that bridge the virus to humans became infected. This is consistent with the mean of the extrinsic incubation period in mosquitoes of 4-12 days (Dohm et al. 2002b) and the intrinsic incubation period in humans. Human intrinsic incubation periods are reported to range from 2-6 days (Goldblum et al. 1954) to 3-15 days (Olejnik 1952), though the exact range is still unknown (Petersen et al. 2002).

The period of likely human infection is preceded by an increase of the kappa value to a maximum and coincides with a downward trend of the kappa values that leads to a drop of more than 30% by day 4 prior (Figure 18, Table 7, 2-day window). This is a statistical manifestation of the temporal characteristics of the amplification cycle. The downward trend in kappa is a result of fewer birds dying close in space and time as the onset of human illness is approached. It is thought that the decreased dead bird activity is due to a localized reduction in the bird population. Because of this reduction, the *Culex* pipiens and other species mosquitoes (that have increased significantly in numbers by now due to peak availability in blood meals) that feed on both birds and mammals (Fonseca et al. 2004) are more likely to turn to dead end hosts like humans and other mammals for blood meals (so-called 'spill-over' effect). These results suggest that by the time a human develops symptoms of West Nile virus disease the amplification cycle has been disrupted in that local area due to a die off of a substantial number of competent hosts.

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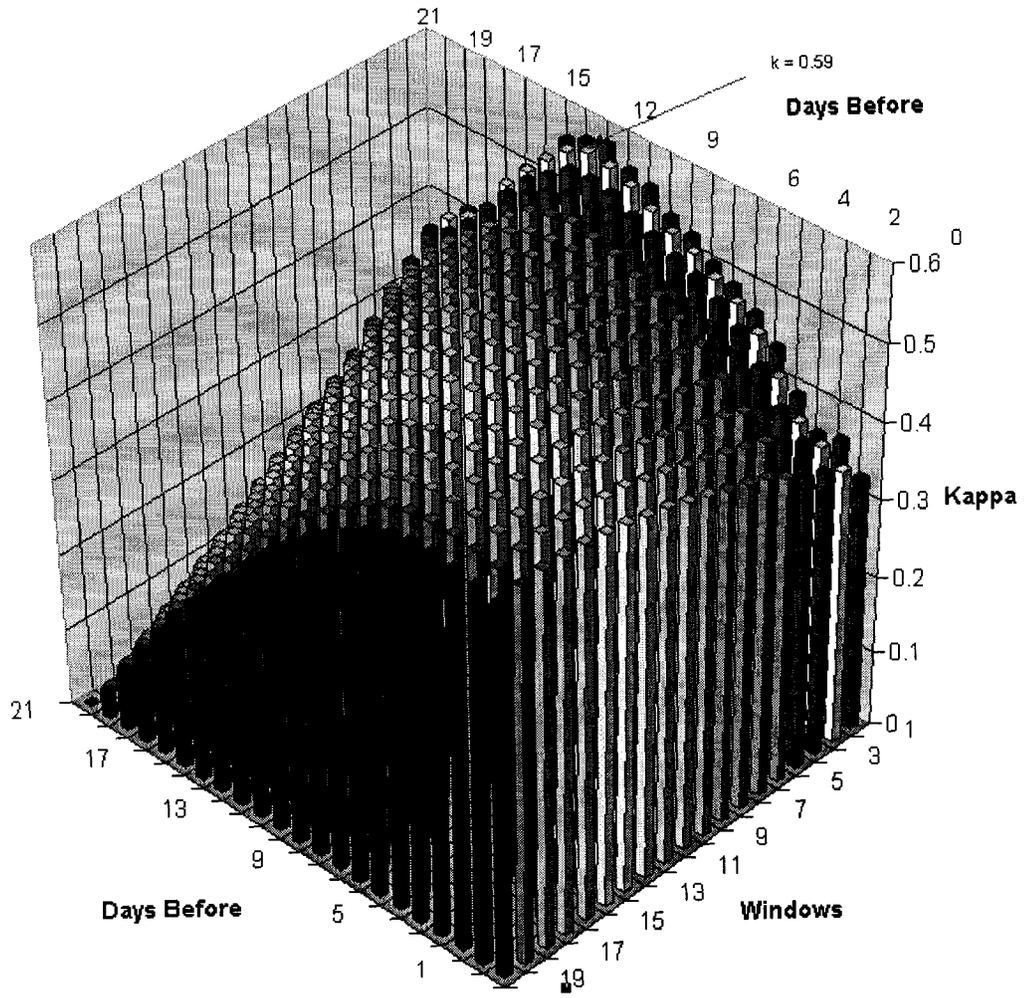

Figure 18. Surface of kappa values for varying combinations of days prior and windows.

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Table 7. Kappa agreement table.

Days prior	Windows 19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
21	-0.01	0.008	0.026	0.044	0.062	0.078	0.096	0.114	0.13	0.141	0.157	0.175	0.192	0.204	0.219	0.255	0.295	0.321	0.358
20	0.037	0.054	0.072	0.091	0.107	0.125	0.143	0.164	0.18	0.197	0.215	0.233	0.251	0.267	0.298	0.333	0.354	0.382	0.381
19	0.082	0.1	0.118	0.135	0.153	0.171	0.192	0.213	0.231	0.25	0.268	0.288	0.307	0.336	0.366	0.387	0.408	0.414	0.41
18	0.124	0.142	0.158	0.176	0.195	0.215	0.236	0.258	0.277	0.295	0.315	0.334	0.361	0.388	0.405	0.423	0.425	0.429	0.419
17	0.166	0.182	0.2	0.218	0.238	0.258	0.28	0.302	0.321	0.34	0.36	0.385	0.409	0.425	0.442	0.446	0.45	0.455	0.456
16	0.205	0.222	0.24	0.26	0.28	0.301	0.323	0.345	0.364	0.384	0.407	0.43	0.445	0.461	0.468	0.475	0.48	0.498	0.503
15	0.237	0.255	0.275	0.295	0.316	0.338	0.359	0.381	0.401	0.423	0.445	0.46	0.474	0.482	0.491	0.499	0.512	0.528	0.526
14	0.27	0.289	0.31	0.33	0.352	0.373	0.395	0.417	0.439	0.46	0.474	0.488	0.496	0.506	0.515	0.529	0.543	0.555	0.564
13	0.302	0.322	0.343	0.364	0.384	0.406	0.428	0.453	0.473	0.486	0.499	0.508	0.517	0.527	0.541	0.554	0.563	0.579	0.577
12	0.334	0.354	0.375	0.395	0.416	0.437	0.461	0.484	0.497	0.51	0.518	0.527	0.536	0.549	0.562	0.57	0.583	0.591	0.584
11	0.363	0.383	0.403	0.423	0.444	0.467	0.488	0.504	0.517	0.524	0.532	0.541	0.552	0.563	0.57	0.58	0.584	0.587	0.566
10	0.39	0.409	0.429	0.449	0.47	0.491	0.506	0.521	0.529	0.536	0.543	0.553	0.561	0.567	0.576	0.578	0.58	0.575	0.558
9	0.413	0.432	0.451	0.472	0.492	0.506	0.52	0.53	0.537	0.543	0.551	0.558	0.562	0.568	0.57	0.571	0.566	0.559	0.535
8	0.435	0.453	0.473	0.491	0.505	0.518	0.527	0.537	0.543	0.549	0.554	0.557	0.561	0.562	0.562	0.557	0.555	0.545	0.526
7	0.453	0.472	0.49	0.503	0.515	0.524	0.532	0.541	0.547	0.55	0.551	0.555	0.554	0.553	0.549	0.546	0.542	0.534	0.503
6	0.471	0.487	0.499	0.511	0.519	0.527	0.535	0.543	0.546	0.546	0.548	0.547	0.545	0.541	0.538	0.534	0.531	0.516	0.49
5	0.484	0.495	0.506	0.513	0.52	0.527	0.535	0.541	0.54	0.541	0.539	0.536	0.531	0.528	0.523	0.52	0.514	0.498	0.468
4	0.49	0.499	0.506	0.513	0.519	0.526	0.531	0.533	0.534	0.531	0.527	0.522	0.517	0.511	0.507	0.5	0.494	0.474	0.441
3	0.493	0.498	0.504	0.51	0.516	0.52	0.521	0.521	0.517	0.513	0.506	0.5	0.493	0.486	0.479	0.471	0.46	0.433	0.393
2	0.491	0.495	0.5	0.505	0.509	0.509	0.507	0.504	0.499	0.492	0.485	0.476	0.469	0.46	0.452	0.441	0.422	0.391	0.359
1	0.488	0.492	0.496	0.499	0.499	0.497	0.492	0.487	0.481	0.473	0.464	0.456	0.447	0.438	0.429	0.412	0.395	0.375	0.365
0	0.483	0.486	0.488	0.488	0.486	0.481	0.475	0.468	0.461	0.452	0.443	0.433	0.424	0.414	0.399	0.383	0.373	0.359	0.332

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Conclusions

Through the use of geostatistical techniques evidence has been provided that corvid deaths are an integral part of the local West Nile virus amplification cycle and that this cycle peaks 15-16 days prior to the onset of human illness. This is followed by a gradual reduction of the amplification activity shown by the reduced number of birds dying close in space and time. This appears to coincide with the time during which humans become infected.

What it is not known is whether this decrease of activity is because of significant bird die-offs, bird migration or a combination of both. However, there is now evidence that crows are dying in significant numbers by West Nile virus (Yaremych et al. 2004), at least in Illinois. In the City of Chicago, corvids populations were reduced by 82% as a result of the 2002 epidemic (Watson et al. 2004); while West Nile virus infections in humans in 2003 were less than five percent of the 2002 human infections. In addition, the amplification process and human infections are highly depended on the predilection of the species of mosquitoes to feed on birds or humans and their feeding behavior needs to be studied further at the local level; however, there is evidence that the *Culex pipiens* are capable on feeding on both humans and birds (Fonseca et al., 2004).

It is cautioned that the findings of this study should not be interpreted as the only process operating behind West Nile virus human infections globally. West Nile virus is a newly introduced agent in the United States and this could be viewed as evidence of a process operating on a naïve amplification host population.

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CHAPTER VI

A COMPARISON BETWEEN THE CHI-SQUARE KNOX TEST AND THE UNCONDITIONAL MONTE CARLO EXTENSION OF THE KNOX TEST

Introduction.

The occurrence of human disease is often presented as point data. Those points can also be labeled with time (or date) of appearance. It is often of interest whether there is clustering in either the space and/or time dimension for these points. Geographers usually examine the spatial clustering component (Haining 2003) and epidemiologists are more interested in the temporal sequence of occurrence of the disease events (Lawson, 2001). Clustering is often only evident if both dimensions are examined simultaneously (Knox, 1963). Knox (1963, 1964a, 1964b) introduced a way of detecting spatial and temporal interaction between disease incidents and inferring an infectious process from this space-time interaction (i.e. Andersson et al, 1995).

The Knox test consists of creating all possible pair combinations of data points found in a spatial and temporal domain and then examining the interpoint distances in space and time and classifying the pairs as close or not in either space or time. The total number of pairs found close in space and time is compared to what would be expected by chance given the individual number of close space and close time pairs. Knox (1963) initially proposed a chi-square test for evaluating the significance of the interaction and then a Poisson test (Knox 1964a). Both of these tests require that the data (pairs) being

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evaluated are part of an independent process. Because the pairs are formed by a finite number of points and they share points this requirement is violated (Knox 1964a). Moreover, excess clustering in either the space or the time dimension of the data can exacerbate this violation, by resulting in underestimation of the variance.

Mantel (1967) proposed a solution to the significance test that involved the Monte Carlo switching of the space-time labels of the data points and then ranking the actual number of close space-time pairs to the Monte Carlo distribution. Barton and David (1966) found this to result in an approximate Poisson distribution but this only applied to that specific case (Williams, 1984). However, with heavy clustering in either the space or time dimension the switching of already close labels tends to influence the variance of the resulting Monte Carlo distribution. In Chapter V, a completely random, unconditional Monte Carlo distribution of points within the spatial and temporal domain of the dataset was introduced which was based on the Knox. The probability of randomness was defined as the proportion of the upper right hand tail of the pair distribution whose number of close space-time, close space and, close time pairs were greater than or equal to the respective actual counts: an unconditional extension of the Knox test.

Most of the applications of the Knox test have been performed retrospectively in single areas, but recently Rogerson (2001) has presented a prospective local area version of the test. Theophilides et al. (2003) implemented the Knox test dynamically over local areas and customized it to model a process, the amplification cycle of West Nile virus in the wild (DYCAST methodology). West Nile virus is a mosquito born agent that under

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favorable environmental conditions cycles and amplifies between birds and mosquitoes. When the cycle of infection is intense, significant amplification of the virus numbers occurs and it spills-over to humans causing illness in the elderly and the immune-weak (Campbell, 2002). In Theophilides et al. (2004), the ability of the modified Knox test (with an unconditional Monte Carlo methodology) to predict human risk for West Nile virus was quantified using a kappa methodology and proved that the method models the amplification and transmission to humans process.

Here the same methods and data are used to compare the Monte Carlo results of Chapter VI with a chi-squared test of the Knox in terms of their discriminatory power to "predict" human risk. The agreement between the two tests in terms of identifying West Nile risk areas is evaluated directly.

Materials and Methods.

Data were provided by the Chicago Department of Health and included the location of dead birds (mostly crows and blue jays, n = 3837, henceforth called the data points) and their date of reporting, and the location of residence and date of onset of illness of humans (n=215) confirmed with West Nile infection.

Methods.

The DYCAST method of Theophilides et al. (2003) and the methods from Chapter V were followed. The DYCAST method partitions space into overlapping local units of ecologically relevant spatial and temporal domains that consist of a 1.5-mile radius and

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the 21 days prior to the current date. This space-time domain was centered about each 0.5x0.5mile grid cell that was laid across the study area (City of Chicago). A Knox chi-square analysis and a Knox based unconditional Monte Carlo analysis was run using all the data points found within the spatial and temporal domains of each cell centroid for each day between June 30th, 2002 and October 5th, 2002. Two probability values were recorded for each day for each cell; one using a chi-square Knox test and one using an unconditional Monte Carlo simulation (Chapter V).

Knox Test.

The mathematical expression of the Knox test is shown Equation 1 as:

$$T(o_{11}) = \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} s_{ij} t_{ij} ,$$

where: s_{ij} and t_{ij} are 1 if the pair formed by the i^{th} and i^{th} point is close in space (interpoint spatial distance <= s) and time (interpoint temporal distance <= t) respectively, and n is the total number of points found in the geographic and temporal space. The critical spatial distance was defined as 0.25 miles (s=0.25m) and the critical temporal distance was 3 days (t=3 days).

Chi-Square test

To assess the significance of the test statistic using a chi-squared test a matrix is formed and the resulting chi-squared value was inverted based on a chi-squared function with one degree of freedom to obtain a probability (Ross 1999, 267).

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Unconditional Monte Carlo test.

For a number of points n found within the Spatial Domain and Temporal Domain, 5000 random sets of n points were generated as independently distributed according to the uniform distribution over the space-time domain. For each set, the number of close space-time pairs (o_{11}), the number close space pairs ($o_{11} + o_{21}$) and, the number of close time pairs ($o_{11} + o_{12}$) were counted. Based on the distribution of these 5000 random runs the probability level was assessed as in equation 3:

$$p = P\{(st \geq ST) \cap (s \geq S) \cap (t \geq T)\}$$

where S, T, and ST are the actual number of points found close in space, time, and both respectively, with s, t, and st being the counts in the random distribution.

Comparison methodology

In this research it is of interest to evaluate the DYCAST results obtained with different statistical tests based on Knox method (chi-square Knox and unconditional Monte Carlo) for assessing (1) the discriminatory power of theses tests to successfully identify areas where human cases occurred prior to their onset of illness and (2) the agreement between them.

To accomplish this the new implementation of the kappa test that has been adapted for a spatial and temporal domain was used (Chapter V). In addition percent success and duration of risk histograms were constructed as in Chapter V for both the unconditional

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extension of the Knox test and the chi-square Knox significance test. The kappa test was introduced by Cohen (1960) as a means of measuring the agreement of classification of data into diagnostic categories between two judges (or raters) after chance agreement was excluded. Later, Congalton and Mead (1983) implemented the kappa over spatial data in Remote Sensing as a means of assessing the accuracy of image classification.

The general form of the kappa statistic was defined in Equation 5 as:

$$\hat{\kappa} = \frac{N\sum_{i=1}^{r} x_{ii} - \sum_{i=1}^{r} (x_{i+} * x_{+i})}{N^2 - \sum_{i=1}^{r} (x_{i+} * x_{+i})},$$

where:

N is the total number of areas considered,

and x_{ii}, x_{i+}, x_{+i} are the elements of the matrix shown in Table 6:

	Rater 1		
Rater 2		Class 1	Class 2
	Class 1	x_{11}	x_{12}
	Class 2	x_{21}	x_{22}

The sum of which amounts to N.

In the spatial and temporal implementation of kappa in Chapter V a kappa value is calculated for all unique combinations of a range of days prior to onset of illness in

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humans and a range of temporal windows in order to find the date and duration of maximum non-chance agreement between DYCAST results and human illnesses. The total number of cells for any unique combination of days prior to onset and a window is expressed as:

$$N = \sum_{d=June30th,2002}^{October5th,2002} G * I \qquad \text{(Equation 4)}$$

where:

G is equal to 1181, the number cells of the Chicago grid and

I is an indicator function which can be evaluated as follows:

(1) *I = 1* if for dates(*d+p days) to(d+p days +(w-1 days)* there is at least one human case. where: *p* is the number of days prior to onset and *w* the window size.

(2) *I = 0* otherwise.

The calculation of the kappa values and their respective chi-square test was done over a combination of *p=0 to 21* and *w=1 to 19.*

To evaluate direct non-chance agreement between the Knox chi-square test and the Monte Carlo test, summed all the cells with agreements (and disagreements) over the whole period were summed and a kappa table as the one shown before was constructed.

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Results

The cumulative percent success and duration of risk histograms are shown in Figure 19 and Figure 20. Figure 19 shows that 10 days prior to the onset of illness in humans, more than 80 percent of the cells in which human cases appeared were shown at risk with the unconditional Monte Carlo extension of the Knox test. For the same 10 days prior, the chi-square Knox test identified less than 50 % of the same cells. The percentage of successful risk identification is consistently less for all days prior with the Knox chi-square test.

Figure 20 shows that the persistence of risk is considerably less with the Knox chi-square test. For example, less than 20% of the cells in which human cases appeared had risk persistence with the chi-square Knox test for 10 days. The persistence of risk with the unconditional Monte Carlo extension of the Knox for the same 10 days was nearly 80 percent.

These figures (Figure 19, Figure 20) indicate that the unconditional Monte Carlo extension of the Knox test is more sensitive in identifying West Nile virus risk and more consistent than the chi-square Knox test.

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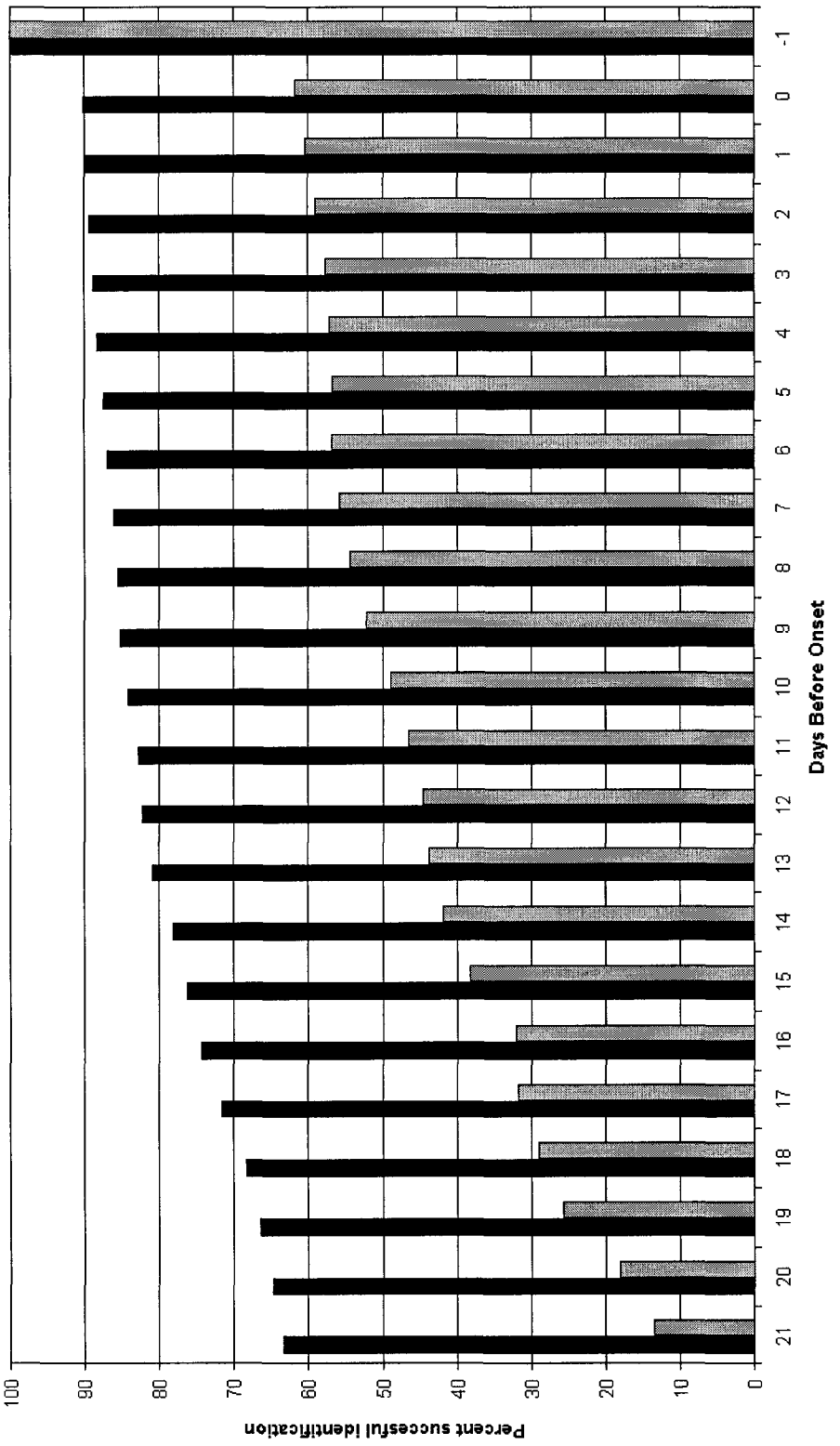

Figure 19. Comparative histogram of cumulative percentage of successful identification of risk for the unconditional Knox test (black) and chi-square Knox test (blue).

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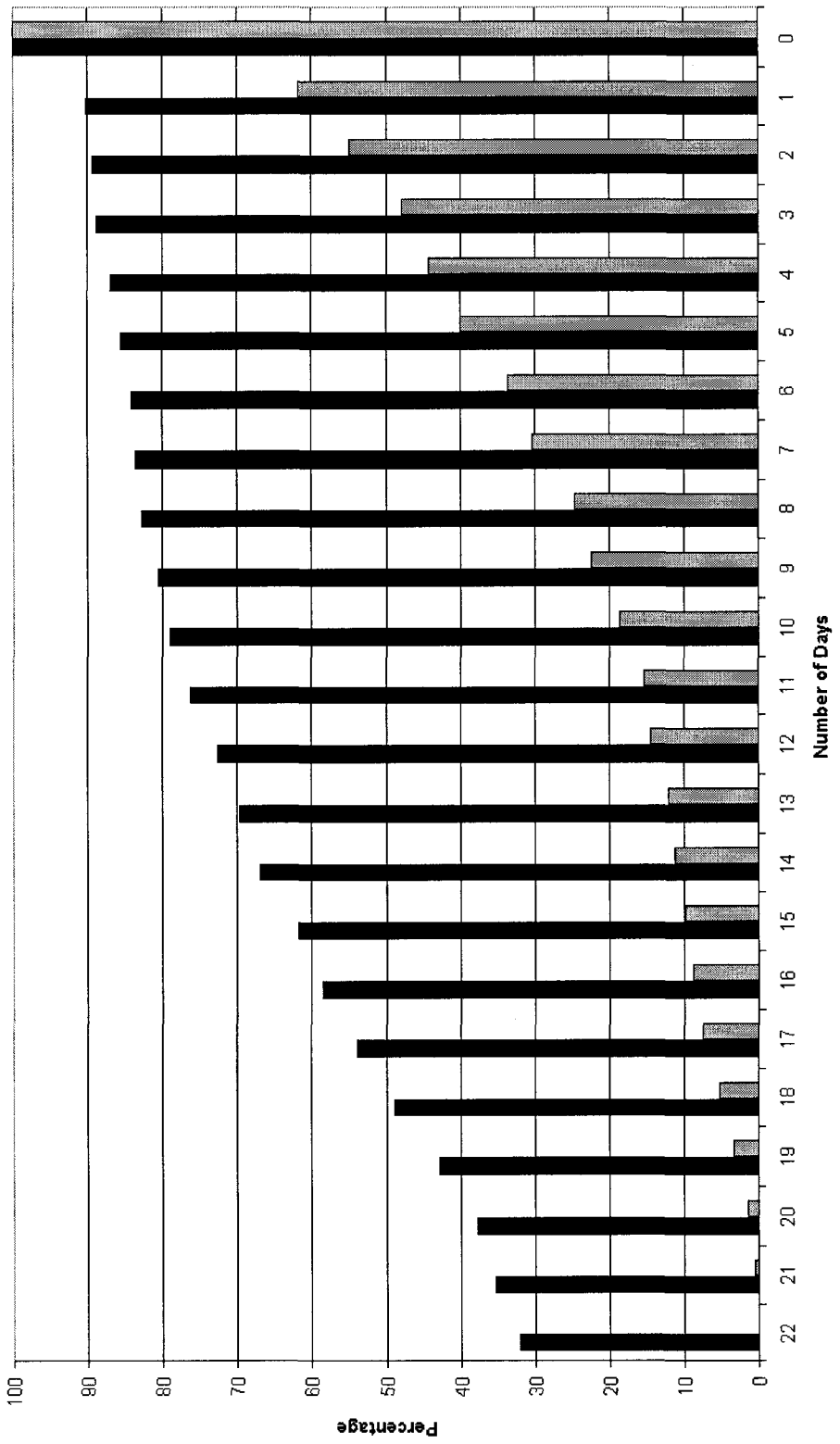

Figure 20. Comparative histogram of persistence of risk identification between the unconditional extension of the Knox test (black) and the chi-square Knox test (blue).

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The results of the non-chance agreement between the human cases and the DYCAST results with the unconditional Monte Carlo method were presented in Chapter V. They were shown as a surface of values for variable size windows and days prior to onset (Figure 18). From those results, it was deduced that peak viremia in the wild birds (corvids) occurs 15-16 days prior to the onset of illness in humans. The structure of the surface also showed that bird deaths start to decrease after the peak period and consistently decrease up to the date of onset. This has attributed the human infections to the decrease of birds available for mosquito blood-meals and the subsequent turn of mosquitoes to humans at approximately day 7 prior to onset and provided evidence for the hypothesis first posed by Despommier (2001).

A similar surface showing the agreement between the DYCAST chi-square results and the human cases is shown in Figure 21 (at a different angle from that of Figure 18). The surface shows that the kappa values are lower than those of the unconditional Monte Carlo DYCAST results by approximately 0.4 (or a chance agreement difference of 40%). The approximate structure of the surface remains the same but the highest kappa value of 0.1592 occurs for a window of 1 day for 11 days prior to onset.

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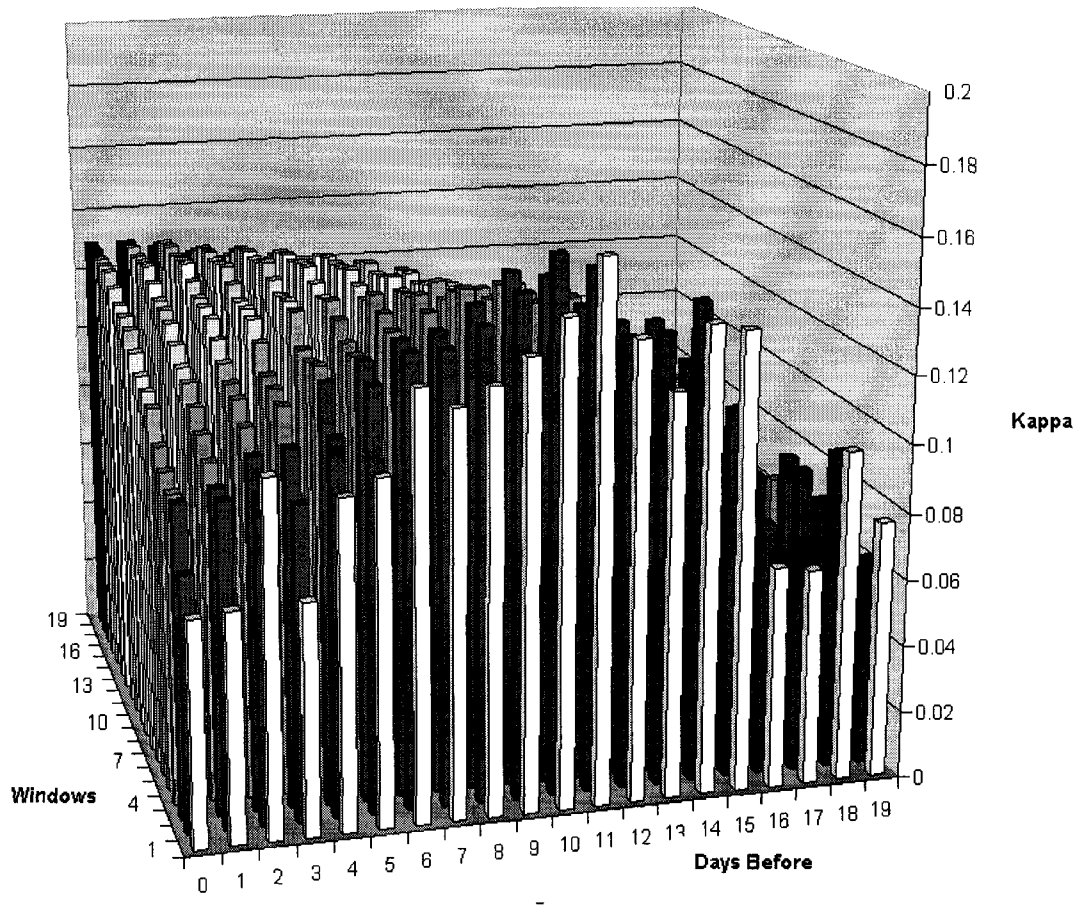

Figure 21. Chi-Square resulting kappa surface.

The surface from the chi-square approximation of the Knox statistic retains the structure of gradual increase and decrease of kappa values. As the window decreases from 19 to 1 days, the peak kappa value shifts to higher days prior to onset, verifying the approximate time of highest viremia to be around 15 days prior. However, as the windows become smaller and reach two and one days the structure of the surface becomes inconsistent, even though the trend of the surface is retained. This is a visual manifestation of the use of a chi-square Knox test which assumes independence for testing a pair dependent dataset. The same probably occurs for larger window sizes, but its only manifestation

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then are the lower kappa values. The analysis with large window sizes includes more temporally autocorrelated cells that average out any fluctuations.

The direct evaluation of non-chance agreement between the Knox chi-square method and the unconditional Monte Carlo Knox extension shows a kappa value of 0.52 ($p < 0.001$). This means that only about half the agreement between the two methods is not due to chance (Table 8).

Table 8. Kappa agreement table between unconditional Knox Monte Calro extension and Knox chi-square

	Monte Carlo		
Chi-square		West Nile risk	No West Nile risk
	West Nile risk	7134	47
	No West Nile risk	10866	97691

Table 8 shows that only 47 cells were shown as at risk with the chi-square method and not with the Monte Carlo method. In contrast, 10886 cells were shown as at risk with the Monte Carlo method and not with the chi-square method. This, combined with the high kappa value with the human cases (Figure 18) is evidence that the Monte Carlo method is a more sensitive measure of evaluating non-random space-time interaction and a more

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accurate one. The largest number of cells in agreement between the two techniques were in the not at-risk category (97691, Table 8).

Conclusion

Quantitative evidence was presented that shows that the widespread use of the chi-square test for evaluating the significance of space-time interaction of the Knox method (1964a) is inappropriate. For smaller granularities of time, it renders results with a high degree of variability. Knox (1964a) first identified the potential pitfall of this statistic due to pair dependencies and in Chapter IV, this was observed from the results of a practical application.

In the case of using the Knox method and the Monte Carlo method with DYCAST, the unconditional Monte Carlo method for evaluating significance, is more sensitive and gives results that more accurately follow West Nile virus activity. This is verified by cross-referencing of the results with human cases. The non-chance agreement of the Monte Carlo DYCAST results with chi-square DYCAST results is almost 50% although both methods accurately show where activity has not occurred.

It is cautioned that more studies may be needed to fully understand the distribution of the Knox statistic both at the theoretical level and for practical applications.

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CHAPTER VII

CONCLUSIONS

The ecology of infectious disease encompasses a complex set of relationships among the environment, the vector and the host across space and time making its modeling a difficult task. This research has demonstrated that the parameterization of statistical techniques and their application in a dynamic geographic model is successful in providing evidence for the West Nile virus transmission process. It was shown that there is significant quantitative evidence to establish the link between local bird deaths with human epidemics of West Nile virus. In the urban environment, amplification (manifested in close space-time bird deaths) peaks 15-16 days prior to the occurrence of human West Nile related disease and is followed by a drop of more than 30 % by day 7 prior and this newly proven knowledge is perhaps the single biggest contribution. These results are consistent with already known incubation periods in mosquitoes, birds and humans. This helps to differentiate between the amplification portion of the cycle, a phenomenon that builds progressively in the bird hosts, and the epidemic portion of the cycle, that culminates in human and mammalian involvement as the result of amplification by bird feeding mosquitoes.

As much as this research aimed at proving this relationship, significant contributions have been made towards Geographic Information Science and took-making. The measure of amplification (close space-time bird deaths) and its implementation is an

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incredibly robust method of identifying West Nile virus activity under different significance tests.

For the first time, the space-time domain and the closeness parameters of the Knox test were based on real world information regarding the ecology and pathology of the disease in and between the vector and hosts. This novel approach solved the problem of arbitrary selections that may produce faulty results. These results could occur when the selection of the parameters leads to the identification of space-time interaction at a scale that may not be meaningful for the problem under study. In addition, the selection of only one combination of space-time parameters (within an ecologically meaningful domain) that produces maximum coincidence of close space-time dead birds, with the human cases (Table 4) ensures that the multiple testing problem of the Knox (1964b) is resolved.

In addition, the use of equally sized overlapping domains as suggested by Tobler (1979) reduces the *change of support* problem. The selection of domain separation distance is adapted from Remote Sensing (Sonka et al. 1998) and ensures that no information is lost during the construction of the space-time interaction significance surface. The dynamic daily increments warrant that West Nile virus activity is tracked accurately over time. The latter is very significant for not only remediation and control purposes, but for future studies that would investigate the factors affecting the origin and spread of West Nile virus in large geographic scale analyses.

The methodology of evaluation of the close space-time bird deaths and human infections is equally significant. While in Remote Sensing the kappa coefficient (Congalton 1991)

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is routinely used in its spatial form, this is the first time it was applied in a space-time domain for identifying a biological relationship. The use of varying size temporal windows and days prior (Figure 12) helped isolate the period of maximum amplification activity and the gradual increase and decrease of the resulting surface of kappa values verifies that this is the result of a physical/biological process.

Last but not least, this research has revived, improved and, applied the Knox test in a computationally intensive environment, for a dynamic process. The approximate parametric and conditional Monte Carlo significance tests that suffer from over or under estimation of the variance due to spatial autocorrelation and lack of pair independence, were replaced by an unconditional Monte Carlo test. This test uses random independently distributed uniform sets of points in the space-time domain that reflects the actual distribution of the close space-time pairs and, their marginal individual space and time distributions. This allows the probability of the margins to be fixed and included in the test rather than allowing them to bias the results. On more practical matter the unconditional extension of the Knox will capture instances where all pairs are close in space and time as an epidemic outbreak whereas old Knox test may not.

Future Research.

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This research has generated a lot more questions that it has answered. There are several issues that have arisen from the results of this research and other that have not been examined. Issues that warrant further investigation and have resulted from this research include the evaluation of public reporting patterns, the patterns of initiation of dead bird activity from certain locations and its spread, and, the examination of the potential success of the dead bird analysis space-time model in other ecosystems including different hosts and vectors. The current research has not allowed for covariates in the process and this should be one of the next steps. Such covariates could take the form of investigation of weather patterns that can lead to West Nile virus outbreaks, the effect that different land covers can have on the abundance of the vector and the host, and the effects that different vectors can have on the amplification cycle and the spill over effect to humans. In addition to all, the re-examination and proof of the theoretical distribution of the Knox statistic is necessary from a statistical standpoint.

The success of any passive surveillance system is dependent primarily on public participation in residential areas and public places. The inclination of the public to report observed dead birds varies perhaps with population density and socioeconomic factors. No study has been conducted to investigate such claims and it could potentially result in tackling with the social fabric of cities as the hypothesis is that the lower socioeconomic classes would be less inclined to report dead birds. It is possible that when the concerns of people with daily living are heavy, they would be less interested in reporting a dead bird. In addition, in neighborhoods where illegal immigrants abound, there is probably less likelihood of people contacting the authorities (even the Health authorities).

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Population density would be less of a concern, but total absence of resident population (such as in parks) could result in serious underreporting. Surveillance in parks should be active. The benefit that would result from monitoring the most likely places of initiation of the West Nile virus amplification cycle outweighs the resource expense.

This research has shown that West Nile virus activity is location and temporally specific and that it initiates from specific locations and spreads in a contiguous mode. This expansion becomes evident at a very large geographic scale (i.e. at the 0.5 grid cell used). Despite this fact, even recent research insists on treating this as a small scale phenomenon (i.e. counties, (Brownstein et al. 2004). This small scale treatment can not provide insights in the biological dynamics of the outbreaks of the disease but could perhaps help explain the factors affecting the geographic spread. Future research should concentrate at a large geographical scale that would allow for covariates such as vegetation cover, type of housing and weather patterns. In addition, it would be of interest to examine, at this large geographical scale, the complicated vector-host relationships. It is most likely that this relationship is defined by a non-linear interplay because as West Nile virus activity reaches a different level its dynamics change (i.e. reduction of birds and human infections). Remote Sensing technology could be very useful as new commercial satellite sensors have the ability to capture sub-meter multispectral images. Furthermore, the warning system could be further optimized to take into consideration the residential locations of high at-risk populations such as retirement communities and neighborhoods.

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The surveillance system developed in this research and its evaluation were implemented and calibrated in one type of environment, the urban of the North East United States. It would be very useful to examine different ecological regions and analyze the cycle in those regions between different types of vectors. For example, it is thought that in the West where the primary vector is the *Culex tarsalis* there would be less of a warning by dead birds as its promiscuous nature does not deter it from seeking blood meals from any available host whether that is birds or humans. Hence, the peak and reduction of kappa values seen in this research could become a peak and then a flat line with data from the Western US.

Last but not least, the distribution of the Knox based Monte Carlo test statistic should be theoretically investigated and validated.

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