Chaoter 3 : Canine Distemper virus in wild mesocarnivores in the Southeastern united states

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# INTRODUCTION

## Intro paragraph on CDV and land use and disease

Urbanization is occurring at an ever-increasing rate worldwide and indeed plays a large part in changing the composition of wildlife communities with some species suffering and others thriving under the new conditions. Not only does this altered landscape influence the macroscopic species, but this consequently has a huge role to play in the pathogen landscape at this wildlife-domestic-human interface (Bradley and Altizer 2007, Gottdenker et al. 2014).There are a number of specific features that are altered by anthropogenic land changes. Urban areas possess abundant resources for anthropophilic species, such as raccoons, that are not prone to seasonal fluctuations. These resources include food supplies (e.g., household waste) but also shelter. As a result, urban and suburban areas are capable of supporting much greater raccoon population densities (Prange et al. 2003). In addition to there being a greater quantity of resources in urban areas there tends to be greater aggregation of resources. This clumping of resources, for example at a large landfill site, results in two factors which are of importance in disease transmission; they result in migration of individuals into the area and in exceptionally high contact rates between not only member of the same species but between members of different species. Contact rates play a vitally important role in disease transmission with higher population density resulting in greater contact rates and consequently greater rates of disease transmission (Hu et al. 2013). One particular study showed this higher population density in response to resource availability resulted in higher parasite richness and increased *B. procyonis* prevalence in raccoons(Wright and Gompper 2005). And canine distemper virus cases are more prevalent in urban and suburban counties than in rural counties, which support a much lower population density of raccoons (Taylor et al. 2021). There is an added potential layer of complexity to additional resource provision as increased birth rates in this situation increase the abundance of susceptible juvenile hosts compared to a natural environment. Finally there is the question on how the quality of this diet (Schulte-Hostedde et al. 2018) affects the immune response of these individuals and whether this may also result in greater amount of pathogen shedding.

With habitat fragmentation, local extinctions occur resulting in a reduced local biodiversity of urban wildlife. This can influence the transmission of some vector-borne diseases through a process termed the ’dilution effect’(Keesing et al. 2006). In this process, high host species richness can lower parasite transmission if vectors feed on multiple host species varying in competence as a pathogen host. The reverse situation could occur in urbanized areas if low host diversity increases the abundance of particular hosts that serve as highly competent reservoirs. Essentially low competent hosts dilute the competent hosts in a highly biodiverse ecosystem resulting in lower pathogen abundance. An example of where the dilution effect comes into play is with West Nile virus whereby a higher non-passerine bird diversity results in significantly lower rates of WNV as these birds are much less competent hosts for the virus than passerine birds (Ezenwa et al. 2006). One can foresee then where in low biodiversity fragmented patches of urban land containing only anthropophilic passeriform birds that WNV transmission could be much higher than in larger less fragmented areas with higher biodiversity where the dilution effect comes into play.

There is also the issue of how urbanization brings native wildlife species into contact with novel pathogens to which they are immunologically naïve. As undisturbed ecosystems reach a state of equilibrium, the same can also be said for their host-pathogen systems. In the face of urbanization, another source of disturbance to this equilibrium is the introduction of “novel” pathogens to that system. This can occur in a number of ways. Either through the accidental introduction or release of invasive species (e.g., gray squirrels in the UK) or through domestic species. Any of these new species introduced to an area have their own set of pathogens with the possibility of transmitting these into the local wildlife population and threatening their health and survival. The example I will use here is from a well-studied system, that of the Golden Gate National Recreation Area in California (Riley et al. 2004). In this system we have a well-established native population of native gray foxes who harbor their own diversity of pathogens to which they have reached a natural equilibrium. This extensive study by Riley et al compared the seroprevalence in rural and urban zones for pathogens of domestic canines in gray foxes. The study showed a significantly higher exposure to parvovirus in gray foxes located in the urban area compared to the rural area. This is of significance as parvovirus is a pathogen with serious morbidity and mortality in canids (Steinel et al. 2001) and particularly in naïve populations, and this study demonstrates that urbanization is bringing wildlife populations into contact with “novel” pathogens that may pose a significant threat.

·How do the dynamics change across rural-urban gradient with change in host density? (Bianco et al., 2020)

·Role of anthropophilic species

·Land use effects on host population size and connectedness of population

oMap spread over time and space

## CDV paragraph

* + - General
    - In Southeast/US
    - Spillover

Canine distemper virus (CDV) is a significant cause of morbidity and mortality in a wide range of species but particulary carnivore species. This makes this virus of is a major conservation concern. CDV has been implicated in severe population declines in multiple species, including the near extinction of the black-footed ferret in the US (Williams et al. 1988). It is also an important disease in domestic dogs. Additionally the virus has been shown to pass back from wildlife to dogs (Kapil and Yeary 2011). CDV has also been proposed as a risk to human health, it has been hypothesized tahr waning population level measles immunity will leave humans susceptible to CDV infection.(Martinez-Gutierrez and Ruiz-Saenz 2016). Morbiliviruses have a tendency to have a narrow host range, but CDV goes against this trend by its ability to infect a wide variety of carnivore hosts. However, there is an incomplete understanding of the dynamics of CDV infection within multi-host systems, such as carnivore communities. The role that particular species plays in the maintenance and spread of the disease in this system is not understood and consequently the targeting of mitigation measures is not well informed. The southeastern US is one such multi-host system, containing a wide variety of potential host species. Raccoons are frequently the most reported wild carnivore species in distemper outbreaks and have been suggested as the possible reservoir host (Roscoe 1993). Preliminary work from necropsy data of wild carnivores diagnosed with CDV has demonstrated that CDV is widely spread in the SE USA with at least 9 carnivore species experiencing mortality as a result of infection. In the most commonly infected species, raccoons and gray foxes, there appeared to be a trend of cases clustering in suburban areas with fewer cases occurring in highly urbanized and in rural areas(Taylor et al. 2021).

information needed to elucidate the transmission dynamics in this multihost system across land-use gradietns. In order to effectively predict the outcome of outbreaks of CDV in wild carnivores and develop effective counter measures, it is vital to understand the dynamics of the disease in this kind of system.

## LAnduse paragraph

* + - Spillover and landiuse
    - Disease spread
    - Exam answer

## Closing paragraph

Here, we analyse the CDV genetic diversity int wild mesocarnivores in the Sotuheastern US. Additiionally we explored the spatiotemporal distribution of CDV in free-ranging mesocarnivores from the same region from 2019 to 2022. Finally we the investigated the environmental/ecological factors which may increase risk of CDV outbreaks. Our findings may help …in this multihost system.

# MATERIALS AND METHODS

## Study area/data set

The southeastern cooperative wildlife disease study incorporates X states generally in the south east of the USA. Here we have used a data set including pathological diagnosis of the cause of death of 270 mesocarvores from January 2019 to December 2022. The data set includes the variables XXX. Additionally the land cover data for each location was extracted from raster maps available from the National Land Cover Database (NLCD). Along with elevation data from XXX and average temperature and precipitation bvalues accessed from the PRISM database. Further variables calculated for each data point were distance to nearest hydrological feature and distance to the nearest other distemper case in the data. The hydrological maps were accessed from XXX. The R script for the data collection, cleaning and analysis is included at XXX.

## SCWDS diagnstics(check other paper)

. Cases of CDV infection were identified by one or more of the following diagnostic features: CDV positive by fluorescent antibody testing (Fairchild et al. 1971) or immunohistochemistry (Palmer et al. 1990) and  characteristic histopathology (including intranuclear and intracytoplasmic inclusions). Many of the animals submitted were found dead or were found moribund and were subsequently euthanized. The data set contained the following variables: case number, state, county, area, sex, species, age, and collection year.

Data were imported into R Studio (version 1.3.1056). A detailed description of data analysis is contained in the scripts within the project repository (https://github.com/JJWilson1991/CDVGA\_Project\_JJW). All analyses were conducted in the R programming environment (version 3.5.3.). References to packages in this methods section indicate specific packages used within the R environment to perform analyses.

Mapping data for US states and counties, used to harmonize data at the county level, was obtained through the *ggplot2* (Wickham 2016) package. As the location data were limited to county level information, county centroid coordinates were used for plotting case points. Individual cases were mapped for the entire dataset

## Nucleic acid detection/sequencing/analysis

GET from riley paper

Samples were submitted between 2010 and 2014 to the UTCVM Clinical Virology Lab for canine distemper virus detection. Submissions were mainly canine from eastern Tennessee, but samples from Canada, Texas, Washington, Kentucky, West Virginia, Virginia, and South Carolina were also tested. Samples were also submitted for wildlife including raccoons and foxes from Tennessee.

RNA was extracted from clinical samples (including nasopharyngeal/conjunctival swabs, urine, and tissues) and cell culture supernatant containing virus with a commercially available extraction kit (QIAamp Viral RNA Mini Kit, Qiagen, Valencia, CA, USA) as previously described [[7](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4683949/#CR7)]. RNA previously extracted was stored at −80 °C.

Samples were tested for CDV by real-time RT-PCR. cDNA from positive samples were genotyped by sequencing the variable M-F intergenic region, and a phylogenetic tree was constructed according to previously described methods [[7](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4683949/#CR7)].

Samples used for genome sequencing included CDV sample number 13–1941, which was isolated from lung that had been stored at −80 °C from a fox that displayed neurologic signs prior to euthanasia and tested negative for Rabies virus. It was submitted to the pathology department for disposal from the Avian and Zoological Medicine service in the UTCVM. The virus was cultured on Vero SLAM cells (kindly provided by Dr. Edward Dubovi, Cornell University, NY, USA). CDV sample number 13–2262 was collected via urine from a 6 month old puppy from South Carolina with clinical signs consistent with canine distemper, and the sample had a real-time RT-PCR Ct value of 13.11. This strain was not isolated and whole RNA from the urine sample was used directly for genome amplification.

Approximately 50 bp overlapping primer sets with ~1000 bp products were designed using Primer 3 [[27](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4683949/#CR27), [28](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4683949/#CR28)] to amplify complete CDV genome sequence (minus the extreme 5’ and 3’ non-coding ends) (Table [2](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4683949/table/Tab2/)). Two μL of RNA per sample were run in 25 μL total volume reactions using a commercially available master mix (SuperScript III Platinum One-Step RT-PCR kit, Invitrogen, Life Technologies, Grand Island, NY, USA) using 300 nM of each primer and one unit of RNAse inhibitor (RNAse Out, Invitrogen, Life Technologies, Grand Island, NY, USA) for RT-PCR. Samples were amplified in a thermal cycler (GenePro, BIOER Technology, China) with a RT step at 50 °C for 30 min., activation step for the hot start Taq polymerase at 94 °C for 2 min., followed by 35 cycles of denaturation at 94 °C for 30 s., annealing at 60 °C for 1 min., and elongation at 72 °C for 3 min., with an additional elongation step at 72 °C for 10 min. The RT-PCR products were electrophoresed on a 1 % TBE agarose gel stained with SYBR Safe®, and visualized by SYBR© Green-filtered UV light with a CCD camera system (UVP, Inc., Upland, CA, USA). Products with a single band were purified using ExoSAP IT (Affymetrix, Santa Clara, CA, USA). Products with more than one band but a single clear product at ~1000 bp (3/15 sets) were excised and gel purified (QIAquick gel extraction kit, Qiagen, Valencia, CA, USA) and all products were capillary sequenced at the UT Molecular Biology Core Facility using the same primers used for the PCR reactions.

Chromatograms for capillary DNA sequence were manually edited and assembled using Geneious©, and all positions in the sequence had at least 2x coverage. Available reference genomes and H genes representing the major CDV lineages were downloaded from GenBank and aligned to CDV 13–1941 and CDV 13–2262 using MAFFT v7.017 [[29](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4683949/#CR29)]. Nucleotide substitution model GTR was selected using ModelGenerator v0.85 [[30](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4683949/#CR30)] and phylogenetic trees were constructed using MRBAYES v2.0.9 (genomes) [[31](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4683949/#CR31)] with 1,000,000 iterations with subsampling every 1000 trees and a burnin of 20,000 iterations [[32](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4683949/#CR32)] or Neighbor-Joining method (H gene). Individual coding sequences (CDS) were predicted using GLIMMER3 v1.4 [[33](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4683949/#CR33)], extracted, translated and aligned to representative sequences from GenBank. Phylogenetic trees for individual protein sequences were generated using UPGMA in Geneious© Tree Builder (version 6.1.4, Biomatters [http://www.geneious.com](http://www.geneious.com/)) with the Jukes-Cantor distance model.

## Statistical analysis GLM

A generalized linear model was developed to identify factors associated with the positive diagnosis of CDV in wild mesocarnviores.

The presence/absence of antibodies against CDV was the response variable, whereas geographical area, sex, age, death cause and sampling year were explanatory factors. A logistic link function was applied, and a binomial error distribution was assumed. Data were analyzed using SPSS 17.0 (IBM, Chicago, IL, USA). Additionally, the 95% confidence interval (95% CI) was calculated for each year and the lower and upper limits were delimited (Kohn & Senyak, [**2021**](https://onlinelibrary.wiley.com/doi/full/10.1111/tbed.14323#tbed14323-bib-0029)). Comparations among the years were performed by the Fisher's exact test using GraphPad Prism v.8.0 (GraphPad Software Inc, San Diego, CA, USA).

# RESULTS

## Describe data

A total of 270 mesocarvores were present in this dataset from January 2019 to December 2022. 158 out of the 270 mesocarnivores (58.5%) were diagnosed as CDV positive. There were four host species present in this data; raccoon, grayfox, striped skunk, red fox. These animals came from 13 states. The breakdown pf the state and species distribution is shown in table X.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Gray Fox | Raccoon | Red Fox | Striped Skunk | Sum |
| AR | 3 | 14 | 0 | 0 | 17 |
| FL | 0 | 6 | 2 | 0 | 8 |
| GA | 9 | 25 | 3 | 0 | 37 |
| KS | 0 | 14 | 3 | 12 | 29 |
| KY | 0 | 18 | 1 | 1 | 20 |
| LA | 4 | 14 | 1 | 3 | 22 |
| MO | 2 | 19 | 2 | 9 | 32 |
| NC | 15 | 53 | 3 | 2 | 73 |
| NE | 0 | 1 | 0 | 2 | 3 |
| PA | 1 | 5 | 0 | 0 | 6 |
| TN | 2 | 1 | 0 | 0 | 3 |
| VA | 0 | 3 | 3 | 0 | 6 |
| WV | 0 | 9 | 5 | 0 | 14 |
| Sum | 36 | 182 | 23 | 29 | 270 |

**PLOT OF MONTHS**

A mean of XXX cases were diagnosed with distemper per month with a standard deviation of 16.96 (**Figure X**).

## Spatial description

## Phylogeny

X CDV partial H-gene (length) isolates from X states were jvndfjvnfjvndkfjv

Large cluster from eastern states most closely related to isolate from eruopean dog. Cases from MO quite genetically distinct from eastern samples. MO skunks separate clade.

Partial H-gene Sequences were obtained from 31 animals. 22 of the isolates from eastern states (NC, FL, GA) all grouped together in one large cluster with isolates from missorui being quite distinct from these Fig X

revealing four different sequences with the following GenBank accession numbers: MZ169061, MZ605429, MZ605430 and MZ605431. The sequences showed 98% similarity with the closest canine morbillivirus strain (KY214447.1), isolated from a wolf in Portugal in 2017. All four sequences clustered together in the phylogenetic tree (Figure [**3**](https://onlinelibrary.wiley.com/doi/full/10.1111/tbed.14323#tbed14323-fig-0003)), and they showed very low distance between them. GenBank accession number, location, species, year and lineage, following Meli et al. ([**2010**](https://onlinelibrary.wiley.com/doi/full/10.1111/tbed.14323#tbed14323-bib-0040)), of the 66 used sequences in the phylogenetic tree can be found in Table [**S1**](https://onlinelibrary.wiley.com/doi/full/10.1111/tbed.14323#support-information-section). Additionally, pairwise genetic distance between sequences is shown in Table [**S2**](https://onlinelibrary.wiley.com/doi/full/10.1111/tbed.14323#support-information-section).

## Univariate analysis

## GLM

The mean annual seroprevalence ranged from 25% in 2020 to 63.64% in 2015. Fisher's tests between years indicated numerous significant differences (Table [**S3**](https://onlinelibrary.wiley.com/doi/full/10.1111/tbed.14323#support-information-section)), suggesting important prevalence fluctuations through time with maxima in 2008, 2012, 2015, 2017 and 2018 and minima in 2014 and 2020 (Figure [**4a**](https://onlinelibrary.wiley.com/doi/full/10.1111/tbed.14323#tbed14323-fig-0004)). The rate of seropositivity varied with geographic region (Figure [**4b**](https://onlinelibrary.wiley.com/doi/full/10.1111/tbed.14323#tbed14323-fig-0004)): 24 of 77 (31.2%) badgers from the Western region were positive, compared to 78 of 221 (35.3%) from the Central area and 195 of 386 (50.5%) from the Eastern area. The rate of seropositivity was 41.2% (124/301) among males and 41.8% (135/323) among females. The rate of seropositivity was 42.7% (197/461) among adults and 38.0% (62/163) among subadults. The results of individual sera distributions of each group can be found in Figure [**S6**](https://onlinelibrary.wiley.com/doi/full/10.1111/tbed.14323#support-information-section).

Results of GLM revealed no sex- or age-related differences in the probability of CDV positive diagnosis. Nevertheless, both sampling year (*χ*2 = 34.77, *p*= .01) and area (*χ*2 = 15.97, *p*< .001) were confirmed to be statistically significant factors according to GLM, with badgers from the Eastern region showing a higher probability of seropositivity than those from Western and Central areas. On the other hand, trapped badgers also showed a statistically significant higher probability of being seropositive than those killed in road traffic accidents (*χ*2 = 41.25, *p*< .001).

-land cover-hgh +med developed 0.05, low 0.1

-precipitation0.1

-age-juveile 0.1

-imperviousness0.05

-month- 0.1

Nearest neighbour- 0.001

-species-red fox 0.01

# DISCUSSION

## Refer back to prelim data on

* + - Species
    - month
    - Landuse
    - Closeness and disease ecology

## Relevance- conservation etc

## Limitations

The limiatiions agaijn mostly apply to the type of sampling. The samples are collected passively through submissions by Georgia DNR and the equivalent departments in other states. This is dependent on a number of factors; a dead or ill animal being reported to the authorities or seen by them and a willingness to submit for necropsy. There are likely to be large numbers of subclinical cases which are missed. This leads to a number of potential areas of sampling bias, with more populated areas and areas with state/national parks likely to have more cases submitted. Additionally, areas with rabies concerns are likely to submit more cases as the two diseases present very similarly.

Additionally the raster data from NLCD is from 2019 which is before any of our samples were taken, however the landcover is unliklely to have changed much in this timeframe, particular with the economic impact of the COVID-19 pandemic, the the effects of this time lag are likely to be minimal.

# CONCLUSION

Sfsfdvsdfsdfsdf

Urbanization is a complex problem when it comes to disease dynamics at the wildlife-domestic-human interface with no solution that fits every disease. The examples here present some of the concepts that demonstrate that social responsibility and responsible urban planning with biodiversity at the forefront of development can mitigate future problems. Additionally, surveillance and control measures particularly regarding diseases in anthropophilic species can also play a crucial role in dynamics of wildlife disease in urban environments.

# References

BENINDE, J., M. VEITH, ANDA. HOCHKIRCH. 2015. Biodiversity in cities needs space: a meta-analysis of factors determining intra-urban biodiversity variation. Ecol Lett 18: 581-592.

BRADLEY, C. A., ANDS. ALTIZER. 2007. Urbanization and the ecology of wildlife diseases. Trends Ecol Evol 22: 95-102.

ELMORE, S. A., R. B. CHIPMAN, D. SLATE, K. P. HUYVAERT, K. C. VERCAUTEREN, ANDA. T. GILBERT. 2017. Management and modeling approaches for controlling raccoon rabies: The road to elimination. PLoS Negl Trop Dis 11: e0005249.

EZENWA, V. O., M. S. GODSEY, R. J. KING, ANDS. C. GUPTILL. 2006. Avian diversity and West Nile virus: testing associations between biodiversity and infectious disease risk. Proc Biol Sci 273: 109-117.

GOTTDENKER, N. L., D. G. STREICKER, C. L. FAUST, ANDC. R. CARROLL. 2014. Anthropogenic land use change and infectious diseases: a review of the evidence. Ecohealth 11: 619-632.

HU, H., K. NIGMATULINA, ANDP. ECKHOFF. 2013. The scaling of contact rates with population density for the infectious disease models. Math Biosci 244: 125-134.

KAPIL, S., ANDT. J. YEARY. 2011. Canine distemper spillover in domestic dogs from urban wildlife. Vet Clin North Am Small Anim Pract 41: 1069-1086.

KEESING, F., R. D. HOLT, ANDR. S. OSTFELD. 2006. Effects of species diversity on disease risk. Ecol Lett 9: 485-498.

KOMAR, N. 2001. West Nile virus surveillance using sentinel birds. Ann N Y Acad Sci 951: 58-73.

MARTINEZ-GUTIERREZ, M., ANDJ. RUIZ-SAENZ. 2016. Diversity of susceptible hosts in canine distemper virus infection: a systematic review and data synthesis. BMC Vet Res 12: 78.

MCCALLUM, H., ANDA. DOBSON. 2002. Disease, habitat fragmentation and conservation. Proc Biol Sci 269: 2041-2049.

PRANGE, S., S. D. GEHRT, ANDE. P. WIGGERS. 2003. Demographic Factors Contributing to High Raccoon Densities in Urban Landscapes. The Journal of Wildlife Management 67.

RILEY, S. P., J. FOLEY, ANDB. CHOMEL. 2004. Exposure to feline and canine pathogens in bobcats and gray foxes in urban and rural zones of a national park in California. J Wildl Dis 40: 11-22.

ROSCOE, D. E. 1993. Epizootiology of canine distemper in New Jersey raccoons. J Wildl Dis 29: 390-395.

SCHULTE-HOSTEDDE, A. I., Z. MAZAL, C. M. JARDINE, ANDJ. GAGNON. 2018. Enhanced access to anthropogenic food waste is related to hyperglycemia in raccoons (Procyon lotor). Conserv Physiol 6: coy026.

SMITH, G. C., ANDD. WILKINSON. 2003. Modeling control of rabies outbreaks in red fox populations to evaluate culling, vaccination, and vaccination combined with fertility control. J Wildl Dis 39: 278-286.

STEINEL, A., C. R. PARRISH, M. E. BLOOM, ANDU. TRUYEN. 2001. Parvovirus infections in wild carnivores. J Wildl Dis 37: 594-607.

TAYLOR, K., J. J. WILSON, A. W. PARK, N. M. NEMETH, M. J. YABSLEY, H. FENTON, M. K. KEEL, ANDN. L. GOTTDENKER. 2021. Temporal and Spatial Patterns in Canine Distemper Virus Cases in Wildlife Diagnosed at the Southeastern Cooperative Wildlife Disease Study, 1975-2019. J Wildl Dis 57: 820-830.

WILKINSON, D. A., J. C. MARSHALL, N. P. FRENCH, ANDD. T. S. HAYMAN. 2018. Habitat fragmentation, biodiversity loss and the risk of novel infectious disease emergence. J R Soc Interface 15.

WILLIAMS, E. S., E. T. THORNE, M. J. APPEL, ANDD. W. BELITSKY. 1988. Canine distemper in black-footed ferrets (Mustela nigripes) from Wyoming. J Wildl Dis 24: 385-398.

WRIGHT, A. N., ANDM. E. GOMPPER. 2005. Altered parasite assemblages in raccoons in response to manipulated resource availability. Oecologia 144: 148-156.