The Use of Geographic Information Systems (GIS) and the Chi-Square Statistic to Spatially and Statistically Analyze Trends in Chronic Wasting Disease (CWD) Across White Tailed Deer Management Units (DMUs) within the Current CWD Management Zone (CWD-MZ) in South Central Wisconsin

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Abstract

The increasing spread of Chronic Wasting Disease (CWD) across Southern Wisconsin has given rise to grave concerns about the spread of the disease, despite the best efforts put forth by the state and federal government and other agencies. With current technological advancements and the use of Geographic Information Systems (GIS), the spread of CWD can closely be monitored. This project focuses on the spread of CWD in deer management units (DMUs) within the current CWD management zone (CWD-MZ) from the years 2005 to 2009 in south central Wisconsin. The current CWD management zone is located in the south central quadrant of the state of Wisconsin. A brief history of CWD, its diagnosis, public health concerns, risk of transmission to humans (Epidemiologic Studies), and transmission to other animals are discussed. There are many aspects of the disease for which information is very limited. The ultimate goal is to keep collecting information on endemic areas in the state, and to spatially and numerically analyzing test results. Results derived from such analyses may then be used for better decision making.

Introduction

According to Medicinenet (2002), Chronic Wasting Disease can be defined as a transmissible spongiform encephalopathy (TSE) of North American deer and elk. It is a progressive neurodegenerative disorder that produces spongiform changes in the brain and causes chronic weight loss leading to the death of the infected animal. CWD is classified as a transmissible spongiform encephalopathy (TSE) and is similar to mad cow disease in cattle and scrapie in sheep. Infectious agents of CWD are neither bacteria nor viruses but are hypothesized to be prions. Prions are infectious proteins without associated nucleic acids (Medicinenet, 2002).

Belay, Maddox, Gambetti, and Schonberger (2003) suggest although CWD is a contagious fatal disease among deer and elk, research reveals humans, cattle, and other domestic livestock are resistant to natural transmission. While the possibility of human infection remains a concern, it is important to note there have been no verified cases of humans contracting CWD (Belay et al., 2003).

CWD reduces the growth and size of wild deer and elk populations in areas where the prevalence is high and is of increasing concern for wildlife managers across North America. The disease was long thought to be

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limited in the wild to a relatively small endemic area in northeastern Colorado, southeastern Wyoming and southwestern Nebraska but has recently been found in several new areas across the North American continent. The disease also has been diagnosed in commercial game farms in several states and provinces (Raymond, Bossers, Raymond, O'Rourke, McHolland, Bryant, 2000).

Brief History of CWD

Chronic Wasting Disease (CWD) has been associated with clinical syndrome of mule deer (*Odocoileus hemionus*) for more than 30 years; modeling suggests the disease may have been present in free ranging populations of mule deer for more than 40 years (Williams, 2005).

Susceptibility to the CWD prion has been documented in sheep through intra-cerebral inoculation. However no studies have been conducted to show that CWD can be transmitted to sheep through oral exposure or other vectors (Williams, 2005).

Timeline

According to MaWhinney, Pape, Forster, Anderson, and Bosque (2006), the origin of CWD is not known and it may never be possible to definitively determine how or when CWD arose. Though of academic interest, determining the origin is probably not very important from a management perspective; nonetheless, speculation continues.

It is possible, though never proven, that deer came into contact with scrapie agent either on shared pastures or in captivity somewhere along the front range of the Rocky Mountains where high levels of sheep grazing occurred in the early 1900s. However, CWD has never been identified in other areas of North America or other parts of the world where cervids and domestic sheep with scrapie must have co-mingled (MaWhinney et al., 2006).

Diagnosis of Chronic Wasting Disease (CWD)

Mathiason, Powers, Dahmes, Osborn, Miller, Warren, Mason, Hays, Hayes-Klug, Seelig, Wild, Wolfe, Spraker, Miller, Sigurdson, Telling, and Hoover (2006) finds clinical signs of CWD alone are not diagnostic. Definitive diagnosis is based on examination of the brain for spongiform lesions and accumulation of CWD associated protease resistant protein (PrPCWD) in brain and lymphoid tissues.

This test is based on use of monoclonal antibodies and chromogens to detect accumulation of PrPCWD in various tissues. The dorsal portion of the medulla oblongata is the most important site to examine for diagnosis of CWD because of its early involvement following infection. It is critically important that the correct portion of the brain be sampled for a meaningful test (Mathiason et al., 2006).

According to Raymond, Hope, Kocisko, Priola, Raymond, and Bossers (1997), CWD affected deer and elk show loss of body condition and changes in behavior. The clinical disease is often more subtle and prolonged in elk than in deer.

Affected animals may walk repetitive courses; they may show subtle ataxia and wide based stance and suffer from subtle head tremors. In addition they may be found near water sources or in riparian areas, may have periods of somnolence, may carry their head and ears lowered (Raymond et al., 1997).

Animals affected by CWD may continue to eat but amounts of feed consumed are reduced thereby leading to gradual loss of body health condition. Excessive drinking and urination are

common in the terminal stages because of specific lesions in the brain. Many animals in terminal stages of CWD have excessive salivation and drooling; this may result in wetting of the hairs of the chin and neck (Raymond et al., 1997).

According to Raymond et al. (1997), death is inevitable once clinical disease occurs. The clinical course of CWD varies from a few days to approximately one year with most animals surviving from a few weeks to several months.

Public Health Concerns

No cases of human prion disease have been associated with CWD. In fact, MaWhinney et al. (2006), confirms the incidence of Creutzfeldt-Jakob disease in humans living within 7 CWD endemic Colorado (U.S.) counties has not significantly increased between the years of 1970-2001 and no case of a human prion disease resulting from CWD exposure has ever been documented.

The tendency toward a natural "species barrier" reducing human susceptibility to CWD and other prion diseases has been demonstrated by in-vitro studies. These studies assess potential risks that CWD may pose to humans. Although there is a long history of human exposure to scrapie through handling and consuming sheep tissues, (including brain), there is no evidence this presents risk to human health. In contrast, British and European citizens exposed to the Bovine Spongiform Encephalopathy **(**BSE) agent resulted in approximately 106 deaths due to variant Creutzfeldt-Jakob disease as of February 2002 (Mathiason et al., 2006).

Risk of Transmission

Humans (Epidemiologic Studies)

According to Belay et al. (2003), Chronic Wasting Disease (CWD) of deer and elk is endemic in a tri-corner area of Colorado, Wyoming, and Nebraska, and new foci of CWD have been detected in other parts of the United States. Introduction of CWD due to translocation or natural migration of animals may account for some new foci of infection. Increasing spread of CWD has raised concerns about the potential for increasing human exposure to the CWD agent.

The food borne transmission of bovine spongiform encephalopathy to humans indicates the species barrier may not completely protect humans from animal prion diseases. Conversion of human prion protein by CWD associated prions has been demonstrated in an in vitro cell-free experiment, but investigations have not identified strong evidence for CWD transmission to humans. More epidemiologic and laboratory studies are needed to monitor the possibility of such transmissions (Belay et al., 2003).

Other Animals

Concerns have been raised about the possible transmission of the CWD prion to domestic animals, such as cattle and sheep, which may come in contact with infected deer and elk or CWD contaminated environments. If such transmissions were to occur, they would potentially increase the extent and frequency of human exposure to the CWD prion. In addition, passage of the agent through a secondary host could alter its infectious properties by increasing its potential for becoming more pathogenic to humans. This phenomenon may have occurred with BSE when a strain of scrapie, a possible original source of the BSE outbreak, changed its pathogenic properties for humans after infecting cattle. However,

the exact origin of BSE remains unknown (Belay et al., 2003).

Discussion

Research conducted by Belay et al. (2003) indicate ongoing studies involving transgenic mice expressing human and cervid prion protein are in progress to further assess the potential for the CWD agent to cause human disease.

Epidemiologic studies have also been initiated to identify human cases of prion disease among persons with an increased risk for exposure to potentially CWD infected deer or elk meat. If such cases are identified, laboratory data showing similarities of the prion to that of the CWD prion would strengthen the conclusion for a causal link (Belay et al., 2003).

In the meantime, to minimize the risk for exposure to the CWD prions, the general public should consult with their state wildlife agencies to identify areas where CWD occurs and continue to follow advice provided by public health and wildlife agencies.

Purpose

The main objective of this project was to spatially and statistically analyze the spread of CWD in the deer management units (DMUs) that are located within the current CWD management zone (CWD-MZ) in south central Wisconsin. This project serves as an instrument to further explore ongoing research in better understanding Chronic Wasting Disease.

Statistical and spatial analyses were used on the number of positive occurrences over a 5 year period to determine levels of significance to help understand CWD within the deer management units (DMUs). This was done to reinforce the spatial analysis portion of the project.

ArcGIS was used for spatial analysis portion, and visualization of the CWD affected areas.

Study Area

The project focused on the south central quadrant of the state of Wisconsin where the current CWD management zone (CWD-MZ) is located.

ArcGIS was used to display a Wisconsin outline displaying the 144 deer management units (DMUs) in the state. A closer look at the lower portion of the state containing the current CWD management zone (CWD-MZ) and the18 deer management units (DMUs) that were used in the study are displayed in (Figure 1).

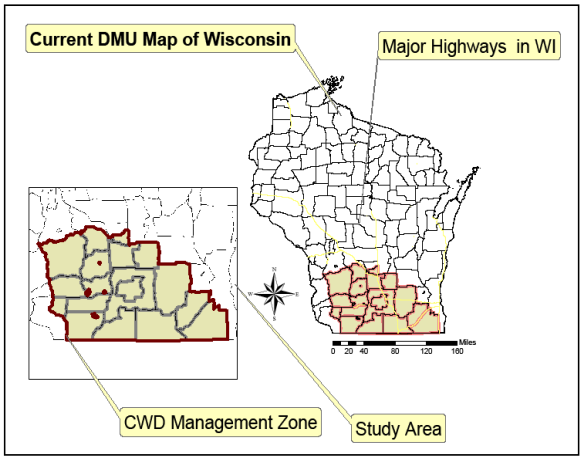

Figure 1. Study area illustrating the south central region in the State of Wisconsin (WI). Study area is approximately 872 square miles.

Methodology

Data Acquisition

Datasets were acquired from the Wisconsin DNR public GIS ftp site. Data included: county boundary, deer management units, the Wisconsin state outline, and Wisconsin roads shapefiles.

Excel spreadsheets of CWD test results for DMU totals for years 2005 to

2009, which contained the columns for deer testing positive for CWD, the number of deer sampled, and the number of deer analyzed for each of the 144 DMUs.

Testing results for 2010 was not available as at the time the project was completed.

Projection

All data used in this project were projected in order to keep datasets aligned. The following projection was used in the study:

NAD_1983_HARN_Transverse_Mercator
Projection: Transverse_Mercator
False_Easting: 520000.000000
False_Northing: -4480000.000000
Central_Meridian: -90.000000
Scale_Factor: 0.999600
Latitude_Of_Origin: 0.000000
Linear Unit: Meter
GCS_North_American_1983_HARN
Datum: D_North_American_1983_HARN

Datasets utilized that did not match this coordinate system were re-projected to match it.

Data Preparation and Analysis

Raw data was utilized in order to create shapefiles of currently infected deer management units (DMUs) and the current CWD management zone (CWD-MZ). Created shapefiles were the deer management units positive for CWD layer, and current management zone layer. All symbology was classified using the Natural Breaks Jenks Method with 4 classes. The 4 classes ranged from 0, which represented no positive occurrence of CWD, to the highest number of positive occurrences within the deer management units (DMUs).

Major highways were extracted from the Wisconsin's roads shapefile that was downloaded from the Wisconsin's DNR GIS FTP website. The extracted highways were: Interstates 39, 43, 90, 94. These were overlain in ArcGIS on the DMU layer.

Data integrity was of utmost importance, hence, excel spreadsheets for each year was joined to the DMUs attribute table. Standardizing data fields ensured all datasets were consistent and accurate.

Only 18 out of a total of 144 deer management units (DMUs) that fell within the current CWD management zone (CWD-MZ) were selected for GIS analysis. Deer management units (DMUs) that fell outside the extent of the current CWD-MZ were excluded because they fell outside the scope of the project.

The 18 DMUs and their corresponding counties included in the analysis are listed in Table 1.

Table 1. Deer management units and the counties they occur within.

Deer Management Units	Counties
54B-CWD	Juneau and Sauk
70-CWD	Richland and Sauk
70A-CWD	Dane and Iowa
70B-CWD	Columbia and Sauk
70E-CWD	Adams, Columbia and Marquette
70G-CWD	Columbia
71-CWD	Crawford, Richland and Vernon
73B-CWD	Grant
73E-CWD	Grant and Iowa
75A-CWD	Green and Lafayette
75C-CWD	Dane, Grant and Iowa
75D-CWD	Grant and Lafayette
76-CWD	Dane, Green, Lafayette and Rock
76A-CWD	Dane, Doge, Jefferson, Rock and Waukesha
76M-CWD	Dane
77A-CWD	Rock and Walworth
77B-CWD	Kenosha, Racine and Walworth
77C-CWD	Jefferson, Walworth and Waukesha

In order to satisfy the project deliverables, general activities were undertaken which

included but were not limited to: using ArcGIS to perform analysis on the raw and created data, pre-processing and mapping of the affected and susceptible deer management units (DMUs) that fell within the current CWD-MZ, and the use of contingency tables for numerical analysis, and using the Chi-Square statistic to perform statistical analysis to test for levels of significance.

Results

In Wisconsin, CWD is largely an issue in the south central area of the state and it is the occurrence of CWD in the 18 DMUs of the area that is examined in this paper. Only one case of CWD is noted to have occurred in the state outside this area.

Geospatial Distribution of Positive CWD Occurrences from 2005 to 2009

Testing results for an individual year are for the CWD year which runs from April 1st through March 31st each year. For example, the results for the 2007 CWD year would be from April 1st, 2007 through March 31st, 2008.

CWD Year 2005

The highest number of positive occurrences was found in deer that were analyzed from the 70A CWD DMU which is located in Iowa and part of Dane county. A total of 19,962 deer were analyzed in 2005 for CWD and 181 tested positive for the disease. One hundred and twenty-eight of 2,812 that were analyzed from 70A CWD DMU tested positive.

Out of the 2,426 deer analyzed from the 76 CWD DMU, 14 tested positive for the disease. The 76 CWD DMU covers parts of Dane, Green, Lafayette, and Rock counties. Both the 70A and 76 CWD DMUs had the highest number of positive cases of CWD for 2005 as seen in (Figure 2).

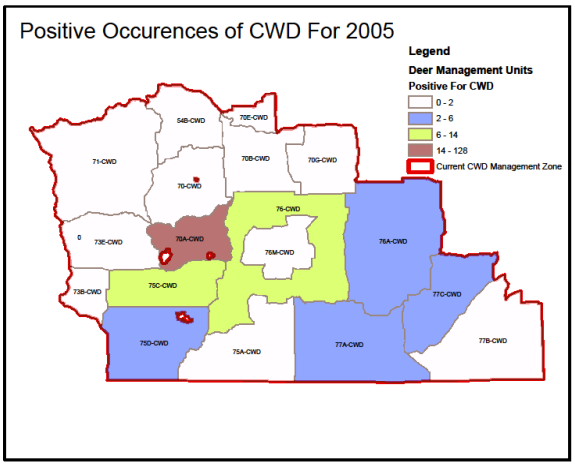

Figure 2. Displays the Positive Occurrences of CWD in the 18 DMUs within the CWD Management Zone for 2005.

CW D Year 2006

The highest number of positive occurrences was found in deer that were analyzed from the 70A CWD DMU which is located in Iowa and part of Dane county.

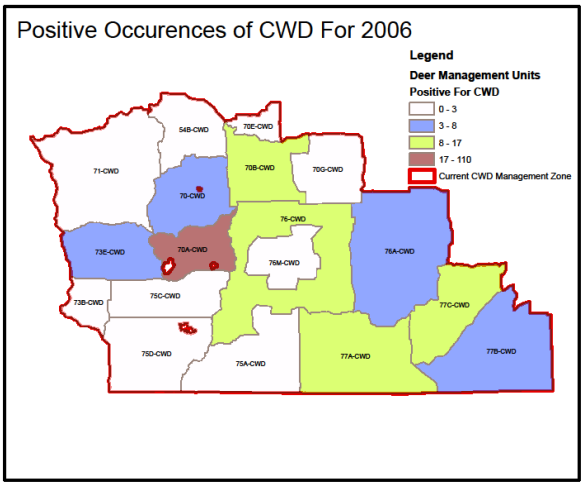

Figure 3. Displays the Positive Occurrences of CWD in the 18 DMUs within the CWD Management Zone for 2006.

A total of 19,869 deer were analyzed in 2006 for CWD and 205 tested positive for the disease. One hundred and ten of 2,552 that were analyzed were from the 70A CWD DMU tested positive.

DMUs 70, 73E, 77B, 70B showed significant increase in the number of positive cases and a spread pattern that covers parts of Sauk, Richland, Columbus, Racine and Kenosha counties. 75D had a decrease in the number of positive cases (Figure 3).

CWD Year 2007

A trend can be seen here, even though fewer deer were tested in 2007, CWD remains high. The highest numbers of positive tests were from the 70A CWD DMU which is located in Iowa and part of Dane county. A total of 7,130 deer were analyzed in 2007 for CWD and 135 tested positive for the disease. Eighty-six deer out of 1,867 that were analyzed were from the 70A CWD DMU tested positive.

DMU 77B showed a lower number of positive cases in 2007 while DMU 75C (which from the previous year had 0 to 3 positive tests from deer analyzed) now had between 4 to 10 positive occurrences. DMU 77B as of 2007 covered parts of Dane, Grant, Green, and Iowa counties (Figure 4).

CWD Year 2008

Again fewer deer were tested this year but there was an increase in the number of positive occurrences in 70A CWD DMU compared to the previous year. A total of 6,142 deer were analyzed in 2008 for CWD and 181 tested positive. One hundred and twenty-one of 1,537 that were analyzed were from the 70A CWD DMU tested positive.

2008 revealed a slight change in DMU 77B which had almost no occurrences in 2007, but in 2008 had in between 1 to 4 deer testing positive for the disease. DMU 76M which is located in the heart of Dane county had been disease free in previous years tested, but now had confirmed cases of CWD (Figure 5).

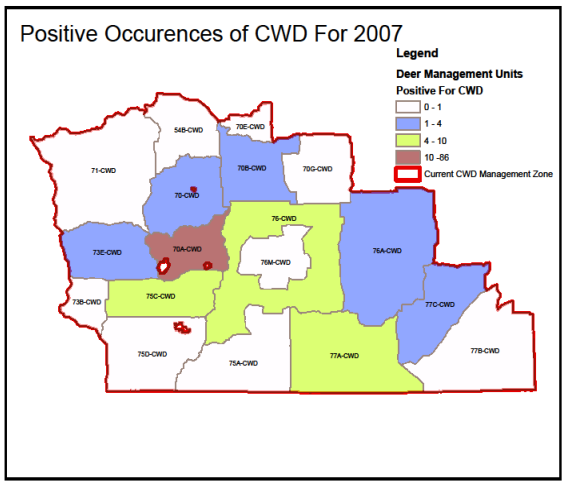

Figure 4. Displays the Positive Occurrences of CWD in the 18 DMUs within the CWD Management Zone for 2007.

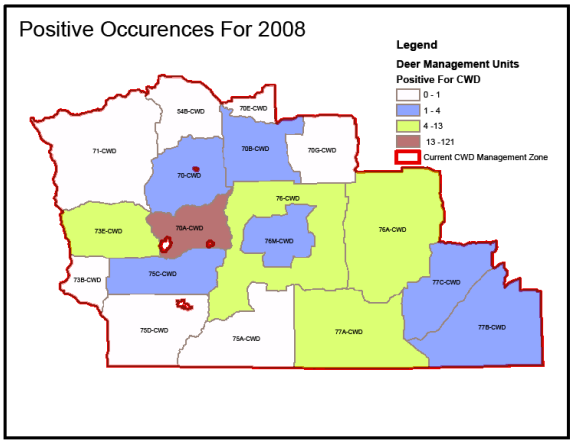

Figure 5. Displays the Positive Occurrences of CWD in the 18 DMUs within the CWD Management Zone for 2008.

CWD Year 2009

The deer analyzed in 2009 totaled 6,857 animals. Of that number 175 tested positive for CWD. Ninety-eight of 1,336 that were analyzed from the 70A CWD DMU tested

positive for CWD as well. 2009 results reveal DMUs 77B, 70B, and 76M had decreased numbers of positive occurrences. The 71 CWD DMU had between 2 to 7 new positive cases in tested deer, an indication of spread of the disease. This area covers parts of Crawford, Vernon and Richland counties (Figure 6).

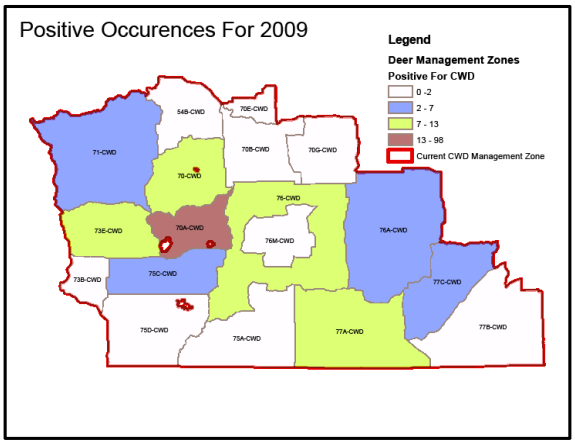

Figure 6. Displays the Positive Occurrences of CWD in the 18 DMUs within the CWD Management Zone for 2009.

Numerical and Statistical Analysis

Contingency Tables

Contingency tables were used to record and analyze the relations between two or more categorical variables for numerical analysis. The variables were the number of deer that tested positive for CWD and the number of deer that tested negative for CWD from the total number of deer harvested for testing in the years 2005 to 2009. The Alpha error (the likelihood of rejecting the null hypothesis when it is true) was set = 0.05 as justification for rejecting the null hypothesis Ho.

Chi-Square Statistic

The Chi-Square statistic was calculated by finding the difference between each observed and expected frequency for each possible outcome, squaring them, dividing each by the expected frequency, and taking the sum of the results.

The Chi-Square statistic was calculated with the following formula:

$$\sum\sum = \frac{(\text{Observed frequency} - \text{Expected frequency})^2}{\text{Expected l frequency}}$$

Degrees of Freedom

A second part of determining the test statistic is to define the degrees of freedom of the test: this is essentially the number of observed frequencies adjusted for the effect of using some of those observations to define the "expected frequencies."

Degrees of freedom (DF) were calculated with the following formula (Zar, 1999):

$$DF = (r - 1)(c - 1).$$

Where

r = number of rows
c = number of columns

Hence

$$(2 - 1)(5 - 1) = 4.$$

Percentage Comparisons

Table 2 shows numbers of deer harvested for testing from 2005 to 2009 from the 18 DMUs used for the study area in the project. It also shows the percentage of cases of Chronic Wasting Disease within the 18 DMUs study area located south central Wisconsin.

Table 2. Percentage of deer testing positive and negative within the 18 DMUs study area from 2005-2009.

Year	2005	2006	2007	2008	2009
% testing Positive	0.93%	1.03%	1.85%	2.95%	2.52%
% testing Negative	99.07%	98.87%	98.15%	97.05%	97.48%
# of deer	19362	19869	7130	6142	6857

The percentage change from year to year reveals that the occurrence of CWD has increased in these 18 DMUs. In the first year of testing when over 19,000 deer were examined, less than 1 % tested positive. By 2008, a three-fold increase was noted when the number testing positive was almost 3 %. The rate of infection observed was slightly lower in 2009 (~2.5 %).

The years of 2005 and 2006 were intensively examined with over 19,000 deer tested each year. For the following years of 2007, 2008 and 2009 only 6,000 to 7,000 deer were examined each year. It is possible that the difference in intensity of deer sampling over the years has influenced the results noted but this is not likely the case, particularly since fewer deer were sampled in more recent years rather than vice versa. More likely, an increase in the occurrence of CWD is being noted.

Hypotheses Calculations

The contingency table below contains the total number of deer that were harvested for testing from the entire state of Wisconsin in the years 2005 to 2009. The hypotheses tested were:

Ho: CWD occurrence in deer is independent of year analyzed.
Ha: CWD occurrence in deer is dependent of year analyzed.

Table 3. A 2 x 5 contingency table for testing the independence of the occurrence of CWD and year in deer.

Occurrence	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Totals
Positive	181	205	135	181	175	877
Negative	24641	30059	9179	12107	6941	82927
Totals	24822	30264	9314	12288	7116	83804

Table 3 shows the proportion of positive and negative occurrences of CWD in the deer tested. The level of significance between years and occurrence of CWD was assessed using the Chi-Square Statistic. The Chi-Square Statistic was used to assess whether an apparent dependency existed in the data between year and occurrence of CWD.

Having calculated the degrees of freedom = 4, and alpha set at 0.05. The critical value of the Chi-Square distribution was 9.488. The calculated Chi-Square value obtained here was equal to 237.1 and the null hypothesis of independence of year and CWD was rejected. In this instance, the data indicate that there was a relationship between the number of deer testing positive for CWD and the year they were harvested.

It is alarming to see the number of deer testing positive for CWD in 2009 is almost the same as 2005 given that there were only as third as many deer tested in 2009 and is 2005 and 2006.

Only deer harvested from the 18 deer management units (DMUs) with higher occurrences of CWD were used in the next analysis. The hypotheses tested were:

Ho: CWD occurrence in deer analyzed within the current CWD-MZ from all 18 deer management units (DMUs) is independent of year.
Ha: CWD occurrence in deer analyzed within the current CWD-MZ from all 18 deer management units (DMUs) is dependent of year.

Table 4 below shows the results for deer harvested for testing from the 18 deer management units (DMUs) that are within the current CWD management zone (CWD-MZ) examined in this project.

Table 4. A 2 x 5 contingency table for testing the independence of the occurrence of CWD and year in deer.

Occurrence	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Totals
Positive	181	205	135	181	175	877
Negative	19182	19664	6995	5961	6882	58484
Totals	19362	19869	7130	6142	6857	59361

Here, the calculated Chi-Square value was 218.8. Again, the null hypothesis of independence was rejected (Chi-Square table = 9.488, alpha = .05).

The Chi-Square value conclusion suggests the occurrence of CWD within the current CWD-MZ for the 18 DMU's units chosen for this study was dependent on year. In fact, most of the deer testing positive came from these deer management units.

In observing both contingency tables, CWD occurrence seems to be localized in the southern portion of the state of Wisconsin. The numbers suggest 2009 positive cases are almost the same as 2005 though almost three times as many deer were examined in 2005 and 2006 as in 2009. There is not much change between 2008 and 2009.

On-Going Constraints

Issues involved in this project were very vast. CWD is an ongoing problem despite the best efforts by researchers who are working on preventive and emergency response plans.

One of the issues encountered during this project was the fluctuation in sample size. That is, the number of deer harvested for testing from the 18 DMUs and the entire state of Wisconsin varied from year to year. For example, in 2005 deer were harvested from 97 of 144 DMUs for testing. In 2009 deer were harvested and tested from 63 of 144 DMUs. This pattern gives rise to fluctuating numbers of deer harvested for testing and might affect the number of positive and negative cases of CWD noted.

Managing CWD in free ranging animals presents even greater challenges. Long term active surveillance programs to monitor CWD distribution and prevalence have been instituted in the affected area to determine the extent of the disease spread and to assist in evaluating the effects of management intervention. Management programs established to date focus on containing CWD and reducing its prevalence in localized areas. Management goals also vary among affected states and provinces (Mathiason et al., 2006).

In Wisconsin areas where CWD may not yet be endemic, eradication could be considered as an ultimate goal for CWD management.

Recommendations

It might be advantageous to correlate other variables between randomly selected deer management units within the current CWD management zone. For example, collateral information about deer management units, and surrounding areas could be collected such as: land cover, topography, terrain, abundance of water, and distance from the Mississippi River etc, to see if sort of predictive model of positive occurrence could be developed.

Conclusion

Strictly evaluating test data from 2005 to 2009, results suggest western Dane and eastern Iowa counties are core endemic areas of the disease and percentages of deer testing positive. The occurrence of CWD has increased almost three fold in the 18 DMUs examined in this study between 2005 and 2008-2009. Based on findings of this project, there is strong evidence to suggest

that CWD poses an increasing threat to the long term health of Wisconsin's deer herd population.

Acknowledgments

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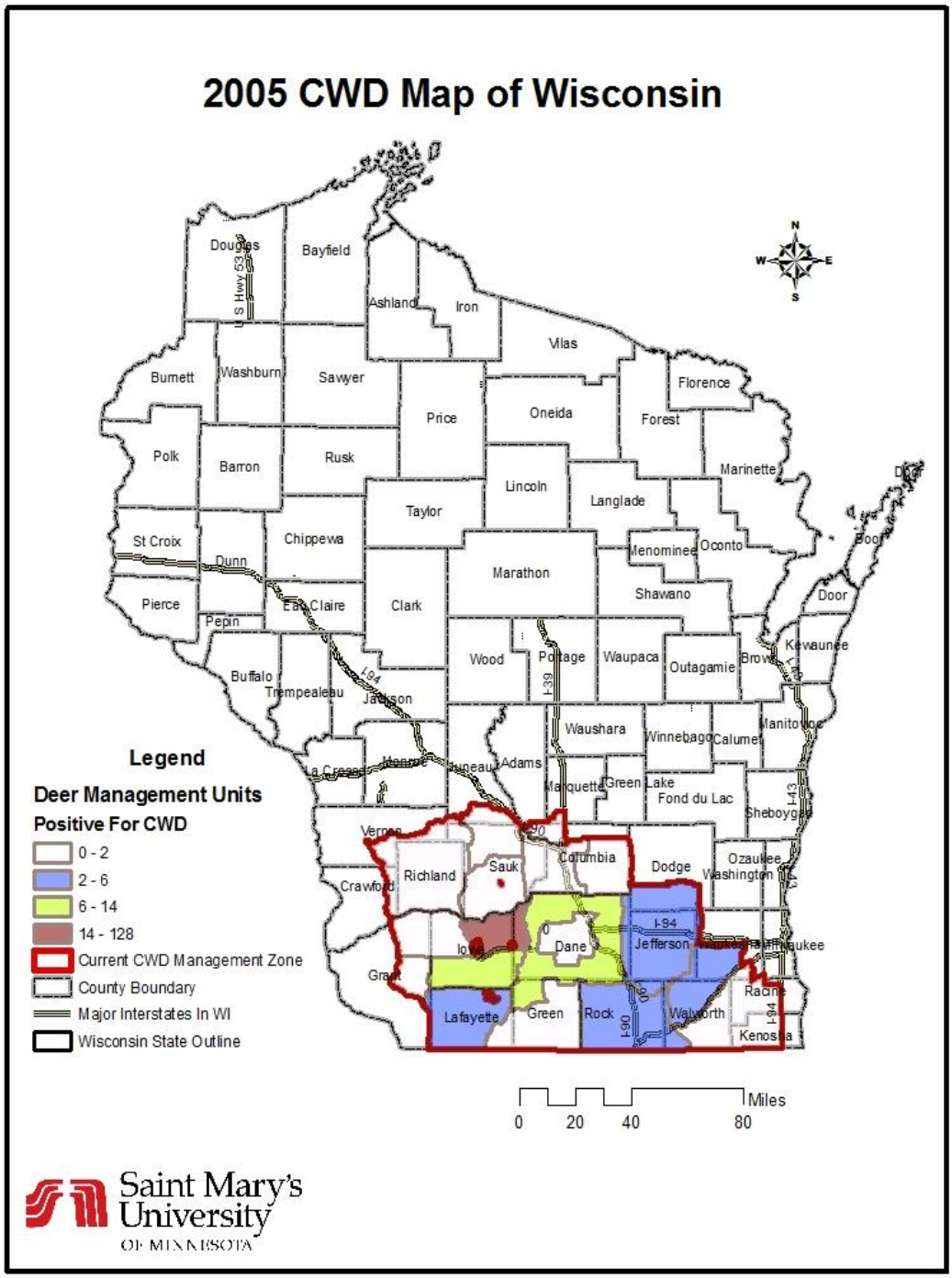
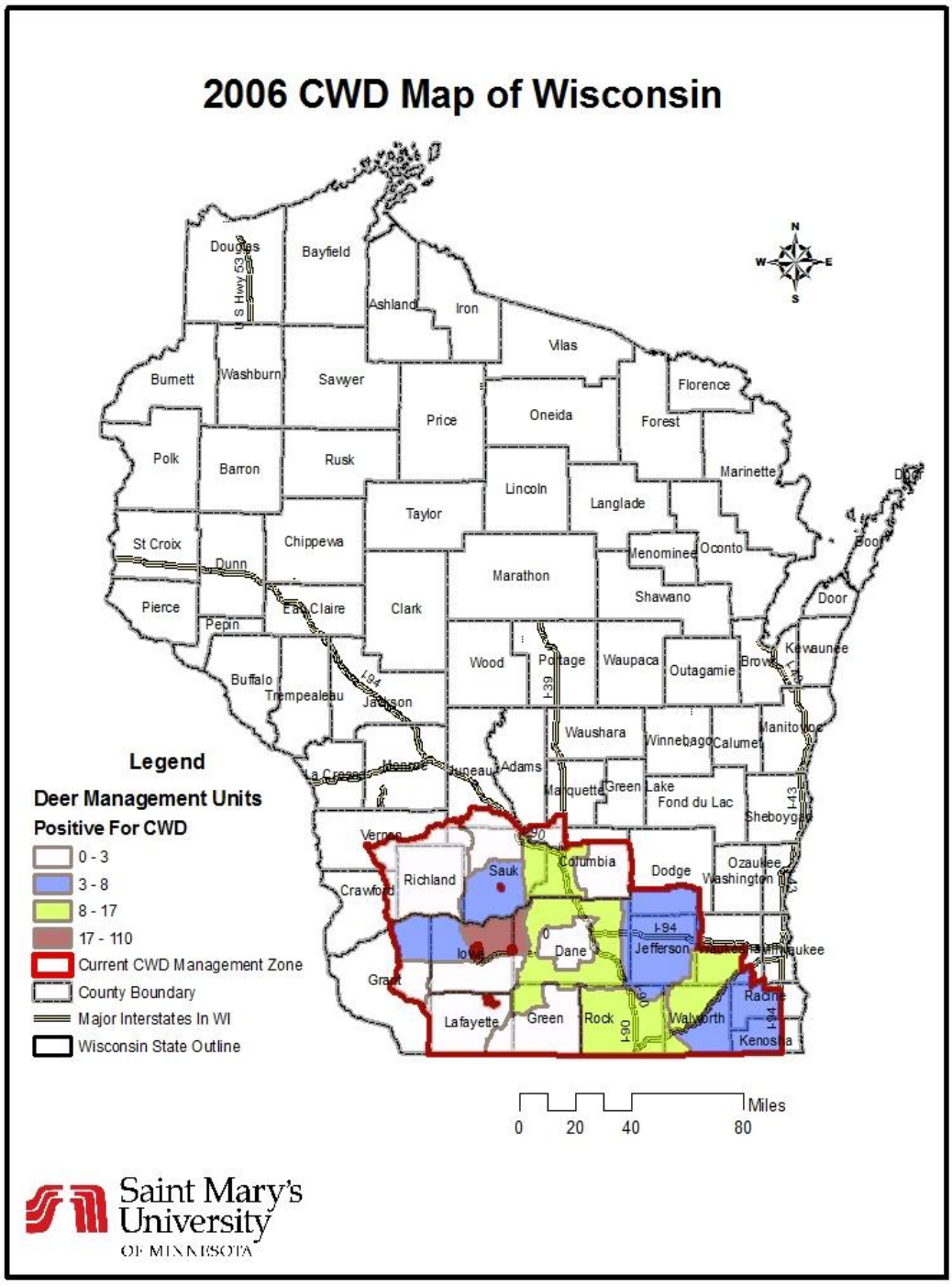
Appendix A. State of Wisconsin (April 1st 2005 through March 31st. 2006) CWD Map. Numbers reflect the number of white-tailed deer testing positive for CWD.



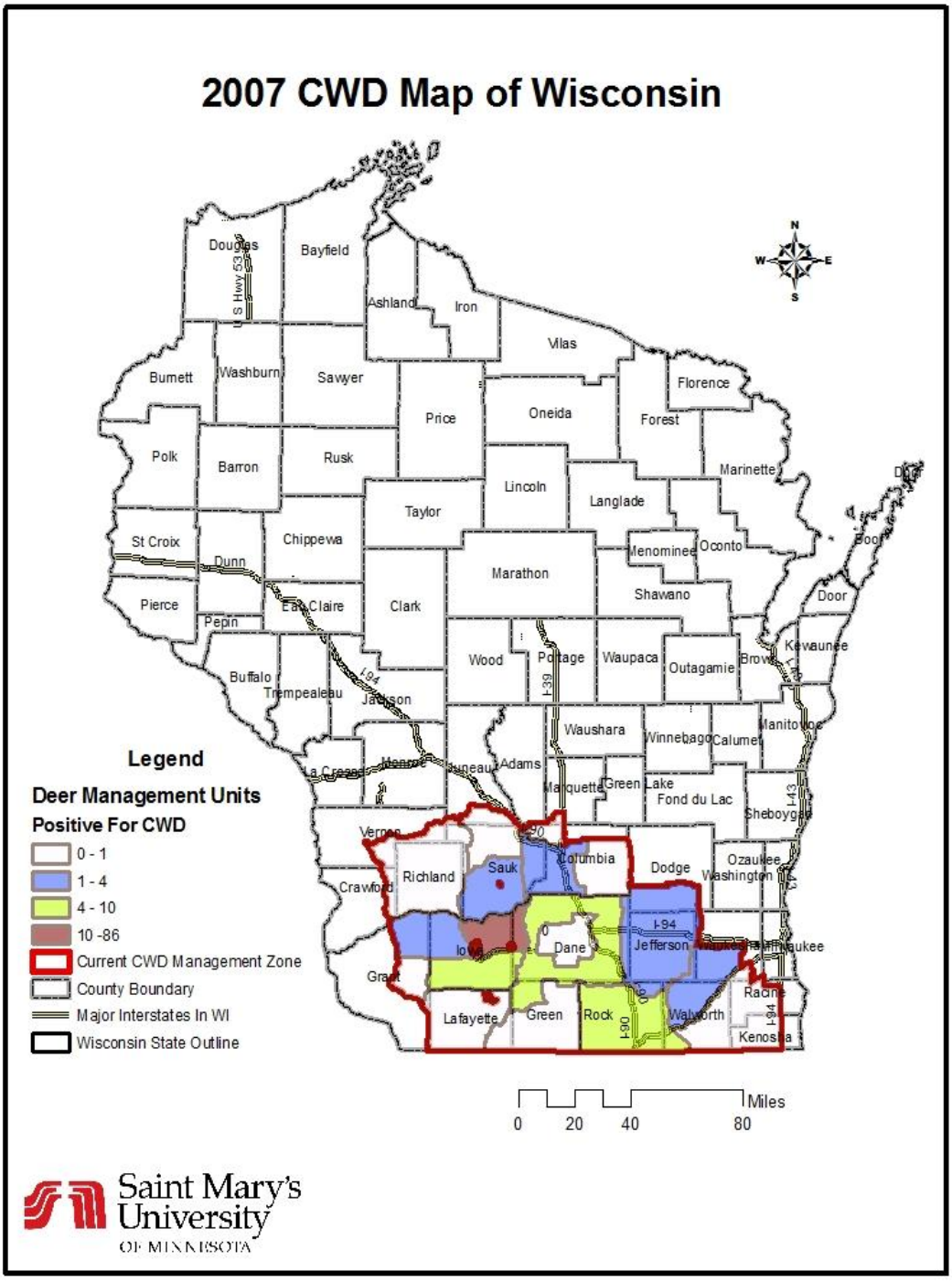
*All symbology was classified using the natural breaks Jenks method.

Appendix B. State of Wisconsin (April 1st 2006 through March 31st. 2007) CWD Map. Numbers reflect the number of white-tailed deer testing positive for CWD.



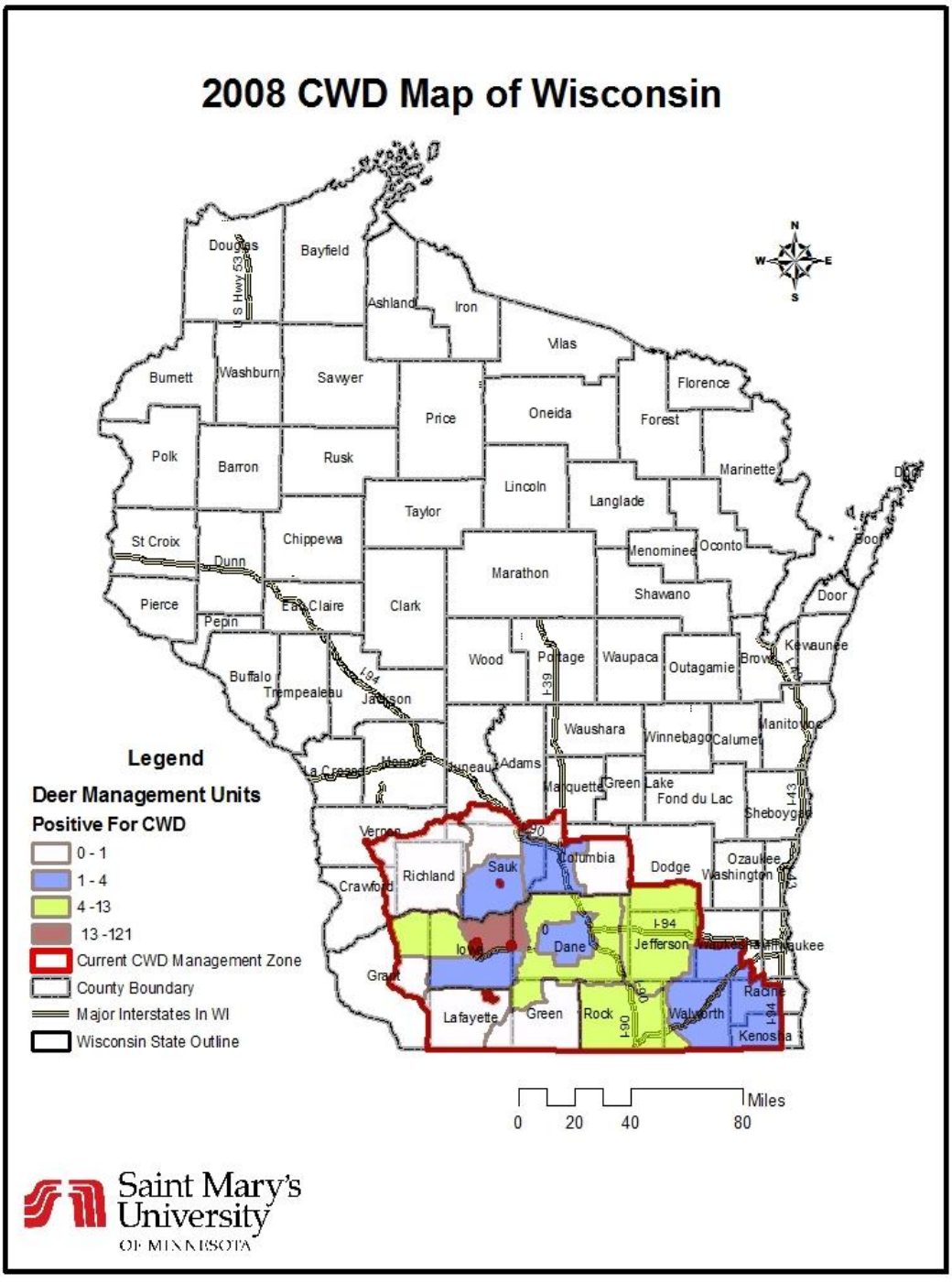
*All symbology was classified using the natural breaks Jenks method.

Appendix C. State of Wisconsin (April 1st 2007 through March 31st. 2008) CWD Map. Numbers reflect the number of white-tailed deer testing positive for CWD.



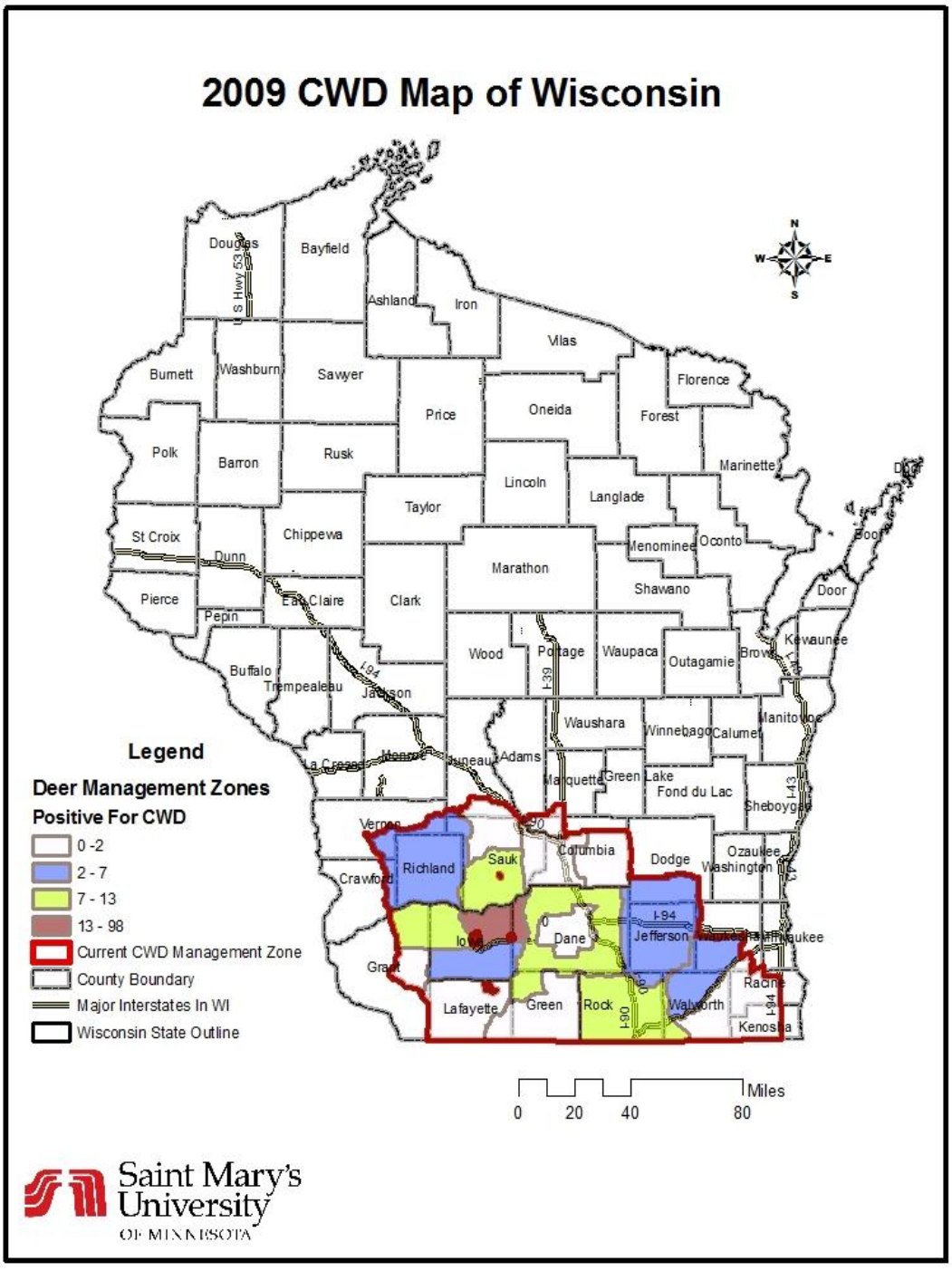
*All symbology was classified using the natural breaks Jenks method.

Appendix D. State of Wisconsin (April 1st 2008 through March 31st. 2009) CWD Map. Numbers reflect the number of white-tailed deer testing positive for CWD.



*All symbology was classified using the natural breaks Jenks method.

Appendix E. State of Wisconsin (April 1st 2009 through March 31st. 2010) CWD Map. Numbers reflect the number of white-tailed deer testing positive for CWD.



*All symbology was classified using the natural breaks Jenks method.