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LAND USE and URBANIZATION PATTERNS IN AN ESTABLISHED ENZOOTIC RACCOON RABIES AREA

by

JOHN E. DUKE

D.V.M., AUBURN UNIVERSITY

A Thesis Submitted to the Graduate Faculty of Georgia State University in Partial Fulfillment of the Requirements for the Degree

MASTER OF PUBLIC HEALTH

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ABSTRACT

We analyzed how land-use patterns and changes in urbanization influence positive raccoon rabies cases in an established enzootic area. County resolution was used and the study area included all 159 counties in Georgia. We obtained data on raccoons submitted from 2006 through 2010 for testing at the state public health labs due to exposure incidents with people or domesticated animals. The land-use patterns were extracted from the US Geological Survey's National Land Cover Database from both 2001 and 2006. Odds ratios were calculated on 16 land-use variables that included natural topography, agricultural development, and urbanization. An additional variable, Submissions/Population density, was used to normalize counties and to account for population bias associated with rabies surveillance. The use of this demographic variable was substantiated by GIS clustering analysis. The outcome variable was heavily right skewed and over dispersed and therefore a negative binomial regression was used in this count statistics technique. The final analysis showed that low intensity residential development is associated with raccoon rabies cases while evergreen forest offers protection. This study supports the hypothesis that the raccoon rabies enzootic is maintained in those edge ecosystems of urbanization. It is advocated here that the public health animal rabies database to include GPS coordinates when reporting wildlife rabies submissions for testing to improve the resolution when studying the disease ecology of enzootic rabies.

Key words: enzootic, raccoon (*Procyon lotor)*, rabies, land-use, count statistics, demographic bias, surveillance, environmental heterogeneity

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Land Use and Urbanization Patterns in an Established Enzootic Raccoon Rabies Area

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I. Introduction

The terrestrial reservoir for rabies in Georgia and the entire eastern seaboard is the raccoon (*Procyon lotor*). Dog rabies *variant* in the U.S. has been eliminated through persistent vaccination laws and policies (Velasco-Villa et al., 2008). In Georgia, the rabies raccoon enzootic has been established for over 50 years and whenever domestic animal rabies cases do occur, we know from molecular studies that the rabies variant is of raccoon origin (Jackson, Wunner, W. H., & Smith, Jean S. - chapter 3, 2002; McQuiston, Yager, Smith, & Rupprecht, 2001; Recuenco, Cherry, & Eidson, n.d.).

Although people are exposed to bite wounds and saliva from a variety of mammals, and greater than 50% of post-exposure prophylaxis (PEP) cases are due to dog and cat bites, rabies disease maintenance in Georgia is mostly attributed to the raccoon enzootic. One study estimates that the average incidence of rabies PEP is about 13.1 per 100,000 persons in those states where raccoon rabies is enzootic. For comparison, the incidence is estimated to be 7.8 per 100,000 where skunk rabies is enzootic, and about 2.2 where there is no terrestrial reservoir (Christian, Blanton, Auslander, & Rupprecht, 2009). For example, after the raccoon rabies epizootic entered the New England states, public health rabies prevention expenditures and annual human exposure to rabid animals rose dramatically (Bretsky & Wilson, 2001; Chang et al., 2002). Wildlife enzoonosis is one factor in the algorithm that health care providers use when determining whether to start PEP (Moran et al., 2000).

It is possible that the enzootic terrestrial species maintains the terrestrial rabies virus in all other carnivore populations (Krebs, Williams, Smith, Rupprecht, & Childs, 2003; Lembo et al., 2008). Bats are a confounding factor in rabies epidemiology and unlike terrestrial reservoirs,

where species-specific carnivores maintain the disease in defined geographical areas, multiple bat species are a continental-wide non-terrestrial reservoir that unlikely spills over into *domestic* animals. Molecular evidence suggests that the raccoon (wildlife) enzootic has originations from a bat variant. (Blanton, Palmer, & Rupprecht, 2010). Therefore, without discounting the evidence that exists for bat rabies variants to potentially evolve and adapt to terrestrial mammals, it is important to know where to concentrate very limited publicly allocated resources to control the raccoon enzootic before the raccoon-adapted variant possibly adapts to another terrestrial species as well, most likely the skunk (Guerra et al., 2003; Leslie et al., 2006; Wang et al., 2009). Land use studies may help to formulate those policies by pinpointing where the raccoon enzootic is maintained.

The purpose of this study is to help understand the geographic distribution of raccoon rabies cases across an established enzootic area and to evaluate the hypothesis that the enzootic might be maintained in high density raccoon populations in those edge ecosystems of urban and suburban development. An edge ecosystem is created when a natural habitat is broken up. This can occur naturally, such as disturbance by fire, or can be part of the geomorphology, such as a lake shoreline or the demarcation line between uplands and wetlands. Or it can occur when both urbanization and agricultural development creates this line between forest and open space. It is analogous to increasing the surface area. Some species, for example, predators at the top of the food chain, decline under these conditions while others, such as blue jays, deer, and raccoons might thrive. Those that thrive utilize increased food resources in the disturbed area while using the natural habitat for protective cover (T. M. Smith & Smith, 2008).

The raccoon is an opportunistic omnivore and it is well documented that they have not only adapted but also probably thrived from urbanization and farming enchroachment into

forested habitats. They will eat anything from crayfish in creeks to acorns to bird eggs to garden vegetables and even garbage. They have been known to devastate corn crops. They are commonly hit by road traffic and they have been known to commute through sewer drains from denning sites to food resources. Therefore, higher densities of raccoon populations may exist in urbanized areas than in other habitats (Riley, Hadidian, & Manski, 1998; H. T. Smith & Engeman, 2002).

A study of 203 counties in Maryland, Pennsylvania, and Virginia retrospectively analyzed 14 land use and demographic variables in association with the raccoon epizootic when it first progressed through that study area. The study showed that human population density, water coverage, agricultural use, and mixed forest were significantly correlated with the initial epizootic and that deciduous forest was a protective variable. (Jones, Curns, Krebs, & Childs, 2003). The study also acknowledged that human population density can have considerable bias due to increased surveillance; there are more cases reported where there are more people due to the rabies database being a passive surveillance system in which cases are reported only when people or domesticated animals are suspected of being exposed to a raccoon. It may be possible to mitigate this bias by including a weighted variable on the right side of the regression equation.

Therefore, this study was undertaken to:

- Detect the influence of land use, both natural and anthropogenic, on cases of raccoon rabies in a long-term established enzootic area.
- Understand the influence of increasing urbanization on rabies cases in an adaptable, omnivorous enzootic species such as the raccoon.
- Explore methodology to decrease the human population bias associated with a passive surveillance database.

II. Literature Review

Rabies is probably nearly 100% fatal in raccoons. Evidence comes from immunological and population studies. It has been demonstrated that raccoon populations fall by as much as 40% after an initial epizootic passes (Riley et al., 1998). It is assumed that very few rabid raccoons survive natural rabies infection because herd immunity has been estimated to be very low, between 1 and 5% (Childs et al., 2000).

Several studies have described the spatial and temporal dynamics of the epizootic as it spreads into new raccoon populations. The spread of the raccoon variant all along the eastern seaboard began in the 1970's when a raccoon incubating the virus was trans-located into Virginia from an enzootic area of the southeastern United States (Baer, 1991; Nettles, Shaddock, Sikes, & Reyes, 1979; J. S. Smith, Sumner, Roumillat, Baer, & Winkler, 1984). The enzootic had previously been contained in Florida and Georgia for approximately 30 years (Kappus, Bigler, McLean, & Trevino, 1970). Once the disease took hold in Virginia, the literature has described the epizootics into new naïve raccoon populations as being "sensational" (Real, Russell, Waller, Smith, & Childs, 2006). An initial epizootic can be so fulminating that natural barriers such as rivers and high elevation will only slow it down (David L. Smith, Lucey, Waller, Childs, & Real, 2002; Wheeler & Waller, 2008).

Generally, the initial epizootic spike is large and lasts for about 48 months. Then the following epizootics are smaller and of shorter duration but possibly occur more frequently. Subsequent epizootics are defined as having number of cases above the median for 2 consecutive months and probably occur on average at 4 year intervals (Childs et al., 2000; Gordon et al., 2004) (Figure 1).

However, as the disease spread outward from the initial node of infection in Virginia, there were significant differences in the characteristic of the epizootics in the southern states as compared to northern states. Almost 65% of counties in the eastern seaboard states have experienced a raccoon rabies epizootic but the epizootics were less frequent, smaller, and temporarily more erratic in those counties such as North Carolina than those counties in the northeast (Childs et al., 2001). Fifteen years after the first case of trans-located raccoon rabies was detected in Virginia, it had reached 700 km north into New Hampshire yet had only progressed to the North Carolina coast, 300 km away from the index case. It is suggested that lower raccoon densities were the reasons for the slower southward spread (Biek, Henderson, Waller, Rupprecht, & Real, 2007).

Most studies consider whether or not human population densities confound their results. Density may influence surveillance in the first place; positive cases might be biased due to increased testing resulting from more frequent human contact with the reservoir species.

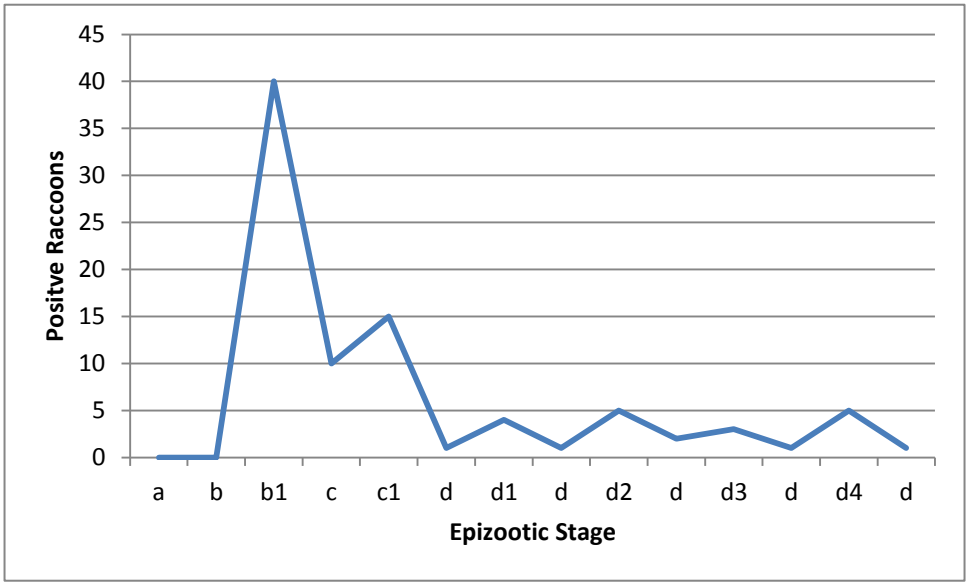

Fig 1: Schematic of the temporal stages of a typical county in New York during the raccoon rabies epizootic from 1992 to 2000. Stage a: Pre-raccoon variant – rare cases in raccoons; cases of raccoon rabies might spill over *from* other wildlife. b1: initial epizootic of raccoon variant rabies as it moves through the county. c1: sequential epizootic. d1: sequential inter-epizootics. (Adapted from Gordon et al, 2004).

There has been much less research done on the enzootic after it has become established. Studies in New York that were done 15 years after the epizootic had passed offer an example for characterizing how land use patterns might influence the raccoon rabies enzootic. Using census tract resolution, there were significant increases in raccoon rabies cases associated with low intensity residential areas. Wetlands were a significant protective variable (Recuenco, Eidson, Cherry, Kulldorff, & Johnson, 2008). Although water is considered a preferred habitat of raccoons, the water sources usually consists of small streams and vernal (temporary) ponds among fragmented forested landscapes, including among agricultural development. (James C. Beasley, Devault, Retamosa, & Rhodes Jr., 2007). The enzootic studies in New York used statistical methodology, using covariates, to adjust for population densities and large scale geographic variations when finding significant clusters of cases. It found that clusters of recurring epizootics were consistently in the Albany area, a city with a metropolitan area of just under 1 million. This study seemed to indicate that epizootics occurred more frequently than normal 4 year raccoon rabies cycles in an urbanized area and that their methods helped mitigate demographic bias to make this conclusion. (Recuenco, Eidson, Kulldorff, Johnson, & Cherry, 2007).

In rural areas, using radio-telemetry on collared raccoons and doing trap and recapture studies, it has been documented extensively, at multiple scales of resolution, that raccoons prefer edge habitat at the agricultural – forest interface when compared to large tracts of deciduous forests (J. C. Beasley & Rhodes, 2010; Pedlar, Fahrig, & Merriam, 1997). Where there are large forest tracts, the spread of epizootics between urbanized areas is significantly slowed and rivers actually become more of an effective barrier (D L Smith, Waller, Russell, Childs, & Real, 2005). In Ontario, when comparing raccoon densities for managing the eastern raccoon enzootic either

through distribution of oral vaccination (rural areas) or through trap-vaccinate-release programs (urbanized areas) it was found that raccoon densities are at least twice as high and possibly even 35 times higher in the populated, developed edge habitats in southern Ontario, especially in the Niagara area near the New York border (R. Rosatte et al., 2010). Without regard to the effects that agricultural crops might have in providing raccoon food resources, urban and suburban sites simply have significantly higher raccoon carrying capacities when compared to rural areas (Prange, Gehrt, & Wiggers, 2003).

Even with the bias that might be associated with increased passive surveillance in higher population centers, the literature clearly establishes that the raccoon is highly adaptable and does quite well in the disturbed habitats caused by development, whether it be agricultural or urbanized. We know that the epizootic seems to fade from a single, large event where the population of susceptible hosts falls significantly due to rabies being 100% fatal, to a more chronic enzoonosis that seems to flare up with fewer cases and of shorter duration. Our analysis of the land use variables from the 159 counties of Georgia hopes to offer some information about how the raccoon rabies enzootic behaves in an area where it has been present for as long as anywhere else in North America.

III. Methods

Description of area:

The state of Georgia includes an area of 153, 911 square kilometers. It is divided into more counties than any other US state, except for Texas (US Census Bureau, 2010) (Figure 2). Texas has 1.6 times the number of counties as Georgia yet is over 4 times larger geographically. This division into smaller units in Georgia gives resolution for analyzing data by counties in the United States.

Georgia consists of ecotones that range from the Blue Ridge Mountains to the north, highest elevation 1458 meters above sea level, where there are 4 seasons, to the Atlantic coast in the southeast. The coastline is 309 kilometers long. The southern half of the state is involved in crop production and paper production, where the dominant land use is agricultural development and cultivated evergreen forests (Georgia Department of Natural Resources). The metro area of Atlanta is the 9th largest in the United States at over 5 million and spreads out over an area of 21, 694 square kilometers (US Census Bureau).

Outcome variable:

The outcome variable is the number of positive raccoon cases per county. The five most recent years of data, from 2006 through 2010, were acquired from the Georgia Department of Community Health, Division of Public Health and the Centers for Disease Control and Prevention (Atlanta, Georgia, USA). This dataset is generated only from raccoon specimens involved in human or domestic animal exposure incidents that are submitted to the public health state diagnostic labs. Testing for the presence of rabies virus is done by direct fluorescent-antibody staining of brain tissue. In previous years, only positive cases were reported and filed

in the database. However, negative cases were reported into the database as well beginning in 2006.

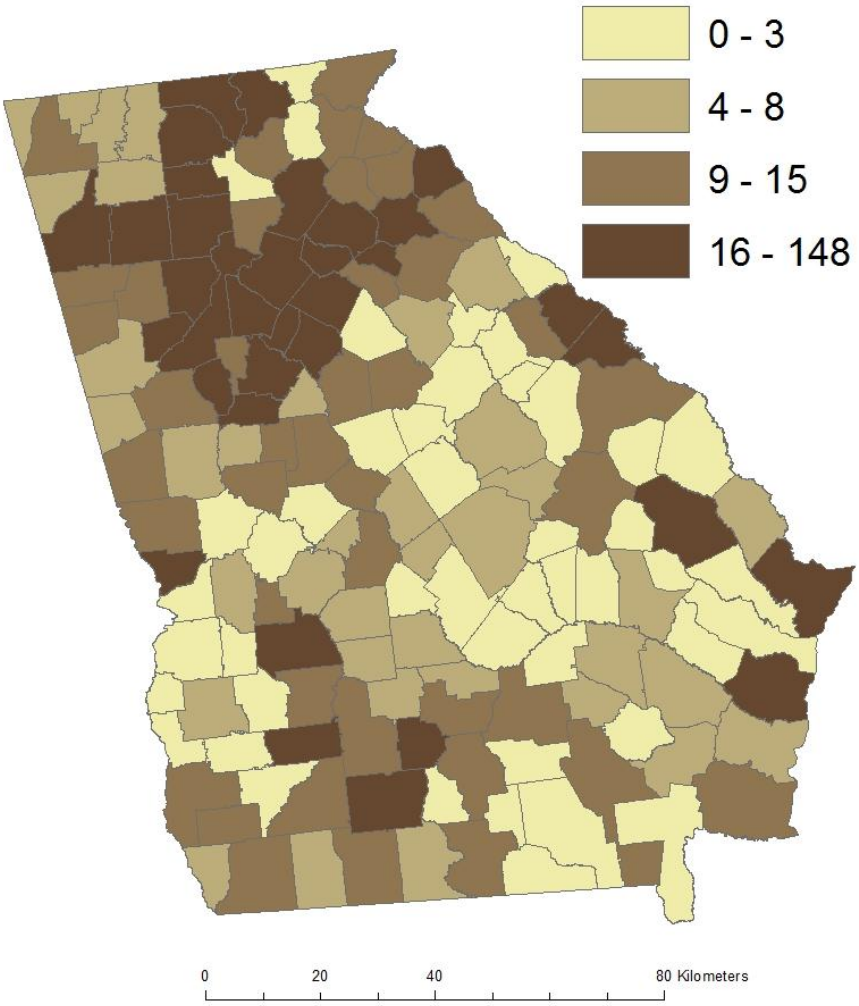

Fig 2: Counties in Georgia and number of raccoons submitted for testing by quartile from 2006 – 2010.

Of the 159 counties in Georgia, there were 9 that submitted no specimens during the five year period but all counties were used in the analysis. There were a total of 2064 raccoon specimens submitted for testing over the 5 year period (Fig 2).

Predictor variables:

Table 1 summarizes the predictor variables. Using Geographical Information Systems (GIS – ESRI ArcMap 10), fifteen land use variables from each county were extracted from the National Land Cover Database 2006 (US Geological Survey). This database is made from satellite imagery and at a spatial resolution of 30 meters; the shapefile classifies every 900 square meter pixel (30 meters X 30 meters) into one of 20 values. Five values do not exist or are not found in large enough areas to be within resolution in Georgia (perennial ice, dwarf scrub, sedge/herbaceous, lichens, and moss). Variable construction was bases on the method of Jones *et al* 2003. It combined two USGS variables, "open space development" and "low intensity development" to form a "low intensity residential" variable. The USGS "medium intensity development" variable became our "high intensity residential" variable and the USGS "high intensity development" variable became our "commercial" variable. These variables are a measure of impervious surface and account for less than 50%, 50 – 79%, and 80% or greater impervious surface area, respectively.

The "agriculture" variable was made by combining the pasture/hay and cultivated crop data. Both census data and land area of each county were obtained from the 2010 US Census (US Census Bureau) to calculate population densities.

Three "change in urbanization" variables to examine change over time were created by also extracting the developed variables from the National Land Cover Database 2001 (US Geological Survey). Again this data were converted into "low intensity residential," "high intensity residential," and "commercial" categories. The change that occurred in each of these categories was calculated according to the following formula: (2006 NLCD – 2001 NLCD) / (Total County Area – Open Water Area).

Variable	Symbol	Units	Source	NLCD value
population density	*density*	persons/km^2	US Census Bureau	n/a
open water	*opn_water*	km^2	USGS NLCD 2006	11
low intensity residential	*res_low*	km^2	USGS NLCD 2006	21 + 22
high intensity residential	*res_hgh*	km^2	USGS NLCD 2006	23
commercial	*com_hgh*	km^2	USGS NLCD 2006	24
change in low intensity res.	*U_res_l*	%	NLCD 06 and 01	n/a
change in high intensity res.	*U_res_h*	%	NLCD 06 and 01	n/a
change in commercial dev.	*U_com*	%	NLCD 06 and 01	n/a
barren land (rock/sand/clay)	*baren*	km^2	USGS NLCD 2006	31
shrub/scrub	*shrub*	km^2	USGS NLCD 2006	52
deciduous forest	*for_dec*	km^2	USGS NLCD 2006	41
evergreen forest	*for_egn*	km^2	USGS NLCD 2006	42
mixed forest	*for_mxd*	km^2	USGS NLCD 2006	43
agricultural use	*agrictre*	km^2	USGS NLCD 2006	81 + 82
natural grasslands	*grasslnds*	km^2	USGS NLCD 2006	71
forested wetlands	*wtlnds_wdy*	km^2	USGS NLCD 2006	90
herbaceous wetland	*wtlnds_eh*	km^2	USGS NLCD 2006	95

Table 1. Independent variables used to predict positive raccoon rabies cases. NLCD: National Land Cover Database (US Geological Survey).

Extraction of Predictor Variables and Statistical Analysis:

GIS was used to combine some of the land use classes as described above and calculate the area of each land use variable in each county. First the state of Georgia was extracted from the entire National Land Cover Database. We obtained both a state shapefile and a shapefile with all 159 county borders from the Atlanta Regional Commission's GIS online database. All independent variable data generated from GIS was imported as dbf files into SAS (version 9.2, Cary, NC). Using SAS data preparation code, each individual land use dbf file was combined with all others to formulate one dataset. This dataset was then exported into Microsoft Excel (2010) to be combined with the US census bureau data and CDC/State Public Health rabies data. Microsoft

Excel was used to calculate the change in urbanization variables. This entire dataset was then imported into SAS to first conduct an analysis of the descriptive statistics, normality, and crude odds ratios.

After determining which independent variables might have significance from crude odds ratio calculations, we used those variables to develop a model of predicting number of rabies cases by backwards stepwise negative binomial regression. We added all significant variables from the odds ratio calculations and then removed any variable that had a significance of $p>0.1$. The final model was derived by removing any variable with a $p>0.05$. The negative binomial regression model is an adaptation of Poisson regression when all the assumptions associated with Poisson distribution are not met. The distribution of the dependent variable meets most requirements for Poisson regression; it is heavily right skewed and has only 14% of zero numbers in the dataset. However, negative binomial regression was used because the variance of the dependent variable was approximately 10 times higher than the mean (Cameron & Trivedi, 1998).

Finally, using the Getis-Ord Gi statistic in GIS, we mapped significant clustering cases first. An inverse distance squared analysis of nearby county neighbor was used in this GIS tool so that the most likely influence of clustering would be attributed to bordering counties. Therefore, to account for testing bias in higher density areas we used a weighted independent variable, Submissions/Density (# submissions per person per square kilometer) in the model that we ran so that the model began as:

"Positive cases" = "Submissions/Density" (+ significant variables, including both land use and density).

IV. Results

Descriptive Statistics

The total number of raccoon specimens submitted for testing, as expected, influenced the number of positive cases. The GIS clustering analysis shows that clustering by raw positive cases occurs mostly in the metro area of Atlanta where there are the highest amounts of submissions. However, when clustering is analyzed as a rate of population density (# positive cases per person per square kilometer) the clustering disperses to rural areas. Clustering of submissions by population density also follows a similar pattern (Fig 3).

The number of positive cases of raccoon rabies by county over the five year period ranged from 0 to 50. The total number of positive cases was 1011 but the mode was 1 (Figure 4). Submissions ranged from 0 to 148 with the median being 8 and the upper quartile demarcated at only 15 (Table 2). The 3 highest submissions came from the core metro area of Atlanta. All three were in the top 4 most populous and the top 5 most densely populated counties. Six of the top sixteen came from the core counties of three of the larger cities in the state (Atlanta, Augusta, and Savannah). However, the city that would rank in size similar to Augusta and Savannah, Columbus, ranked 27th in number of submissions yet attained high enzootic status (8 positives) as well. Dichotomization of variables on the upper quartile was based on the Jones, *et al* 2003 study and therefore, a high enzootic county had 8 positive cases (Table 2).

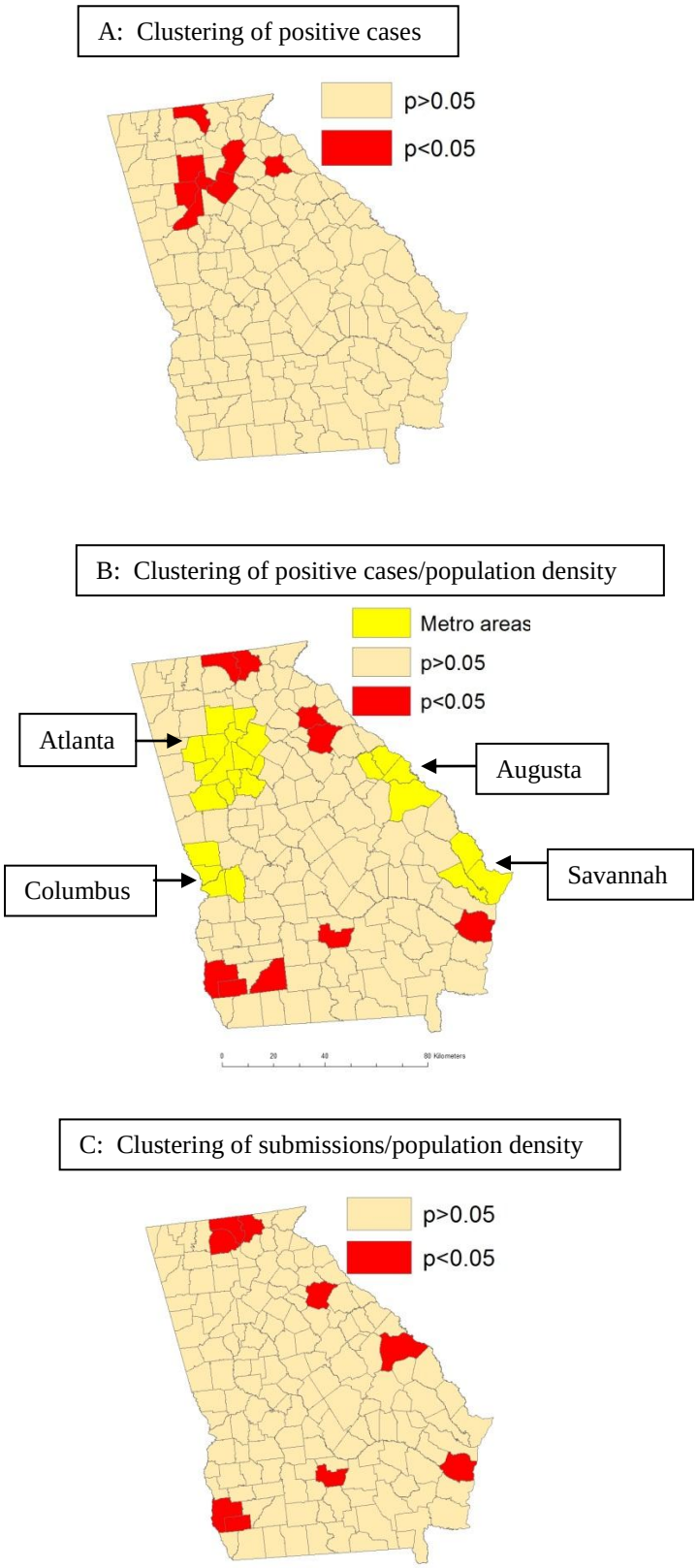

Fig 3: Clustering analysis. Significance is $p \leq 0.05$ or z-score of greater than 1.96.

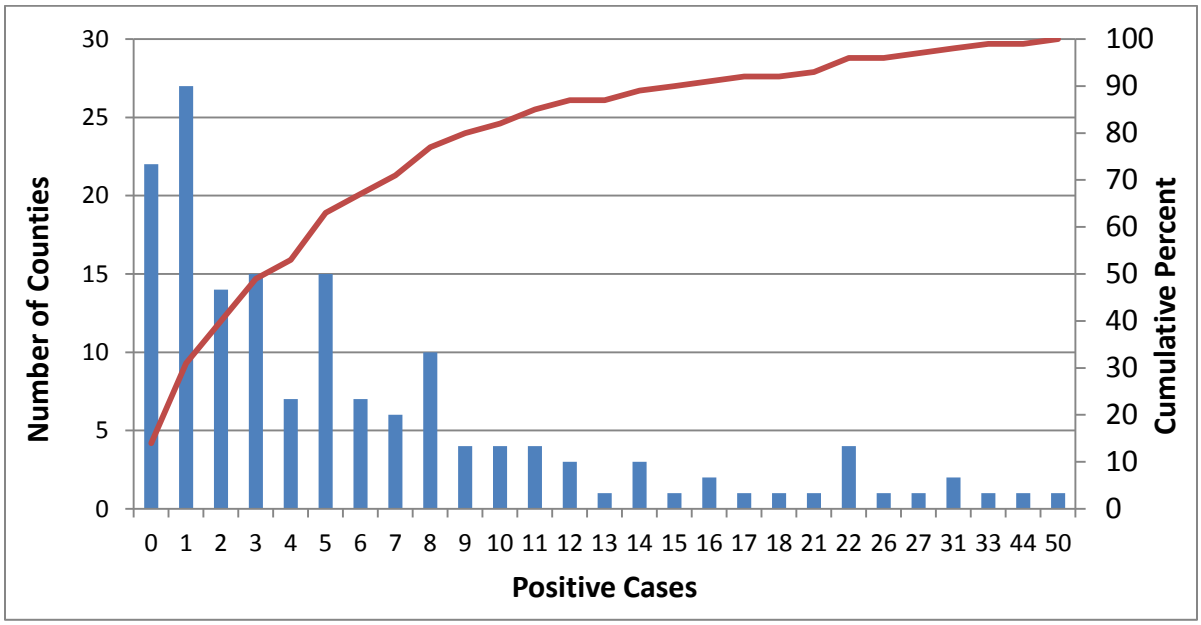

Figure 4. Number of counties (n=159) by positive raccoon cases and the cumulative percent. Over half of the counties had fewer than 5 positive cases over the 5 year period (2006 – 2010). The 75th percentile falls at 8 positive cases (n=46).

The size of the counties in Georgia range from 314 to 2347 square kilometers and the average county is just less than 1000 square kilometers. Fulton County has a large range of land uses because it incorporates most of downtown Atlanta and also has rural farmland in the south as well as low density suburban areas to the north. It ranks 21st in size and is fifth for the percentage of low intensity residential development, ranks third in percentage of commercial development, yet still ranks 111th (out of 159) in percentage of total forested land.

DeKalb County incorporates a small part of the city of Atlanta. However, because its land use patterns are not quite as disparate as Fulton, it is the most densely populated county. Clinch, the least dense county and the 3rd largest geographically, had only one submission over the 5 year period (positive). It also has the 3rd highest amount of total wetlands and the largest area of evergreen forest in the state.

Miller, the county with the highest percentage of agricultural development in the state, over half its total area, had only 11 submissions. However, 9 were positive and therefore became ranked as a high enzootic area. Another county to note is Fannin. It is located in the Blue Ridge Mountains, has the most forested area, consisting of mostly deciduous, and its county seat is the town of Blue Ridge. It submitted 30 specimens over the 5 year period, of which 90% were positive.

Table 3 summarizes the crude odds ratio from changes in urbanization between 2001 and 2006 and table 4 shows the crude odds ratios from the 13 land-use patterns, including agricultural and urban use, and natural topography. We used population density (Table 2), all urbanization variables, and barren land, in addition to the protective variables evergreen forest, shrub, and woody wetlands in our initial regression model.

Variable	Median	3rd Quartile	Range	Enzootic high	Enzootic low	OR	95% CI
density	24.9	58	3.2 - 986.1	22	17	**5.2**	**2.4 - 11.2**
Submissions	8	15	0 - 148	----------	---------	----------	---------
Positive	4	8	0 - 50	---------	---------	----------	----------
Sub/density	0.238	0.432	0 - 1.66	---------	---------	---------	---------

Table 2: Demographic data, number of submissions for testing, and positive raccoon cases from 2006-2010 for 159 Georgia counties. Density is persons/km^2. Sub/density is # of all raccoons submitted for testing per person per square kilometer and is the weighted variable used in the regression model. High enzootic counties have $\geq$ 8 positive cases.

Variable	Median	3rd Quartile	Enzootic high	Enzootic low	OR	95% CI
Low density residential	0.07	0.37	23	17	**5.6**	**2.6 - 12.3**
High density residential	0.01	0.06	23	17	**5.6**	**2.6 - 12.3**
Commercial	0.003	0.02	22	18	**4.8**	**2.2 - 10.4**

Table 3: Percent change in urbanization from 2001 to 2006 for 159 Georgia counties.

Variable	Median	3rd Quartile	Enzootic high	Enzootic low	OR	95% CI
Open water	7.4	13.7	14	27	1.5	0.7 - 3.3
Low density residential	58.1	98	18	21	**2.8**	**1.3 - 6.0**
High density residential	2.4	6	21	19	**4.2**	**1.9 - 8.9**
Commercial	0.7	2.3	23	17	**5.6**	**2.6 - 12.3**
Barren	2	4.34	17	23	**2.3**	**1.1 - 4.9**
Shrub	23	54.2	3	37	**0.14**	**0.04 - 0.5**
Deciduous forest	135.5	277.1	12	27	1.1	0.5 - 2.5
Evergreen forest	198.9	318.9	5	35	**0.3**	**0.1 - 0.7**
Mixed forest	25.7	46	10	30	0.8	0.3 - 1.7
Agricultural	132	237	11	29	0.9	0.4 - 2.0
Grasslands	56.2	86.4	8	32	0.5	0.2 - 1.3
Woody wetlands	54	149.2	6	34	**0.3**	**0.1 - 0.9**
Herbaceous wetlands	1.5	11.8	8	32	0.5	0.2 - 1.3

Table 4. Current land-uses patterns (km^2) and relative risk of having larger numbers of raccoon rabies cases for 159 Georgia counties. Enzootic status is number of counties at or above the 3rd quartile land-use pattern and also having $\geq$ 8 positive cases (high) or < 8 positive cases (low). Bold lettering indicates significant odds ratio.

Open water, deciduous forest, mixed forest, agriculture, grasslands, and emergent wetlands had a confidence interval that encompassed 1 and therefore were not considered in the negative binomial regression model.

Our final model was:

ln(Pos cases) = 0.8968 + 1.7239(Sub/Dens) - 0.0026(Evergn forest) + 0.0077(Low Dens Res).

All independent variables have a significance of $p<0.0001$. The model always over-predicts by an average of 2 when there are no positive cases in the county. If there are between 1 and 15 cases in the county, the model predicts with a margin +/- 3. When there are 16 or greater positive cases in the county, the model usually under-predicts (Table 5). However, in Fulton and

Gwinnet counties it over-predicted by a factor of about 4 and in Dekalb and Cobb counties by a factor of 2 (Fig 5).

Actual Positive Cases	Average Difference Predicted by Model	Average Absolute Difference	Variance (absolute difference)
0	2	2	0.796
1 - 15	0	3	13.85
>15 (16 – 50)	10	26	1227

Table 5: The accuracy of the model to predict at 3 different levels of positive cases. Variance is calculated from the residuals of the regression line.

Fig 5: Fulton, Cobb, and Gwinnett counties: The low residential value accounts for over 80% of the *predicted* cases in the model

Actual	31	50	31
Predicted	141	100	144

In this study, evergreen forest offers as much protection as low density residential development provides risk for high incidence of enzootic raccoon rabies. This occurs because, although the evergreen forest coefficient absolute value is 3 times smaller than low density residential coefficient, coverage of evergreen forest in this study area is approximately 3 times larger than low density residential development on average. Therefore both types of land coverage contribute to the overall model in equal absolute values.

V. Discussion

No other study from our search indicates that a weighted variable to account for submission bias has ever been used. The submission/density variable essentially acted as an addition to the y-intercept; all counties were normalized before the other independent variables were analyzed. Even so, testing bias has not been avoided in this study. The weighted variable contributed about the same to the model on average as either of the two land use variables. But because this study accounted for demographic bias as part of the independent variable analysis in the model itself, it provides greater confidence in our findings of significant land-use patterns associated with enzootic raccoon rabies.

The Recuenco, et al 2007 enzootic study did use a covariate to adjust for population density and found the highest relative risk for clustering of raccoon cases around the Niagara Falls area, where the highest densities of raccoon populations were found in the Rosatte, et al 2010 study. If higher raccoon populations are assumed to be correlated with higher rabies cases, the protection associated with evergreen forest cover found in this study seems to be supported by the natural history literature. For example, pine forests that are managed for timber production have lower raccoon utilization as compared to other forested habitats in general. Specifically, middle aged managed pine forests between 9 and 15 years old are the least preferred habitat selected by raccoons when compared to other forested habitats (Chamberlain, Conner, & Leopold, 2002).

The reason that the model has such high variability in those counties with high submissions numbers (Fulton, Gwinnett and Cobb) is not because of the additional weighted variable. The weighted variable can equalize the counties because the submissions/density value

is very low in Fulton, Gwinnett, and Cobb counties as compared to the other counties (0.119, 0.125, and 0.192, respectively). The low density residential value in these counties is what pushes the predicted positive cases so high; the number of submissions accounts for less than 8% of the model's value while the amount of low density residential area accounts for over 80% of the model's value in these three counties. This finding may indicate that in fact there are more cases of rabies in the raccoon population of these counties than we are finding by current methods. This hypothesis could be tested with a road kill surveillance study that is analyzed concurrently with submissions data and concurrently with mark and recapture studies that estimate raccoon populations.

This study supports the possibility that the raccoon rabies reservoir is maintained in low intensity urbanization areas but fails to support the idea that the same trend might occur from edge effects in agriculturally developed areas as well. This low density development effect on positive cases may be due to higher raccoon population densities in urbanized areas as described in the literature. Using multi-logistic regression, the Jones, et al 2003 study found agriculture as a significant variable, yet found no significance from any urbanization in their final analysis. However, because the distribution of positive cases in both their study and ours is very similar, we feel this study is more compelling because our statistical technique (negative binomial regression) was better adapted for right skewed and over dispersed data.

The challenge of any rabies study is the low number of data points for the outcome variable. To further complicate it, the data we have available, which uses advanced molecular laboratory confirmation of raccoon rabies cases, allows for high specificity for determining potentially high enzoonosis or episodes of epizootics yet sensitivity will be low. This poor sensitivity is due to the large-scale resolution that comes from aggregating the data at the county

level. If there is no database that holds data at a finer resolution, it becomes impossible to know where to begin to investigate an outbreak across an entire county. Excessive reporting at a certain hospital or clinic might trigger an investigation of an area but for the purposes of understanding the disease ecology of rabies, a street level resolution at best or a zip code level resolution at worst would hold much more value in these type studies. We advocate collaboration between the legal and public health professions to help establish such a database in the public health system.

A recent improvement in the database system that began in 2006 is what determined the 5 year period of reporting that was used. If this study were done in a different 5 year span or continued further, there might be different outcomes since this dataset is only sensitive to populations of raccoons that might have experienced an epizootic during this study period. In established enzootic areas, it seems that epizootic trends eventually dissolve into sporadic patterns. Therefore, replicating this study in other established endemic areas would also help in understanding how environmental heterogeneity influences reservoir rabies ecology where the raccoon rabies enzootic has been present long term.

These enzootic studies could help formulate cost-benefit analysis and policies in controlling enzootic rabies in the wildlife reservoir. At a minimum, this study re-enforces the idea that a nuisance raccoon in an enzootic area that is trapped should be euthanized as opposed to being relocated to prevent the spread of disease from a carrier animal to a naïve population. If euthanasia is not desirable or feasible, the trapped raccoon would probably need to be vaccinated at a minimum to afford some herd immunity in the population. However, there is the risk that the animal has already been exposed and one vaccination would not prevent the natural course of disease in a carrier animal.

Because public health rabies prevention costs stay elevated even after the initial epizootic has passed (Gordon, Krebs, Rupprecht, Real, & Childs, 2005), evaluating how to allocate resources in an enzootic area is imperative. Currently, oral vaccination control methods is the standard in rural areas and where there are natural barriers to help cordon the spread of the epizootic into naïve populations to the west. (Robbins et al., 1998; Roscoe et al., 1998). Trap-vaccinate-release programs have been used in urbanized enzootic areas to help establish herd immunity where there are high density raccoon populations and where distribution of oral food packs is less efficacious due to interference from both people and domestic animals (R. C. Rosatte, Power, MacInnes, & Campbell, 1992). Ecologists, public health officials, and wildlife managers are increasingly aware of using integrative strategies to address disease hot spots linked to anthropogenic environmental change (Paull et al., 2012). If there is hope of eliminating rabies in a wildlife reservoir that adapts to human disturbance as well as the raccoon, resources would need to be allocated towards enzootic urbanized areas more heavily. Those tactics might include:

- Public health educational advertising campaigns that are intended to discourage raccoon population growth in urbanized areas
- Providing herd immunity, especially in urbanized epizootic areas, through a trap-vaccinate-release program
- Using GIS to understand and define natural barriers' role in containing the reservoir population. This includes not only using mountains and rivers as barriers but also possibly pure stands of evergreen forests as a potential natural cordon.
- Using GIS to define human population density contour intervals where a "ring" of outer exurb zones of oral vaccination enhance a trap-vaccinate-release program

- Funding studies to determine the lowest level of herd immunity that confers protection
- Improving the public health database by reporting at a finer resolution. This includes providing GPS equipment to animal control officers and other personal that collect raccoon specimens for testing so that coordinates can be logged into the database. With this resolution GIS buffering techniques around the urban-forested habitat interfaces could be used to analyze land patterns in relation to positive rabies cases.
- Consistent monitoring of the variants in other wildlife species through molecular typing to assess the adaptability of the reservoir variant

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