**Chapter 3: Canine Distemper virus in wild mesocarnivores in the South-eastern united states**

# INTRODUCTION

Human land use change has a significant impact on the dynamics of infectious diseases at the wildlife-domestic-human interface (Bradley and Altizer 2007, Gottdenker et al. 2014). The destruction and fragmentation of natural habitats disrupts the ecological balance and can lead to the displacement of wildlife, changes in the population dynamics can also lead to overcrowding and poor sanitation, which can facilitate the spread of diseases. Furthermore, changes in land use can alter the distribution and abundance of disease vectors, such as mosquitoes and ticks, leading to an increased incidence of vector-borne diseases. These impacts are further compounded by climate change, which can further change the range of disease vectors and timing of disease outbreaks.

Therefore, it is crucial to understand the relationship between urbanization and infectious disease in wildlife in order to develop effective management and conservation strategies to mitigate the negative impacts and protect affected species and their ecosystems

Canine distemper virus (CDV) is a significant cause of morbidity and mortality in a wide range of species but particularly carnivore species. This makes this virus of 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 that waning population level measles immunity will leave humans susceptible to CDV infection.(Martinez-Gutierrez and Ruiz-Saenz 2016). Morbilliviruses 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 canine distemper virus 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). Studies in other parts of the world have suggested that the dynamics of CDV outbreaks can vary over time and space (Bianco et al., 2020). Given the propensity of CDV to infect anthropophilic mesocarnivores I, it is important to investigate whether there are human land use features which affect the likelihood of the virus occurring in wildlife.

Here, we studied CDV infection in wild mesocarnivores in the south eastern United states using, using carcasses submitted to the Southerastern cooperative wildlife disease trust between January 2020 and December 2022. Animals were diagnosed as CDV positive or negative during postmortem examination at SCWDS. From the positive cases a subset were sampled for CDV and had a partial H gene sequence isolated for phylogentic analysis. The objectives of this study were to;

(1) explore the CDV genetic diversity int wild mesocarnivores submitted to SCWDS

(2) investigate the spatial distribution of CDV in free-ranging mesocarnivores from the same region from 2019 to 2022

(3) develop a model to idenitify specific ecological factors with may increase the rosk of CDV

Specifically we aimed to investigate how different land use types influence the likelihood of wild mesocarnivores being diagnosed with CDV at necropsy.

# MATERIALS AND METHODS

The south-eastern cooperative wildlife disease study incorporates X states generally in the southeast of the USA. Here we have used a data set including pathological diagnosis of the cause of death of 270 mesocarnivores from January 2019 to December 2022. The raw data from SCWDS includes the variables state, county, area, coordinates, species, date, sex, age, weight, diagnosis. 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 the `elevatr` package and average temperature and precipitation values 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 TIGRIS database. The R script for the data collection, cleaning and analysis is included at XXX.

**SCWDS diagnostics**

Cases of CDV infection were identified at necropsy 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..

**Nucleic acid detection/sequencing/analysis**

A subset of 31 of the CDV positive necropsy cases had clinical samples taken, mostly brain, but also lung, liver, spleen, for viral RNA extraction. CDV RNA was extracted from necropsy samples with a commercially available extraction kit (RNeasy Mini Kit, Qiagen, Valencia, CA, USA) according to manufacturer’s instructions. Extracted RNA was stored at −80 °C. Primer pair to amplify approximately 1000bp region of H-gene were synthesized based on REF. A single step process was used for CDNA production and PCR amplification in this case using a commercially available master mix (SuperScript III Platinum One-Step RT-PCR kit, Invitrogen, Life Technologies, Grand Island, NY, USA). Two μL of extracted RNA per sample were run in 25 μL total volume reactions 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 with a RT step at 50 °C for 30 min., activation step 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 2 % TAE agarose gel stained with SYBR Safe® and visualized. Products with a single band at ¬1000 bases were purified using QIaquick PCR purification kit (Affymetrix, Santa Clara, CA, USA). All products were capillary sequenced at the Eurofins Genomics, KY USA, using the same primers as in the PCR reactions. Chromatograms edited and assembled using Geneious© software.

Further available H-gene sequences for the USA were downloaded from GenBank and aligned in Geneious with the study isolates using the MUSCLE algorithm. Geneious tree builder was then used with the Jukes-Cantor genetic distance model and the UPGMA method with 1000 bootstraps to generate a phylogenetic tree from this alignment.

**Statistical analysis**

Diagnostic data from SCWDS cases were imported into R Studio (version 1.3.1056). A detailed description of data analysis is contained in the scripts within the project repository (XXX). All further 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 programming environment.

A generalized linear model was developed to identify factors associated with the positive diagnosis of CDV in wild mesocarnivores in R using the `stats` package.

A positive or negative diagnosis of CDV was the response variable, whereas Species, location, sex, age, month received, distance to nearest distemper case, elevation, precipitation, temperature, distance to water source, surface imperviousness and land cover type were explanatory factors. A logistic link function was applied, and a binomial error distribution was assumed.

# RESULTS

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

Table X: Summary table of necropsy cases by species and state submitted to SCWDS between January 2019 and December 2022

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | 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 |

**Graphical user interface

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Figure X: Map of distribution of necropsy cases submitted to SCWDS by species

**CDV Phylogeny**

31 CDV partial H-gene (¬1200 bp) isolates from 5 states, in addition to 55 additional sequences downloaded from GenBank were aligned in Geneious and used to build a phylogenetic tree.22 of the isolates from eastern states (NC, FL, GA) grouped together in one large cluster. Isolates from Missouri and Arkansas clustered quite distinctly from this large eastern clade although, one NC and one GA isolate did group with these isolates.The large cluster from eastern states was most closely related to an isolate from a European dog.

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Figure X: Phylogenetic tree of…

Sequences obtained in this work are in coloured text.

**Generalized Linear Model of CDV diagnosis**

The results of the GLM did not include location, sex, elevation, temperature, distance to water source as significant explanatory variables. However, age, month received, distance to nearest distemper case, precipitation, surface imperviousness and land cover type were confirmed to be statistically significant factors according to GLM (Table X).

Animals closer to another CDV case were significantly more likely to be positive for CDV, as were animals that were juveniles. Additionally those found in areas of high. medium and low intensity areas of human development. There was a less strong relationship, but still significant between greater surface imperviousness.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Explanatory Variable | Estimate | Std. Error | z value | Pr(>|z|) |
| Age: Juvenile | -1.671e+00 | 6.345e-01 | -2.633 | 0.008461 \*\* |
| Month received | -1.500e-01 | 7.228e-02 | -2.075 | 0.038006 \* |
| Distance to CDV case | -1.689e-05 | 5.013e-06 | -3.369 | 0.000756 \*\*\* |
| Developed, High Intensity | -5.562e+00 | 2.639e+00 | -2.107 | 0.035100 \* |
| Developed, Low Intensity | -2.096e+00 | 9.891e-01 | -2.119 | 0.034116 \* |
| Developed, Medium Intensity | -4.680e+00 | 1.939e+00 | -2.413 | 0.015808 \* |
| Imperviousness | 6.201e-02 | 3.212e-02 | 1.930 | 0.053591 . |

Table X

Summary statistics of significant explanatory variables from GLM

# DISCUSSION

Data from wild mesocarnivores submitted to the SCWDS was analyzed based on their CDV diagnosis at necropsy with a subset of these cases being sampled for CDV for phylogenetic analysis of the virus in wild mesocarnivores in the southeast. Ecological variables including human land use type at the location of each case submitted were included in the data with a generalized linear model developed to identify factors that increased the risk of CDV in these animals.

The phylogenetic analysis of CDV isolates showed a very distinct cluster from samples taken from animals in states east of the Mississippi river. The vast majority Isolates from NC, GA and FL clustered closely together within the phylogenetic tree. Isolates from western states, AR and MO clustered quite separately from these eastern isolates and in a much less clearly defined cluster. This suggests the eastern isolates are all quite closely related and given that the states of GA, FL and NC are contiguous, may all have originated from one large ongoing outbreak in the area. Secondly, that the Mississippi river forms a much more difficult barrier for the virus to traverse than other smaller rivers. This would make sense given that the virus is not transmitted by birds or airborne transmission, so would need to be brought across the river by an infected mammal. This results in distinct strains evolving on each side of the river. The Mississippi as a barrier to disease dispersal in wildlife has been demonstrated before with rabies (Kuzmina et al. 2013). However, based on this study, and others, it is not an impenetrable barrier as there was one GA isolate and one NC isolate that clustered with isolates from Missouri suggesting that at some point in time these were able to spread across the river, whether this be through an infected wild mammal swimming or inside a truck, or perhaps via an infected dog or even via a fomite.

The GLM for CDV diagnosis and ecological variables revealed a number of significant results. The most significant explanatory variable was distance to another positive CDV case. This makes perfect sense from a disease ecology standpoint as it is a directly transmitted pathogen and does not persist in the environment for any great length of time so an animal is not going to spontaneously contract the virus from the environment. Being close to another existing case likely means being close to an existing outbreak.

The most important result of the GLM from the objectives of this study are that land cover type was a significant factor in CDV diagnosis, specifically High, medium and low intensity developed were a risk factor for CDV diagnosis. There are a number of reasons why urban areas could be resulting in higher likelihood of CDV in wild mesocarnivores. Urban areas often possess abundant resources for anthropophilic species, such as raccoons, which may not be 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 prevalence of the zoonotic nematode B. procyonis  in raccoons(Wright and Gompper 2005). A Study by Hwang et al showed higher prevalence of severe fever with thrombocytopenia syndrome virus antibodies in urban dwelling feral cats than in their rural counterparts (Hwang et al. 2017). 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. There is also the possibility that closer contact with domestic dogs in an urban setting results in more CDV spillover events into wildlife populations than in rural areas. Howver there may also be potential sourcres of bias in urban area skewing the results. As this is passively collected data that generally comes from animals found dead or moribund, or exhibiting neurologivcal signs (in which case they are first submitted for rabies testing) then these are more likely to be seen and reported to the appropriate state authority in an urban area as there are more people there who would see the animal.

Some of the less significant factors in the model were surface imperviousness, age of animal, month animal received. Whilst one would think that imperviousness would correlate with level of development discussed above, it is probably less significant because of the fact that many of these animals would have been found on roads and carparks etc., which even in rural areas are an impervious surface which may have reduced the significance of this factor. The age of the animals, specifically being a juvenile showed some significance. This may be due to waning maternal antibodies making them susceptible to diseases (REF) or that these young animals were more likely to be killed by other means and also happened to have CDV concurrently. As discussed in Taylor and Wilson 2021, there maybe I higher risk for CDV during the breeding season, which may explain the significance of the month received

The results of this study may have important implications for surveillance and conservation efforts. By identifying areas of intense human development as areas of highest risk for disease it may be possible to focus surveillance efforts in these areas, allowing outbreaks to be identified earlier. It may even be possible in these urban areas to instigate a citizens science program using a reporting application similar to that used for rabies in skunks in Colorado (Pepin et al. 2017). This may allow for vaccination programs in the face of outbreaks which threaten more vulnerable mammal species.

**Limitations**

The limitations of this study mostly apply to the type of sampling. The samples are collected passively through submissions to SCWDS by Georgia DNR and the other equivalent departments in other states. This is dependent on a number of factors; a dead or ill animal being reported to the authorities or being seen by them and a willingness to submit for necropsy, so there are likely to be large numbers of subclinical cases which are missed. This also 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 as there are more people and/or officers present in these areas. Additionally, areas with rabies concerns are likely to submit more cases as the two diseases present very similarly and frequently a case will in fact have been submitted as a query rabies case and after that has been ruled out will then be tested for CDV.

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

# CONCLUSION

The conclusions of this study are twofold. Firstly, it provides further evidence of widespread CDV infection in wild mesocarnivores within the southeastern US and that there is significant genetic diversity within this virus in the area, particularly divided by the Mississippi river. Secondly, human land use may play an important role in the disease ecology of this virus with areas of intense human development being shown to be of higher risk for CDV infection in wild mesocarnivores. Land use change is a complex problem when it comes to disease dynamics at the wildlife-domestic-human interface with no solution that fits every disease. Social responsibility and responsible urban planning with biodiversity at the forefront of development can mitigate future problems. Additionally, surveillance and control measures, such as vaccination particularly regarding diseases in anthropophilic species can also play a crucial role in dynamics of wildlife disease in urban environments.

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