Longitudinal analysis of raccoon rabies in West Virginia, 2000-2015

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Animal borne rabies is a source of infection in humans, and raccoons (*Procyon lotor*) are the primary terrestrial reservoir in West Virginia (WV). To assess the behavior and status of raccoon variant rabies virus (RRV) in WV, a longitudinal analysis for the period 2000-2015 was performed, using data provided by the state Bureau of Public Health. Analytic approaches included linear-mixed, Poisson, and zero inflated Poisson regressions. Each of these approaches indicated that there had been a reduction in numbers of RRV positive animals over the study period, predominantly due to a decrease in raccoon infections. Non-raccoon species did not appear to have a similar decline, however. This has implications for the preventive measures currently being implemented. Spatial analyses of RRV and further examination of the virus in non-raccoon species are warranted.

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Abstract

Animal borne rabies is a source of infection in humans, and raccoons (*Procyon lotor*) are the primary terrestrial reservoir in West Virginia (WV). To assess the behavior and status of raccoon variant rabies virus (RRV) in WV, a longitudinal analysis for the period 2000-2015 was performed, using data provided by the state Bureau of Public Health. Analytic approaches included linear-mixed, Poisson, and zero inflated Poisson regressions. Each of these approaches indicated that there had been a reduction in numbers of RRV positive animals over the study period, predominantly due to a decrease in raccoon infections. Non-raccoon species did not appear to have a similar decline, however. This has implications for the preventive measures currently being implemented. Spatial analyses of RRV and further examination of the virus in non-raccoon species are warranted.

Introduction

Rabies causes an almost invariably fatal infection in any mammal, including humans. This neurotropic virus is a lyssavirus, within the *Rhabdoviridae* family.(Prevention 2011a) Rabies induced fatal encephalomyelitis is endemic throughout the Americas, with higher numbers of human deaths in Mexico, Central America and South America.(Prevention 2011c)

Over the past 100 years, the species distribution of rabies and risk of human exposure in the United States have changed dramatically because of coordinated vaccination efforts in domestic animals, especially dogs and cats.(Prevention 2011b; Prevention 2011c) More than

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90% of all animal cases reported annually to the CDC now occur in wildlife; whereas before 1960, the majority were in domestic animals.(Prevention 2011b) The principal rabies hosts in the U.S. today are wild mesocarnivores and bats,(Prevention 2017) and most human exposures remain from carnivores. Although rabies kills thousands of people each year worldwide, human deaths have decreased dramatically in the United States, (Lozano et al. 2012) primarily due to successful preventive efforts.

Affected wildlife animals, including raccoons (*Procyon lotor*), often lose their fear of humans and become active during daylight hours, drastically increasing the potential for human and domestic animal exposures.(Kappus et al. 1970) When a dog or cat is reported to have bitten a human, the animal is quarantined for an established observation period of 10 days.(webmaster@wvlegislature.gov 2017) In the event of a wild animal bite, if the animal in question is deceased, or the quarantine period is not established, the brain of the animal is submitted for rabies confirmation.(Brown et al. 2016) Often, the person bitten must undergo a post exposure prophylactic (PEP) regimen that entails an injection of anti-rabies immunoglobulin in addition to three doses of human rabies vaccine.(Prevention 2015) In the US, 40,000 to 50,000 PEP treatments are given to people annually, suggesting rabies remains a significant problem.(Prevention 2015) In the event of a local outbreak, or when the disease becomes established in a region, the number of PEP treatments administered increases to meet the local need, putting a burden on local resources. While costs vary, a course of rabies immunoglobulin and three doses of vaccine typically exceed $3,000.(Prevention 2015)

There are a number of strains of rabies virus, with many being associated with a specific host species, although spillover into other hosts frequently occurs.(Wallace et al. 2014) In fact, the labelling of the strains by host species (raccoon, bat, fox, etc.) is only reflective of the species that acts as the primary reservoir for that strain of virus at that time.(Baer 1991) Either bat or carnivore origin rabies infection can be transmitted to any other mammalian host. It is almost always lethal in all species, including humans, once symptoms have developed.

In the United States, effective rabies vaccination protocols for domestic animals have resulted in near elimination of the disease in that population.(Prevention 2011b) However, non-domesticated animals are still a problem and effective prevention does not come without an economic burden. The cost of rabies prophylaxis, treatment and control programs is estimated to be between $250 and $500 million dollars annually.(Prevention 2011b) This includes prophylaxis for both domestic and wild animals. Additionally, rabies infection in agricultural settings has significant cost burdens for animal producers.(Chipman et al. 2013)

In West Virginia (WV), the viral strains known to be present are the bat and raccoon rabies virus variants.(WV Dept of Health and Human Services 2017) Bat strain rabies virus has caused sporadic cases reported in all counties. Even so, bat cases represent less than 5% of the animals found to be positive for virus.(West Virginia Department of Health and Human Services 2017) Raccoon strain rabies virus (RRV) is currently enzootic in the Southeast United States, and it has expanded its range in the eastern part of WV subsequent to the inadvertent introduction of translocated rabid raccoons along the WV – Virginia border near Greenbrier County in the late 1970s.(Nettles et al. 1979) Since then, RRV infection has expanded geographically at a rate between 30 and 60 km/yr.(Sterner et al. 2009) This has become a major issue because, despite the success of immunization programs in domestic animals, rabies infection is on the rise in WV wildlife, particularly in raccoons.(West Virginia Department of Health and Human Services 2017) Raccoons are of special concern since they readily coexist in peridomestic settings close to humans; for example, they commonly raid refuse containers and pet food left outdoors, along with other food sources such as bird feeders around homes.

Prior to the introduction of RRV in WV, there were a handful (5-10) of rabies positive raccoons reported annually, all of which were infected with bat strain virus.(West Virginia Department of Health and Human Services 2017) Once the RRV front moved through, numbers of RRV positive raccoons increased dramatically, peaking in 2002 with 126 positive animals.(West Virginia Department of Health and Human Services 2017) Moreover, spillover species added 37 positive animals (23% of the total positives) in that year, including eight domestic animals (cats, horses and cows).(West Virginia Department of Health and Human Services 2017) RRV is especially associated with spillover into other species, and has been described as a "super spreader" organism.(Wallace et al. 2014) There has been some suspicion that RRV has the potential to undergo host shifts more readily than other viruses, because it easily adapts to a new host species and begins independent circulation within that species.(Wallace et al. 2014) This tendency could result in establishment of a viral reservoir in previously uncontaminated species, and potentially even in domestic animals, if vaccination and control practices are not maintained. The importance of RRV in WV is highlighted by the fact that over 95% of rabies positive animals identified from 2000-2015 were infected with RRV.(West Virginia Department of Health and Human Services 2017)

Of particular interest are cats which represent roughly 5% of the RRV positives identified in peridomestic settings in WV.(West Virginia Department of Health and Human Services 2017) This is of particular concern, because many remain unvaccinated for rabies, even though rabies prophylaxis is mandatory in WV.(webmaster@wvlegislature.gov 2017) In addition, cats have a propensity to establish viable feral populations.(Rupprecht et al. 2006) These factors, and others, contribute to the current situation, where over 2500 animal bites and other potential rabies exposures are reported annually in WV.(WV Dept of Health and Human Services 2017) Unfortunately, data are not available regarding how many of these are true exposures (defined as a bite or a scratch), nor is a complete species breakdown of the animals involved available.

In order to assess the public health risk of rabies to humans in WV and the current state of rabies prevention efforts, the aim of this paper is to determine whether there has been a significant change in the number of RRV cases over the period 2000-2015 in WV. The novel software techniques employed evaluate the data accounting for the uneven distribution of rabid animals, in addition to including all types of affected animal species in the analysis.

Materials and Methods

Data collection and database structure

Data used here were from the annual state rabies cases of RRV by county, provided by the WV State Bureau of Public Health.(West Virginia Department of Health and Human Services 2017) This is a complete dataset, including all cases of RRV identified in the state during the study period and the county where they were collected, as well as the species of positive animals and viral strain. Disease was diagnosed by submission of the heads of suspect animals to the state diagnostic laboratory by veterinarians and animal control personnel. Positive animals were then submitted to the CDC for viral strain identification. Unfortunately, the database does not provide location data more specific than county. Only those cases specifically identified to have RRV infection were retained for evaluation, with cases showing unspecified viral strain or bat strain excluded. All data were compiled in Microsoft Excel spreadsheets.

Data analyses

The open source statistical programming platform R was used to evaluate the data, employing the glmmADMB package (version0.8.3.3).(Skaug et al. 2016; Team 2017) Initial analysis involved generating descriptive statistics. Next, linear mixed models were developed to optimize the longitudinal nature of the data and examination of the data distribution revealed that it was not normally distributed (Figure 1). The data were seen as following a Poisson distribution, so a Poisson model was fit. However, Poisson modelling requires an offset, to permit different weighting of the data clusters. The offset is the variable used to denote the population at risk in each cluster for the Poisson regression. Although the preferred offset would be total raccoon population in each county, these data are not readily available. Several other factors were considered as possible offsets, and potential offsets evaluated in exploratory analyses. Offsets examined were area (in square kilometers) of individual counties, county population and county human population density. It became evident that these potential offsets were essentially equivalent, both in coefficient value as well as p-value. Given the known behavior of raccoons and their propensity to inhabit areas in close proximity to human activity, human population density was selected as the offset, as it was believed to be most likely to be proportional to actual raccoon populations.(Erb et al. 2012) Finally, due to the large number of zero case entries in many counties, an analysis incorporating a zero inflated Poisson (ZIP) approach was performed. The ZIP approach allows for separation of the two components of the distribution for separate analyses, separating out excess zero outcomes for analysis under a binomial distribution, while non-zero outcomes are evaluated using a Poisson distribution.(Lambert 1992) All models were run using $\alpha = 0.05$ as the significance threshold.

Results

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There were 1464 RRV positive animals during the study period from 2000-2015. These were comprised of 962 raccoons, 391 nondomestic non-raccoons (NDNR) (predominantly striped skunks (*Mephitis mephitis*) and red foxes (*Vulpes vulpes*)), and 111 domestic animals. Preliminary examination of the state RRV data from 2000-2015 seems to show a decreasing trend in absolute number of animal cases over that timeframe, as shown in Figure 2.

The mixed linear modeling provided a regression coefficient of -0.061, with a p-value of 0.0024, a highly significant result. The Akaike Information Criterion (AIC) for the linear-mixed model was 4432.4 however, indicative of a poor fit of the model to the data. Linear mixed models assume normally distributed data, which was not true in this study, and the AIC likely reflects this. Examination of the data histogram (Figure 1) bears this out, as previously discussed.

The Poisson model was fit using log human population density as the offset, yielding a regression coefficient of -0.050 with a p-value of <0.001 and an AIC of 2377.5, as shown in Table 1. This indicates that there is a significant negative trend in RRV numbers and that the model provides an improved fit to the data as compared to the linear mixed model.

The data appeared highly right skewed, as shown in Figure 1, with substantial numbers of zero values. Once the ZIP model was fit, a regression coefficient of -0.044 was obtained, with a p-value of <0.001 and a 2260.2 AIC. This result was highly significant and resulted in an AIC that indicated improved model fit. In the ZIP model, the coefficient can be interpreted as follows: the mean number of cases in log-scale was reduced by 0.044 per year for 16 years, which is equivalent to a reduction of 1.045 cases per year for 16 years.

Finally, ZIP models were fit for each of the three animal types in this study, and the results are shown in Table 2. Raccoons were found to have a regression coefficient of -0.053, with a p-value of <0.001 and an AIC of 1924.3, all of which are comparable to the results obtained for the total numbers of RRV positive animals. Nondomestic, non-raccoon (NDNR) species had a regression coefficient of -0.007, p-value of 0.55 and an AIC of 1057.1. Domestic animals had a regression coefficient of 0.002, p-value of 0.93 and an AIC of 584.6. These results indicate that while there was a significant (p<0.001) reduction in raccoon infection, no such significant reduction was detected in non-raccoon species, both nondomestic and domestic (p=0.55 and 0.93, respectively).

Discussion

An examination of the current literature indicates that there have been several studies regarding rabies infection, with many specific to RRV. Rabies is frequently reported in the Eastern United States, where the primary reservoir for the virus is the raccoon. Raccoons represented 32% of the positive animals nationwide in 2012, 2013 and 2014, although there was a reduction in total numbers of positive raccoons detected of 1.4%, 2.8% and 4.0%, respectively.(Dyer et al. 2013; Dyer et al. 2014; Monroe et al. 2016) Prior studies have focused on cases in raccoons, while disregarding cases in domestic and non-raccoon, non-domestic animals. The study includes a novel examination of all species affected with RRV. There is a relative paucity of published studies regarding longitudinal analysis of RRV. This is unfortunate, since such information is useful for determining the optimum allocation of limited resources for rabies prevention and control.

Since the inadvertent introduction of RRV into the Mid-Atlantic States, the disease has spread throughout the region and into New England and Canada. There have been few studies published that examine the numbers of RRV positive animals over time, especially ones that consider all positive animals as opposed to raccoons alone. The primary finding of the current

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analysis is that there has been a significant decline in all RRV positive animals in WV during the study period, with the bulk of the decline in positives occurring in raccoons. Substantial resources have been used in WV to control rabies in raccoons,(Nelson 2010; Slate et al. 2009) and have apparently been successful only with regard to raccoons. The decline does not extend to non-raccoon species. Control efforts have not deterred spread to, and among, other species. This suggests that the virus is becoming established in non-raccoon species, and may be beginning to circulate independent of the raccoon reservoir. This is plausible given the propensity of RRV to spillover into non-raccoon species and establish itself in new reservoir host animals.(Wallace et al. 2014)

These findings are consistent with the available literature. Ma et al noted a general reduction in numbers of RRV positive raccoons recovered in areas of WV where oral rabies vaccination (ORV) occurred, subsequent to the commencement of the ORV program.(Ma et al. 2010) Their study was concerned only with raccoons and examined counties where ORV had been provided, however, and compared them to the eastern counties of the state, rather than the state as a whole. Likewise, their data only extended up through 2007. They did identify 2002 as the peak of RRV positives in the state, with a subsequent smaller peak in 2006, but their study period ceased prior to the peaks in 2009 and 2011 shown in Figure 1. Here we also considered disease in non-raccoon animals, both domestic and non-domestic.

There are several potential reasons for the reduction in RRV incidence over the study period. The ORV project is well established in the state, and may be having a significant effect on overall RRV numbers. Additionally, given the rapidly fatal progression of the infection in affected animals, it is possible that the disease is "burning itself out" and has reached, or is reaching, a self-limiting steady state. Fluctuations in state and local human populations may be affecting raccoon numbers, with concomitant changes in animal contacts with affected animals.

The temporal pattern of RRV infection in non-raccoon animals may be cause for concern. One would anticipate that as numbers of RRV positive raccoons decline, numbers in non-raccoon species would experience a similar decline. This is not borne out by the data examined here. NDNR and domestic species had no significant changes in RRV positive animals. The fact that both are not declining tends to decrease the likelihood that this is simply a reflection of diminished domestic animal vaccination practices. This would indicate that RRV is not experiencing a decline in these animal species, and could be indicative of the virus becoming independently established in another reservoir where baits are not having an effect. This is of particular concern in the case of domestic animals, as these are most likely to have close contact with humans. Additionally, there were a number of cases in livestock species (such as horses, cows, sheep and goats). Although less frequently encountered than in domestic carnivores, these cases may actually represent higher risk to humans due to a lowered index of suspicion among farmers or veterinarians caring for these animals. This could cause significant delay in proper diagnosis of these infections, potentially allowing owners and others to have greater risk of infection.

Limitations: The data available constrain the current study. Given that the data provided are limited to county and year of collection and/or analysis, it is impossible to examine seasonal patterns or perform more detailed geographic analysis. Additionally, it is difficult to assess whether the animals submitted for testing are truly representative of the disease as it exists in the larger natural population of these species. It is possible that these animals represent a biased sample of the population as a whole. Finally, the limited number of submissions of non-raccoon animals, both domestic and wild, may place constraints on accurate assessment of the longitudinal trend in these species. The analyses suggested the need for an analytic approach that accounts for the high number of zeros in the data. Thus, a ZIP model was more appropriate given the data distribution; correspondingly, the fitted model had a lower AIC.

Conclusion

This study demonstrates that numbers of RRV positive animals declined significantly over the study period throughout the state of WV, particularly in the primary viral reservoir host, raccoons. There is no reason to assume that diagnostic or recovery methods have changed during this same period. Further examination of RRV in non-raccoon species seems warranted to explain why these diverse groups are not trending down also. Future directions for this work include a spatial analysis of those factors that may be associated with RRV and raccoon populations, including land use, human population density and availability of surface water as well as ambient weather conditions. Additionally, cluster analysis of RRV positives would provide useful information to use as guidance for RRV control and other public health measures.

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Table 1 (on next page)

Comparison of regression models fit for RRV in West Virginia, 2000-2015.

Comparison of the regression models evaluated in this study. All models showed significant reduction in RRV for the study period (2000-2015), with the ZIP regression providing the best fit to the data.

[a] Lower value indicates better fit

Analysis type	Coefficient (SE)	p-value	AIC[a]
Linear-mixed model	-0.061 (0.02)	0.0024	4432.4
Poisson model	-0.050 (0.006)	<0.001	2377.5
Zero inflated Poisson model	-0.044 (0.006)	<0.001	2260.2

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Table 2(on next page)

ZIP regression coefficients, p-values and AIC for the different animal types in this study,West Virginia, 2000-2015.

A comparison of the regression coefficients for the animal types during the study period, 2000-2015. Note the lack of significance of the regressions for the NDNR and domestic species. (NDNR = Nondomestic, non-raccoon)

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Animal type	*Regression coefficient (SE)*	*p-value*	*AIC*
Raccoon	-0.053 (0.008)	<0.001	1924.3
NDNR	-0.007 (0.012)	0.55	1057.1
Domestic	0.002 (0.024)	0.93	584.6

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Figure 1 (on next page)

Histogram of total RRV positives by county (n=55) in West Virginia, 2000-2015.

The histogram demonstrates the preponderance of zero count entries in the study period, necessitating zero inflated Poisson analysis.

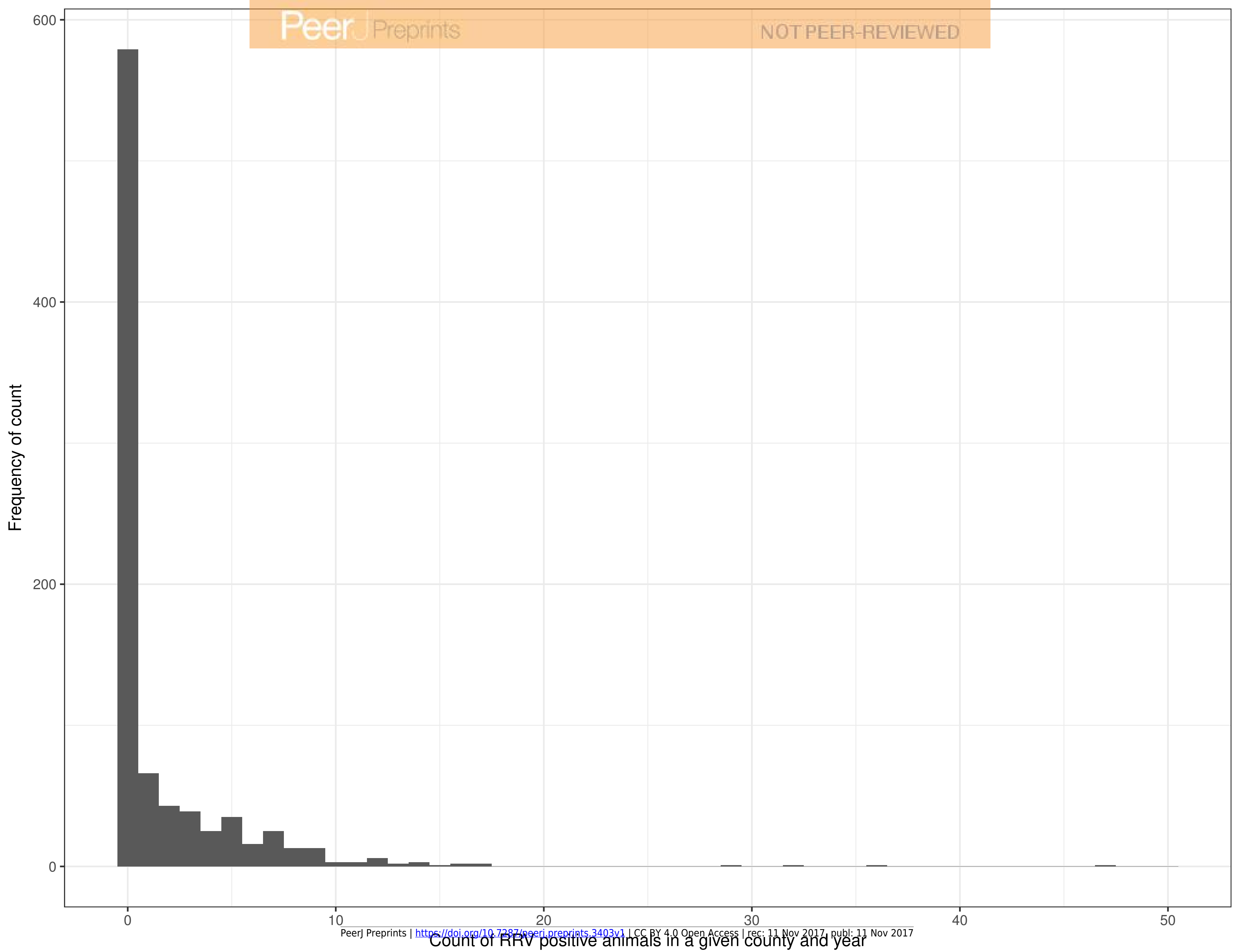

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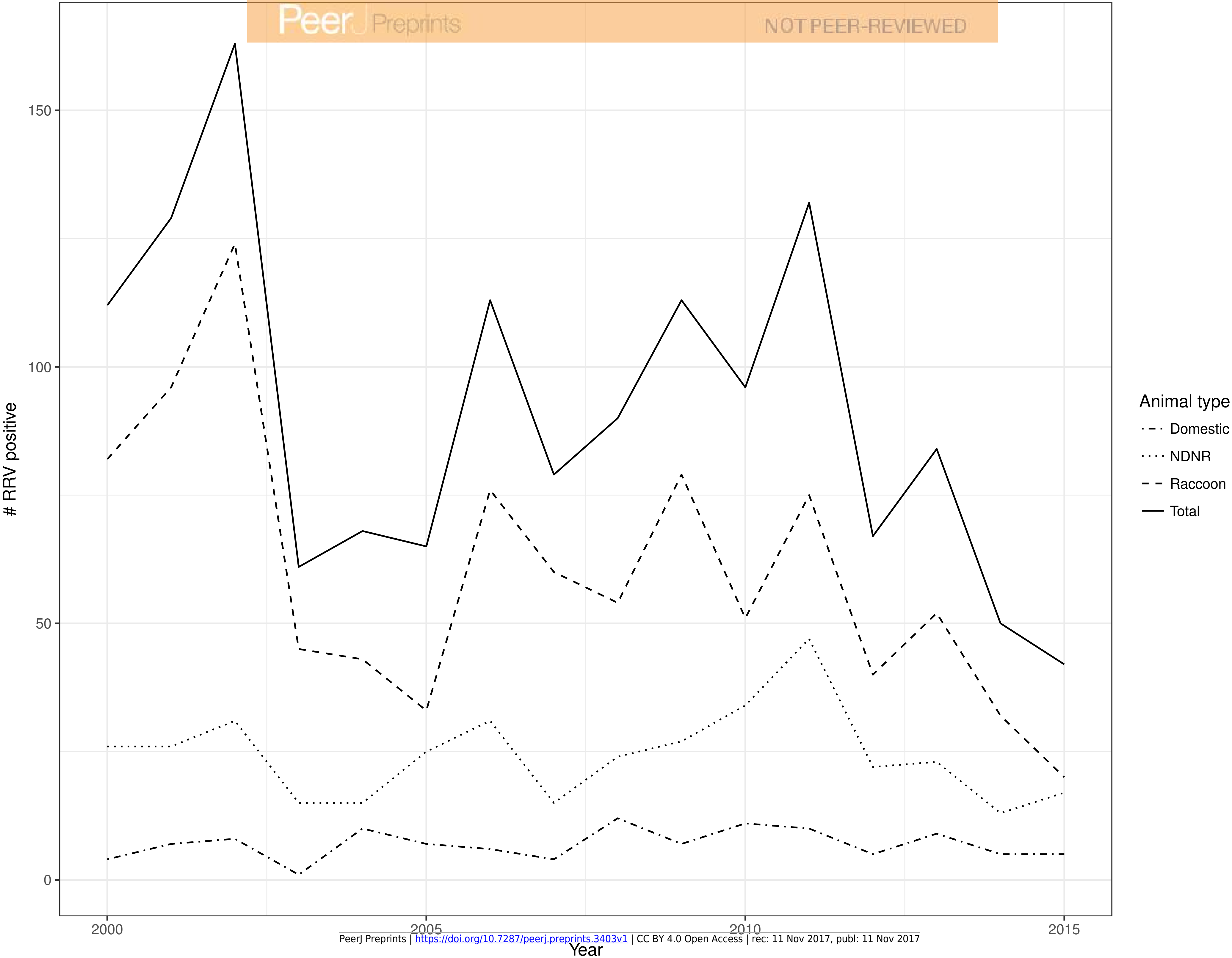
Figure 2(on next page)

Numbers of RRV positive animals by animal type, West Virginia, 2000-2015 .

Figure 2 depicts the decline of RRV in raccoons over the study period, 2000-2015. There was no concomitant decline in non-raccoon species.



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