



2014

# Distribution of Wildlife Rabies in Central Appalachia and Analysis of Factors Influencing Human Exposure

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**Distribution of Wildlife Rabies in Central Appalachia and Analysis  
of Factors Influencing Human Exposure**

Capstone Final Paper

A paper submitted in partial fulfillment of the requirements  
for the degree of  
Master of Public Health  
in the  
University of Kentucky College of Public Health  
by  
Sara J Reilly

Lexington, KY July 28,2014

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## Abbreviations and Definitions

ORV	Oral Rabies Vaccine
PEP	Post Exposure prophylaxis
CDC	Centers for Disease Control and Prevention
USDA	United States Department of Agriculture
APHIS	Animal and Plant Health Inspection Service
US	United States
SCSK	South Central Skunk rabies virus variant
RAC	Raccoon rabies virus variant
AVMA	American Veterinary Medical Association
Forest land	Land at least 10-percent stocked by trees of any size, including land that formerly had such tree cover and that will be naturally or artificially regenerated. Forest land includes transition zones, such as areas between heavily forested and nonforested lands that are at least 10-percent stocked with trees and forest areas adjacent to urban and builtup lands. Also included are pinyon-juniper and chaparral areas in the West and afforested areas. The minimum area for classification of forest land is 1 acre and 120 feet wide measured stem-to-stem from the outer-most edge. Unimproved roads and trails, streams, and clearings in forest areas are classified as forest if less than 120 feet wide. (USDA USFS)
Metropolitan Area	Region consisting of a densely populated urban core and its less-populated surrounding territories sharing industry, infrastructure, and housing
Non-Metropolitan Area	Area including some combination of open countryside, rural towns (<2,500 residents), and/or urban areas with populations ranging from 2,500 to 49,999 that are not part of a larger labor market area.

## Abstract

**Background:** The rabies virus is a Lyssavirus of the family Rhabdoviridae which affects all mammals and causes progressive encephalomyelitis that is fatal in nearly one hundred percent of untreated cases. In the United States, wildlife act as the primary reservoir for rabies and prevention, surveillance, and control costs remain high. The purpose of this study is to understand the current distribution of wildlife rabies in Central Appalachia, as well as identify any demographic or geographic factors which may affect the risk of human exposure at the county level.

**Methods:** A spatial statistical analysis using StatScan was performed to identify county clusters with apparently high or low rates of raccoon rabies. A Negative Binomial Regression Analysis was then performed to identify potential demographic and geographic factors associated with these varying rates of rabies.

**Results:** 100 North Carolina counties, 118 Virginia counties and independent cities, and 55 West Virginia counties submitted a total of 12,516, 15,556, and 2,642 animals respectively to their state health departments for rabies testing. In North Carolina, raccoons constituted 50% of positive tests, in Virginia, 49%, and in West Virginia 50%. A final model was developed for raccoon rabies rates and then used to model all other species separately. Compared to those living in West Virginia counties, citizens of North Carolina counties had 1.67 times the risk of exposure ( $p < .0001$ ) to a rabid raccoon, while citizens of Virginia counties and independent cities have 1.82 times the risk of exposure ( $p < .0001$ ) to a rabid raccoon. Compared to those counties where farmland makes up less than seventeen percent of total area, citizens of counties with 17-28% farmland have 1.32 times the risk of exposure ( $p = 0.013$ ) to a rabid raccoon, counties with 28-39% farmland have 1.84 times the risk of exposure ( $p < .0001$ ), and counties with 39-100% farmland have 1.64 times the risk of exposure ( $p < .0001$ ). Compared to those counties designated non-metropolitan and non-adjacent to a metropolitan area, citizens of counties designated non-metropolitan adjacent to a metropolitan area have 1.56 times the risk of exposure ( $p = .005$ ) to a rabid raccoon while those in areas designated as metropolitan have 1.41 times the risk of exposure ( $p = .024$ ). This model did not appear to be the best predictor for rabies exposure from other species.

**Conclusions:** Holding all other factors constant, state, rurality, and percent of area designated as farmland were the best predictors of risk of raccoon rabies exposure. Further expansion of this research is needed to better understand other reservoir species, as well as better identify the effect of the ORV zone in controlling the risk of human exposure to raccoon rabies.

## I. Introduction

The rabies virus is a non-segmented, negative strand RNA Lyssavirus of the family Rhabdoviridae<sup>1</sup>. Rabies as a disease is an acute viral infection, causing progressive encephalomyelitis that is fatal in nearly one hundred percent of untreated cases<sup>2</sup>. All mammals are susceptible to rabies, and transmission typically occurs through the bite of affected animals or from direct exposure to contaminated saliva through the nose, mouth, eyes, or an open wound<sup>1</sup>. All Lyssaviruses are antigenically related, but the use of monoclonal antibodies and nucleotide sequencing demonstrates that there are different variants of the virus dependent on species or geographical region<sup>2</sup>. Worldwide, the canine rabies variant is most prevalent and of biggest concern, causing approximately 90% of human cases and 99% of human deaths<sup>3,4</sup>. In the United States and Canada, where canine rabies variant has been eradicated, wildlife act as the primary reservoirs for rabies<sup>5</sup>. In the United States the four primary reservoirs are the skunk, the fox, the raccoon, and the bat<sup>1</sup>.

Although the number of human deaths due to rabies in the United States has fallen to less than four per year, rabies remains a significant public health concern because of its high case fatality rate when improperly treated and its continued presence in the wildlife population<sup>1</sup>. Because rabies is 100% preventable through prompt medical care, public health officials in the US are not as concerned with human deaths as human exposures. Although there is no surveillance system in place, the CDC estimates that 40,000 post-exposure prophylaxes (PEP) are given each year in the US at an average of \$1,000 per course<sup>1</sup>. The best form of human prevention is to

vaccinate pets and avoid contact with wildlife, but as the human population expands to overlap with wildlife habitats, the latter has become much more difficult.

The high costs associated with surveillance, diagnostic testing, and post-exposure treatment of humans potentially exposed to rabies have resulted in coordinated efforts to control the expansion of rabies, particularly in raccoon populations<sup>6</sup>. In 1990 the USDA began using oral rabies vaccines (ORV) to reduce the prevalence of rabies in specific wildlife species in targeted states<sup>7</sup>. Beginning in 2005, the ORV program established a barrier along the Appalachian Mountains to prevent the westward expansion of raccoon variant rabies, considered to be more pathogenic than other strains<sup>7</sup>. Surveillance, then, is performed conditionally at the State Health Departments when animals are submitted for testing due to a potential human exposure and randomly by the USDA Wildlife Services to track prevalence around the ORV zones.

There are two primary objectives of this study. First, we will use the USDA enhanced surveillance data and state health department data (North Carolina, Virginia, West Virginia) to focus on the current distribution of raccoon rabies in Central Appalachia and identify any clusters of unusually high or low rates of rabies at the county level. We will then investigate what characteristics of these counties may act as indicators of increased exposure risk to rabies. We hypothesize that those areas with greater farmland coverage and greater population size will have higher rates of raccoon rabies.

## II. Literature Review

Rabies has been a part of human history for as long as there has been written word<sup>8</sup>. The disease is caused by a *Lyssavirus* of the family *Rhabdoviridae*<sup>9</sup>. This bullet-shaped genus of negative sense RNA viruses includes various strains of the rabies genotype designed for either general or host-specific invasion<sup>2</sup>. Clinically, it is a form of acute progressive encephalitis with symptoms and human responses similar to meningitis or poliovirus<sup>9</sup>. Little is still known about the molecular pathogenesis of rabies, but a number of studies over the years have contributed to our understanding of the virus<sup>10</sup>. Once the virus has entered the body, it accesses the muscles and attaches to some target cell, most commonly acetylcholine receptors or neural cell adhesion molecules<sup>10,11</sup>. There, it is believed that the virus remains dormant under the control of muscle-specific microRNA until enough particles build up to invade the peripheral nerve endings and enter the CNS<sup>12</sup>. By way of motor nerve axons, the virus moves through the CNS via retrograde fast axonal transport<sup>10</sup>. Once the virus has successfully infected the CNS, rapid dissemination of viral particles occurs along neuroanatomical pathways to allow spread into the corneal tissue, salivary glands, and certain organs<sup>10</sup>. All mammals are susceptible to rabies, although not equally susceptible; certain canids, herpestidae, mustelids, procyonids, and bats tend to be the most susceptible to infection<sup>10</sup>. Monoclonal antibody studies reveal that strains from the same species and region may be similar, while strains from different species, or even the same species but different regions, vary in their makeup<sup>13</sup>.

The transmission capability of the rabies virus has been widely debated throughout history. Once the virus establishes itself in the CNS, it begins to spread

outwardly to infect almost all organs of the body, including the heart, lungs, gastrointestinal tract, bone marrow, cornea, kidneys, and salivary glands<sup>14,15,16</sup>. Because of this, detection of rabies virus RNA is possible and can be used for ante mortem diagnostic purposes in suspected human cases<sup>14</sup>. There have been rare reports of transplant-induced infection in hospitalized patients<sup>2,14,17</sup>, leading many individuals to believe that rabies can be successfully transmitted through contact with blood or urine, but this has not been supported by laboratory studies<sup>1,18</sup>. Instances of aerosolized transmission have occurred, primarily in bat populations where the extreme density of bats and their subsequent respiratory and salivary secretions provide enough virus particles to cause infection<sup>1</sup>. Only two cases of human rabies resulting from aerosolized particles have ever been reported, both accidental lab exposures<sup>2,13</sup>. Current research demonstrates that the virus itself is only infectious in relatively fresh saliva or neural tissue; this material easily becomes non-infectious when dried out or exposed to sunlight<sup>1</sup>.

The CDC and WHO define two primary categories of potential exposure to rabies. The first is bite exposure, where an infected animal breaks the skin of a susceptible person and saliva has the chance to enter nervous tissue<sup>1,3</sup>. This is the most common source of transmission in both humans and animals, although it does not guarantee infection. The virus particles are shed intermittently, and towards the end of the incubation period, so the saliva of a rabid animal is not always infectious to others<sup>2,13</sup>. The second category is non-bite exposure. Non-bite exposures include contamination of wounds, cuts, scratches, or mucous membranes with potentially infectious material such as saliva or nervous tissue<sup>1,3</sup>. Neither the CDC nor WHO

recognizes contact with skin, fur, blood, urine, or feces of a rabid animal to be a source of exposure to rabies, although this is still widely disputed in clinical settings due to the stigma and poor prognosis associated with the disease<sup>1</sup>.

In humans, rabies initially presents with general flu-like symptoms including weakness, discomfort, fever, and headache that may last for several days<sup>1</sup>. Patients who may suspect rabies based on previous exposure to a bite often become nervous or anxious inherently given the poor prognosis, but mood changes including restlessness, anxiety, nightmares, a sense of foreboding or tension, and depression have been recorded<sup>13</sup>. The patient may experience tingling, discomfort, itching, or numbness at the site of the bite<sup>1,13</sup>. Experimental infection with rabies shows some viral attack on the limbic system, which could correlate with the characteristic aggressiveness, abnormal sexual behavior, and loss of timidity<sup>19</sup>. The furious form of rabies occurs in about 80% of human cases<sup>13</sup>. Furious rabies presents with hyper-aggressive behavior, hallucinations, alternating periods of arousal and lucid calmness, myocarditis, and pneumonia<sup>1,13</sup>. Most characteristically, the patient develops hydrophobia, aerophobia, and hyperaesthesia due to spastic paralysis of the throat muscles and hyper-stimulation of the nervous system<sup>13</sup>. Patients with furious rabies are considered terminal once they lapse into a coma and typically die within a week of symptom onset<sup>13</sup>. The paralytic or dumb form of rabies occurs in less than 20% of human cases, but in the majority of animal cases<sup>13</sup>. Patients experience spreading paralysis throughout their bodies and lack the characteristic hydrophobia, eventually succumbing to the disease in about thirty days as vital organ systems become paralyzed and cease functioning<sup>13</sup>. No pathogenetic explanation has yet been found

for the two different clinical forms of rabies<sup>20</sup>. Despite the severity of the clinical symptoms, the neural response to rabies infection is fairly mild, suggesting that neuronal dysfunction rather than structural damage within the body may be the cause of clinical manifestations<sup>10</sup>.

Despite the designation of rabies as a neglected tropical disease, it is still widely feared around the world due to its high case fatality and lack of proven treatment after symptoms occur<sup>21</sup>. Rabies is 100% preventable given proper primary prevention measures, wound treatment, and administration of post exposure prophylaxis. The current CDC protocol for non-immunized individuals exposed to rabies involves immediate and thorough cleansing of wounds with soap and water, or a virucidal agent if available, followed by the full course of post exposure prophylaxis (PEP)<sup>1</sup>. PEP involves the local infusion of rabies immunoglobulin (RIG) around the exposure site followed by the intramuscular administration of either the Human Diploid Cell Rabies Vaccine (HDCV) or the Purified Chick Embryo Cell Rabies Vaccine (PCECV) at days 0, 3, 7, and 14, where day 0 is the first day of PEP<sup>1</sup>. For patients previously vaccinated against rabies, the protocol remains the same except that RIG is not administered and vaccines are only required on days 0 and 3<sup>1</sup>. Treatment should be sought immediately after exposure to increase likelihood of PEP effectiveness, but the patient is still treatable during most of the incubation period. Once symptoms begin to appear in humans, there is no proven treatment and the case fatality is upwards of 96%<sup>1,\*</sup>.

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\* In 2004, 15 year old Jeanna Giese of Fond du Lac, WI became the first previously unimmunized, symptomatic case to survive rabies. She was treated with a novel therapy developed by a team of physicians at the Children's Hospital of Wisconsin. This therapy, known as the "Milwaukee Protocol", involved induction of a coma and

Rabies is still a major zoonotic problem around the world, particularly in undeveloped countries. The WHO and CDC report the global burden of rabies to be an estimated 55-60,000 human deaths per annum<sup>3,4</sup>. More deaths occur in Asia than any other continent, exceeding 30,000 deaths per annum, with India accounting for nearly two-thirds of that estimate<sup>3</sup>. Africa follows closely behind, with reported deaths nearing 24,000 per annum<sup>3</sup>. In both circumstances, dogs act as the primary reservoir. Dogs are presumed to account for greater than 90% of exposures and 99% of deaths worldwide<sup>1</sup>. Absent or weak canine vaccination programs, large numbers of stray dogs, and the close contact these dogs share with both humans and wildlife account for much of the problem in these developing nations<sup>3</sup>. This is demonstrated in regions like Latin America and the Caribbean, where successful dog vaccine programs have reduced the number of rabies deaths from 250 in 1990 to less than 10 in 2010<sup>3</sup>.

In the United States and Canada, where canine variant rabies has been eradicated,

**Distribution of Major Terrestrial Reservoirs of Rabies in the United States**



Centers for Disease Control and Prevention. *Wildlife Reservoirs for Rabies*. Available at: [http://www.cdc.gov/rabies/exposure/animals/wildlife\\_reservoirs.html](http://www.cdc.gov/rabies/exposure/animals/wildlife_reservoirs.html)

wildlife acts as the primary reservoir, accounting for more than 90% of reported cases of rabies<sup>5,22,23</sup>. According to the CDC, the major rabies variants in the United States vary by region and are associated with skunks, foxes, raccoons, and bats<sup>1</sup>.

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administration of antiviral medications. Although this case was successful, the protocol has not proven successful in subsequent attempts and is not considered a treatment for rabies<sup>24</sup>.

Fewer than four cases of human rabies are reported annually and almost all of these are a result of bat exposure<sup>1</sup>. Despite these numbers, many people in the United States still associate the image of rabies with dogs. According to the 2012 CDC Rabies Surveillance Report, of the 102,963 samples successfully submitted and tested for rabies 6,162 were positive, and of those only 4.2% were cats and 1.4% were dogs. This report includes not only state health department requests due to possible human exposure, but additional surveillance testing performed by the USDA APHIS Wildlife Services, which does not involve human exposure. This creates a major economic problem in the US, as it is further estimated that over 40,000 PEP are administered each year at a cost of nearly \$1,000 US per course<sup>1</sup>. One Kentucky study from 1994 suggested that the vast majority of instances where PEP is administered are unnecessary due to lack of compliance with public health protocols, including absence of animal for testing and inaccurate determinations of exposure where the patient did not actually come into contact with infectious material<sup>25</sup>. The need for improved rabies surveillance in wildlife as well as the development of a PEP surveillance system in the US is evident from these and other findings<sup>21,26</sup>.

The National Rabies Management Program was established through the USDA APHIS Wildlife Services in recognition of the changing scope of rabies<sup>7</sup>. The goal of this program is to prevent the further spread of wildlife rabies and eventually eliminate terrestrial rabies in the US<sup>7</sup>. In order to accomplish this, oral rabies vaccines (ORV) are distributed in pre-determined locations around the US to produce some rabies immunity in wildlife populations. ORV was first demonstrated to be a feasible means of rabies management in captive red foxes in the US in 1969, then in Europe

from 1977 onward<sup>27</sup>. Canada initiated an ORV program in 1989 that strives to eliminate both arctic and red fox variant rabies<sup>27</sup>. Following extensive field safety and efficacy trials, the US began its official ORV program in the 1990's specifically targeting gray fox variant rabies and the prevention of canine variant rabies reintroduction from Mexico into southern Texas and the raccoon variant rabies (RAC) along the east coast<sup>7</sup>.

The latter bait region, which follows the natural barrier provided by the Appalachian Mountains, has been of primary focus for the program in order to prevent the further spread of RAC, which began in Florida and Georgia and spread up the east coast primarily through translocation of wild raccoons<sup>27</sup>. Raccoons are the primary reservoir for rabies in the east and RAC is now endemic throughout the eastern seaboard; this poses a serious threat, as raccoon populations are present in all of the 48 contiguous states<sup>7</sup>. In the 2012 Rabies Surveillance Report, 31.7% of submitted animals testing positive for rabies were raccoons. Raccoons are very curious in nature and commonly interact with humans and their pets in both rural and urban settings. The magnitude of noted rabies outbreaks in raccoons, as well as the number of animals tested for possible infection, is highly correlated with human population density at the county level<sup>28</sup>.

Currently the only ORV licensed for use in the US is the Raboral V-RG<sup>®</sup>, which is coated in fishmeal to seem more attractive to target species<sup>7</sup>. However, this particular vaccine has not produced sufficient levels of population immunity in skunks in the wild, nor has it proven very effective in skunks in laboratory settings<sup>27</sup>. Cases of raccoon rabies variant and bat rabies variant appearing in skunks around the

US are quite common, suggesting the increased potential of skunks to harbor different forms of rabies<sup>29</sup>. In response to this, several studies have been conducted testing the feasibility of other ORV types which could be used as a more all-encompassing approach to wildlife rabies prevention, such as the use of CAV2 to reach skunks, dogs, and raccoons with equal efficacy, or the use of ONRAB to vaccinate foxes, skunks, and raccoons while assuming the smallest risk of side effects on non-target species<sup>30,31</sup>. Other studies have looked at the possibility of combining immunocontraceptives with rabies vaccines in order to both immunize and control the size of the existing populations, an approach which has so far shown considerable success in countries where canine rabies variant is still endemic in stray dog populations<sup>32,33</sup>.

The ORV program in the US is a noteworthy project in wildlife disease history with enough potential to overshadow its challenges as long as the focus continues to shift from treatment and testing to surveillance. Human cases of rabies in the US remain at an all time low, primarily due to vaccination programs for domestic and wild animals, and yet the overall cost of rabies prevention and surveillance in the US is estimated to be around \$300 million per year<sup>1</sup>. As critical federal funding becomes limited, the USDA must strategically reduce both the number of baits dropped over a given region and the size of the regions being covered<sup>27</sup>. The primary issue with the ORV program is that progress is slow, and in order for the program to see sustained or increased access to state and federal funding, more rapid successes are needed to show that elimination of terrestrial rabies is still an attainable goal<sup>27</sup>. As well, current rabies surveillance programs are inadequate for efficient management of the ORV

program<sup>22</sup>. The recently developed RabID program, a GIS-based, real time internet mapping tool used by the CDC to conduct nationwide rabies surveillance, has demonstrated this increased need<sup>22</sup>. The system provides rapid and timely analysis for the management and assessment of ORV programs in the US, which is critical for the success of total terrestrial rabies elimination<sup>22</sup>.

This shift in focus toward increased surveillance over the years has already revealed a serious issue in the US. The CDC's 2010 Rabies Report shows a dramatic decrease in the number of confirmed rabid raccoons, and only slight variations in the number of confirmed cases in other species since the early 1990's<sup>34</sup>. However, the number of PEP distributed each year seems to be increasing, from 1981 estimates of 16,000 PEP annual to 1996 estimates of 39,000 annual to current estimates of over 40,000 PEP annual<sup>1,35</sup>. Given this costly and unusual trend in the distribution of PEP in the US, there is a surprising lack of literature investigating how rabies prevalence in wildlife affects the potential for human exposure. This paper aims to explore the issue, focusing specifically on the central Appalachian region where the ORV bait zone has been present since 2005<sup>7</sup>. We hypothesize that county social factors such as income and socioeconomic status will be more closely associated with the number of potential exposures than the actual prevalence of rabies in wildlife. To study this, we used the USDA enhanced surveillance data to map out the counties with the highest rates of wildlife rabies in terms of overall rabies and, more specifically, raccoon rabies. We then overlaid this information with the state health department data, comparing how total submission hotspots and high rabies prevalence hotspots compared with the USDA hot zones. For those counties with high numbers of state

health department submissions and high rates of rabies, we then evaluated specific factors which might indicate why some counties submit high numbers of animals for testing and why some counties had higher numbers of potential exposures (indicated by rates of positives in the submitted animals).

### III. Methods

#### *Data Collection*

This is an ecologic study of wildlife rabies in the counties and independent cities of North Carolina, Virginia, and West Virginia from 2010-2013. This research was ruled exempt by the University of Kentucky Institutional Review Board as well as by the Institutional Review Boards of the three participating states, as it did not meet the federal definition of human subjects (45 CFR 46.102(f)).

The primary data involving rabies rates were collected from two sources: state health departments and USDA Wildlife Services. State health department data were collected from North Carolina, Virginia, and West Virginia. The CDC has listed rabies as a notifiable disease in both humans and animals. As a result, any potentially rabid animal that has been in contact with humans and thus poses a threat for human rabies exposure can be submitted to the state for rabies testing. States archive these tests each year to monitor rates of rabies within their borders. Each state provided data on rabies testing from 2010-2013 which included submitting county, total number of animals submitted, total number of raccoons submitted, total animals testing positive, and total raccoons testing positive. The USDA Wildlife Services branch provided similar rabies surveillance data for North Carolina, Virginia, and West Virginia. As part of the oral rabies vaccine program, the USDA regularly traps and tests animals around the designated oral rabies vaccine zone to determine the efficacy of the vaccine and the success of the program. The Rabies Management Program of the USDA provided data on rabies testing from 2010-2012 which included county where animal was trapped, total number of animals tested, total

number of raccoons tested, total animals testing positive, and total raccoons testing positive.

County-level demographic variables were obtained from the 2010 US Census. County-level total land area and farmland area were collected from the USDA 2012 Agriculture Census. County-level forest land area was collected from the USFS 2012 Census.

### *Spatial Analysis*

County-level rates of rabies in both in raccoons and overall were mapped using ArcGIS v10.1<sup>40</sup>. County and state borders were downloaded from the US Census, TIGER/Line website. State health department rates were designated with graduated colors, while USDA rates were designated with graduated symbols. Areas of potentially low or high rates of raccoon and overall rabies at the county level were identified using Kulldorff's spatial scan statistic implemented in SaTScan v9.3<sup>41,42</sup>. Because our data were collapsed across four years, a purely spatial cluster analysis using the discrete Poisson probability model was used to scan for non-overlapping counties with either significantly higher or lower rates of raccoon rabies compared to the rest of the study area. Given the relatively small sample size of this study and the need to identify smaller county clusters of extreme rabies rates, a maximum spatial cluster size of 25% of the total population at risk was used.

### *Regression Analysis*

Given the high percentage of overall rabies submissions that were raccoons, and the historical focus on raccoon rabies in the eastern United States, the primary outcome of interest in this ecologic analysis was rates of raccoon rabies by county.

Distributions of the percent farmland and percent forest land were examined to determine cut points for categorical interpretation and to explore dose-response. Educational attainment for each county was described by the category of educational attainment achieved by the greatest percentage of people greater than 25 years of age. The original Census data contained seven categories which were collapsed into 3 categories in this study for increased sample size per category and to simplify interpretation. These categories were high school diploma or less, some college or an associate's degree, and bachelor's degree or greater. A county or independent city was assigned to the education category in which the highest percentage of its respondents claimed. Similarly, the original rural urban continuum code (RUCC) defined by the USDA Economic Research Service contains nine codes depending on metropolitan or non-metropolitan status and population size. To reduce collinearity with population, the nine categories were collapsed into three, ignoring population size. These categories were metropolitan area, non-metropolitan area adjacent to a metropolitan area, labeled "non-metro", and non-metropolitan not adjacent to a metropolitan area, labeled "rural".

Regression analyses were performed using proc genmod in SAS v9.3<sup>41</sup>. Both Poisson regressions and negative binomial regressions were initially considered for interpretation. Due to the high number of counties with zero submissions or zero positive tests, the negative binomial regression proved better for controlling the resulting zero inflation factor. The first model included all seven demographic and geographic variables, and in each subsequent model the variable with the highest non-significant p-value (where alpha = 0.05) was removed. This was continued until the

final model contained only significant variables. One variable, median household income, was removed from the final model in place of RUCC despite it being statistically significant when RUCC was not. This decision was made because income produced a negligible log effect on the rabies rate, so while it was statistically significant it was not practically significant. We assessed goodness of fit using Deviance, AIC, and BIC. All counties with submissions to the state health department were included in both the cluster analysis and the regression analysis.

#### IV. Results

Table 1 displays the raw testing counts from the USDA and SHD by county collapsed into the three states. In North Carolina, 12,516 animals were submitted to the state health department for testing, of which 1,504 were raccoons. Approximately 41.2% of raccoons submitted tested positive for rabies, while only 5.6% of all other species submitted tested positive for rabies. In Virginia, 15,556 animals were submitted to the state health department, of which 2,481 were raccoons.

Approximately 44.3% of raccoons submitted tested positive for rabies, while only 8.7% of all other species submitted tested positive. In West Virginia, 2,642 animals were submitted to the state health department, of which 565 were raccoons.

Approximately 28.5% of raccoons and 7.6% of all other species submitted tested positive for rabies. In the USDA animal samples from North Carolina rabies was present in 9.2% of raccoons and 4.8% of other species; from Virginia, rabies was

present in 7.1% of raccoons and 6.5% all other species; and from West Virginia, rabies was present in 2.1% of raccoons and 2.5% of all other species.

<b>Table 1: Summary of Animals Submitted to the State Health Department and Collected by the USDA for Rabies Testing</b>			
	<b>North Carolina</b>	<b>Virginia</b>	<b>West Virginia</b>
<b>Total Number of Counties &amp; Independent Cities</b>	100	134	55
<b>State Health Department</b>			
Counties with Submissions	100	118	55
Positive Raccoons	619	1,099	161
Total Raccoons	1,504	2,481	565
Positive Other Species	622	1,140	159
Total Other Species	11,012	13,075	2,077
<b>Positive Submissions</b>	<b>1,241</b>	<b>2,239</b>	<b>320</b>
<b>Total Submissions</b>	<b>12,516</b>	<b>15,556</b>	<b>2,642</b>
<b>USDA Wildlife Services</b>			
Counties with Submissions	11	28	45
Positive Raccoons	13	45	54
Total Raccoons	141	636	2,604
Positive Other Species	2	38	11
Total Other Species	42	586	442
<b>Positive Submissions</b>	<b>15</b>	<b>83</b>	<b>65</b>
<b>Total Submissions</b>	<b>183</b>	<b>1,222</b>	<b>3,046</b>

Figure 1 provides a map of the current rabies rates in raccoons according to both the USDA and each of the SHDs, as well as the 2011 ORV zone. Higher rates of raccoon rabies according to the USDA are indicated by larger circles, while higher rates according to the SHD are indicated by darker shades of blue.

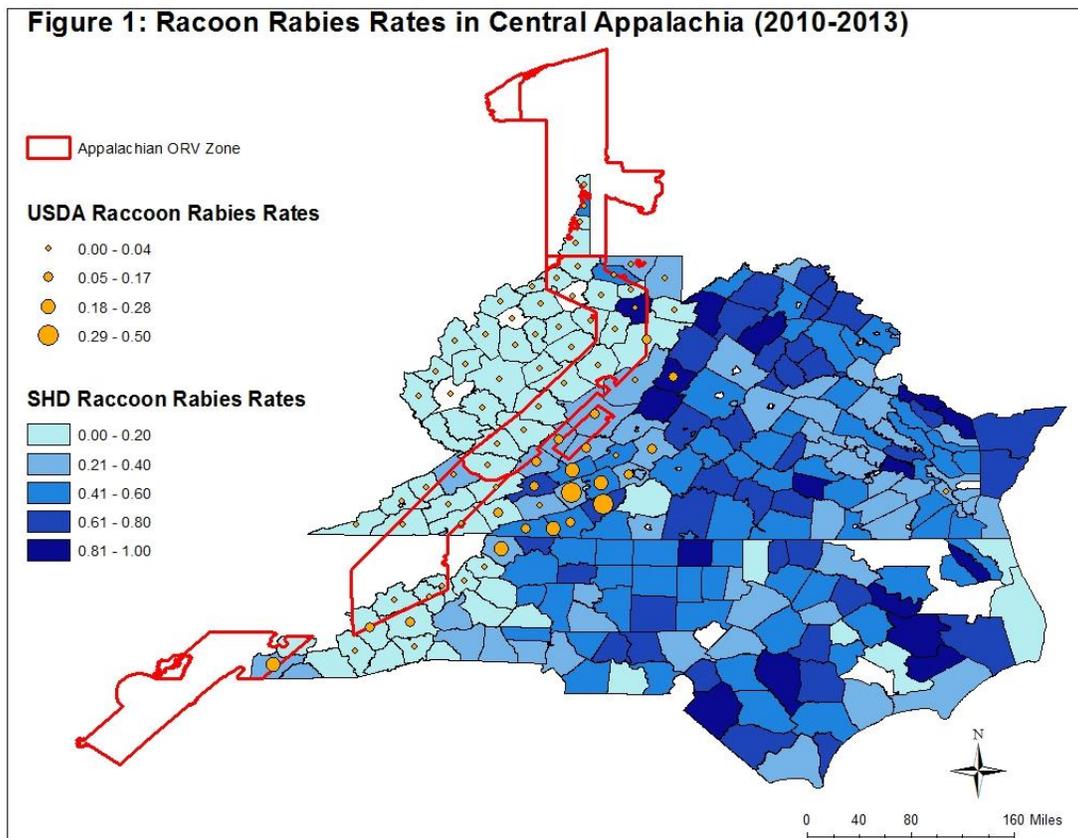


Figure 2 displays the spatial cluster analysis of SHD raccoon rabies rates. Two significant high rate and two significant low rate clusters were identified. The most likely high rate cluster consisted of 23 counties and independent cities. There were 493 confirmed positive raccoons, while only 356 were expected. Residents of counties within this cluster are at 1.52 times the risk of being exposed to a raccoon which is positive for rabies relative to residents of counties outside the cluster. A secondary high rate cluster was found among 67 counties and independent cities throughout south-central Virginia and central North Carolina. This cluster had 577 confirmed positive raccoons, with 458 expected positives, a rate 37% higher than outside the cluster (RR=1.37,  $p < .0001$ ). Meanwhile, two low-risk clusters were identified along the ORV zone. The first cluster, containing 25 counties and independent cities along the West Virginia-Virginia border,

had 0 confirmed positives with 68 expected. The second cluster, containing 22 counties and independent cities in southwest Virginia and western North Carolina, had 52 confirmed positives with 146 expected and rate 66% lower than outside the cluster (RR=0.34, p<.0001).

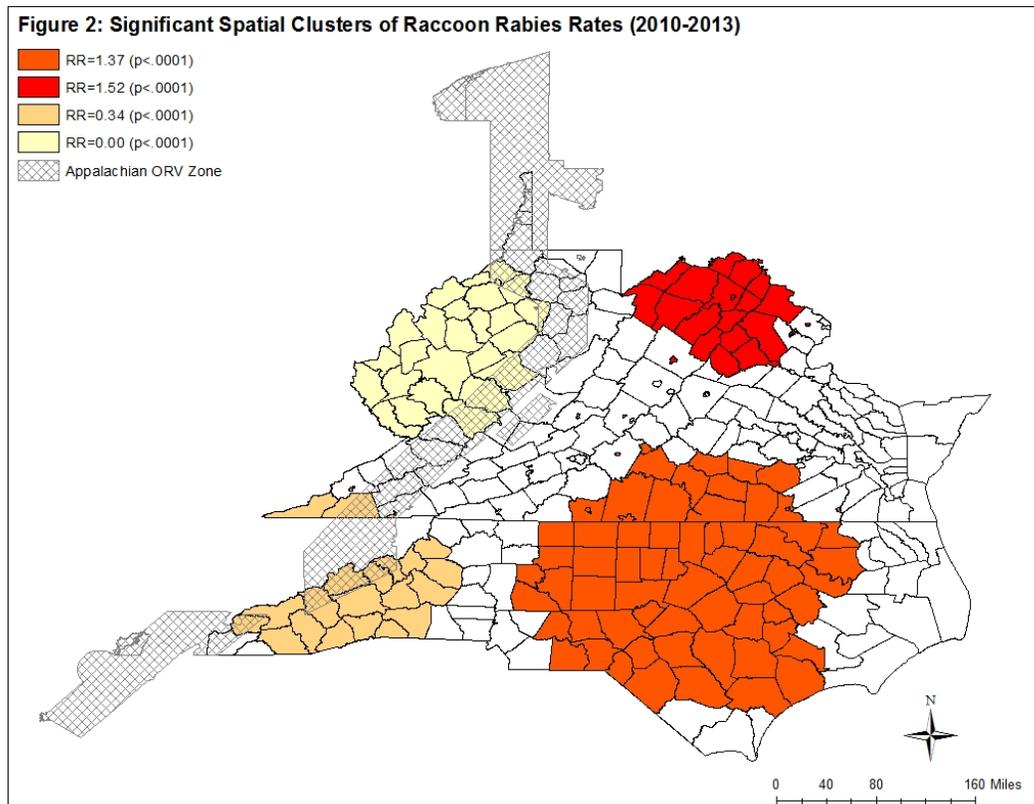


Table 2 shows the distribution of geographic and demographic attributes of each county averaged or summed by state. While North Carolina is the largest state both in total population and total land area, Virginia has the highest average median household income. 81 (60.4%) of Virginia’s counties and independent cities are classified as urban, while only 46 (46%) and 21 (38.2%) counties are classified as urban in North Carolina and West Virginia, respectively. In West Virginia, a majority of citizens in every county claimed a high school diploma as their highest educational attainment. In Virginia, 28

counties and independent (20.8%) cities claimed “some college” as their majority educational attainment, while 9 (6.7%) claimed “at least a bachelor’s degree”. In North Carolina, 40 counties (40%) claimed “some college” as their majority educational attainment, while 3 (3%) claimed “at least a bachelor’s degree”. The distribution of farmland and forest land is fairly even in North Carolina counties. In Virginia, the majority of counties and independent cities contain between 50 and 75% forest land, while the distribution of farmland is fairly equal. In West Virginia, the majority of counties contain more than 75% forest land and farmland is distributed fairly evenly.

<b>Variable</b>		<b>North Carolina</b>	<b>Virginia</b>	<b>West Virginia</b>
<b>Number of Counties and Independent Cities</b>		100	134	55
<b>Total Population</b>		9,535,483	8,001,024	1,852,994
<b>Average Median Household Income (\$US)</b>		\$41,710.54	\$52,464.96	\$36,968.36
<b>RUCC (# of counties or independent cities)</b>	<b>Metro</b>	46	81	21
	<b>Non-Metro</b>	40	38	22
	<b>Rural</b>	14	15	12
<b>Educational Attainment</b>	<b>≤ HS Diploma</b>	57	97	55
	<b>Some College</b>	40	28	0
	<b>≥ Bachelor's Degree</b>	3	9	0
<b>Total Land Area (acres)</b>		31,115,830	25,273,881	15,386,409
<b>Percent Forest Land (# of counties or independent cities)</b>	<b>0-49.5%</b>	29	18	3
	<b>50-64.9%</b>	30	42	9
	<b>65-74.9%</b>	25	20	9
	<b>75-100</b>	16	17	30
<b>Percent Farmland (# of counties or independent cities)</b>	<b>0-16.9%</b>	26	21	18
	<b>17-27.9%</b>	25	24	12
	<b>28-38.9%</b>	24	22	17
	<b>39-100%</b>	23	31	8

Table 3 describes the bivariate analysis of each independent variable against our outcome variable of interest. According to these analyses, population, educational attainment, and forest

land are not statistically significant ( $p < 0.10$ ) predictors of raccoon rabies rates. There appears to be some dose-response relationship between raccoon rabies rates and farmland coverage; as farmland coverage increases, raccoon rabies rates appear to increase as well. Those variables with statistical significance were retained for analysis in the final model.

<b>Table 3: Bivariate Analyses to determine significance of independent variables against outcome alone</b>				
<b>Variable</b>		<b>Parameter Estimate</b>	<b>95% CI</b>	<b>P-value</b>
<b>State</b>	North Carolina	0.482	(0.224-0.740)	0.0003
	Virginia	0.524	(0.275-0.772)	<.0001
	West Virginia	0.00	---	---
<b>Population</b>		0	0	0.78
<b>Income</b>		0	0	0.07
<b>RUCC</b>	Metro	0.273	(-0.033-0.578)	0.08
	Non-Metro	0.42	(0.100-0.741)	0.01
	Rural	0.00	---	---
<b>Education Attainment</b>	≤ HS Diploma	0.033	(-0.325-0.390)	0.86
	Some College	-0.023	(-0.208-0.162)	0.81
	≥ Bachelor's Degree	0.00	---	---
<b>Forest Land</b>	0-49.5%	-0.345	(-0.605--0.085)	0.009
	50-64.9%	-0.292	(-0.559--0.025)	0.03
	65-74.9%	-0.102	(-0.322--0.117)	0.36
	75-100%	0.00	---	---
<b>Farmland</b>	0-16.9%	0.529	(0.312-0.745)	<.0001
	17-27.9%	0.573	(0.114-0.350)	<.0001
	28-38.9%	0.296	(0.119-0.062)	0.01
	39-100%	0.00	---	---

Table 4 describes the final model developed with negative binomial regression analysis to fit raccoon rabies rates, which was of primary focus in this region. Compared to a those living in West Virginia counties, all other factors being held constant, residents of North Carolina counties who reported a potential exposure were 1.67 times more likely to have actually encountered a rabid raccoon ( $p < .0001$ ), while residents of Virginia

counties and independent cities who reported exposure were 1.82 times more likely to have actually encountered a rabid raccoon ( $p < .0001$ ). Compared to those counties where farmland makes up less than seventeen percent of total area, all other factors held constant, residents of counties with 17-28% farmland who reported exposure were 1.32 times more likely to have actually encountered a rabid raccoon ( $p = .013$ ), counties with 28-39% farmland were 1.84 times more likely ( $p < .0001$ ), and counties with 39-100% farmland were 1.64 times more likely ( $p < .0001$ ). Compared to rural counties, all other factors held constant, residents of non-metropolitan counties who reported exposure were 1.56 times more likely to have actually been exposed to a rabid raccoon ( $p = .005$ ), while those in metropolitan areas were 1.41 times more likely ( $p = .024$ ).

**Table 4: Negative Binomial Model for Positive Raccoon Rabies and Other Species Rabies- Relative Risk and 95% Confidence Intervals, Ecologic Analysis by County**

Variable		Raccoon Rabies			Other Species		
		RR	95% CI	P-value	RR	95% CI	P-value
State	North Carolina	1.67	1.31-2.12	<.0001	1.13	0.82-1.55	0.4516
	Virginia	1.82	1.43-2.31	<.0001	2.05	1.51-2.79	<.0001
	West Virginia	1.00	--	--	--	--	--
Percentage Farmland	39-100%	1.64	1.34-2.02	<.0001	1.58	1.18-2.12	0.0021
	28-38.9%	1.84	1.49-2.27	<.0001	1.97	1.47-2.64	<.0001
	17-27.9%	1.32	1.06-1.65	0.0131	1.55	1.14-2.09	0.0045
	<17%	1.00	--	--	--	--	--
Rural Urban Continuum Code	Metro	1.41	1.05-1.89	0.0240	0.66	0.48-0.92	0.0137
	Non-Metro	1.56	1.15-2.13	0.0045	0.99	0.69-1.36	0.8600
	Rural	1.00	--	--	--	--	--
Goodness of Fit		Deviance = 1.18 AIC = 1080.0 BIC = 1111.3			Deviance = 1.16 AIC = 1246.5 BIC = 1278.2		

The same model used for all other species of animals submitted for testing did not yield as many statistically significant associations. Compared to those citizens in West Virginia counties, residents of Virginia counties who reported exposure have 2.05 times the risk of actual

exposure to a rabid animal other than raccoons, all other factors held constant ( $p < .0001$ ). Compared to those counties where farmland makes up less than seventeen percent of total area, all other factors held constant, residents of counties with 17-28% farmland have 1.55 times the risk of actual exposure ( $p = .005$ ) to a rabid animal other than a raccoon, counties with 28-39% farmland have 1.97 times the risk of actual exposure ( $< .0001$ ), and counties with 39-100% farmland have 1.58 times the risk of actual exposure ( $p = .002$ ). Residents of metropolitan counties have 44% lower risk of actually being exposed to a rabid animal other than raccoons compared to those of rural counties, all other factors held constant.

## V. Discussion

The purpose of this study was to identify the current distribution of wildlife rabies in central Appalachia, as well as identify potential demographic factors which might be associated with variation in this distribution. As expected from the initial spatial overview of the rates of raccoon rabies in central Appalachian counties, the spatial scan statistic identified several county clusters with unexpectedly high or low rates of rabies compared to the area as a whole. Those clusters with low rates were located near the USDA's ORV zone, while those clusters with high rates tend to be more centrally located. It is also evident from the Figure 1 that there are several counties where USDA random surveillance is suggesting low rates of rabies, while the state health departments are reporting upwards of 50% positive test results. The negative binomial regression analysis found that the variation in the distribution of raccoon rabies rates by county can be best explained by percent of area designated as

farmland, the state in which the county is located, and the rurality of the county as classified by the USDA Economic Research Service's Rural-Urban Continuum Codes.

As far as we know, this is the first ecological analysis of wildlife rabies rates in the US which investigates potential factors explaining the variation in rabies rates at the county level. The study contributes to understanding of the current distribution of raccoon rabies in a highly endemic region and highlights potential factors associated with varying rates of rabies by county. This is especially important in a time when wildlife rabies, particularly in raccoons, has reached historically high percentages<sup>36,37</sup>. The results of this study suggest that while there are strong variations in the rates of raccoon rabies in central Appalachian counties, these variations are not easily explained by demographic characteristics. It might be that the higher rates of rabies in more metropolitan counties could be explained by the greater number of people available to contact a wild animal and submit it for testing. However, the regression analysis for county population alone resulted in a relationship that was not statistically significant ( $p=0.78$ ). The finding that some measure of population density and percent farmland were both related to risks of raccoon rabies rates is supported by the natural tendency of raccoons to live in close proximity to humans<sup>38</sup>, as well as by findings of another study focusing on potential rabies epizootics in the tidewater region<sup>39</sup>.

The most interesting finding from this study is the statistical significance of state as a variable. Given the somewhat scattered distribution of raccoon rabies rates across this region, as well as the widely varied demographics of each county, we expected to see more statistically significant demographic factors in the model. However, it does not appear that county demographics have a large impact on the rates of raccoon rabies, other than the rurality of each county. It is also interesting to note that given the statistical significance of

the state variable, both North Carolina and Virginia counties had higher relative risks of rabies exposure than West Virginia counties. The USDA ORV zone runs along the spine of the Appalachian Mountains, almost cutting West Virginia in half as well as covering a large portion of its surface area. The combination of lower raccoon rabies rates reported by both the USDA and the SHD in West Virginia, as well as the lack of explanation from demographic factors, suggests that the ORV zone may play a bigger role in raccoon rabies control than previously realized.

In future studies, it would be interesting to perform a similar analysis with an independent variable measuring proximity to the ORV zone. This would allow us to better analyze the true impact this ORV program has on the reduction of raccoon rabies and thus the decreased risk of exposure to humans. It would also be beneficial to conduct a comparative analysis of USDA surveillance rates and SHD surveillance rates where the USDA was able to trap over all counties, not just those in proximity to the ORV zone. A more comprehensive study such as this would allow us to compare models with raccoons trapped randomly to those voluntarily submitted to the SHD. It could also be beneficial to perform a similar study analyzing the effect of natural borders, such as rivers, mountains, lakes, or gorges, as wildlife tend not to follow political county barriers. Finally, it may be beneficial to expand this study to states on both sides of the ORV zone to not only explore how the ORV zone affects raccoon rabies, but also to provide a better model for other reservoir species, such as the southeastern skunk.

This study did have some limitations which should be mentioned. There were several counties with no submissions to the SHD, which does not indicate that rabies is not present or that humans are not at risk of being exposed. It could be that there were exposures but the

animal was not available for testing, or the animal was available but was delivered in such a condition that it was inadequate for testing. Low numbers of submissions at the county level result in smaller sample sizes and thus a weaker analysis, although this cannot necessarily be helped without further collapsing rates across counties. This study also relies on the accuracy of the reporting individual. When animals are submitted to the state health department for rabies testing, the CDC and most state protocols dictate that it should be due to an actual exposure, defined by the CDC as either a bite exposure (where the skin is broken and saliva is allowed to enter the wound) or a non-bite exposure (where potentially infectious material, such as saliva or nervous tissue directly contacts open wounds, nervous tissue, or mucous membranes)<sup>1</sup>. The exact type of exposure is not always indicated in the rabies report when an animal is submitted, and these particular data do not include any information about the potentially exposed individual. Therefore, true exposure is not guaranteed and the rates calculated in this analysis may not accurately portray human exposure. The use of SHD data to observe rabies rates is very reliable, as rabies is a notifiable disease in both humans and animals and thus is actively surveyed in every state. The addition of the USDA data allows for a more unbiased surveillance of rabies rate in the central Appalachian region.

## VI. Conclusion

This study contributes to knowledge about the current distribution of wildlife rabies in central Appalachia, and provides a novel view of the variation in the distribution of rates. Expansion of this study across more states, as well as with more demographic variables, would be beneficial to our understanding of how to reduce the risk of human exposure to

rabies in the United States. From this study, it appears that the best predictors of raccoon rabies exposure risk at the county level are percent farmland, state, and rurality. The results of this study as well as the lack of literature exploring the zoonotic trends of rabies in the US demonstrate a need for further research concerning this topic. Only through this understanding will we be able to focus our efforts on rabies prevention and education and thus reduce the overall cost of rabies prevention and control.

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## Acknowledgements

This work would not have been possible without the constant help and guidance of my committee. Thank you for seeing this project through.

Dr Wayne Sanderson

Dr Steve Browning

Dr W. Jay Christian

Dr Brent Shelton

Thanks to all the officials who provided me with data for this research. The creation of this dataset would not have been possible without the collaboration of each of you.

Dr. Julia Murphy, DVM, MS, DACVPM, State Public Health Veterinarian, VDH

Mr. Waqas Humayon, Virginia Department of Health Office of Epidemiology

Ms. Jordona D. Kirby, Rabies Field Coordinator, USDA APHIS WS

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A special thanks to my friends and family for encouraging me throughout this program. I could not have made it without your unwavering love and support.

## Appendix

### SatScan v 9.3

Purely Spatial analysis scanning for clusters with high or low rates using the Discrete Poisson model.

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#### SUMMARY OF DATA

Study period.....: 2000/1/1 to 2000/12/31

Number of locations.....: 289

Total population.....: 4550

Total number of cases.....: 1879

Annual cases / 100000.....: 41211.2

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#### CLUSTERS DETECTED

1. Location IDs included.: 54053, 54079, 54035, 54011, 54087, 54105, 54039, 54107, 54043, 54099, 54013, 54005, 54015, 54085, 54073, 54021, 54045, 54007, 54059, 54019, 54067, 54095, 54017, 54081, 54041, 54109, 54101, 54103, 54033, 54097, 54047

Overlap with clusters.: No Overlap

Coordinates / radius.: (38.769520 N, 82.026550 W) / 158.00 km

Gini Cluster.....: Yes

Population.....: 164

Number of cases.....: 0

Expected cases.....: 67.73

Annual cases / 100000.: 0

Observed / expected...: 0

Relative risk.....: 0

Log likelihood ratio.: 68.977306

P-value.....: < 0.000000000000000001

2. Location IDs included.: 37115, 37021, 37199, 37087, 37121, 37089, 37111, 37175, 37011, 37099, 37149, 37173, 37161, 37023, 51169, 51520, 37189, 51105, 37113, 37027, 37075, 51191, 37045, 51720, 51195, 37009, 37043, 51167, 37035

Overlap with clusters.: No Overlap

Coordinates / radius.: (35.858100 N, 82.705620 W) / 136.23 km

Gini Cluster.....: Yes

Population.....: 353

Number of cases.....: 52

Expected cases.....: 145.78

Annual cases / 100000.: 14700.4

Observed / expected...: 0.36

Relative risk.....: 0.34

Log likelihood ratio..: 42.666241

P-value.....: 0.0000000000000048

3. Location IDs included.: 51069, 51840, 51043, 54027, 51187, 54003, 54037, 54065, 51171, 51107, 54031, 51157, 54057, 51061, 51139, 54023, 51047, 51683, 51685, 51113, 51153, 51600, 51165, 51059, 51610, 51660, 51079, 51013, 51137, 51510, 54071

Overlap with clusters.: No Overlap

Coordinates / radius.: (39.204360 N, 78.262520 W) / 110.62 km

Gini Cluster.....: Yes

Population.....: 862

Number of cases.....: 493

Expected cases.....: 355.98

Annual cases / 100000.: 57074.2

Observed / expected...: 1.38

Relative risk.....: 1.52

Log likelihood ratio..: 29.876217

P-value.....: 0.00000000042

4. Location IDs included.: 37183, 37063, 37101, 37069, 37085, 37135, 37037, 37077, 37105, 37127, 37195, 37181, 37145, 37001, 37191, 37185, 37051, 37033, 37125, 37163, 37079, 37065, 37093, 51117, 37083, 37151, 37081, 37107, 51590, 51083, 37061, 37147, 37157, 37123, 51025, 37165, 37017, 37153, 37131, 51143, 51111, 37155, 51037, 51081, 37117, 37057, 51595, 37103, 51690, 51089, 37067, 37167, 37015, 37141, 37169, 51135, 37007, 51147, 37049, 51031, 37091, 37133, 37013, 51053, 37047, 37159, 37059, 51175, 51067, 51011, 51141, 51183, 37025, 51007, 51620, 51680, 37197, 51019, 37187, 37041, 51515, 37073, 37179, 37129, 37137, 51730, 51049, 37171

Overlap with clusters.: No Overlap

Coordinates / radius.: (35.790100 N, 78.650100 W) / 195.62 km

Gini Cluster.....: Yes

Population.....: 1110

Number of cases.....: 577

Expected cases.....: 458.39

Annual cases / 100000.: 51874.4

Observed / expected...: 1.26

Relative risk.....: 1.37

Log likelihood ratio..: 19.264553

P-value.....: 0.0000054

5. Location IDs included.: 37119

Overlap with clusters.: No Overlap

Coordinates / radius.: (35.246460 N, 80.832630 W) / 0 km

Gini Cluster.....: No

Population.....: 185

Number of cases.....: 49

Expected cases.....: 76.40

Annual cases / 100000.: 26431.7

Observed / expected...: 0.64

Relative risk.....: 0.63

Log likelihood ratio..: 5.842828

P-value.....: 0.551

6. Location IDs included.: 51017, 51091, 51163

Overlap with clusters.: No Overlap

Coordinates / radius..: (38.058610 N, 79.740940 W) / 37.38 km

Gini Cluster.....: No

Population.....: 34

Number of cases.....: 28

Expected cases.....: 14.04

Annual cases / 100000.: 82182.5

Observed / expected...: 1.99

Relative risk.....: 2.01

Log likelihood ratio..: 5.419734

P-value.....: 0.686

---

## Analysis

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Type of Analysis : Purely Spatial

Probability Model : Discrete Poisson

Scan for Areas with : High or Low Rates

## Spatial Neighbors

-----

Use Non-Euclidian Neighbors file : No

Use Meta Locations File : No

Multiple Coordinates Type : Allow only one set of coordinates per location ID.

### Spatial Window

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Maximum Spatial Cluster Size : 25 percent of population at risk

Window Shape : Circular

Isotonic Scan : No

### Space And Time Adjustments

-----

Adjust for known relative risks : No

### Inference

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P-Value Reporting : Default Combination

Number of Replications : 999

Adjusting for More Likely Clusters : No

### Spatial Output

-----

Report Hierarchical Clusters : Yes

Criteria for Reporting Secondary Clusters : No Geographical Overlap

Report Gini Optimized Cluster Collection : Yes

Gini Index Based Collection Reporting : Optimal Only

Report Gini Index Cluster Coefficients : No

Spatial Cluster Maxima : 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, 25

Restrict Reporting to Smaller Clusters : No

## Other Output

-----

Report Critical Values : No

Report Monte Carlo Rank : No

## Run Options

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Processor Usage : All Available Processors

Suppress Warnings : No

Logging Analysis : Yes

## SAS v 9.3 Regression code

```
proc genmod data=temp1;
  class state farmland_cat rucc;
  model SHDPOSrac = state farmland_cat rucc /
    dist=nb link=log offset=ltotalrac type3 dscale;
  estimate 'Beta' state 1 0 -1 / exp;
  estimate 'Beta' state 0 1 -1 / exp;
    estimate 'Beta' farmland_cat 1 0 0 -1 / exp;
    estimate 'Beta' farmland_cat 0 1 0 -1 / exp;
    estimate 'Beta' farmland_cat 0 0 1 -1 / exp;
  estimate 'Beta' rucc 1 0 -1 / exp;
    estimate 'Beta' rucc 0 1 -1 / exp;
title "Dr Browning's Favorite Model for raccoons";
run;
proc genmod data=temp1;
  class state farmland_cat rucc;
  model SHDPOSother = state farmland_cat rucc /
    dist=nb link=log offset=ltotalother type3 dscale;
  estimate 'Beta' state 1 0 -1 / exp;
  estimate 'Beta' state 0 1 -1 / exp;
    estimate 'Beta' farmland_cat 1 0 0 -1 / exp;
    estimate 'Beta' farmland_cat 0 1 0 -1 / exp;
    estimate 'Beta' farmland_cat 0 0 1 -1 / exp;
  estimate 'Beta' rucc 1 0 -1 / exp;
    estimate 'Beta' rucc 0 1 -1 / exp;
title "Dr Browning's Favorite Model for all other species";
run;
```