DYNAMICS OF INTERSPECIES VIRAL TRANSMISSION IN MULTIHOST SYSTEMS

by

JONATHAN WILSON

(Under the Direction of Nicole Gottdenker)

ABSTRACT

Understanding the dynamics of multihost pathogens and interspecies pathogen transmission can go some way to alleviating the risks posed. In this dissertation several techniques have been employed to investigate both theoretical and applied questions relating to interspecies viral transmission.

The synthesis of cross species transmission events and pathogenesis raised two main points. Firstly, the analysis suggests that distinct patterns can be observed in the pathogenesis data, particularly in spillover events involving humans. Secondly, the study emphasizes the insufficient availability of precise and comprehensive virus-host pathogen interaction data, particularly at the cellular and subcellular levels. It underscores the importance of increased collaboration and concentrated efforts among the scientific community to identify receptors and pathogenic pathways that may signal spillover risk. Simulations of viral epizootics demonstrated that the highest transmissibility and genetic diversity occur at different stages of an epizootic. Moreover, the study suggests that viral populations may adopt a bistable strategy, wherein they either evolve into a moderately

transmissible population with a slower epizootic or become maximally transmissible with a very rapid epizootic, depending on certain conditions.

Canine distemper virus (CDV) in wildlife in the southeastern USA was used as a case study to investigate the dynamics of a virus in a multihost system and the role that human development may play. Analysis revealed distinct temporal and spatial patterns of distemper cases in multiple host species. The study also showed that past cases in gray foxes and raccoons can predict the number of gray fox and raccoon cases. The final chapter draws two main conclusions. Firstly, it presents additional evidence of the widespread occurrence of CDV infection in wild mesocarnivores in the southeastern US and highlights the significant genetic diversity of CDV in the region, especially on either side of the Mississippi River. Secondly, the study suggests that human land use may be a crucial factor in the disease ecology of CDV, with wild carnivores in areas of high human development facing a greater risk of CDV infection. Ultimately, there were sufficient potential relationships in these two studies to support more targeted active surveillance for canine distemper virus in wildlife.

INDEX WORDS: Disease ecology; Pathology; Canine distemper virus; wild carnivores; disease dynamics; wildlife disease ecology; morbillivirus

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Cross species transmission of pathogens is a complex and ever evolving issue. These events are dependent on a wide range of factors including, ecological, epidemiological, microbiological, behavioral, and physiological interactions (Parrish et al. 2008, Plowright et al. 2017, Becker et al. 2019, Ellwanger and Chies 2021). Additionally, cross species transmission can be viewed from the perspective of the reservoir host, the recipient host, or the pathogen itself. As such, there are many areas of cross species transmission that merit investigation, particularly given that it is such a relevant and pressing concern for humankind in the 21st century (Grange et al. 2021, Ruiz-Aravena et al. 2022, Ozili and Arun 2023).

RNA viruses are overrepresented in cases of interspecies pathogen transmission (Woolhouse and Gaunt 2007, Olival et al. 2017, Mollentze and Streicker 2020, Williams et al. 2021). There are a number of specific features of RNA viruses that may lead to this. Generally they revolve around the ability of these viruses to quickly adapt to new hosts and this is predominantly due to their genetic plasticity and ability to develop genetic diversity in their populations (Elena et al. 2006). RNA viruses are particularly adept at this due to the low fidelity of their RNA dependent RNA polymerase and the lack of proofreading inherent in being single stranded (Drake 1993, Elena et al. 2006). This creates a scenario where mutations occur at a very high rate in the viral genome, allowing

them to evolve and adapt to new hosts rather quickly (Bashor et al. 2021), making RNA viruses of particular interest to cross species pathogen transmission.

When considering host and pathogen factors related to cross species transmission, it is important to realize that the interplay of these factors relates to the pathogenesis of an infection. Pathogenesis is defined as the manner in which a pathogen infects, replicates within a host, and is transmitted between hosts. Whether the pathogenesis of a disease in a reservoir host influences how likely a given virus is to spill over into a new host is an important question when considering the risk of interspecies transmission (Pulliam and Dushoff 2009). Although there has been much investigation of specific pathogen and host traits involved in cross species transmission, there has been little research on the interplay between host and pathogen in disease spillover models. The potential role of infectious disease pathogenesis, in cross species disease transmission has proven to be a difficult question to answer (Pulliam and Dushoff 2009). Thus, we recognize there is a knowledge gap when it comes to the role of disease pathogenesis in viral spillover.

Another factor involved in cross species transmission is the timing of these events and whether there are particular periods during an epizootic when the risk of cross species transmission is higher. It has been shown on multiple occasions that epizootics in one species can lead to spillover into others (Chua 2003, Weckworth et al. 2020). It has been hypothesized that these events correspond to shedding peaks in the reservoir species (Peel et al. 2019). This supports the notion that the key factor in cross species transmission is having an overwhelming quantity of virus in the system. A recent modeling approach has also suggested that seasonal peaks in reservoir population sizes

results in not only more prevalence of infection but also greater genetic diversity in the pathogen population (Remien and Nuismer 2020). Viral population genetic diversity plays a major role in viral evolution (Sanjuán and Domingo-Calap 2021). There are a number of factors influencing pathogen genetic diversity and consequently the probability that a pathogen population contains generalist strains with the ability to cross species barriers effectively (Retel et al. 2019). Experimental evidence has shown that genetic diversity can drive emergence in a novel species (Dennehy et al. 2010). However, not all genetic changes are beneficial, these changes may come at a cost to other aspects of the pathogen's fitness, and this may sometimes prevent host shifts from occurring (Longdon et al. 2014). As such, it is not clear what drives the timing and extent of spillover within individual epizootics.

Anthropogenic land use has a significant impact on the population structure and dynamics of infectious diseases at the wildlife-domestic-human interface (Bradley and Altizer 2007, Patz et al. 2008, Gottdenker et al. 2014, Plowright et al. 2021). Proposed mechanisms by which anthropogenic land use (e.g., deforestation, silviculture, agricultural activities, urbanization, suburbanization) can alter structure and spread of infectious disease within and between wildlife populations and domestic animals and humans include altered host density and behavioral changes, and impaired host immune function (Slingenbergh et al. 2004, Lambin et al. 2010, Zylberberg et al. 2013, Guo et al. 2019). For directly transmitted pathogens, host density drives contact rates and thus pathogen spread (Tompkins et al. 2011). High densities usually result in increased contact within and between species and individuals and subsequently greater prevalence of infection (Ditchkoff et al. 2006, Almberg et al. 2009). This concept of higher density

populations and greater contact rates leading to more disease is of particular importance in synanthropic species. These species thrive in human disturbed environments where supplemental resource availability, shelter and lack of predation allow the environment to harbor large populations (Cavallini 1996, Prange et al. 2003, Contesse et al. 2004). Despite this, some studies have suggested that highly urbanized areas may dampen spread of infectious diseases among wildlife due to reduced populations in these areas (Gras et al. 2018). Thus, it remains unclear how continued urban development affects the dynamics of directly transmitted pathogens in synanthropic species. Therefore, it is crucial to understand the relationship between land use change and in particular the role of human development and infectious disease transmission in synanthropic wildlife in order to develop effective management and conservation strategies to mitigate the negative impacts and protect affected species and their ecosystems.

When considering viruses or pathogens in general it is important to look at their life history and decide if a pathogen is specialized to a specific host species or whether it has a more generalist life history with multiple potential hosts (Cleaveland 2001). In domesticated animals, 77% of pathogens of livestock and 90% of pathogens of domestic carnivores are known to be multi-host pathogens (Cleaveland 2001) and over 60% of known human pathogens are zoonotic (Taylor et al. 2001). Thus understanding the infection dynamics of multihost pathogens is of critical importance.

Canine distemper virus (canine morbillivirus) is an example of a multihost pathogen, affecting a wide range of wild and domestic mammals, principally carnivores (Deem et al. 2000, Martinez-Gutierrez and Ruiz-Saenz 2016). Canine morbillivirus results in a highly immunizing, acute infection that typically requires high densities and

large populations of hosts for long-term persistence (Williams 2001). The virus is maintained among wild carnivores by multi-host transmission, which overcomes obstacles of population size and host density within a single host species (Almberg et al. 2010). There is evidence of CDV infection in all terrestrial carnivore families and some marine carnivore families (Deem et al. 2000) and distemper has been implicated in severe population declines in multiple species, including the near-extinction of the black-footed ferret (*Mustela nigripes*) in the USA (Williams et al. 1988). It is also an important disease in domestic dogs, and CDV can be transmitted between wildlife and dogs, and vice-versa (Kapil and Yeary 2011). CDV has also been proposed as a risk to human health, and it has been hypothesized that waning population level measles immunity may leave humans susceptible to CDV infection (Martinez-Gutierrez and Ruiz-Saenz 2016). However, there is an incomplete understanding of CDV infection dynamics within many multi-host systems, such as carnivore communities. The role that different carnivore species play in the maintenance and spread of CDV is not understood, and consequently the targeting of mitigation measures is not well informed. In addition to being a multihost pathogen, CDV is an important pathogen of synanthropic wildlife, such as raccoons and foxes (Hoff et al. 1974, Davidson et al. 1992, Roscoe 1993, Lednicky et al. 2004, Nouvellet et al. 2013), making it a useful study system for investigating the role of human land use in the dynamics of multihost pathogen systems.

The research questions investigated in this dissertation fall into two main areas; those relating to cross species pathogen transmission in general, and those relating to canine distemper in wildlife. The general questions relating to cross species transmission being addressed in this dissertation are;

- Does disease pathogenesis play a role in the cross species transmission of RNA viruses?
- Are there times during a viral epizootic when prevalence and genetic diversity of the viral population lead to greater risk of cross species transmission?

The questions specific to canine distemper in wildlife;

- Are there patterns in canine distemper outbreaks in multiple wild species that may be as a result of cross species transmission?
- Are there human land use factors that make infection with canine distemper virus more likely?

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CHAPTER 2

WHAT DOES PATHOLOGY HAVE TO DO WITH DISEASE ECOLOGY? LINKING PATHOGENESIS TO VIRAL SPILLOVER

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Abstract

While there has been great attention paid to investigating specific pathogen and host traits involved in cross species transmission, there has been little research on the interplay between host and pathogen in disease spillover models. There appears to be a knowledge gap when it comes to the role of disease pathogenesis in cross species transmission among wildlife, domestic animals, and humans. The question arises if trends exist in the broader literature of pathological traits influencing a virus's ability to infect novel species and whether general patterns exist that can at least partially explain the role that host-pathogen interaction or 'pathogenesis' traits may play in cross species transmission; this is a seldom studied area of cross species transmission and there are likely significant gaps in the available literature. Here we investigated the nature of available pathogenesis data as it relates to cases of cross species RNA virus transmission events. The study identifies some gaps in the scientific literature, particularly at a molecular level when it comes to understanding the cellular level pathogenesis of cross species transmission events. Secondly, we analyzed the results of a literature search for cross species transmission events in RNA viruses using multiple correspondence analysis and identified trends in the pathological traits associated with these infections, particularly as they related to spillover events involving humans. Thirdly, hierarchical cluster analysis identified the existence of "pathological groups" of viruses which cluster based on their pathogenesis traits which tend to have other similar traits, especially hosts involved and transmission mechanisms. Elucidating the potential role of pathogenesis in cross species transmission can raise the possibility of expanding research in this area and trying to include these data in predictive frameworks of cross species transmission risk.

INTRODUCTION

Cross species pathogen transmission is a multifactorial process with both extrinsic drivers, and intrinsic pathogen and host factors playing major roles in the process (Plowright et al. 2017, Becker et al. 2019, Ellwanger and Chies 2021). Viral traits, such as having an envelope (Valero-Rello and Sanjuán 2022) and being able to replicate in the host cytoplasm (Pulliam and Dushoff 2009) likely play important roles in cross species transmission. Recent work has looked at the genotypes of viruses as a predictor of zoonotic potential (Mollentze et al. 2021). Frequently studied host factors include phylogenetic distance between hosts (Streicker et al. 2010, Guth et al. 2019) and phylogenetic aggregation (Park 2019). Phylogeny is likely to capture a broad range of host biological traits that facilitate cross species transmission. Both host and viral factors have been included in predictive models of viral spillover (Olival et al. 2017). Although these host and viral factors are important spillover drivers, they must be viewed through the lens of ecological viability of a spillover event happening, and these extrinsic drivers are just as important (Engering et al. 2013, Borremans et al. 2019). While there has been great attention paid to investigating specific pathogen and host traits involved in cross species transmission, there has been little research on the interplay between host and pathogen in disease spillover models (Figure 2.1). Recent advances in metagenomics has allowed for the discovery of large numbers of viruses in animals (Cui et al. 2023). Whilst these novel viruses are often viewed as potential risks for cross species transmission, understanding how pathogenesis relates to the viability of these viruses becoming a threat is an important piece of information that can help further direct research into these newly discovered pathogens. We believe there is a knowledge gap when it comes to the role of

disease pathogenesis in cross species transmission among wildlife, domestic animals, and humans.

The potential role of infectious disease pathogenesis, defined as the manner in which a pathogen infects, replicates within a host, and is transmitted between hosts in predictive models of cross species disease transmission was raised by Pulliam et al, but has proven difficult to elucidate (Pulliam and Dushoff 2009). For instance, comparative analysis of viral-host receptor distribution may provide insights to the probability of inter-species viral transmission. The distribution of the ACE2 receptor in humans is postulated to lead to the multisystem pathology of SARS-CoV-2, but there is a lack of detailed studies in other species (Ruiz-Aravena et al. 2022). The outcome of infections can be drastically different depending on the anatomy of receptor distribution; for example, the ACE2 upper respiratory distribution in humans compared to DPP4 lower respiratory may partly explain differing transmissibility between SARS and MERS in humans (Widagdo 2016, Hou et al. 2020). In addition to distribution of receptors, virulence mismatches in hosts can shape the outcomes of cross species transmission, with increased virulence potentially acting as a limiting factor preventing onward transmission (Mollentze et al. 2020). Pathogenesis therefore may be playing a role in the outcome of cross species transmission events. Major factors influencing pathogenesis include the cellular receptor used by the virus for *attachment* to host cells and the distribution of those receptors within different tissues and organs (Norkin 2010). The cellular-level mechanisms of pathogenesis are known in well-studied viral systems such as influenza (Bender and Small Jr 1992, Zambon 1999, 2001, Fukuyama and Kawaoka 2011). However, much of that detailed knowledge is likely to be absent in many cases, therefore

cell tropism and organ systems affected can serve as a proxy to study how infectious disease pathogenesis is related to cross species transmission. Additionally, the method of within-host spread of the pathogen may play an important role in determining whether disease spread can occur in the ‘recipient’ host species.

The important steps in a viral lifecycle within a host are :

1. Primary transmission
2. **Entry and local replication**
3. **Dissemination within host**
4. **Secondary replication**
5. Shedding/secondary transmission

All these steps can shape pathogenesis in some way. However, the major stages that play a role in pathogenesis are the within-host stages of **entry and local replication, dissemination within the host, and secondary replication**. Pathogenesis therefore plays a key role both in the link from primary transmission to infection and to that of secondary transmission. This is also the case when it comes to cross species transmission or spillover of viral pathogens. Knowledge of viral-host interactions can infer if transmission to a new host will be productive (Escudero-Pérez et al. 2023). Similarly, dissemination within a host and organ tropism can predict shedding and likely secondary transmission (Louten 2016). Clearly the pathogenesis of a viral infection could play a critical role in the disease ecology and transmission dynamics of a pathogen and determine cross species transmission potential.

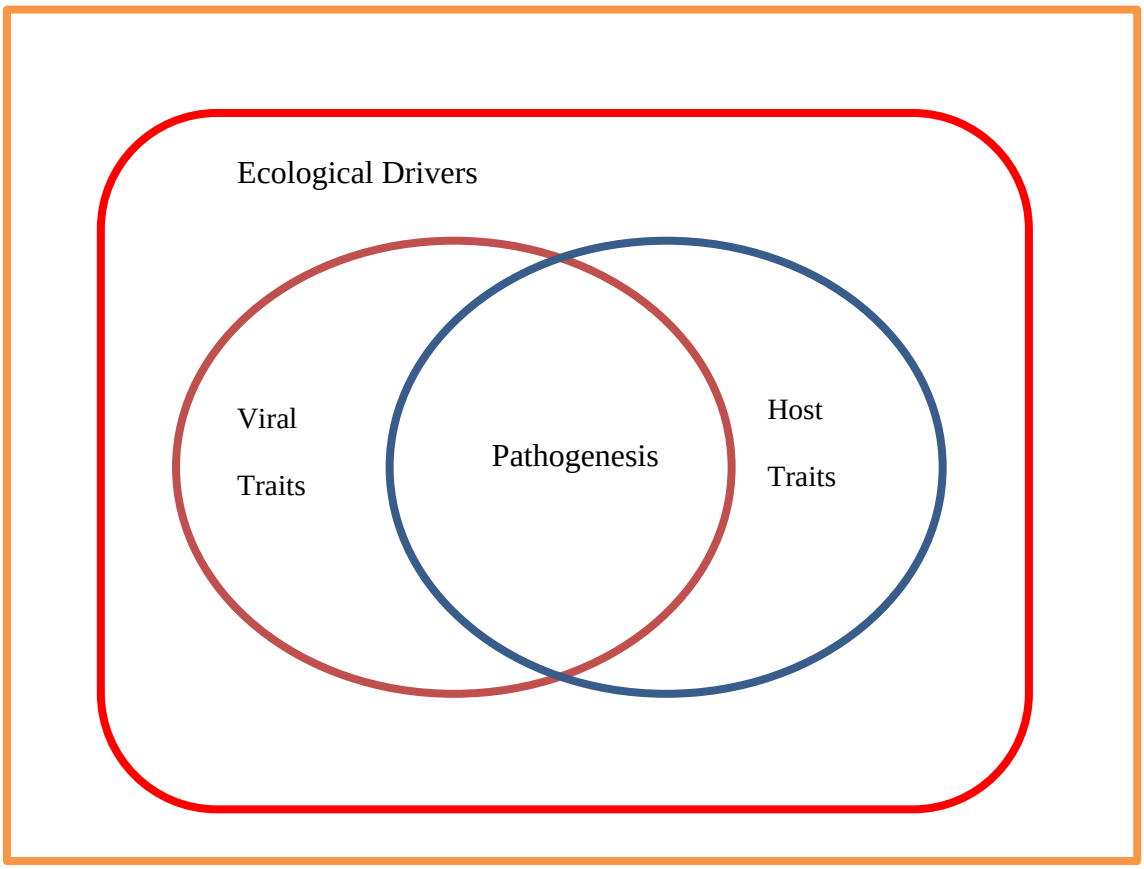

Figure 2.1: Visual representation of the factors involved in cross species pathogen transmission.

Viral entry and local replication

The initial "decision" for a virus upon encountering a new potential host is that of receptor selection and attachment. Contacting a compatible receptor allows the virus to attach to and gain entry to the host cell and consequently access the cellular machinery it requires for its replication cycle. Viruses that can infect multiple hosts tend to use evolutionarily conserved receptors (Woolhouse et al. 2012). A good example of this is rabies virus, which uses the nACh-receptor which is very conserved across all mammals; and rabies has been shown to possess the ability to infect all eutherian mammals (Marston et al. 2018). Another option is to have multiple potential receptors that can be

used for viral attachment to a host cell. Foot-and-mouth disease virus has at least three potential integrin receptors along with Fc receptors it can use, giving it the ability to infect most known cloven-hoofed mammals (Duque and Baxt 2003). This ability to attach to multiple receptors increases the likelihood of encountering a suitable receptor when the virus contacts a novel host.

Dissemination within the host

With regards to viral dissemination within a host and its potential role in cross species transmission, canine distemper can serve as an illustrative example. Canine distemper virus is considered a multi-cell and multi-host pathogen that can infect three different types of host cells; epithelial, lymphoid, and neurological cells (Rendon-Marin et al. 2019). During the first stages of infection within the host, resident dendritic cells and alveolar macrophages in the respiratory tract are infected along with other cells that express CD150 in the alveolae. Infected cells carry the virus to draining lymph nodes, where resident activated T-cells and B-cells are infected through the CD150 receptor, resulting in virus amplification and the initiation of primary viremia (Rendon-Marin et al. 2019). The virus then disseminates to secondary lymphoid organs and is spread through the entire immune system, and finally disseminates to brain, liver, skin, gastrointestinal tract, genitals, and respiratory mucosal surfaces. This systemic spread, particularly to the respiratory mucosa, results in the potential for rapid transmission dynamics through close contact populations. This strategy of using the lymphatic system to spread systemically is likely involved in this virus' ability to infect a wide range of species (Beineke et al. 2015).

Secondary Replication

In addition to receptor selection, a large part of cellular pathogenesis and within-host infection dynamics are controlled by the distribution of the receptor used by the virus within or across organ systems that allow secondary replication at distant sites. The most studied system of receptor distribution across different host species is that of influenza virus. Avian and human influenza virus strains preferentially use differently terminated sialic acid receptors with SAα2,3Gal (avian receptor) and SAα2,6Gal (mammalian receptor) terminated saccharides distributed in the upper respiratory tract of birds and humans, respectively (Kumlin et al. 2008). However, both types of receptor are also present in the upper respiratory tract of pigs, giving rise to the pig as a "mixing vessel" theory of flu recombination and evolution (Ma et al. 2008). The pig upper respiratory tract expresses both types of receptors, allowing for coinfection with an avian and human strain and recombination to produce a highly pathogenic strain that is more transmissible in people than an avian strain, such as occurred in the 2009 swine flu epidemic (Vijaykrishna et al. 2010). Tissue tropism of avian influenza virus has been shown to influence spillover from wild birds to pigs (Zhang et al. 2020). Additionally, distribution of receptors in human spillover hosts is important to cross species influenza virus transmission. Humans possess SAα2,3Gal receptors, but only in their lower respiratory tract (de Graaf and Fouchier 2014). So, while people occasionally become infected with avian influenza virus, this subtle difference in receptor distribution plays a huge role, both in disease pathogenesis and in transmissibility of infection. The presence of the virus and subsequent replication in the lower respiratory tract results in a much more severe infection with higher morbidity and mortality than a typical human strain of

influenza virus. Additionally, the fact that the virus cannot replicate in the upper respiratory tract makes it difficult for the virus to be transmitted via aerosol. Waterfowl along with shorebirds are considered as the primary wild reservoir of avian influenza strains and their role in this partly comes down to receptor distribution. The SAα2,3Gal receptors used by the influenza virus are present in large amounts in the intestinal tract of many species of migratory waterfowl (Costa et al. 2012). Waterfowl belonging to the *Anatidae* family (ducks, geese, and swans) are the primary reservoir of all 16 hemagglutinin and 9 neuraminidase subtypes of avian influenza viruses (Hansbro et al. 2010). Migration of these birds results in the inoculation of waterways, namely bodies of water where birds congregate, with live influenza virus, which can be relatively stable in the water for long periods, depending on conditions (Blagodatski et al. 2021). Additionally, there is potential for free-ranging domestic fowl to be exposed to faeces containing avian influenza from these birds. This illustrates how pathological features of infection and ecology can interact to directly influence viral transmission dynamics.

These are well-studied examples of infection pathogenesis playing a significant role in the ability to infect a novel host. The question arises of whether similar examples exist in the broader literature of pathological traits influencing a virus's ability to infect novel species and whether general patterns exist that can at least partially explain the role that host-pathogen interaction or 'pathogenesis' traits may play in cross species transmission, this is a seldom studied area of cross species transmission and there are likely significant gaps in existing literature. Here, we synthesize available literature on cross species transmission events along with viral pathogenesis data to determine how aspects of viral pathogenesis affect cross species transmission in RNA viruses.

The objectives of this study are two-fold; firstly, we investigate the availability and quality of existing virus pathogenesis data as it currently stands and how it is suited to answering questions related to pathogenesis and cross species transmission and where gaps in this knowledge may lie. Secondly we aimed to analyze the available host-virus pathogenesis data and identify pathological traits associated with different types of cross species transmission events, in particular whether these events involve humans as a host or spillover species.

METHODS

Data acquisition and overview

A search of the PubMed database was performed as described below. These search results were then screened using the *metagear* package in R according to the PRISMA guidelines (Figure 2.3). Following this a secondary search was conducted to provide known pathological data for each virus.

The resulting data were imported into R Studio (version 2022.12.0+353) (Posit team 2022).. A detailed description of data analysis is contained in the scripts within the project repository (https://github.com/JJWilson1991/Pathogenesis_project). All analyses were conducted in the R programming environment (version 4.2.0.)(R Core Team 2022). References to packages in this methods section indicate specific packages used within the R environment to perform analyses.

Literature search

A literature search was performed of the PubMed data base according to PRISMA guidelines (Page et al. 2021). The search terms described in Figure 2.2 were used with the results being screened for eligibility using the *metagear* package in R (Lajeunesse 2016). Eligibility criteria are described in Table 2.1.

(((RNA virus) AND (((((Spillover) OR (cross-species transmission)) OR (host-switching)) OR (interspecies transmission)) OR (zoonosis))) NOT (Covid)) NOT (Review[Publication Type])

Figure 2.2: List of search terms used in PubMed for initial literature search.

Following inclusion in the study, data relating to the cross species transmission event were recorded from each article, listed in Table 2.2.

Table 2.1: List of exclusion and inclusion criteria for literature search screening

Exclusion criteria	Inclusion criteria
Experimental Data Review article Irrelevant	Directly transmitted. Evidence of transmission from reservoir to spillover species. (In descending strength of support) -molecular evidence (virus recovery, PCR etc.) -phylogenetic -serological -epidemiological

The pathological traits recorded from the secondary search are also listed in Table 2.2. Events were defined as zoonotic, anthroponotic or not zoonotic. “Not zoonotic” is

defined as a virus that is not considered to infect humans. An “anthroponotic” event is defined as a virus that has been transmitted from a human reservoir to another species. Spillover is defined as the transmission of a parasite from one host species to another, regardless of whether onwards transmission in the recipient host is successful (Borremans et al. 2019). Reservoir host is defined as a species responsible for shedding the parasite and causing a spillover exposure event, either by shedding the parasite into the environment or through direct contact with the recipient host (Borremans et al. 2019). Spillover host is defined as a species that is infected by a parasite originating from a different host species (Borremans et al. 2019).

Table 2.2: List of data recorded from each qualifying piece of literature on a cross species transmission event and the corresponding pathological data recorded from reference material.

Transmission event data recorded	Pathological data recorded
Virus (Species, genus, family, order)	Within host spread mechanism
Reservoir host (Species, genus, family, order)	Cellular receptor used
Spillover host (Species, genus, family, order)	Receptor distribution
Zoonotic traits	Cellular tropism
	Organs/systems affected

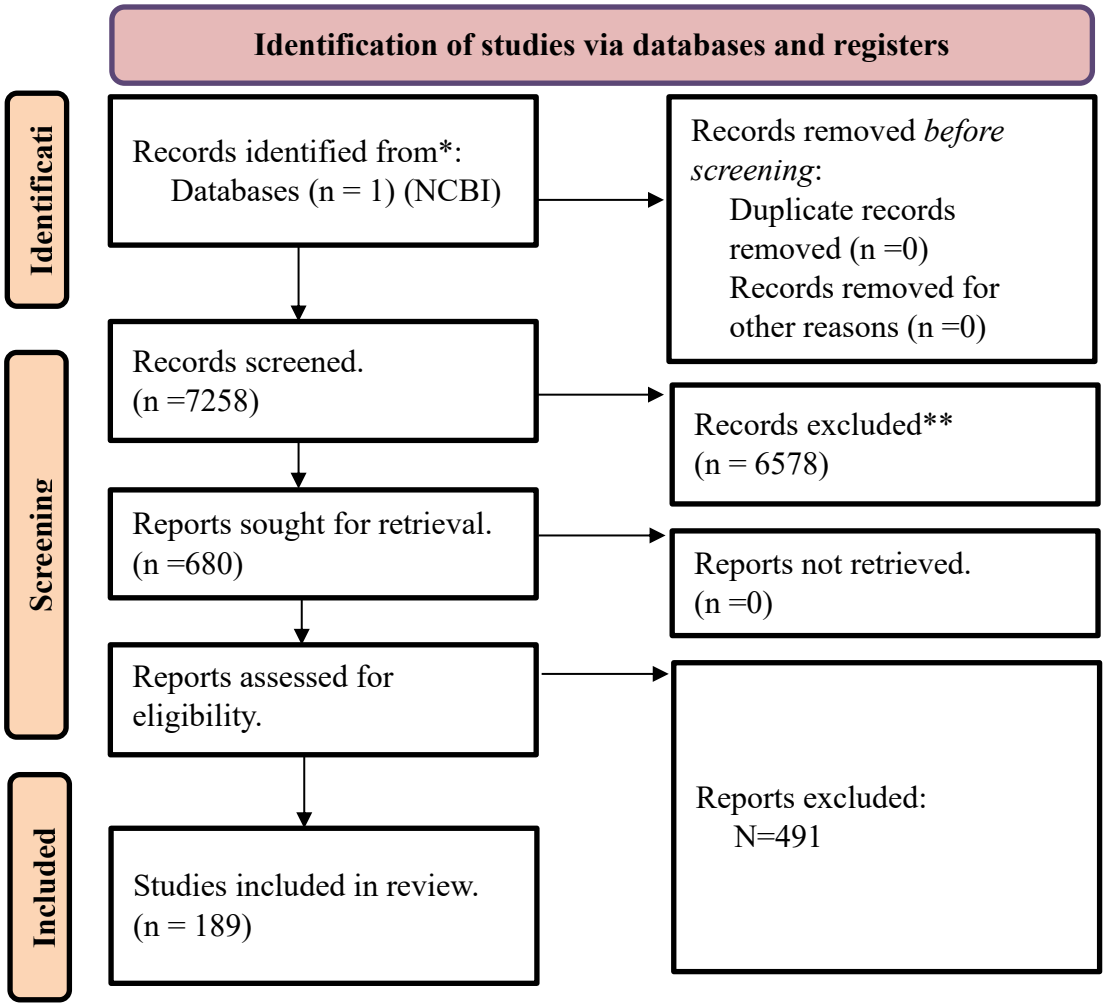

Figure 2.3: Flow diagram for screening and reviewing search results for this study based on PRISMA guidelines.

Multiple correspondence analysis

To analyze the relationships between pathology parameters, multiple correspondence analysis (MCA) was conducted. MCA is a descriptive technique designed to measure correspondence between the rows and columns in tables of data. The objective of MCA is to visualize the relationship of categorical variables. Correspondence analysis is used to explore the relationship between variables by comparison with distance in multiple dimension space. The first two dimensions can

usually explain most of the variation seen in the data. Following data processing a total of n=213 rows along with 31 columns were used for MCA. The MCA was also repeated with just the unique viruses (n=52), to remove bias in the correspondence caused by viruses that occur more frequently in the literature of spillover events between multiple different species, e.g., influenza A viruses. Multiple correspondence analysis was conducted on the data using the package *FactoMineR* (Le 2008).

Hierarchical cluster analysis

We used hierarchical cluster analysis to group viruses into pathologically similar clusters. Specifically, we constructed a dissimilarity matrix using the Gower distance (Gower 1971) with the `daisy` function from the *cluster* package (Maechler 2022). This matrix was then used with the 'hclust' function in the *stats* package (R Core Team 2022) to perform agglomerative clustering on this distance matrix. We next visualized the results of our hierarchical cluster analysis as a dendrogram with the *dendextend* and *factoextra* packages (Galili 2015, Kassambara and Mundt 2017).

RESULTS

A total of 189 articles were entered into the database and following processing, this resulted in n=213 unique entries of virus-reservoir host-spillover host interactions, comprised of n=52 unique viruses. The unknown data for pathology-related variables for each unique virus are summarized in Table 2.3. The most frequent reservoir host was humans (34/213) followed by pigs and dogs. Spillover host species were dominated by humans with 103/213 recorded. The nature of interactions between reservoir hosts and

spillover hosts is represented in Figure 2.3. From the 52 viruses recorded, there were 33 different cellular receptors plus a further 14 unknown receptors.

Table 2.3: Proportions of viruses with missing data for their cellular receptors, spread mechanisms, cellular tropism and organ system affected following searches of databases and reference material.

Pathology variable	**Unknown data proportion**
Cellular Receptor	14/52 (27%)
Within-host spread mechanism	14/52 (27%)
Cell tropism	5/52 (10%)
Organ systems affected	3/52 (6%)

Multiple correspondence analysis

The first two dimensions of the MCA explain 35% of the variance in the data. The major variables that contribute to the first two dimensions are shown in supplementary Figure 2.1. The MCA for individual host interactions (Figure 2.5) clusters epithelial spread and respiratory pathology with anthroponosis, suggesting an association between these variables. There also appears to be an association between lymphocytic tropism and not being zoonotic. 95% confidence ellipses for “anthroponotic” cluster separately from “not zoonotic”, suggesting pathological features differ (Figure 2.5).

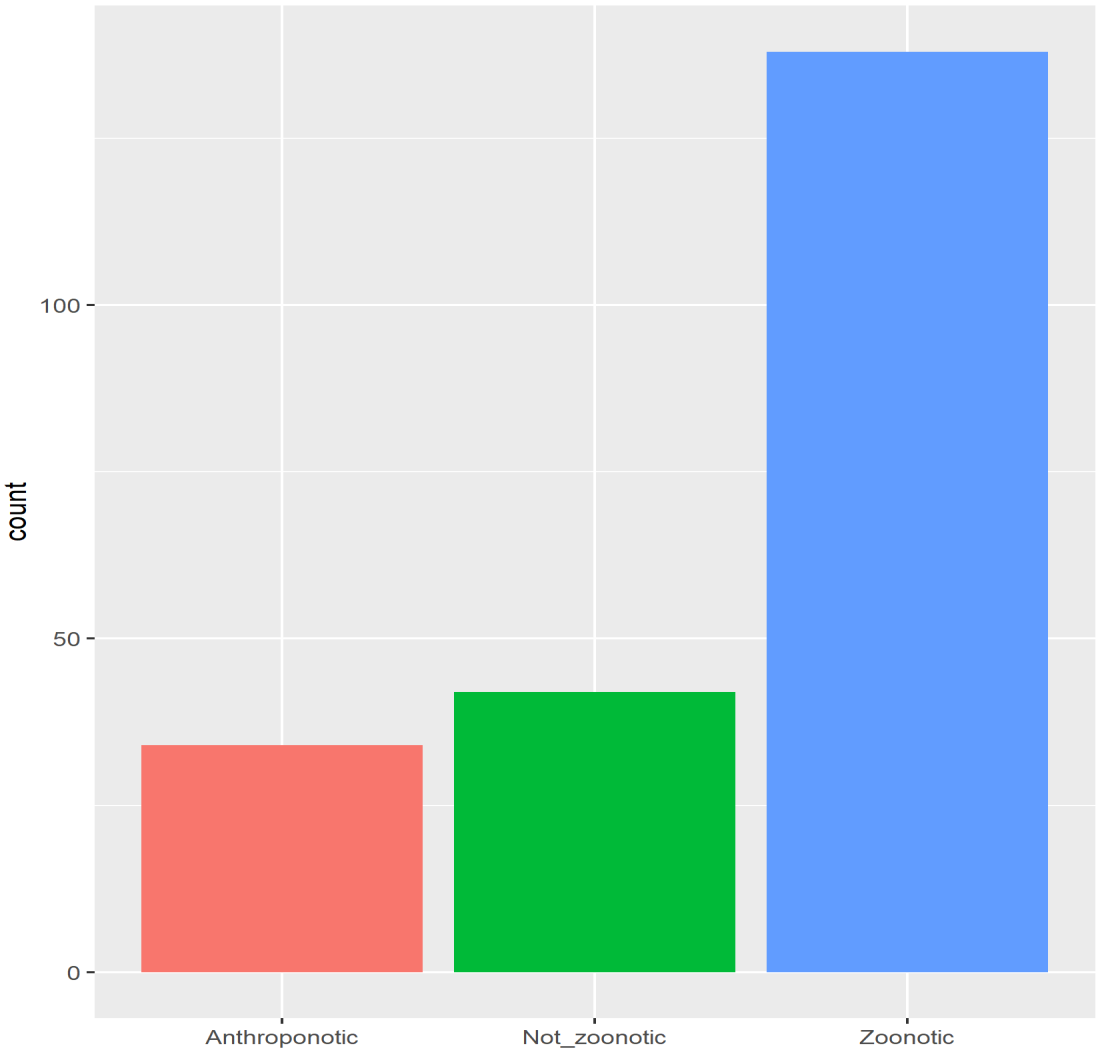

Figure 2.4: Proportion of each individual cross species transmission event that were recorded as being anthroponotic (orange), zoonotic (blue) or neither (green).

The MCA was repeated with the unique viruses in the dataset (Figure 2.6). Unique viruses being a viral species that has been involved in a spillover event, so that there is one entry per virus. Whereas in the original search and analysis, many viruses had multiple entries as they were involved in multiple spillover events involving different host species. In this case, gastrointestinal pathology is associated with “not zoonotic” in addition to lymphocyte tropism. The “zoonotic” ellipses for the unique virus MCA have

the “zoonotic” ellipse as a smaller ellipse within a larger ellipse for “not zoonotic” (Figure 2.5). This suggests that the zoonotic interactions have a narrower range of pathologies compared to the not zoonotic.

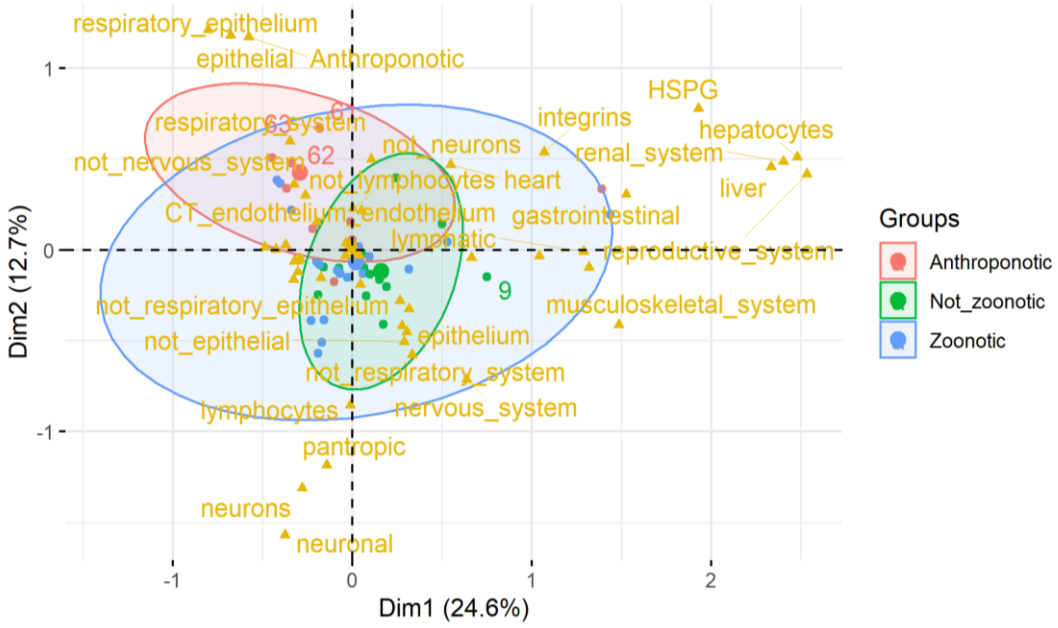

Figure 2.5: Multiple correspondence analysis biplot for the n=213 individual observations of cross species transmission of RNA viruses from the literature search. *The yellow points represent the variables. 95% confidence ellipses for cross species transmission events classified as zoonotic, anthroponotic or not zoonotic have been drawn over the individual observations in the plot.*

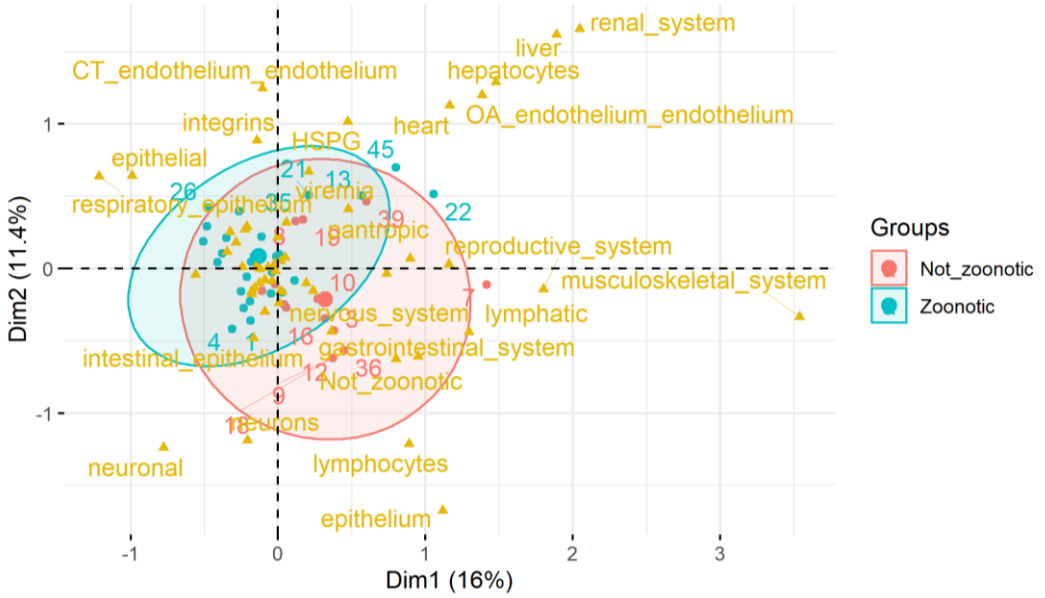

Figure 2.6: Multiple correspondence analysis biplot for the n=52 unique RNA viruses from the literature search. *The yellow points represent the variables. 95% confidence ellipses for cross species transmission events classified as zoonotic, or not zoonotic have been drawn over the individual observations in the plot.*

Hierarchical cluster analysis

When hierarchical cluster analysis is performed on the second data set of 52 viruses and their typical pathogenesis, they tend to cluster into distinct groups based on the pathology they induce in the host. Six main clusters were formed, with five of these based on pathology and morbilliviruses forming their own cluster. There were also an additional six “orphan viruses” that did not cluster with any others based on pathology (Figure 2.7).

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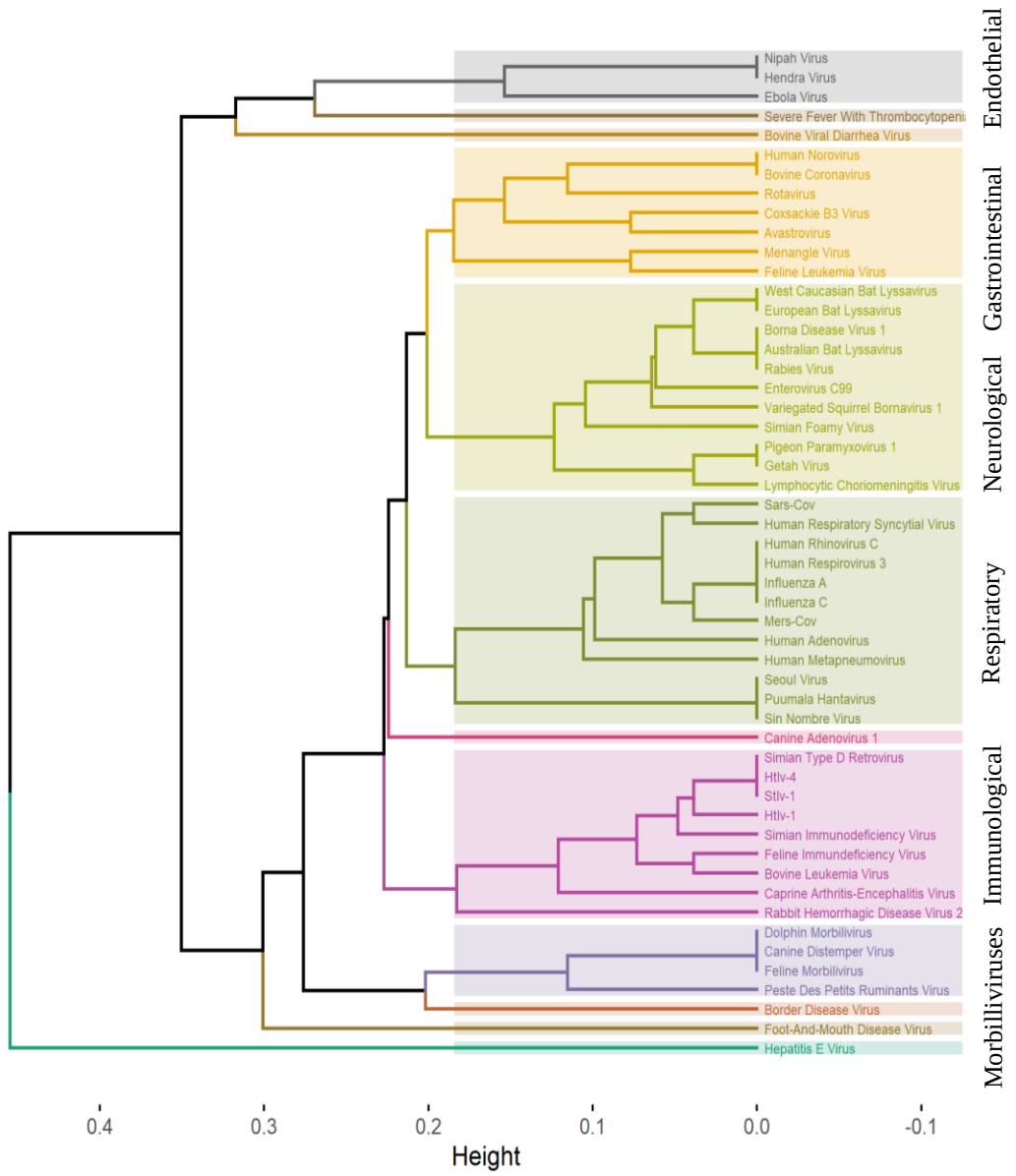


Figure 2.7: Dendrogram generated from 52 unique viruses and their pathological data. *To generate the dendrogram, we constructed a dissimilarity matrix using the Gower distance. We performed a hierarchical cluster analysis on the matrix to group similar pathological data. The dendrogram was cut to divide the tree into 12 clusters, each of which is represented by a different color. The six major clusters are named based on the predominant pathology caused in the host (in addition to the morbillivirus cluster).*

DISCUSSION

The current study investigated the nature of available pathogenesis data as it related to cases of cross species RNA virus transmission events. The study identified major gaps in the scientific literature, particularly at the molecular level, where it came to understanding the cellular level pathogenesis of cross species transmission events. Secondly, we analyzed the results of a literature search for cross species transmission events in RNA viruses using multiple correspondence analysis and identified trends in the pathological traits associated with these infections, particularly as they related to spillover events involving humans. Thirdly, hierarchical cluster analysis identified the existence of "pathological groups" of viruses which cluster based on their pathogenesis traits that tend to have other similar traits, especially in associated hosts and transmission mechanisms.

Data availability

With respect to the availability of pathogenesis data, there was a trend between the microscopic level of the data and level of unavailability, with microscopic traits like cellular receptor being less available than relatively more coarsely scaled data such as organ systems affected. The percentage of unknowns for pathological data increased the finer scale (cellular or molecular level) of the pathological variable, with cellular receptors being the most unknown. Whilst one of the aims was to study cellular receptor trends from the 52 viruses recorded there were 33 different cellular receptors for virus infections known plus a further 14 unknown receptors. While many of the cellular receptors for viral entry in the host are unknown, equally many of the recorded receptors are putative or just one piece of the cellular attachment and entry puzzle, making this

difficult to analyze. Some basic types of receptors associated with viral-host interaction did occur regularly for integrins and heparan sulfate proteoglycans, suggesting that these may be a conserved group of receptors for a number of viruses, and are known to be widely prevalent receptors in hosts. Recent work has also begun to look at the potential role of sialic acid receptors as a conserved receptor involved in zoonotic infections (Kuchipudi et al. 2021). As this may be a promising area of future investigation, the major barrier highlighted by this study is the absence of available data at the *cellular receptor level*. Additionally, there may be even less understanding of cellular receptors in viruses involved in novel spillover events, compared to other diseases that have been endemic in humans or certain domestic species for a longer period of time, as they have been less studied.

An additional area of interest in cross species transmission is that of codon usage bias in viruses (CUB). As CUB varies per virus and the host species they are adapted to this may provide important information regarding abilities to infect new species (Bahir et al. 2009). However, the relative lack of studies conducted in this area emphasizes that cellular and molecular factors are often under investigated in the context of pathogenesis and cross species transmission.

Pathological trends

In the hierarchical cluster analysis, whilst there was some clustering based on viral phylogeny, this was not strict, with some phylogenetically related viruses not clustering together, but instead clustering based on the pathology they induced. With the HCA, it is interesting to note that there were still several “orphan viruses” that did not

cluster well, highlighting part of the challenge here in that the interactions between virus and host that produces pathology is complex and can produce some unique outcomes. Could the clustering of viruses in groups be used to predict whether certain viruses in the group will be of risk to hosts that are infected by other viruses in the group? For example, the respiratory group all involved humans as reservoir or spillover hosts. Could the neurological group, which contained several viruses known to infect humans, in fact all possess this ability based on their shared pathological traits?

Those viruses with primary neuronal tropism have been known to have a broad host range (Brunker 2018). The neurological cluster of the dendrogram featured more variety in terms of hosts and viruses than other clusters. The receptor for rabies virus and possibly other lyssaviruses is the nicotinic acetylcholine receptor, which is widely conserved between mammalian species (Le Novere and Changeux 1995, Gotti and Clementi 2004). This feature of the pathogenesis of this virus helps to explain its broad host range and ease of transmission into novel species.

With the exception of influenza virus, respiratory tropic viruses tended to be transmitted between more closely related species. High barriers to infection due to mucosal immunity may require more conserved receptors, i.e., between more related species for successful cross species transmission (Sato and Kiyono 2012).

Despite endotheliotropic viruses being associated with cross species transmission events causing severe illness, such as hemorrhagic fever viruses (LeDuc 1989), there were not many records of viruses that induce endothelial pathology and there was not a strong correspondence with any other factors. This may be due to the difficulty in accessing this receptor due to its location, making it a more specialized virus trait.

Lymphoid tropism was associated with viruses categorized as ”not zoonotic”. This greater host range may be related to co-opting the host immune system to bypass some of these interspecific barriers or perhaps there may be conserved immunological virus receptors between species.

There are some outlying variables in the MCA, e.g., muscle, caused by individual recordings, which are likely not important to overall trends in pathogenesis but highlight the importance of referring to the actual data when interpreting MCA.

Human related features

64% of articles involved humans as either reservoir or spillover hosts, highlighting the inherent human bias in cross species transmission research. Humans are in close proximity to spillover events involving themselves as reservoir or spillover hosts and can report symptoms, etc. They also have a vested interest in research involving these diseases that affect them directly. This is reflected in the four most common viruses in the dataset; influenza A, rabies, hepatitis E and simian foamy viruses, which are all known to infect humans. In addition to cases involving humans themselves, 66% of records involved what would be considered a domestic species (either livestock or pet) as reservoir or spillover host, with only 8% of records involving neither humans nor domestic species. This demonstrates the inherent bias that exists in this type of data with either the proximity to these species resulting in reporting biases or the interest in surveillance of diseases in these species.

In addition to domesticated species, where contact is likely an important factor, the other major group of species with which humans exchanged viruses are non-human

primates. This is likely due to lower barriers to cross species transmission in this case due to phylogenetic relatedness (Olival et al. 2017, Guth et al. 2019).

The association between anthroponosis, i.e., those cases of virus transmission from human to other species and respiratory pathology, may have to do with human behaviors. Respiratory pathology is generally caused by viruses with a respiratory droplet mode of transmission(Wang et al. 2021). Other methods of transmission are less likely from humans due to human behaviors related to hygiene reducing the risk of faeco-oral transmitted pathogens and those transmitted by direct contact (Penakalapati et al. 2017). This was reflected in the MCA where zoonosis and anthroponosis formed a subset within a larger more variable group containing cross species events not involving humans. This may also reflect a narrower range of pathologies. The occurrence of the “anthroponotic” ellipsis as a smaller ellipsis within the “zoonotic” ellipsis in the MCA for the individual host MCA and the “zoonotic” ellipsis as a smaller ellipsis within a larger “not zoonotic” in the unique virus MCA seems to reflect this (Figures 2.5 and 2.6). The importance of respiratory pathology in humans was shown again in the HCA with the respiratory cluster including only viruses with humans as spillover or reservoir hosts. Much of the driving force behind this trend is the repeated high profile of anthroponotic spillover of human respiratory viruses into great apes (Muehlenbein 2016, Devaux et al. 2019). The strength of this association suggests that whilst hygiene measures are in place to prevent spillover of other pathogens that require closer contact, it appears ecotourism and other activities that encroach on wild primates are still getting close enough to transmit respiratory pathogens.

CONCLUSION

There are two main points to take from this study. Firstly, there appear to be trends in pathogenesis data at the virus-host interface which correspond to certain types of spillover events, particularly those events involving humans. Specifically, viruses involved in cross species transmission events tend to group together based on certain pathological/pathogenesis traits that often corresponded with types of hosts involved. The trends that have emerged from this study demonstrate that further work on cellular and molecular pathology of cross species transmission events is merited and should be better integrated with disease ecology and epidemiological approaches.

Secondly, this study highlights the current absence of high-quality and fine-scale virus-host pathogen interaction data in some areas, particularly at the cellular/subcellular level. This calls for more collaboration and focused efforts among the scientific community to identify receptors that may be indicative of spillover risk. Elucidating the potential role of pathogenesis in cross species transmission can raise the possibility of trying to include this data into predictive frameworks of cross species transmission risk.

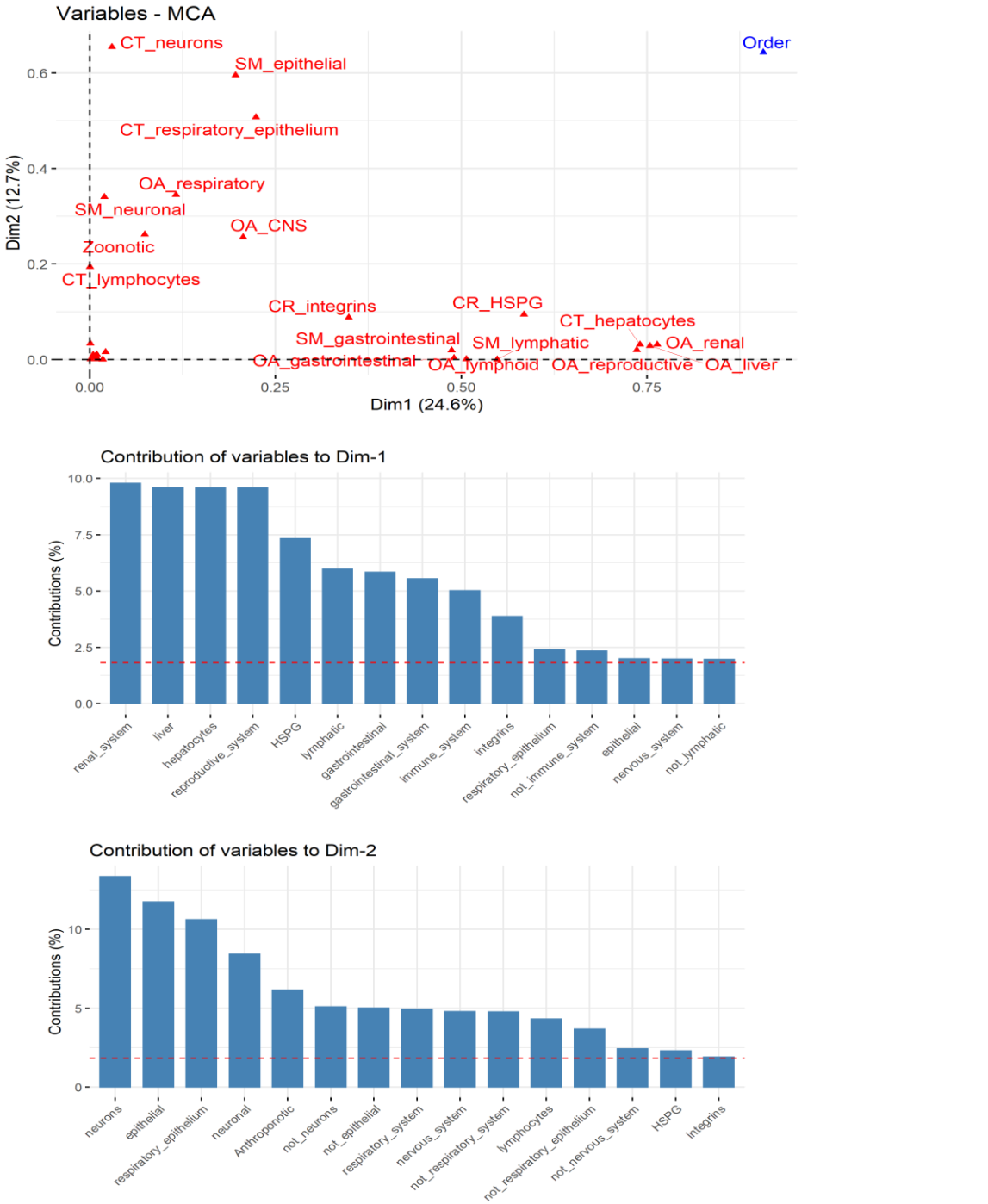
SUPPLEMENTARY MATERIALS

Supplementary Table 2.1: List of virus name, order, family, and genus for each unique virus included in the database of spillover events.

Virus_Name	Order	Family	Genus
Australian Bat Lyssavirus	Mononegavirales	Rhabdoviridae	Lyssavirus
Avastrovirus	Stellavirales	Astroviridae	Avastrovirus
Border Disease Virus	Amarillovirales	Flaviviridae	Pestivirus
Borna Disease Virus 1	Mononegavirales	Bornaviridae	Orthobornavirus
Bovine Coronavirus	Nidovirales	Coronaviridae	Betacoronavirus
Bovine Leukemia Virus	Ortervirales	Retroviridae	Deltaretrovirus

Bovine Viral Diarrhea Virus	Amarillovirales	Flaviviridae	Pestivirus
Canine Adenovirus 1	Rowavirales	Adenoviridae	Mastadenovirus
Canine Distemper Virus	Mononegavirales	Paramyxoviridae	Morbillivirus
Caprine Arthritis-Encephalitis Virus	Ortervirales	Retroviridae	Lentivirus
Coxsackie B3 Virus	Picornavirales	Picornaviridae	Enterovirus
Dolphin Morbilivirus	Mononegavirales	Paramyxoviridae	Morbillivirus
Ebola Virus	Mononegavirales	Filoviridae	Ebolavirus
Enterovirus C99	Picornavirales	Picornaviridae	Enterovirus
European Bat Lyssavirus	Mononegavirales	Rhabdoviridae	Lyssavirus
Feline Immundeficiency Virus	Ortervirales	Retroviridae	Lentivirus
Feline Leukemia Virus	Ortervirales	Retroviridae	Gammaretrovirus
Feline Morbilivirus	Mononegavirales	Paramyxoviridae	Morbillivirus
Foot-And-Mouth Disease Virus	Picornavirales	Picornaviridae	Aphthovirus
Getah Virus	Martellivirales	Togaviridae	Alphavirus
Hendra Virus	Mononegavirales	Paramyxoviridae	Henipavirus
Hepatitis E Virus	Hepelivirales	Hepeviridae	Hepevirus
HTLV-1	Ortervirales	Retroviridae	Deltaretrovirus
HTLV-4	Ortervirales	Retroviridae	Deltaretrovirus
Human Adenovirus	Rowavirales	Adenoviridae	Mastadenovirus
Human Metapneumovirus	Mononegavirales	Pneumoviridae	Metapneumovirus
Human Norovirus	Picornavirales	Caliciviridae	Norovirus
Human Respirovirus 3	Mononegavirales	Paramyxoviridae	Respirovirus
Human Rhinovirus C	Picornavirales	Picornaviridae	Enterovirus
Influenza A	Articulavirales	Orthomyxoviridae	Alphainfluenzavirus
Influenza C	Articulavirales	Orthomyxoviridae	Gammainfluenzavirus
Lymphocytic Choriomeningitis Virus	Bunyavirales	Arenaviridae	Mammarenavirus
Menangle Virus	Mononegavirales	Paramyxoviridae	Pararubulavirus
MERS-CoV	Nidovirales	Coronaviridae	Betacoronavirus
Nipah Virus	Mononegavirales	Paramyxoviridae	Henipavirus
Peste Des Petits Ruminants Virus	Mononegavirales	Paramyxoviridae	Morbillivirus
Pigeon Paramyxovirus 1	Mononegavirales	Paramyxoviridae	Avulavirus
Puumala Hantavirus	Bunyavirales	Hantaviridae	Orthohantavirus
Rabbit Hemorrhagic Disease Virus 2	Picornavirales	Caliciviridae	Lagovirus
Rabies Virus	Mononegavirales	Rhabdoviridae	Lyssavirus
Human Respiratory Syncytial Virus	Mononegavirales	Pneumoviridae	Orthopneumovirus
Rotavirus	Reoviridae	Reoviridae	Rotavirus
SARS-CoV	Nidovirales	Coronaviridae	Betacoronavirus
Seoul Virus	Bunyavirales	Hantaviridae	Orthohantavirus

Severe Fever With Thrombocytopenia Syndrome Virus	Bunyavirales	Phenuiviridae	Bandavirus
Simian Foamy Virus	Ortervirales	Retroviridae	Spumavirus
Simian Immunodeficiency Virus	Ortervirales	Retroviridae	Lentivirus
Simian Type D Retrovirus	Ortervirales	Retroviridae	Betaretrovirus
Sin Nombre Virus	Bunyavirales	Hantaviridae	Orthohantavirus
STLV-1	Ortervirales	Retroviridae	Deltaretrovirus
Variegated Squirrel Bornavirus 1	Mononegavirales	Bornaviridae	Orthobornavirus
West Caucasian Bat Lyssavirus	Mononegavirales	Rhabdoviridae	Lyssavirus



Supplementary Figure 2.1: Contribution of each variable to first two dimensions of multiple correspondence analysis. *Contributions of each variable to the variance shown in dimensions 1 and 2 of the multiple correspondence analysis are shown in figure A , with greater distance from the origin demonstrating a greater contribution to the variance. The scree plots B and C, show individual contribution of variables to each dimension.*

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CHAPTER 3

MODELING PATHOGEN PREVALENCE AND GENETIC DIVERSITY OVER THE COURSE OF AN EPIZOOTIC AS IT PERTAINS TO SPILLOVER RISK.

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Abstract

Cross species transmission of pathogens is a multifactorial process requiring a combination of extrinsic factors including ecological opportunity for contact and intrinsic biological characteristics including virus–host molecular and cellular compatibility. There are two major variables within a viral population that influence how likely the virus is to enter a new species at a given point in time: the force of infection, i.e., the viral prevalence in the reservoir population, and the probability that a strain capable of infecting a new species is present, i.e., genetic diversity of the viral population. Here we use an individual based SIR model and stochastic simulations to track the prevalence and genetic characteristics of a viral population within a reservoir host population throughout an epizootic. Analysis of the results showed that optimum transmissibility evolves in the viral population early in the epizootic with peak genetic diversity following later, after the peak of the outbreak. This has important repercussions for spillover risk, with spillover between closely related species being related to transmissibility and the greatest risk through the upslope and peak of the epizootic when the pathogen population has reached maximum transmissibility. However, the later development of genetic richness would point to spillover risk between distantly related host species being greater later in the epizootic, after the peak has passed. Additionally, we show the existence of a bistable evolutionary strategy under certain conditions whereby either high transmissibility or moderate transmissibility may be the life history path of a virus population.

INTRODUCTION

Cross species transmission of pathogens is a multifactorial process requiring a combination of extrinsic factors including ecological opportunity for contact and intrinsic biological characteristics including virus–host molecular and cellular compatibility (Parrish et al. 2008, Plowright et al. 2017, Becker et al. 2019, Ellwanger and Chies 2021). Phylogenetic distance has often been used as a proxy for biological compatibility between two species, with spillover more likely between closely related species (Streicker et al. 2010, Olival et al. 2017). An additional critical component for cross species transmission is geographic range overlap (Davies and Pedersen 2008, Albery et al. 2020). This is of particular importance with climate change potentially placing more species in proximity to one another and facilitating this ecological opportunity for pathogen sharing (Carlson et al. 2022). However, based solely on these two major factors of biological compatibility and ecological opportunity, there should be many more instances of cross species transmission, so the picture is even more complex. Research into pathogen sharing often does not include information on the force of infection, as would generally be measured by pathogen prevalence. When prevalence is measured it is usually measured independently of the actual potential for cross species transmission, in this case being the likelihood that a pathogen population contains genotypes that are able to switch hosts. This raises the question of whether there are predictors during a single outbreak when a virus is more likely to spillover to a new species.

Grenfell et al. attempted to unify some of these epidemiological and evolutionary ideas in their synthesis of pathogen phylodynamics (Grenfell et al. 2004). RNA viruses are known for their ability to rapidly produce diverse viral populations. Viruses have the

intrinsic capacity for genetic change, and ability to adapt to diverse fitness landscapes (Elena et al. 2009). These innate capabilities for mutation create genetically diverse populations of viruses upon which selection acts (Domingo 2010). Generating maximum diversity may provide the ideal combination of mutants to facilitate cross species transmission (Woolhouse et al. 2001, Borderia et al. 2011).

There are two major variables within a viral population that influence how likely the virus is to enter a new species at a given point in time: the force of infection, i.e., the viral prevalence in the reservoir population, and the probability that a strain capable of infecting a new species is present, i.e., the genetic diversity of the viral population. These correspond to two broad scenarios under which cross species transmission occurs. In the first, there are two closely related species to which the pathogen is preadapted, and here pathogen prevalence may be key. Secondly, we have two distantly related species. In this second scenario, cross species transmission is more likely to happen if the reservoir population contains a genetically diverse population of pathogens (Dennehy et al. 2010). It has been shown on multiple occasions that viral epizootics in one species can lead to spillover into others (Chua 2003, Weckworth et al. 2020). It has been hypothesized that these events correspond to shedding peaks in the reservoir species (Peel et al. 2019). This supports the notion that the key factor in cross species transmission is having an overwhelming quantity of virus in the system. A recent modeling approach has also suggested that seasonal peaks in reservoir population sizes results in not only more prevalence of infection but also greater genetic diversity in the pathogen population (Remien and Nuismer 2020). Increased R_0 can result in increased pathogen evolution and likelihood of disease (Antia et al. 2003). However, despite R_0 maximisation seemingly

favouring the pathogen, this has been shown to rarely be the case in the long term, with R_0 increasing but not reaching the maximum potential level (Cortez 2013). R_0 maximisation only appears to occur in models with simple forms of environmental feedback and in more realistic parasite-host interactions, ecological processes will prevent R_0 maximisation (Lion and Metz 2018). Therefore, tracking changes in transmissibility throughout an epizootic may be of importance in the context of spillover risk.

Viral population genetic diversity plays a major role in viral evolution (Sanjuán and Domingo-Calap 2021). There are a number of factors that can influence pathogen genetic diversity and consequently the probability that a pathogen population contains generalist strains that can effectively cross species barriers (Retel et al. 2019). Experimental evidence has shown that genetic diversity can drive emergence in a novel species (Dennehy et al. 2010). However, not all genetic changes are beneficial and this may sometimes prevent host shifts from occurring (Longdon et al. 2014).

Recent work has investigated the idea of a specialist-generalist spectrum among parasites (Park et al. 2018). This work assumes each pathogen species has a single measure of specificity (the average relatedness of all their host species). This is a relatively coarse metric and within any species there are genotypes that are more likely to infect new species. Additionally, this may be a dynamic process with movement across the spectrum over time. This research approach raises the question of how a population of parasites would move along this spectrum over the course of an epizootic.

Traditional frameworks for studying pathogen evolution focus on making long-term predictions and assume a decoupling of epidemiological and evolutionary dynamics.

However, this assumption is overly restrictive for many parasites that have short generation times, large population sizes, and high mutation rates (Cressler et al. 2016). Both population genetics and adaptive dynamics approaches assume low mutation rates (Metz et al. 1992, Geritz et al. 1998), which does not align with empirical evidence for viruses, particularly RNA viruses, that mutate and evolve rapidly. Thus, it is not possible to uncouple demographic and evolutionary processes for RNA viruses with these techniques.

Several studies have attempted to couple epidemiological and evolutionary timescales during the epidemic phase, often with a focus on the evolution of virulence (Lenski and May 1994, Day and Proulx 2004, Day and Gandon 2007, Bull and Ebert 2008, Bolker et al. 2010). Studies suggest that a transmission-virulence trade-off leads to higher virulence at the beginning of an epidemic when there are many susceptible hosts, followed by virulence evolving to lower levels as the endemic equilibrium is reached and transmissibility is paramount.

There are several alternative hypotheses as to when the risk of cross species transmission is highest. In the SARS epidemic, spillover into humans was believed to have occurred through a bridge species, with the virus retaining some generalist traits on initial entry into a population, making a second jump easier to make (Wang and Eaton 2007). Similarly, at the start of an epizootic, a viral population may be more genetically diverse before the influence of purifying selection by passage in the same host species (Pybus et al. 2007), making it more of a generalist early in an epizootic.

In several rodent and bat reservoir models it has been suggested that seasonal peaks in population size lead to peaks in viral prevalence, which correspond with

spillover events (Amman 2012, Plowright et al. 2015, Akhmetzhanov et al. 2019). These cases would suggest that prevalence is the most important factor and that the peak of an epizootic is when the risk is greatest. However, as mentioned above, there can be a case whereby at peak transmission, a small number of dominant strains with the greatest R_0 in the current host species, but potentially not for other host species, exclude other strains. Finally, we consider a situation where the risk may be greatest in the later stages of an epizootic. We can consider that immune pressure on the whole virus population increases over time. Does this produce a situation where this selection pressure on the viral population selects for generalist traits, i.e., viruses with the ability to host switch, towards the end of an epizootic? There is likely to be a point during the epizootic whereby viral prevalence and genetic diversity reach a state that is most likely to result in cross species transmission.

Viral epizootics occur under different starting conditions and with different fitness landscapes. Broadly speaking, a virus population may be preadapted to a species, therefore relatively fit, or not preadapted so less fit in that species. Additionally, the fitness challenge represented by the host species influences the outcome of an epizootic. Different fitness landscapes are more or less difficult for a viral population to survive and reproduce. In a challenging fitness landscape, such as that represented by a healthy host with a fully functioning immune system, a relatively small proportion of potential virus genotypes will be fit under these conditions, with only the best adapted being able to thrive. In these conditions there is a lot of pressure for the viral population to evolve towards this narrow range of fit genotypes. Under less challenging fitness landscapes, more genotypes are fit under these conditions, resulting in a greater diversity of viruses

being able to replicate in the host population. These conditions present less of an evolutionary challenge for the viral population as it is not required to reach the optimum genotypes in order to survive and reproduce.

To address these questions, we use individual based models and simulations so that we can simultaneously track prevalence and viral genetic and phenotypic diversity, and consequently the likelihood of cross species transmission change over the course of an epizootic. The objectives of this study are to:

1. Create an individual based SIR model that tracks the prevalence and genetic characteristics of a viral population within a reservoir host population throughout an epizootic.
2. Run model simulations with different conditions to represent different fitness scenarios that may occur and track the outcome.
3. Analyse the results of these simulations to infer under what combination of prevalence and genetic diversity are cross species transmission events likely to occur under different scenarios.

METHODS

Model/simulation development in C++

In order to investigate how pathogen prevalence and pathogen genetic diversity change over the course of an epizootic, we employ an individual-based SIR epidemiological model without demography, assuming the epizootic occurs in a time frame in which births and deaths of hosts are negligible. The model features susceptible (S), infected (I), and recovered (R) hosts, and new infections occur at a rate of $(\beta/T)SI$, ,

with β representing the combined effects of contact rate and the probability of pathogen transmission (Keeling and Rohani 2008). We assume complete cross-immunity and no coinfection, such that hosts become immune to all reinfections, regardless of strain type, after recovery. Recovery time is fixed at seven days for all hosts regardless of infecting strain. Our model incorporates mutation through a strain space with each mutation changing the genotype by one hamming distance (Bornberg-Bauer 1997), at rate 0.01 per allele per infection. Consequently, pathogen mutation within infected individuals can produce new strains, and rare strains may become extinct through stochastic processes. The parameters of the model included a finite population size of 100 individuals and a time scale of four years. The pathogens were characterized by different combinations of ones and zeros and had a genome length of 10 (giving rise to 1024 possible genotypes). In terms of transmissibility, more 'ones' in the genome (at any location) results in higher transmissibility. The simulation was always initiated with five infected individuals. The model was developed in C++ (Stroustrup 1995). We analyse this model by simulating the model to determine how pathogen prevalence and pathogen genetic diversity vary over the course of the epizootic.

Experimental Design

Broadly, we wanted to design an experiment that had scenarios that represented pre-adapted and not pre-adapted pathogens in the host population (starting conditions) and fitness landscapes where fit genotypes ranged from rare to common.

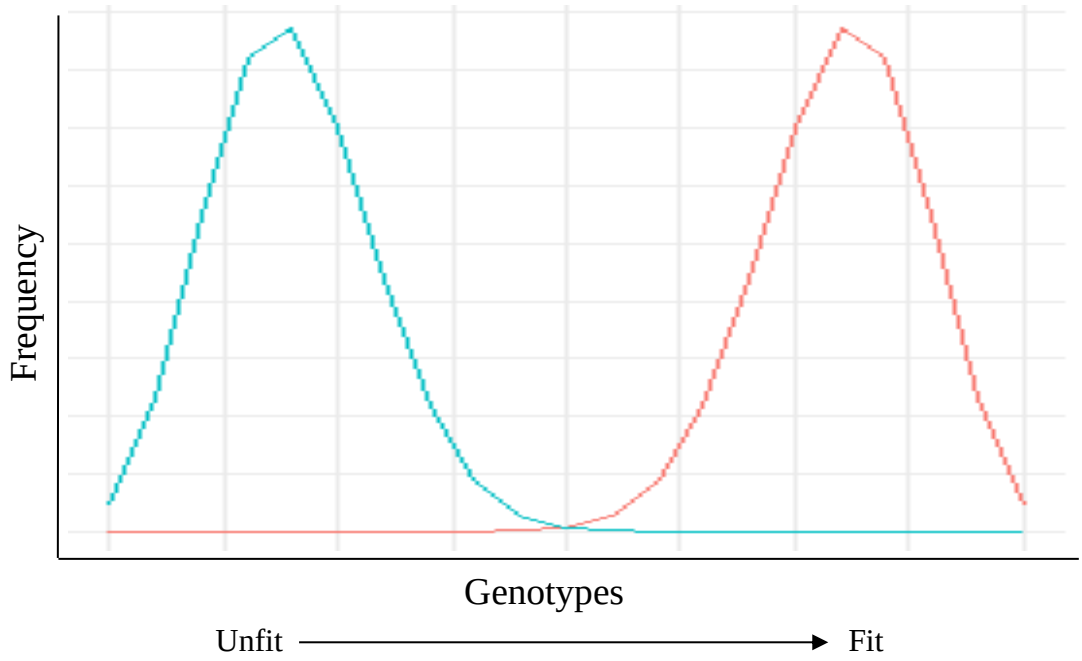

Figure 3.1: The two alternative distributions that the initial five viruses in a simulation are chosen from. *Distribution A contains predominantly unfit genotypes and represents a non-pre-adapted virus in the host population. Whereas distribution B contains predominantly fit genotypes and represents a pre-adapted virus population.*

The simulations were repeated under six different conditions representing all combination of two sets of starting conditions and three sets of fitness landscapes. For the initial conditions, the first 5 viruses to infect individuals from the population were selected from one of two distributions (Figure 3.1). They were either selected from a distribution of fit (transmissible) viruses which represent a preadapted virus in a population. Or they were selected from a distribution of less fit viruses which represent a novel virus in the host. The simulation was run using one of three fitness landscapes of decreasing challenge (Figure 3.2). In the most challenging landscape, fitness map 0, only a small proportion of potential genotypes will be fit under these conditions. As the landscape becomes less challenging a greater proportion of potential genotypes will be fit

and therefore more pathogen genotypes are likely to be successful in the host population. For each combination of initial conditions and fitness landscape the simulation was repeated 1000 times.

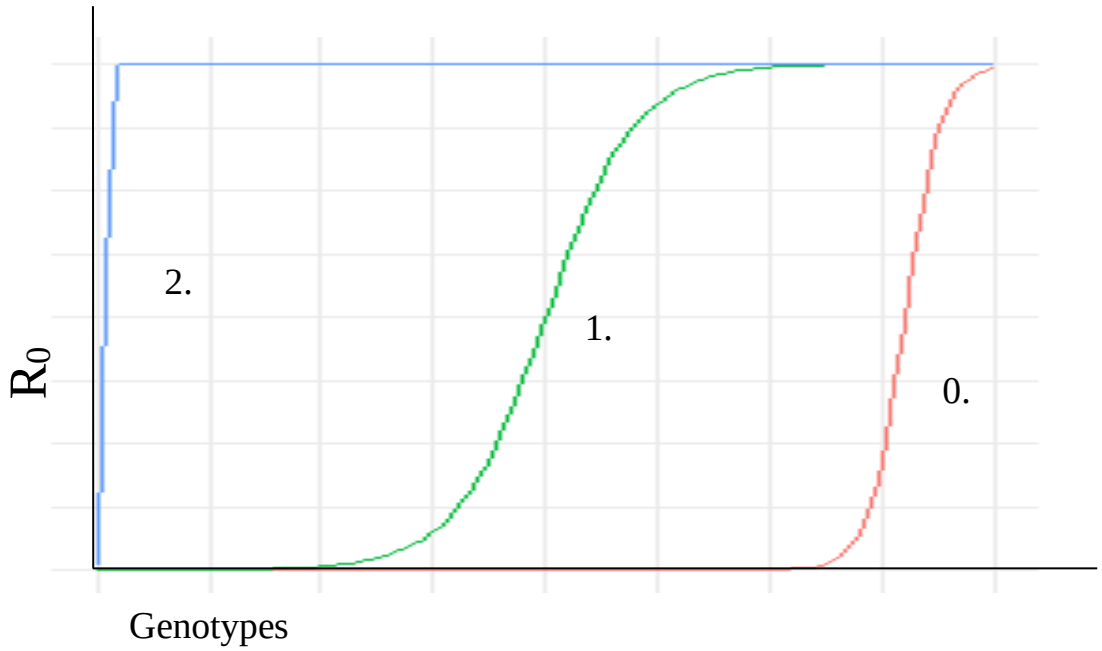

Figure 3.2: The three alternative fitness landscapes used in the epizootic simulation experiments. *Landscape 0 is the most challenging for the viral population, with only a small number of genotypes being fit. Landscapes 1 and 2 get progressively less challenging for the viral populations as more genotypes are fit.*

Analysis of Results in R

The outcome of the simulations were saved in CSV format and imported into R Studio (version 2022.12.0+353) (Posit team 2022). A detailed description of the model and data analysis is contained in the scripts within the project repository (https://github.com/JJWilson1991/Chapter2_model_2). All analyses and data visualization described below were conducted in the R programming environment (version 4.2.0.) (R Core Team 2022). References to packages in this methods section indicate specific packages used within the R programming environment.

Simulations were sampled and the dynamics were visualized in *ggplot2*. Summary statistics for pathogen prevalence, mean transmissibility, pathogen richness (number of genotypes) and pathogen evenness (relative abundance of each genotype) were calculated and visualized for each set of experimental conditions. Spearman's correlation coefficients were calculated for each simulation between prevalence and richness, prevalence and evenness, and evenness and richness using the `cor` function from the *stats* package (R Core Team 2022).

RESULTS

Prevalence

The mean prevalence, measured between 0 and 1 with 0 being not prevalent and 1 maximum prevalence, across the simulations varied under different sets of experimental conditions. When considering the different fitness landscapes, map 0 is the most challenging for the virus population as only a small proportion of potential genotypes will be fit in this scenario. This proportion increases as the fitness landscapes become progressively less challenging through maps 1 and 2 (Figure 3.2). There is less of a fitness challenge for the viral population in these scenarios as more genotypes will be fit in the host population and will survive and reproduce. In the unfit virus initial conditions there is a significant difference in the prevalence between all three fitness landscapes (Figure 3.3a). There is very low mean prevalence with low variation in the most challenging landscape (map 0) (these viruses are unsuccessful at establishing epizootics so there is no prevalence). There is an increase in mean prevalence and variation in the medium. landscape (map 1), but the median prevalence is still very low, indicating that

many of these simulations fail to produce successful epizootics. In the least challenging fitness landscape (map 2), the prevalence is very high with relatively low variance. When initial conditions represented fit starting viruses, all of the fitness landscapes displayed a high mean prevalence. There was, however, a significant difference in the mean prevalence between the most challenging fitness landscape (map 0) and the two less challenging (maps 1 and 2) landscapes (Figure 3.3b). There is little variation in the two least challenging fitness landscapes (fitness map 1 and 2), however there is a high degree of variation in the most challenging fitness landscape (map 0). There is no significant difference between the medium (map 1) and least challenging (map 2) fitness landscapes from fit initial viruses suggesting that prevalence plateaus, close to maximum prevalence of 1. In summary, high prevalence is expected in the less challenging fitness landscapes and when the starting viruses are fitter. Additionally, there is greater variance in prevalence under greater fitness challenge with fit starting viruses.

Transmissibility

We see a very similar pattern with mean transmissibility across the different initial starting conditions and fitness landscapes with transmissibility increasing as the fitness landscape becomes less challenging (from map 0 to map 2) and when the initial viruses are fitter (Figure 3.4). However, there is a significant difference between the mean transmissibility in the least challenging (map 2) fitness landscape and the medium (map 1) fitness landscape for fit starting viruses (Figure 3.4b) suggesting that transmissibility continues to develop in this less challenging landscape,

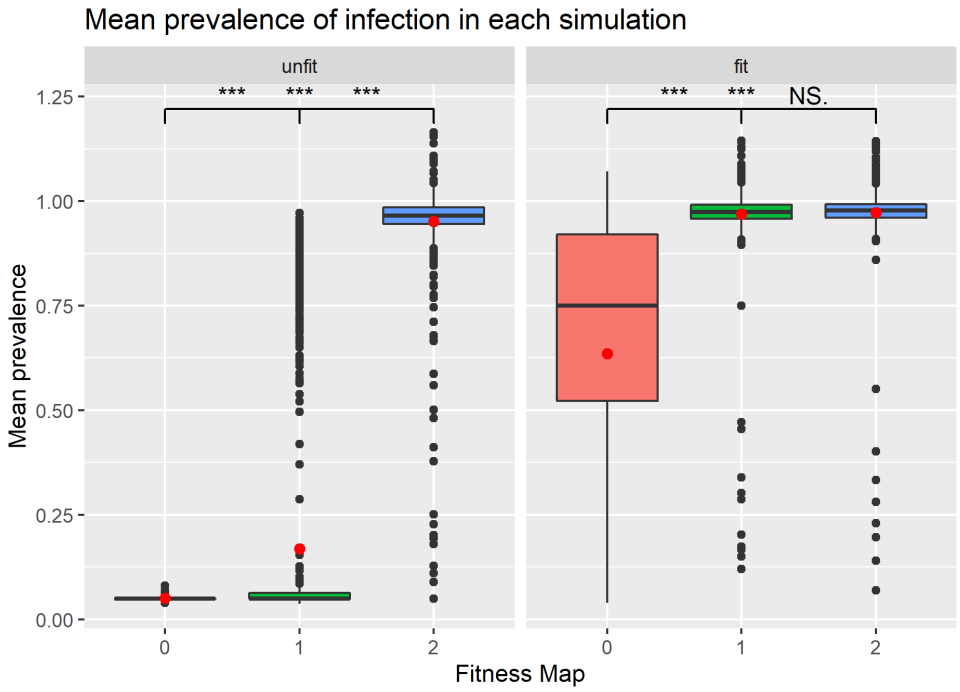

Figure 3.3: Boxplots of the mean prevalence of infection for each of the 1000 repetitions of the epizootic simulation for each set of starting virus and fitness landscape conditions. *The solid bold line represents the median value, and the red dot represents the mean.*

whereas prevalence has plateaued. There is also virtually no variation in transmissibility in this scenario. There only appears to be meaningful variation in transmissibility in the case of fit starting viruses in fitness map 0, the most challenging fitness conditions.

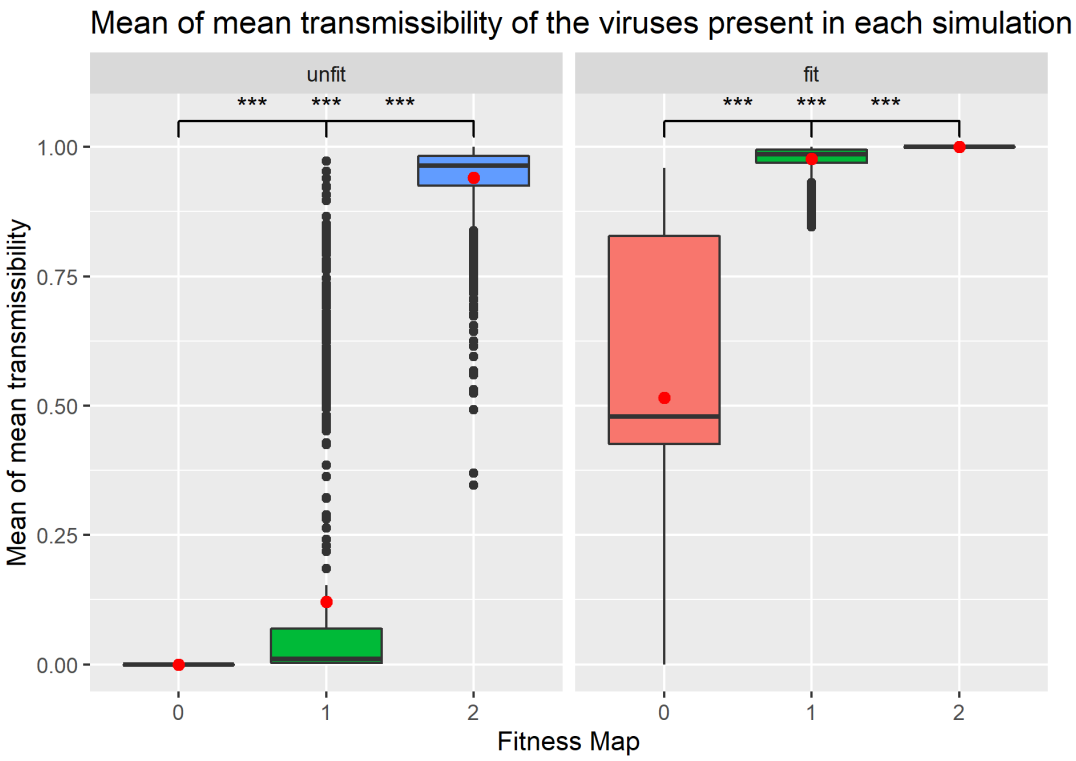

Figure 3.4: Boxplots of the mean of the mean strain transmissibility for each of the 1000 repetitions of the epizootic simulation for each set of starting virus and fitness landscape conditions. *The solid bold line represents the median value, and the red dot represents the mean.*

Richness

Similar to prevalence, mean viral richness increases as the fitness landscape becomes less challenging and viral fitness in the starting conditions increases (Figure 3.5). Again, this effect plateaus with the fit initial viruses in the two less challenging fitness landscapes (maps 1 and 2) (Figure 3.5b).

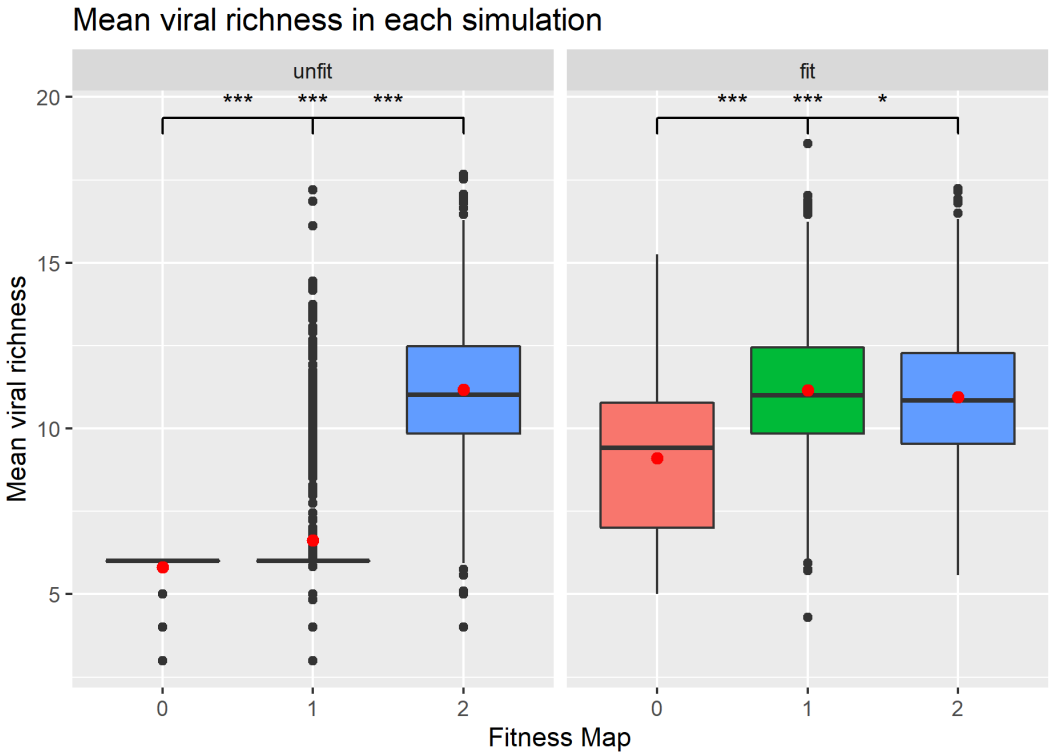

Figure 3.5: Boxplots of the mean viral strain richness for each of the 1000 repetitions of the epizootic simulation for each set of starting virus and fitness landscape conditions. *The solid bold line represents the median value, and the red dot represents the mean.*

Evenness

Pathogen strain evenness was measured using Pielou's J (Pielou 1966). With evenness we see a different pattern emerge; evenness declines as the fitness landscape gets less challenging in the unfit virus starting conditions (Figure 3.6a), but evenness increases as the fitness landscape gets less challenging with our fit virus initial conditions (Figure 3.6b). With fit initial conditions, we start off with less evenness because we have some strains which are initially fit and dominate the population. As the fitness landscape becomes less challenging the evenness becomes greater as strains are equally successful.

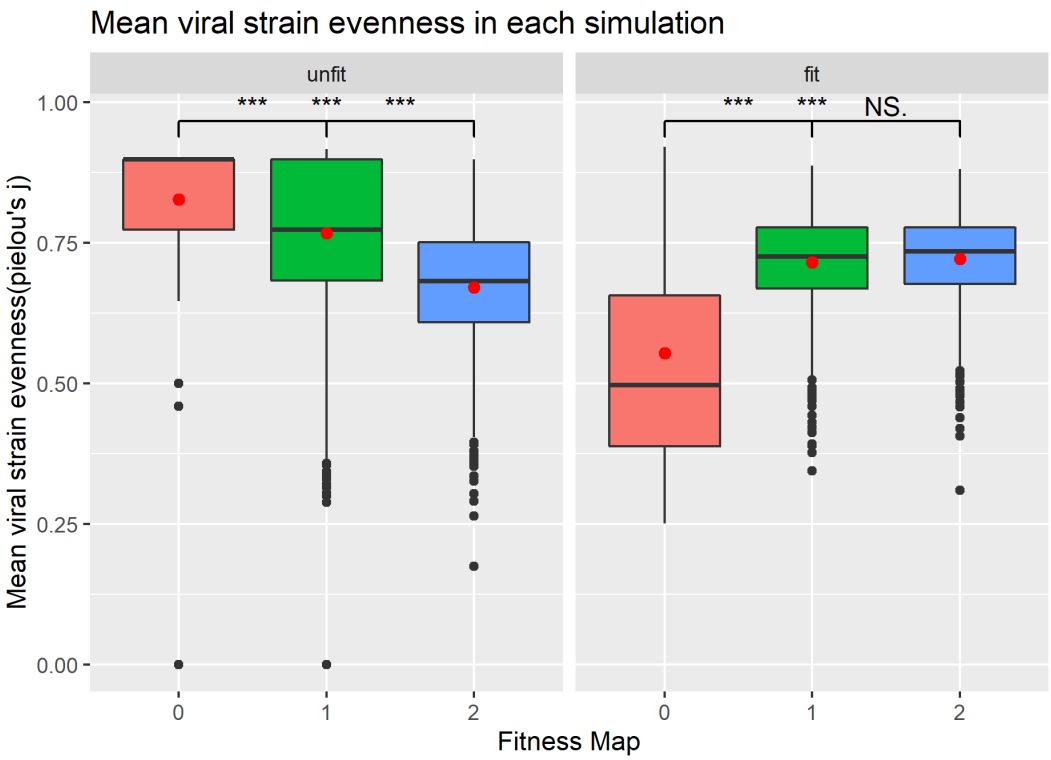

Figure 3.6: Boxplots of the mean viral strain evenness (Pielou's J) for each of the 1000 repetitions of the epizootic simulation for each set of starting virus and fitness landscape conditions. *The solid bold line represents the median value, and the red dot represents the mean.*

Spearman's Correlation Coefficients

For all the correlation plots, there is no plot for the unfit starting viruses in the most challenging fitness landscape as they fail to survive. For the other scenarios there is a negative correlation coefficient between evenness and richness (Figure 3.7). The correlation is significantly more negative in the least challenging fitness landscape (map 2) for the unfit starting viruses (Figure 3.7a). There is no significant difference between any of the fitness landscapes with the fit starting viruses.

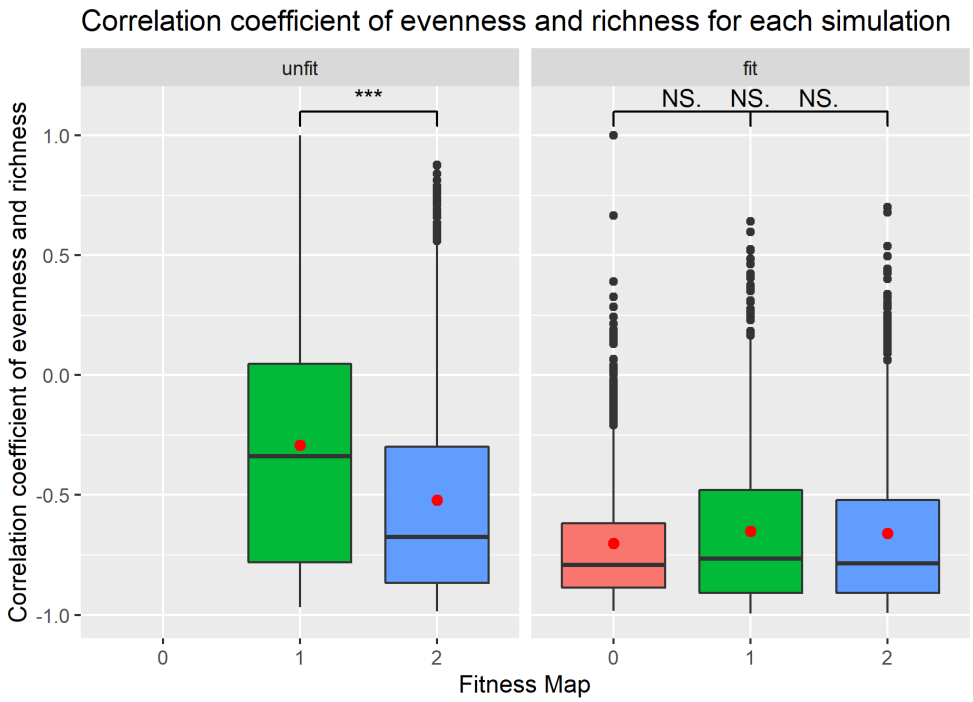

Figure 3.7: Boxplot of the Pearson's correlation coefficients between mean richness and mean evenness for each of the 1000 repetitions of the epizootic simulation for each set of starting virus and fitness landscape conditions. *The solid bold line represents the median value, and the red dot represents the mean.*

There is a positive correlation coefficient between prevalence and richness for each scenario (Figure 3.8). This effect is stronger in the less challenging fitness landscapes, i.e., map 2 for the unfit starting conditions and maps 1 and 2 for the fit starting conditions. There is a negative correlation coefficient between prevalence and evenness for each scenario (Figure 3.9). In the unfit initial conditions there is a much less strong negative correlation between prevalence and unevenness for the medium fitness landscape (map 1), additionally there is much greater variation in this scenario (Figure 3.9a). In the fit starting conditions, the negative correlation between prevalence and evenness is less strong in the most challenging fitness landscape (map 0) (Figure 3.9b).

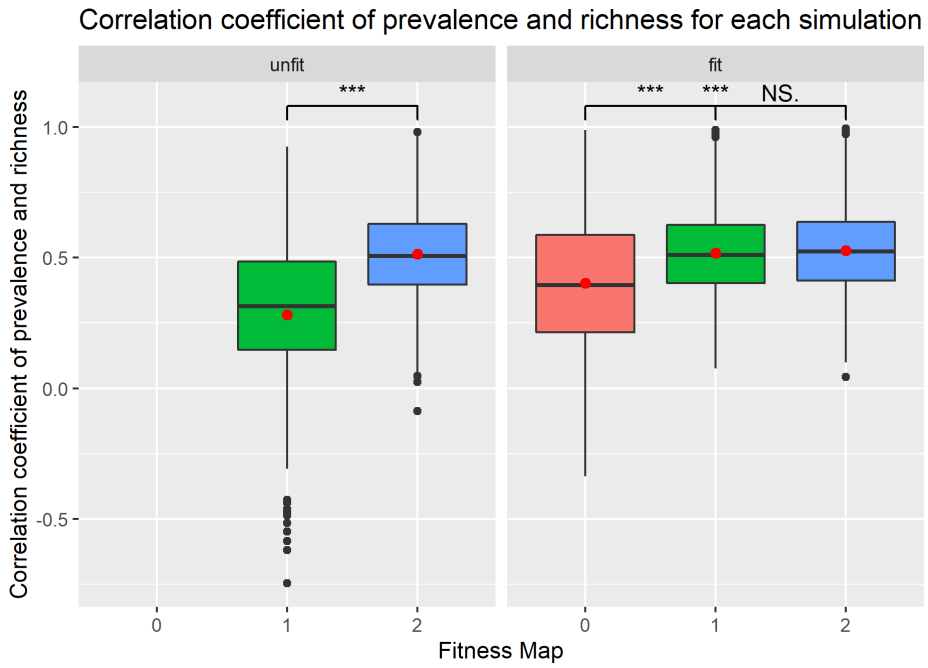

Figure 3.8: Boxplot of the Spearman's correlation coefficients between mean prevalence and mean richness for each of the 1000 repetitions of the epizootic simulation for each set of starting virus and fitness landscape conditions. *The solid bold line represents the median value, and the red dot represents the mean.*

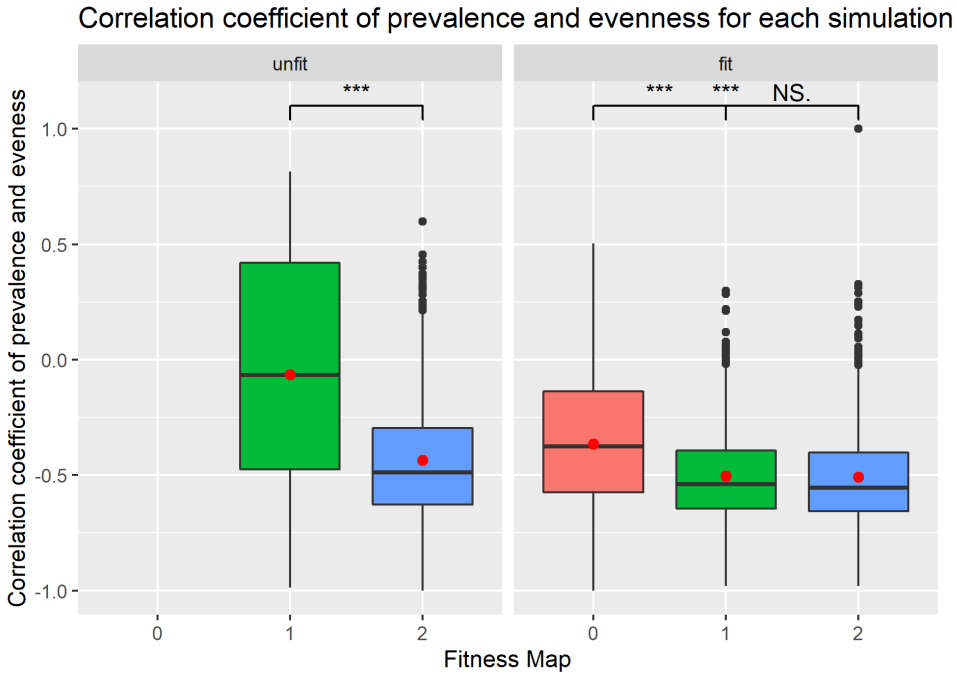

Figure 3.9: Boxplot of the Spearman's correlation coefficients between mean prevalence and mean evenness for each of the 1000 repetitions of the epizootic simulation for each set of starting virus and fitness landscape conditions. *The solid bold line represents the median value, and the red dot represents the mean.*

Epizootic Dynamics

Plotting the dynamics of the epizootics and transmissibility for each scenario reveals different outcomes under each combination of starting conditions and fitness landscape. With unfit starting viruses in the most challenging fitness landscape (map 0), there viruses fail to produce productive epizootics (Figure 3.10a). With the unfit starting viruses in the medium challenge landscape (map 1) we have much variation in terms of the dynamics of the epizootic simulations (Figure 3.10b). Lastly, for the unfit starting viruses in the least challenging landscape (map 2) we have a much narrower range of epizootic types, with them generally being rapid, explosive epizootics with maximal transmissibility (Figure 3.10c). The simulations for fit starting viruses in the most challenging fitness landscape (map 0) reveals the existence of different subgroups, with there being a group with rapid epizootics and high transmissibility and a second group with slower epizootics and moderate transmissibility (Figure 3.10d). Finally, with our fit starting viruses in the two less challenging fitness landscapes (maps 1 and 2) we see very little variation, with all of the epizootics being explosive and short lived with maximal transmissibility (Figures 3.10e and 3.10f).

Histograms of mean transmissibility (Figure 3.11) show a single mode in every scenario except in the case of fit starting viruses in the most challenging fitness landscape. There is a trimodal distribution in this scenario with a non-transmissible group, a moderately transmissible group, and a high transmissibility group (Figure 3.11d).

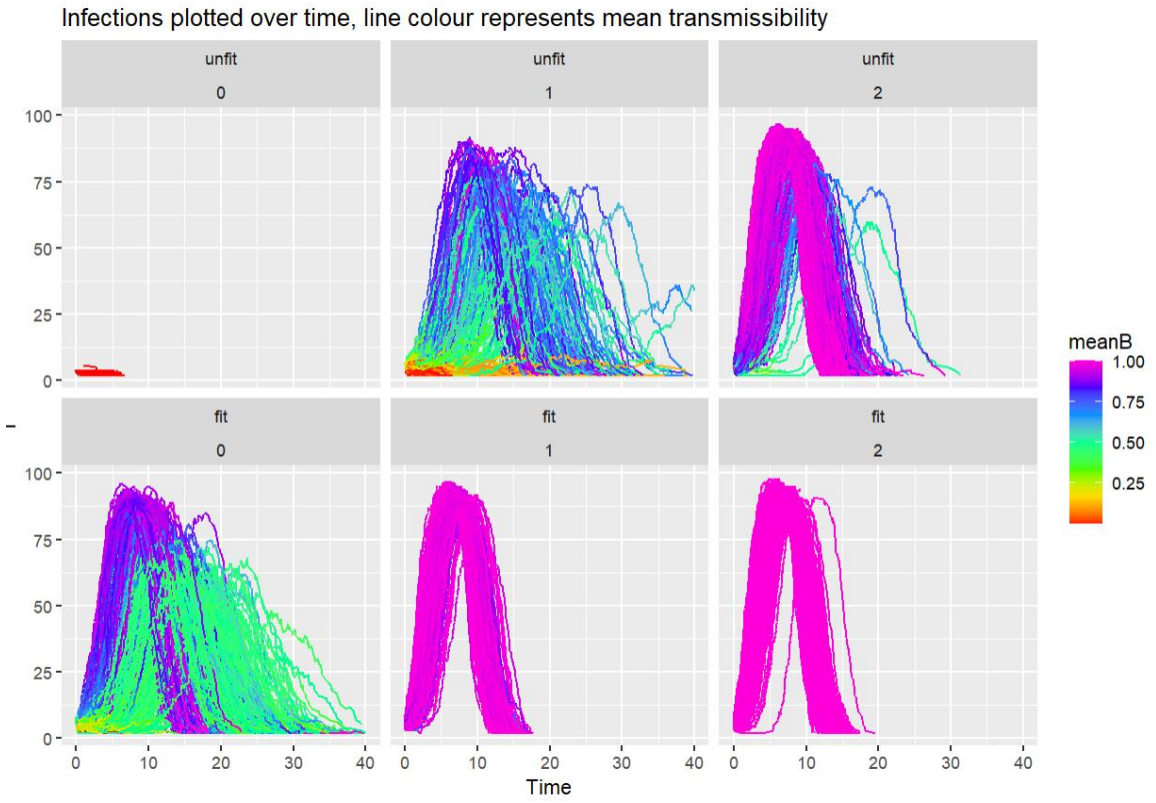

Figure 3.10: Epizootic dynamics for each simulation, plotted as number of infection individuals over time, for each pair of initial starting conditions and fitness landscapes. *The color of the lines represents the mean transmissibility of the viruses in the simulation.*

Splitting the simulations into a high transmissibility group and moderately transmissible group for comparison of their population metrics shows differences between the two groups (Figure 3.12). Comparing the dynamics of the epizootic, the high transmissibility group are very uniform and have explosive and short-lived epizootics whereas the moderately transmissible group has more variation and a slower burning epizootic. When the epizootic curves are coloured to show changes in mean viral transmissibility over time, there is a stark contrast between the two groups, with the high transmissibility group rapidly evolving to high transmissibility and maintaining this for

the duration of the epizootic. The moderately transmissible groups maintain a moderately transmissible state for the duration of their epizootic. Differences are also apparent

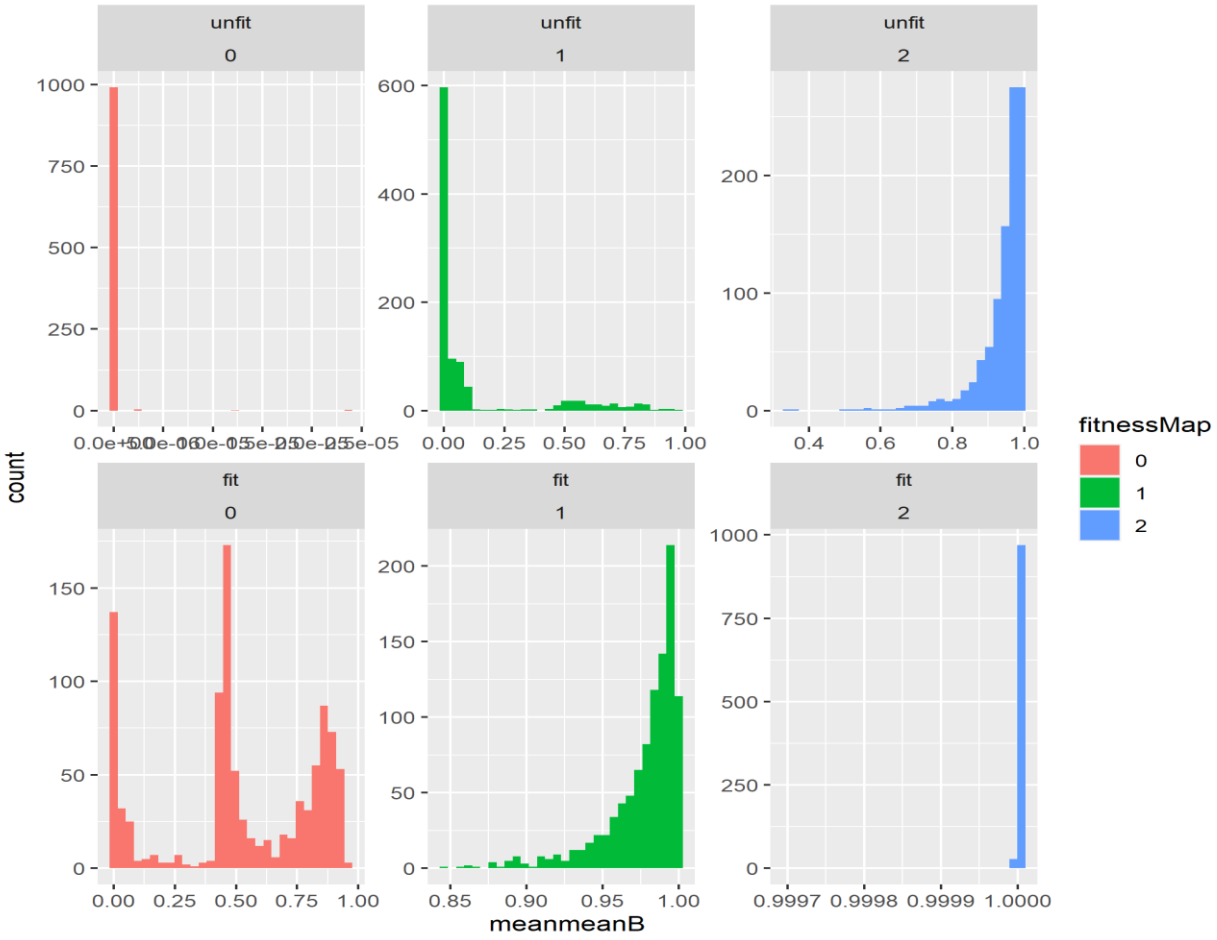

Figure 3.11: Histograms of the mean transmissibility for each of 1000 epizootics under each set of starting conditions and fitness landscapes. *The X axis corresponds to the mean of the mean transmissibility for all of the virus strains within an epizootic simulation, with 1.00 being maximally transmissible and 0.00 being not transmissible.*

between the two groups when comparing richness and evenness. The high transmissibility group develops richness earlier with peak richness corresponding with peak prevalence whereas the moderately transmissible group develops richness later and continues to develop greater richness even beyond the peak of the epizootic. With

evenness, in the high transmissibility group evenness is rapidly lost early in the epizootic whereas evenness is maintained and lost at a slower rate in the moderately transmissible group.

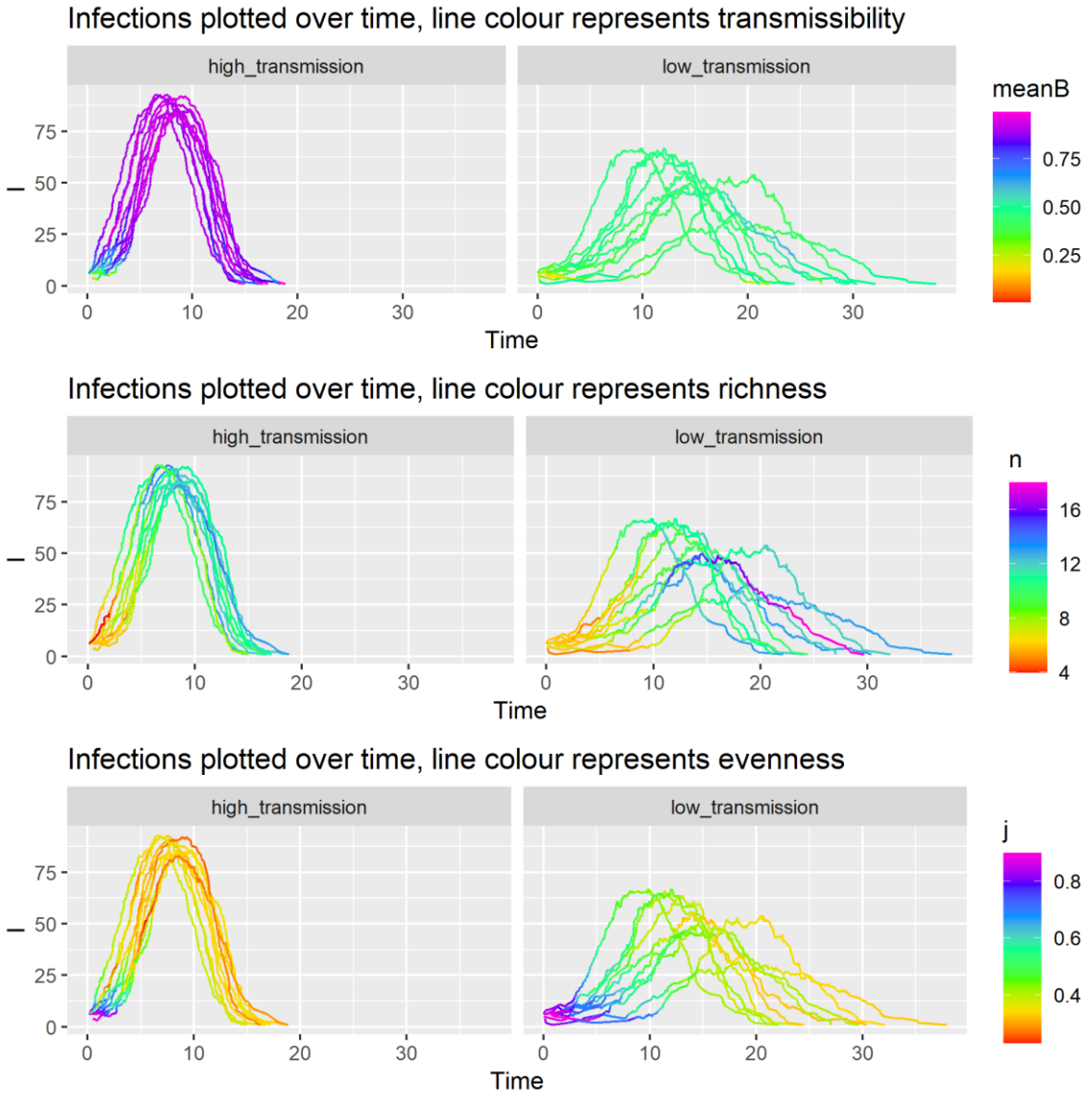

Figure 3.12: Epizootic dynamics for each simulation, plotted as number of infected individuals over time, for high transmissibility and moderate transmissibility groups. *The color of the lines represents: A-the mean transmissibility of the viruses in the simulation; B- the viral strain richness in the simulation; C- the viral strain evenness in the simulation.*

DISCUSSION

In this study we have created an individual based SIR model to track the prevalence and genetic characteristics of a viral population within a reservoir host population throughout an epizootic. We then simulated the epizootics under different conditions to represent different fitness scenarios. Analysis of the results showed that optimum transmissibility evolves in the viral population early in the epizootic with peak genetic diversity following later, after the peak of the outbreak. This has important repercussions for spillover risk, with spillover between closely related species being related to transmissibility and thus the greatest risk being through the upslope and peak of the epizootic when the pathogen population has reached maximum transmissibility. However, the later development of genetic richness would point to spillover risk between distantly related host species being greater later in the epizootic, after the peak has passed. Additionally, we show the existence of a bistable evolutionary strategy under certain conditions whereby either high transmissibility or moderate transmissibility may be the life history path of a virus population.

General trends

There are some general trends relating to prevalence, richness, and evenness. These trends varied depending on the fitness challenge presented by the fitness landscape. In a challenging fitness landscape, such as that represented by a healthy host with a fully functioning immune system, a relatively small proportion of potential virus genotypes will be fit under these conditions, with only the best adapted being able to thrive. Under less challenging fitness landscapes, more genotypes are fit under these conditions, resulting in a greater diversity of viruses being able to replicate in the host

population. Richness was greater in the less challenging fitness landscapes, suggesting that richness maybe a function of prevalence. This is due to increased prevalence resulting in more reproduction, resulting in more mutations and more strains being created hence there is greater richness with increased prevalence. As richness increased there was a subsequent reduction in evenness. This is likely because as richness increases there is an increase in the number of both fitter and less fit strains; these are more or less successful in the population with the least fit strains being less prevalent and the fit strains being more prevalent. This creates an unevenness in the distribution of strains in the population. This relationship is stronger in the less challenging fitness landscape. This is likely due to less fit strains being more likely to survive in the less challenging fitness landscape resulting in even greater richness and unevenness.

In all fitness/initial condition scenarios that result in productive epizootics, a similar pattern emerges; transmissibility increases rapidly in the early stages of the epizootic and remains high. Meanwhile, genetic diversity, in the form of viral strain richness, develops later, but remains high even as the epizootic wanes. This early development of increased transmissibility appears to be vital in the explosive growth of the epizootic with spread amongst the same host species. Referring to our initial question of spillover risk and the two major scenarios of between closely related and distantly related hosts, it seems plausible that this high early transmissibility with lower diversity should pose a greater risk in the scenario of two closely related species. This initial increase in prevalence has a secondary effect in that it also results in an increase in diversity purely because of there being a larger number of viruses present in the system. This is also demonstrated by the strong positive correlation between prevalence and

richness. However, diversity does not peak with prevalence and continues to develop throughout the epizootic. Other experimental approaches have shown that genetic diversity increased most significantly once fitness became asymptotic (Papadopoulos et al. 1999). Working under the assumption that pathogen diversity is the greatest risk for transmission between two more distantly related species (Dennehy et al. 2010), it would seem that the later development of diversity through the epizootic would put the risk for this scenario higher later in the epidemic. However, in both of these scenarios, it is likely that high prevalence is an important factor in terms of a novel host actually being exposed to the virus, in which case highest risk in either scenario is likely to be around the peak of the epizootic (Amman 2012, Plowright et al. 2015).

Understanding the risks of optimal genetic richness in a pathogen population is important when considering the timing of some spillover events. Spillover events in some species such as bats and rodents (Amman 2012, Akhmetzhanov et al. 2019) have often been described as seasonal and as a result of population booms at certain times of year leading to spillover as a result of increased contact between species and increased prevalence within these naïve individuals. In fact, some of this apparent increased spillover risk may be due to the optimal genetic richness in the pathogen population occurring at this time and not exclusively be due to increased host population and pathogen prevalence.

We can consider that these different combinations of initial starting conditions and fitness landscape scenarios are analogous to some real-world situations. As mentioned previously, the fit starting viruses are analogous to pre-adapted viruses, which already exist in a reservoir host species versus the unfit starting viruses, which are more

akin to a novel virus in a species where the virus is not pre-adapted. An unchallenging fitness landscape is akin to an immunocompromised host population or a genetically ‘homogenous’ group of organisms, such as a flock of commercial chickens (Zekarias et al. 2002, Gul et al. 2022), whereby a virus such as avian influenza virus will have a very rapid and catastrophic outbreak, with very high transmissibility. The greatest richness appears to develop in the least challenging fitness scenarios. This is a similar concept to antibiotic resistance emerging in the more favourable fitness landscape of an immunocompromised host (Margolis and Rosch 2018). The more challenging fitness landscapes are analogous to a fully immune healthy host population. In this scenario, with our unfit starting viruses in the most challenging landscape, we do not see productive outbreaks. This is reflected in reality where most times when a novel virus comes in contact with a host there is no productive outbreak as the fitness challenge is too great (Parrish et al. 2008). As the challenge becomes lesser, we have a range of different outbreaks in the medium landscape with our unfit, not pre-adapted strains. This is supported by the literature whereby a risk factor for spillover events is an immunocompromised host such as you may see in a commercial farm setting with hosts such as chickens and pigs (van der Most et al. 2011). Returning to the scenario of fit starting viruses in the most challenging landscape, selection pressure is driving evolution in the virus population whereby there is selection for a bistable state in which there can be survival as a highly transmissible virus or as a moderately transmissible virus.

Life history strategy

With the fit initial viruses in the most challenging fitness landscape, we have two scenarios that emerge: a rapid explosive epizootic with high transmissibility or a slower

developing, slower burning epizootic with a more moderate transmissibility. The branch point as to whether a viral population follows one track or the other appears to be very early on in the outbreak. Both of these different life history strategies can be valid in different scenarios. This may be an example of a bistable evolutionary strategy (Dercole et al. 2002, Martins and Pinto 2017, Walworth et al. 2020). In the scenario of a large population of closely-related, susceptible individuals, the explosive epizootic is a valid life history strategy as it allows the virus to spread rapidly through an in-contact population and this is a strategy which is used by a number of viruses, the classic example of this being morbilliviruses (Bolker and Grenfell 1995, Taylor 2005). However, a caveat to this strategy being that a very large or essentially infinite population is needed, otherwise the virus will face extinction in that population. This is seen in the case of measles whereby a population of at least 250,000 to 400,000 is needed to maintain virus transmission (Keeling and Grenfell 1997, Pearce-Duvet 2006). In this simulation, which has a finite population, this strategy entails the virus population reaching extinction quickly. The alternative life history of being a slower burning epizootic with lower transmissibility is more applicable to a more finite population size as it does not burn through its pool of susceptibles as quickly and allows for demographic factors to replace susceptibles. It has also been suggested that a slow and steady epizootic is preferable in finite populations (Parsons et al. 2018) . Additionally, and importantly to our questions, the simulations suggest that this strategy drives higher genetic diversity in the population of viruses. This raises the question of whether this could be considered a more generalist life history strategy with greater genetic diversity within the viral population being more likely to allow it to infect alternative host species (Borucki et al.

2013). With this in mind, these two life history strategies can be considered either as a specialist strategy or a more generalist strategy. The specialist strategy requiring a large population of susceptibles for survival as it is more reliant on one host species, whereas the generalist will be outcompeted within that species by a specialist strain, its ability to persist for longer allows the development of genetic diversity which could allow for it to adapt and spillover into new species. Multihost experimental evolution studies on plant RNA viruses could not verify the existence of a cost for generalists, as expected to arise from antagonistic pleiotropy and other genetic mechanisms generating a fitness trade-off between hosts (Bedhomme et al. 2011). The fact that this scenario only arises when fit starting viruses are in the most challenging fitness landscape suggests that there is a requirement of quite strong selective pressure so that the viral population does not take the high transmissibility route as it does in every other scenario where epizootics occur.

Optimization of virus transmissibility can result in a trade-off with diversity (Papadopoulos et al. 1999, Wahl and Krakauer 2000, Frank and Bush 2007). Viruses with high R_0 are usually very stable, such as measles, whilst a low R_0 is usually found amongst rapidly evolving, diverse viruses, like influenza (Rodpothong and Auewarakul 2012). Viruses tend to undergo the strategy of either replicating at the maximum rate for a short period, or to extend their period of replication but at lower levels. To survive for an extended period a virus needs to be able to escape immune responses, and escape mutations lead to antigenic diversity. Viruses that have a high R_0 tend to be antigenically conserved, suggesting they are subject to strong negative selective pressure. This pressure likely arises from the need to maintain an optimal structure for maximal transmission and replication fitness. Since protective epitopes frequently overlap with virus receptor-

binding domains, escape mutations can impact binding affinity to viral receptors and hinder viral fitness (Watabe and Kishino 2010).

CONCLUSION

The conclusions of this study are that maximal transmissibility and genetic diversity develop at different stages of an epizootic and do not synchronise with peak prevalence of infection. This is significant in terms of when the risk of a cross species transmission event would be highest, with high transmissibility in the early stages of the epizootic being a risk factor between closely related species and high genetic diversity in the later stages being a risk factor in less closely related species. Additionally, we show that under certain conditions, viral populations appear to exhibit a bistable strategy whereby they evolve into a moderately transmissible population with a slower epizootic or become maximally transmissible with a very rapid epizootic.

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CHAPTER 4

TEMPORAL AND SPATIAL PATTERNS IN CANINE DISTEMPER VIRUS CASES IN WILDLIFE DIAGNOSED AT THE SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY, 1975–2019[1]

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Abstract

Canine distemper is a high impact disease of many mammal species and has caused substantial carnivore population declines. Analysis was conducted on passive surveillance data of canine distemper (CDV) positive wild mammal cases submitted to the Southeastern Cooperative Wildlife Disease Study, Athens, Georgia, US, between January 1975 and December 2019. Overall, 964 cases from 17 states were CDV positive, including 646 raccoons (*Procyon lotor*, 254 gray foxes (*Urocyon cinereoargenteus*, 33 striped skunks (*Mephitis mephitis*), 18 coyotes (*Canis latrans*), four red foxes (*Vulpes vulpes*), three gray wolves (*Canis lupus*), three American black bears (*Ursus americanus*), two American mink (*Mustela vison*), and one long-tailed weasel (*Mustela frenata*). Raccoon and gray fox case data from the state of Georgia (*n*=441) were selected for further analysis. Auto-regressive integrated moving average models were developed predicting raccoon and gray fox case numbers. The best performing model for gray foxes used numbers of gray fox CDV cases from the previous 2 mo and of raccoon cases in the present month to predict the numbers of gray fox cases in the present month. The best performing model for raccoon prediction used numbers of raccoon CDV cases from the previous month and of gray fox cases in the present month and previous 2 mo to predict numbers of raccoon cases in the present month. Temporal trends existed in CDV cases for both species, with cases more likely to occur during the breeding season. Spatial clustering of cases was more likely to occur in areas of medium to high human population density; fewer cases occurred in both the most densely populated and sparsely populated areas. This pattern was most prominent for raccoons, which may correspond to high transmission rates in suburban areas, where raccoon population densities are

probably highest, possibly due to a combination of suitable habitat and supplemental resources.

INTRODUCTION

Canine distemper affects a wide range of wild and domestic mammals, principally carnivores (Deem et al. 2000, Martinez-Gutierrez and Ruiz-Saenz 2016). It has the second highest case fatality rate among canine diseases, after rabies (Swango 1995). The causative agent, canine morbillivirus (or canine distemper virus; CDV) is an enveloped, single-stranded, negative-sense RNA virus in the *Morbillivirus* family. The major route of transmission is through aerosolization of virus from respiratory secretions (Deem et al. 2000). Canine morbillivirus is highly infectious and may be shed for 60-90 d post infection (Greene and Appel 1990, Loots et al. 2018).

Canine morbillivirus results in a highly immunizing, acute infection that typically requires high densities and large populations of hosts for long-term persistence (Williams 2001). The virus is maintained among wild carnivores by multi-host transmission, which overcomes obstacles of population size and host density within a single host species (Almberg et al. 2010). There is evidence of CDV infection in all terrestrial carnivore families and some marine carnivore families (Deem et al. 2000). The Mustelidae family includes some of the species with the highest fatality rate, while the domestic dog (*Canis lupus familiaris*) can be a subclinical carrier (Deem et al. 2000). Canine distemper has been responsible for substantial population declines in the African lion (*Panthera leo*) (Roelke-Parker et al. 1996), Amur tigers (*Panthera tigris altaica*) (Seimon et al. 2013), and the endangered black-footed ferret (*Mustela nigripes*) in the US (Williams et al. 1988).

In North America, raccoons (*Procyon lotor*), red and gray foxes (*Vulpes vulpes* and *Urocyon cinereoargenteus*), coyotes (*Canis latrans*), wolves (*Canis lupus*), striped

skunks (*Mephitis mephitis*), American badgers (*Taxidea taxus*), American mink (*Mustela vison*) and ferrets (*Mustela* spp.) are among the wild species susceptible to CDV infection (Beineke et al. 2015). Distemper is endemic in raccoon populations in the eastern US, and raccoons are thought to be a reservoir for other wild animals and domestic dogs (Roscoe 1993). Canine morbillivirus is also a major cause of disease among gray foxes in the southeastern USA; in one study, 78% of animals that underwent postmortem diagnostic evaluation from 1972 to 1989 was diagnosed with the disease (Davidson et al. 1992). It can persist in areas with diverse carnivore populations, including Yellowstone National Park, where multiple outbreaks have occurred in wolf, coyote, and cougar (*Puma concolor*) populations within the park (Almberg et al. 2009, Almberg et al. 2010).

While there is a large body of work identifying distemper outbreaks in wild carnivores in the US (Roscoe 1993, Deem et al. 2000, Martinez-Gutierrez and Ruiz-Saenz 2016), there has been limited work on the spatio-temporal dynamics of CDV, particularly in raccoon populations. Furthermore, most studies have been short term and often have focused on systems such as the Yellowstone National Park system, which differs from the southeastern US in a number of ways, including the absence of major urban centers (Almberg et al. 2009, Almberg et al. 2010). It is well established that urbanization can play a role in wildlife disease dynamics, particularly with multi-host pathogens such as CDV (Bradley and Altizer 2007). Outside the US, time series data from the wildlife-domestic animal interface has demonstrated that infection dynamics in one population can significantly impact the other, resulting in spatially-structured incidence (Viana et al. 2015). As host species vary considerably in their clinical signs (Deem et al. 2000), determining the extent to which cross species transmission drives

epizootics within wildlife species could improve estimated risk of infection in subclinical hosts and facilitate integrated management strategies that include consequences of cross species transmission (Haydon et al. 2002).

The primary objective of our study was to identify long-term spatial and temporal patterns in CDV cases submitted for diagnostic evaluation to the Southeastern Cooperative Wildlife Disease Study (SCWDS) at the University of Georgia. We also analyzed how outbreaks in two different species (raccoons and gray foxes) may be related, because raccoons are considered to be the primary wildlife reservoir of CDV, and epizootics in this species may be followed by epizootics in others. Finally, we identified spatial patterns of infection within the southeastern US, including potential associations with human activity.

METHODS

Data acquisition and overview

We acquired data from wild mammals submitted to SCWDS between 1975 and 2019 that were diagnosed with CDV infection on postmortem examination. 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. Cases were submitted from many southeastern states; however, the greatest proportion of

confirmed cases came from gray foxes and raccoons in Georgia (46%); all other reporting states had confirmed case numbers below 100 for the entire 44-year period and thus did not provide sufficient cases for the time course analysis as they are temporally sparse. Additionally, the states with the next highest cases (Louisiana, Kansas and North Carolina) are not geographically contiguous with the state of Georgia, making spatial relationships unlikely. Therefore, analyses focused on confirmed cases in the state of Georgia.

Human population and land area data for Georgia counties from 1975 to 2019, were accessed and downloaded from census.gov. Data were imported into R Studio (version 1.3.1056). A detailed description of data analysis is contained in the scripts within a project repository (Wilson 2020). All analyses were conducted in the R programming environment (version 3.5.3.; (R Core Team 2020)). References to packages in this methods section indicate specific packages used within the R environment to perform analyses.

Spatial analysis

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 and the presence of CDV infection in raccoons and gray foxes was mapped at the county level in Georgia. Presence was defined as at least one CDV diagnosis in raccoon or gray fox in a particular county in Georgia in a particular year. Analysis of spatial clustering of cases in Georgia was performed using Ripley's K from the *spatstat* package (Baddeley et al. 2015). These

analyses identified if, and at what spatial scale, spatial point data were more clustered or dispersed compared to a random distribution.

Temporal analysis

Cases were also analyzed in relation to the raccoon and gray fox reproduction cycles. The breeding season was defined as the period from January to March, and the period of lactation was defined as April to June. The remainder of the year was designated as the non-breeding season (Zeveloff and Dewitte 2002). A chi-square test (McHugh 2013) was performed on these data to determine if cases occurred disproportionately in certain phases of the reproduction cycle.

Time-series analysis and auto-regressive integrated moving average (ARIMA) model construction was conducted using the "fpp2" package (Hyndman and Athanasopoulos 2018). The three components of an ARIMA model are auto-regression, differencing, and the moving average. Autoregression uses previous data (i.e., cases) in the time series from present month, *t*, and previous months, *t-n*, for *n* number of previous time points, up to n=12 months, to predict future data; differencing computes the difference between observations in non-stationary data to remove the influence of trends or seasonality; and the moving average uses the past forecast errors (ε_{t-n}) in the model to make future predictions. In addition to these basic components of the ARIMA model, lagged case data of the other species were included in models. In the gray fox predictive model, raccoon cases, y_{t-n}, were used along with past gray fox cases, x_{t-n}, to predict the present months gray for cases, x_t, with the opposite being used in the raccoon model to predict present raccoon cast, y_t.

For time series analysis, the data were pooled into raccoon or gray fox distemper cases in Georgia for each month from April 1975 to December 2019. For training and testing of the ARIMA models, the sequence of months from April 1975 to December 2019, *n*=524 time points, was divided approximately 80:20 into the training set (up to *n*=423; i.e., July 2011) and the testing set (*n*=423 to *n*=524; i.e., August 2011-December 2019). The training set was used for building the model and the testing set, the latter of which was withheld during model building, is used for testing the accuracy of the model's predictions compared to the real data. The stationarity of the time series was confirmed using both the augmented Dickey–Fuller test (Dickey and Fuller 1979) and the Kwiatkowski–Phillips–Schmidt–Shin test (Kwiatkowski et al. 1992). The best fit models for each species were evaluated using the Akaike Information Criterion (AIC) (Akaike 1974). The best model from each species was tested using the later part of the data set, and the root mean squared error (RMSE) was used to evaluate model performance. Predictors were considered significant if the coefficient was more than two standard errors from zero.

RESULTS

A total of 964 cases were diagnosed with canine distemper among nine host species; these cases were submitted from 17 states over the 45-year period (Table 4.1). A mean of 21.42 cases were diagnosed with distemper per year with a standard deviation (SD) of 16.96. The highest annual number of cases was reported in 1988(Fig. 4.1).

Table 4.1. Summary table of the species and state of wildlife cases diagnosed with canine distemper submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019, Athens, Georgia, USA.

	Raccoon	Gray Fox	Striped Skunk	Coyote	Red Fox	Black Bear	Gray Wolf	Mink	Long-tailed Weasel	Sum
GA	302	139	6	5	2	0	3[a]	0	0	457
LA	59	21	1	2	0	0	0	0	0	83
KY	50	4	10	2	1	0	0	1	0	68
NC	29	32	0	0	1	1	0	0	1	64
KS	38	2	2	7	0	0	0	0	0	49
TN	46	2	1	0	0	0	0	0	0	49
VA	29	16	4	0	0	0	0	0	0	49
SC	30	10	0	1	0	0	0	0	0	41
WV	20	10	0	0	0	0	0	0	0	30
FL	19	9	0	0	0	0	0	1	0	29
PA	8	3	7	0	0	2	0	0	0	20
MO	7	1	2	0	0	0	0	0	0	10
AR	2	2	0	0	0	0	0	0	0	4
MS	3	1	0	0	0	0	0	0	0	4
AL	3	0	0	0	0	0	0	0	0	3
MD	1	2	0	0	0	0	0	0	0	3
NE	0	0	0	1	0	0	0	0	0	1
Sum	646	254	33	18	4	3	3	2	1	964

State Key: GA- Georgia, LA- Louisiana, KY- Kentucky, NC- North Carolina, KS- Kansas, TN- Tennessee, VA- Virginia, SC- South Carolina, WV- West Virginia, FL- Florida, PA- Pennsylvania, MO- Missouri, AR- Arkansas, MS- Mississippi, AL- Alabama, MD- Maryland, NE- Nebraska

Species: raccoon (*Procyon lotor*), gray fox (*Urocyon cinereoargenteus*), striped skunk (*Mephitis mephitis*), coyote (*Canis latrans*), red fox (*Vulpes vulpes*), gray wolf (*Canis*

lupus), black bear (*Ursus americanus*), mink (*Mustela vison*), long-tailed weasel (*Mustela frenata*).
[a.]*The gray wolf cases were from captive specimens at Atlanta Humane Society.*

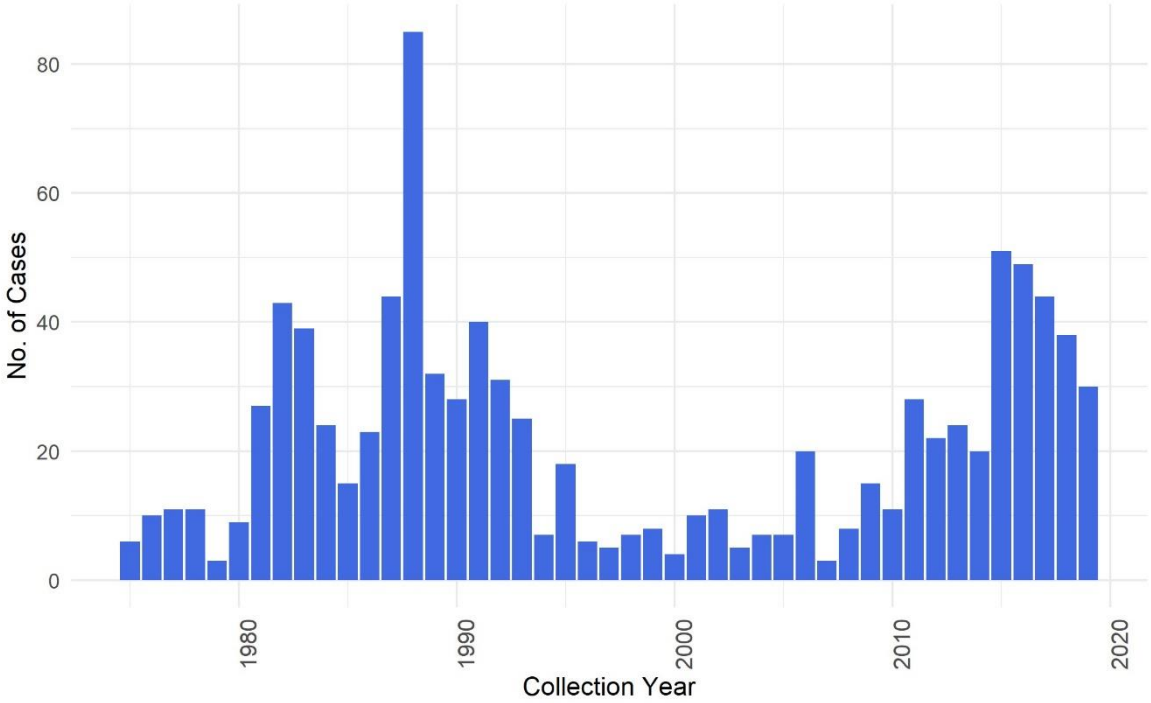


Figure 4.1. Total number of wildlife cases diagnosed with canine distemper for all species and all states per year submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019.

Canine distemper cases in all species most often originated in southeastern states (93%), and cases tended to occur in the same or adjacent counties to those with raccoon cases (Supplementary Fig. 4.1). The greatest number of cases throughout all years of study were submitted from Georgia, although from 2010 to 2019, the highest number was from Louisiana (Supplementary Fig. 4.3).

From 1975 to 2019, numbers of raccoon and gray fox distemper cases varied significantly over time; the greatest numbers per year, for both species, were seen in the years from 1980 to 1990 (n=441, mean=9.8, SD=11.82). The years 2000, 2007, 2008 and 2010 had no reported cases in gray foxes or raccoons in Georgia (Fig. 4.2).

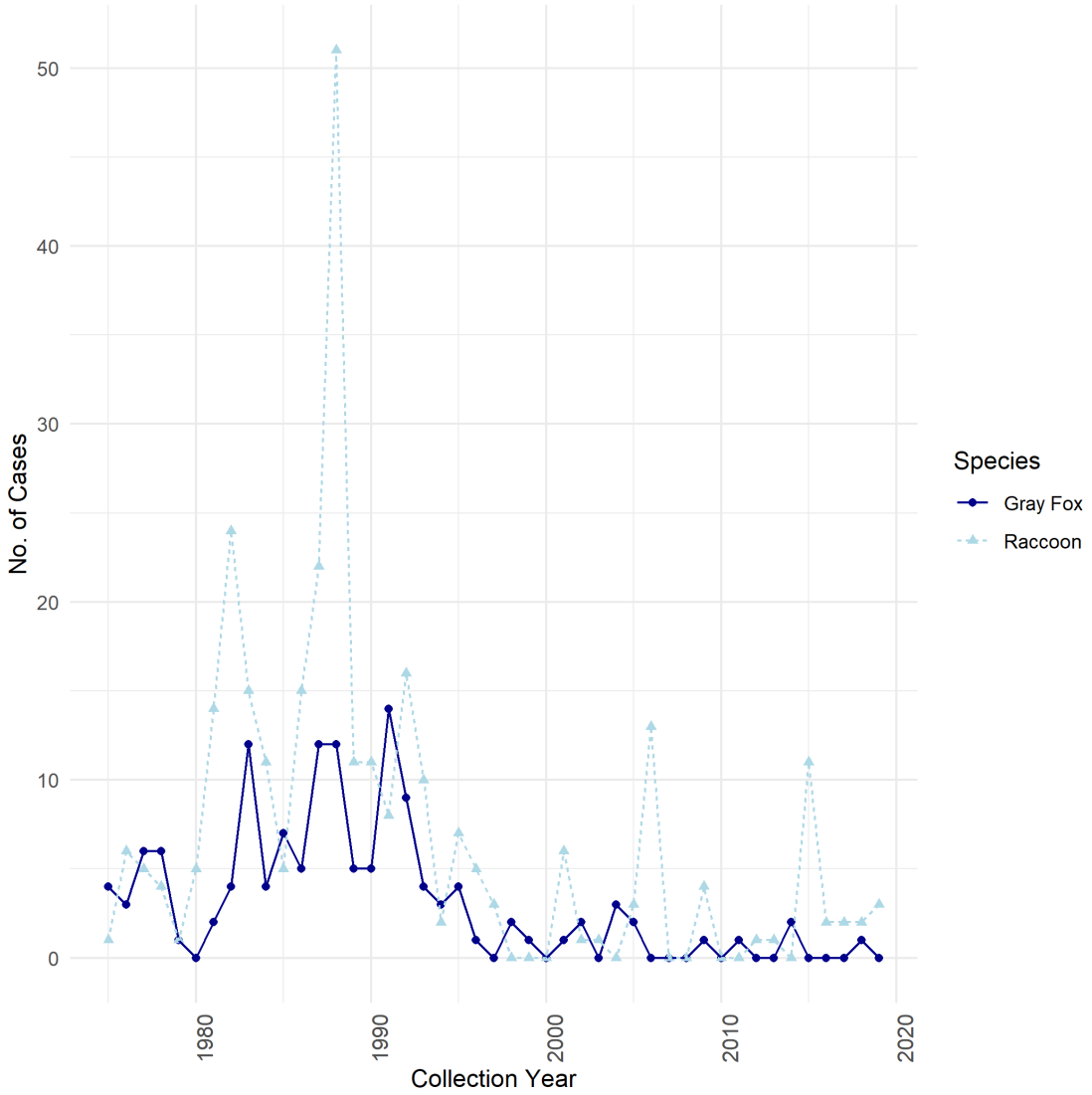


Figure 4.2. Total number of raccoons *(Procyon lotor)* and gray foxes *(Urocyon cinereoargenteus)*, diagnosed with canine distemper per year from Georgia that were submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019.

Most of the raccoon and gray fox cases in Georgia were received during the breeding seasons for these species (Zeveloff and Dewitte 2002). There was a significant

association between breeding season and the number of cases, particularly in raccoons (Supplementary Fig. 4.4). There was a significant association between breeding season and number of distemper cases in both raccoons (χ^2=21.20, p=2.49e$^{-05)}$ and gray foxes (χ^2=11.46, *P*=0.003) in Georgia; cases occur disproportionately during the breeding season, with fewest cases occurring in the non-breeding season.

Spatial analysis

The general pattern over the entire study period showed most cases occurring in counties in the northern part of Georgia around the population centers of Atlanta, Athens, and Augusta (Fig. 4.3). Additionally, high case numbers were documented in the southeastern part of the state, around Savannah. Ripley's K analysis of CDV cases in raccoons and gray foxes in Georgia showed that these cases are significantly clustered (Fig. 4.4). This pattern appeared again in case data stratified by year (Fig. 4.5**)**, with cases occurring near these population centers, particularly in the 1980s. The northern part of the state had a cluster of cases involving many counties during the 1980s and early 1990s, with a smaller number of counties involved in the southeast. The data indicated increased frequency of distemper case submissions in medium to medium-high human-population-density counties (suburban), with fewer cases diagnosed in very high (urban) and very low (rural) density counties. This relationship was more pronounced for raccoons than for gray foxes (Fig. 4.6**)**.

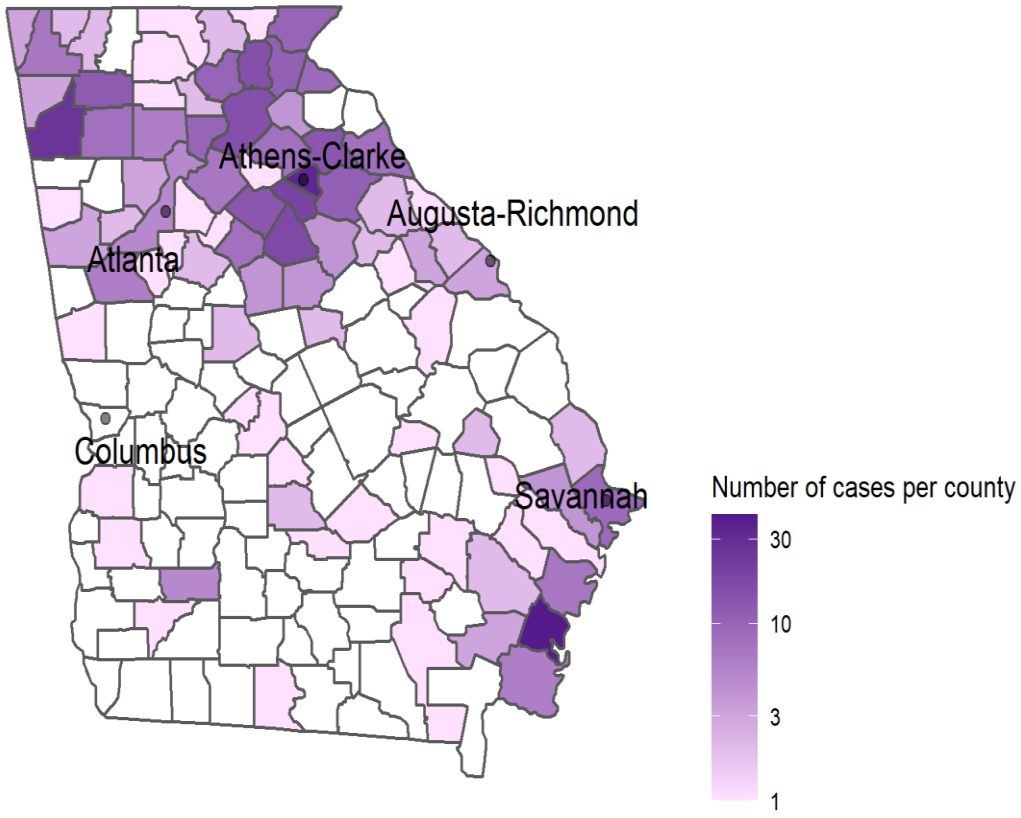


Figure 4.3. Total number of raccoons (Procyon lotor), and gray foxes (Urocyon cinereoargenteus), diagnosed with canine distemper per county in Georgia that were submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019.

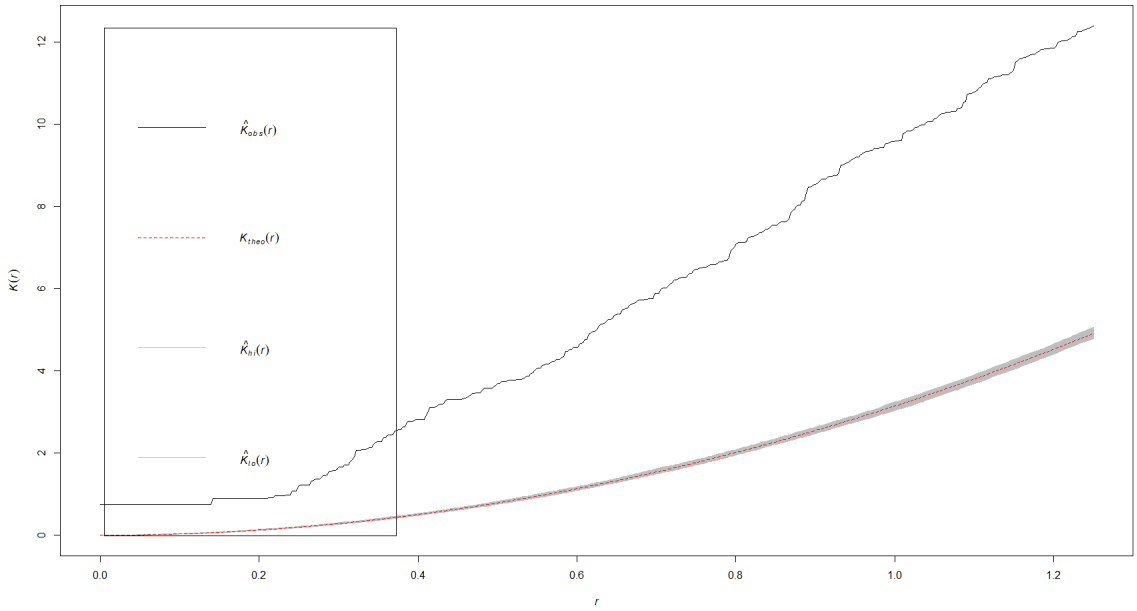


Figure 4.4. Ripley's K analysis of the location of raccoons *(Procyon lotor*) or gray foxes (*Urocyon cinereoargenteus*), diagnosed with canine distemper in Georgia that were submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019. $\widehat{K}_{obs}(r)$ *(solid line) is the Ripley's K statistic for the observed cases of raccoons and gray foxes combined.* $K_{theo}(r)$ *(dashed line) is the K statistic for a completely random (Poisson) point process.* $\widehat{K}_{hi}(r)$ *and* $\widehat{K}_{lo}(r)$ *(shading around dashed line) are the upper and lower envelopes for the Poisson simulation.*

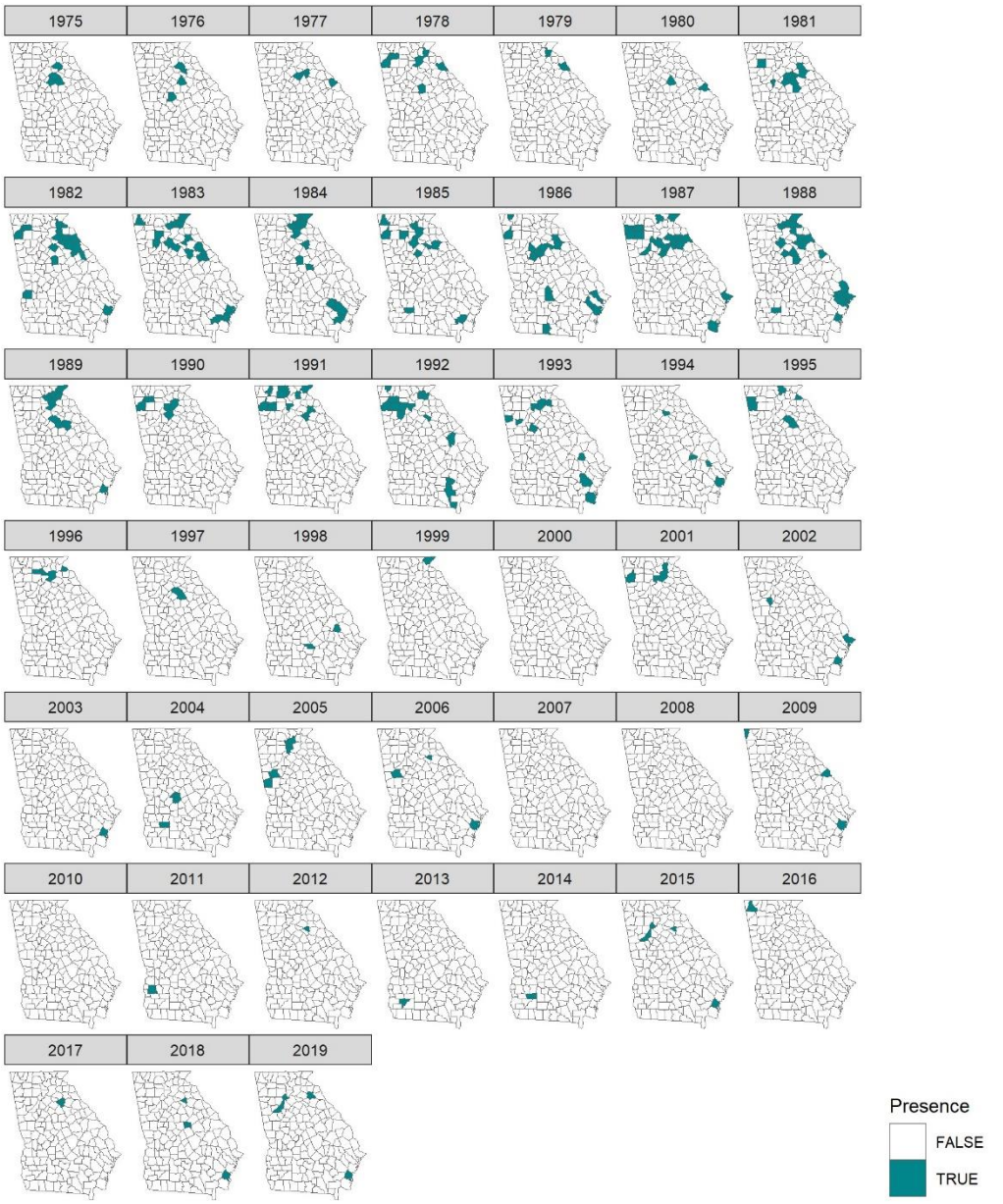


Figure 4.5. Presence of raccoons *(Procyon lotor)* or gray foxes *(Urocyon cinereoargenteus)*, diagnosed with canine distemper per county in Georgia, USA per year that were submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019.

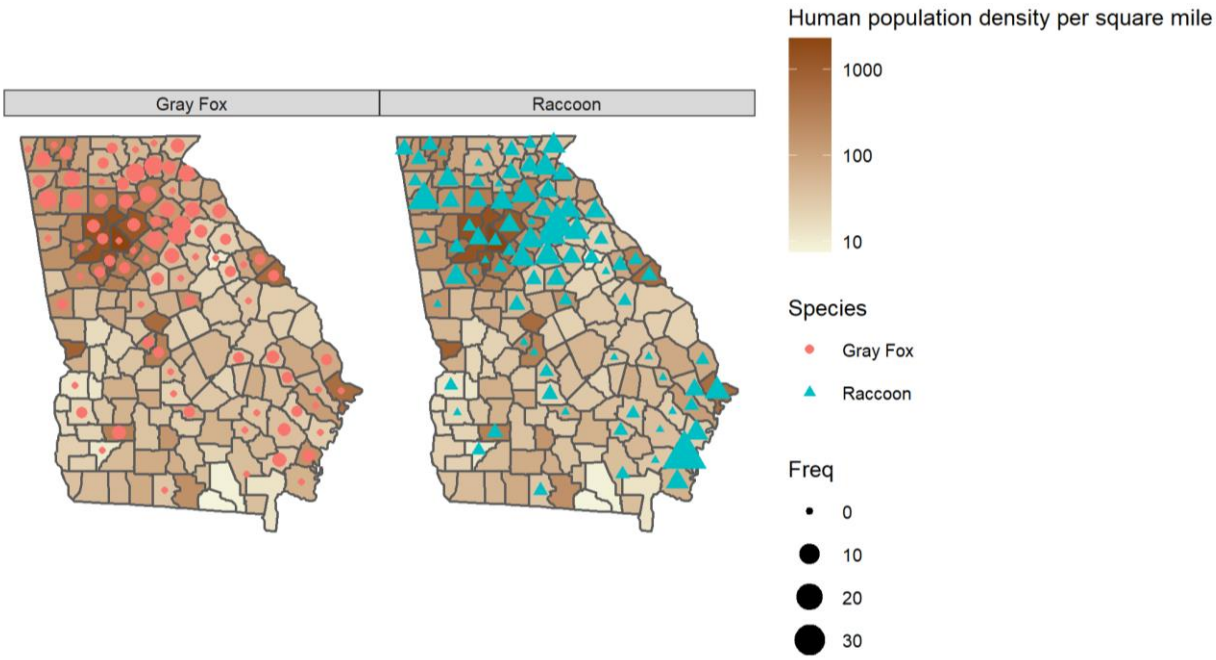


Figure 4.6. The average human population density per square mile for each county in the state of Georgia, USA from 1975-2019 and the number of raccoons *(Procyon lotor*) and gray foxes (*Urocyon cinereoargenteus*), diagnosed with canine distemper, plotted as a county centroid coordinate, submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019.

Predictive Model development

The best performing model for predicting the number of gray fox cases, x_t, used gray fox case numbers from *t*-1 and *t*-2 months, the *t*-1 predictive error, and the current month's raccoon cases, y_t (Fig. 4.7A). The best performing model for predicting raccoon cases, y_t used the *t*-1 raccoon cases and *t*-1 predictive error in addition to the number of gray fox cases from *t*, *t*-1, *t*-2 months (Fig. 4.7B). The gray fox predictive model was the most accurate model with an RMSE of 0.2669 for the test data, meaning it more accurately predicted gray fox distemper cases from these data. The raccoon prediction had a higher RMSE (0.7290).

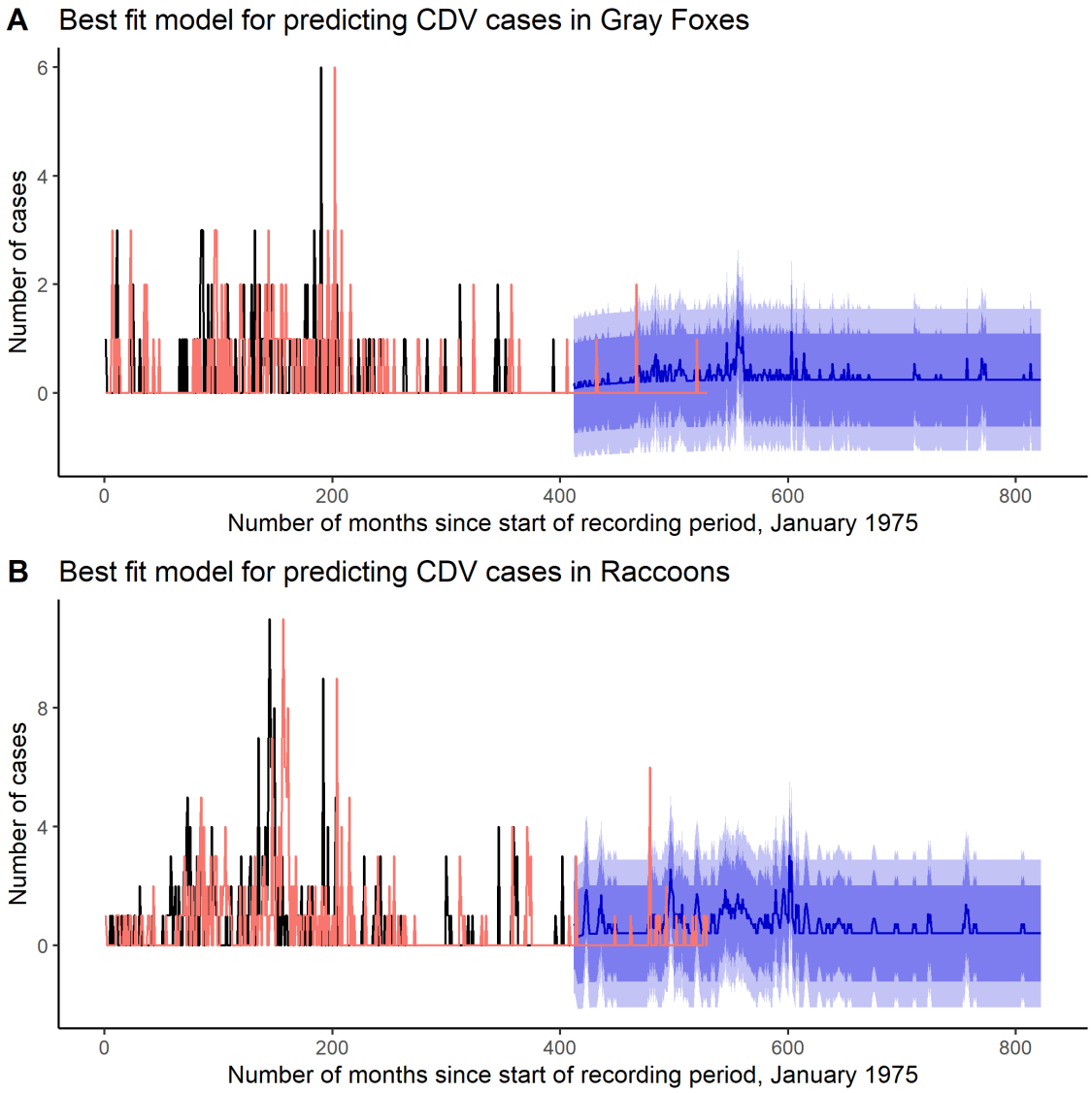


Figure 4.7. Best fit auto-regressive integrated moving average model for predicting canine distemper cases in gray foxes (*Urocyon cinereoargenteus*), (4.7A) and raccoons *(Procyon lotor*) (4.7B) in the state of Georgia, USA, using the numbers of raccoons and gray foxes diagnosed with canine distemper per month, which were submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019. *The case data for gray foxes and raccoons are shown by the gray line, with the model prediction for the training period in black on the left of the figure. The predictions for the test period and beyond are the black line surrounded by shading on the right of the figure. The shading represents 80% and 95% prediction intervals.*

DISCUSSION

Analysis of this 45-year diagnostic data set of canine distemper in wild carnivores for spatial and temporal trends found that nine species were diagnosed with canine distemper in 17 states, primarily concentrated in the Southeastern US. Distemper cases in different species tended to cluster spatially. Ripley's K analysis carried out on raccoon and gray fox data from Georgia showed significant clustering of reported cases and an apparent association between urban gradient and number of cases, with more cases occurring in suburban counties surrounding major urban areas. There was a significant temporal association with the overlapping breeding season of foxes and raccoons (March-June) and higher numbers of cases.

Georgia reported the most cases among the 17 states, especially in the 1980s. The cause(s) of this upswing in submitted cases is not known but may include interest in rabies in wildlife at the time (Parham 1983, Davidson et al. 1992). Louisiana submitted the most cases in the 2010s;whether this was due to an actual increase in CDV epizootics or other factors related to willingness to collect and submit carcasses, detection or reporting is unknown.

Spatial analysis showed significant clustering of distemper cases, probably reflecting viral transmission ecology. Viral persistence generally requires dense populations of susceptible hosts to facilitate viral spread, leading or contributing to epizootics (Williams 2001). The primary mode of CDV infection is aerosol inhalation, suggesting that habitat overlap and direct contact is important in transmission (Hoff et al. 1974). The immunizing nature of infection is useful for spatial and temporal analysis, as cases occurring in the same area but years later are likely to be due to a new outbreak that

has spread back into the area after an increase in the susceptible population has exceeded the threshold required for an outbreak. This has been demonstrated with CDV in Serengeti lions as the number of susceptible animals increased during the years following an epizootic (Packer et al. 1999). Disease presence can be used for analysis in this scenario, as one can assume that a case corresponds to an outbreak (Nouvellet et al. 2013).

Cases of CDV in raccoons appeared to be more commonly submitted (and diagnosed) from suburban counties in Georgia, with Atlanta and more rural counties submitting fewer animals. This may be due to suburban areas being a hotspot for disease circulation, with these areas being attractive to raccoons due to the available habitat and easy scavenging opportunities. Raccoon populations can reach higher densities within urban and suburban environments (Prange et al. 2003). A similar pattern was reported from studies in Germany: suburban and urban red foxes had a higher prevalence of CDV infection compared to their rural counterparts, but the CDV risk was reduced in highly urbanized areas in Berlin (Frolich et al. 2000, Gras et al. 2018). This is of particular relevance as spillover of CDV infection from raccoons into domestic dogs has been suggested (Kapil and Yeary 2011).

There was a much greater number of distemper cases in the northern and northeastern part of Georgia than in other areas of the state, followed by the southeastern part. There are numerous possible reasons for this. Reporting biases may have played a role. For example, the northern part of the state is more densely populated by humans; it is also in closer proximity to SCWDS at the University of Georgia, and thus it may be more convenient to deliver carcasses. Further, this region may have more suitable habitat

and thus more robust populations of raccoons and gray foxes. In addition, distemper cases in our study were possibly skewed toward those that involved obvious illness or death of wildlife. In contrast, one study reported that gray foxes and raccoons in Tennessee (November 2013 – August 2014) were frequently infected, but passive surveillance only detected those with clinical signs; this failed to account for subclinically infected animals, with 55% of subclinically infected animals testing positive by real-time reverse transcription -PCR assay (Pope et al. 2016).

The data also suggested a correlation between breeding season and the number of distemper cases diagnosed in raccoons and gray foxes, with cases more likely to occur during the breeding season. This could have been due to more frequent contact between individual animals as they search for mates, thereby promoting aerosol spread of virus. Similarly, rabies virus infection can spread quickly among raccoons when the virus is introduced during the breeding season (Reynolds et al. 2015). During the breeding season, susceptible animals are introduced to the population and can be infected when they interact for the first time with infected established members of the population. In addition, the physiological stress of reproduction may leave animals more susceptible to the virus (Hawley and Altizer 2011) or, as the cases in our study were from animals found moribund or dead, it could be that the increased movement during the breeding season leads to higher mortality rates from a variety of causes, such as vehicular collision, for which CDV may have contributed but was not the primary cause of death.

The ARIMA model for predicting monthly distemper cases in gray foxes could accurately predict this using the numbers of distemper cases in gray foxes from the previous 2 mo and in raccoons from the current month, suggesting that there was an

association between cases in the two species. Initial introduction of CDV into wild carnivores in the US in the 1960s was through gray foxes with subsequent spread to raccoons (Hoff et al. 1974). Similarly, a distemper outbreak in raccoons in Berlin, Germany appears to have originated in foxes, with transmission seeming to readily occur among wildlife species (Renteria-Solis et al. 2014).

The raccoon prediction model also was accurate in predicting raccoon distemper cases, albeit with a larger predictive error than the gray fox prediction model. This suggested a possible association between gray fox cases in previous months and raccoon cases in the current month. However, as the data for the time series analysis and ARIMA model building were pooled for the whole state, it was possible that the cases contributing to the overall number per month could have been separated by hundreds of miles; thus, the cases may not have been related to each other. While this was rare, the long-term data set covered a wide geographic region (i.e., the southeastern US and statewide across Georgia), and there were limitations to using such a large area to generate meaningful conclusions about temporal trends.

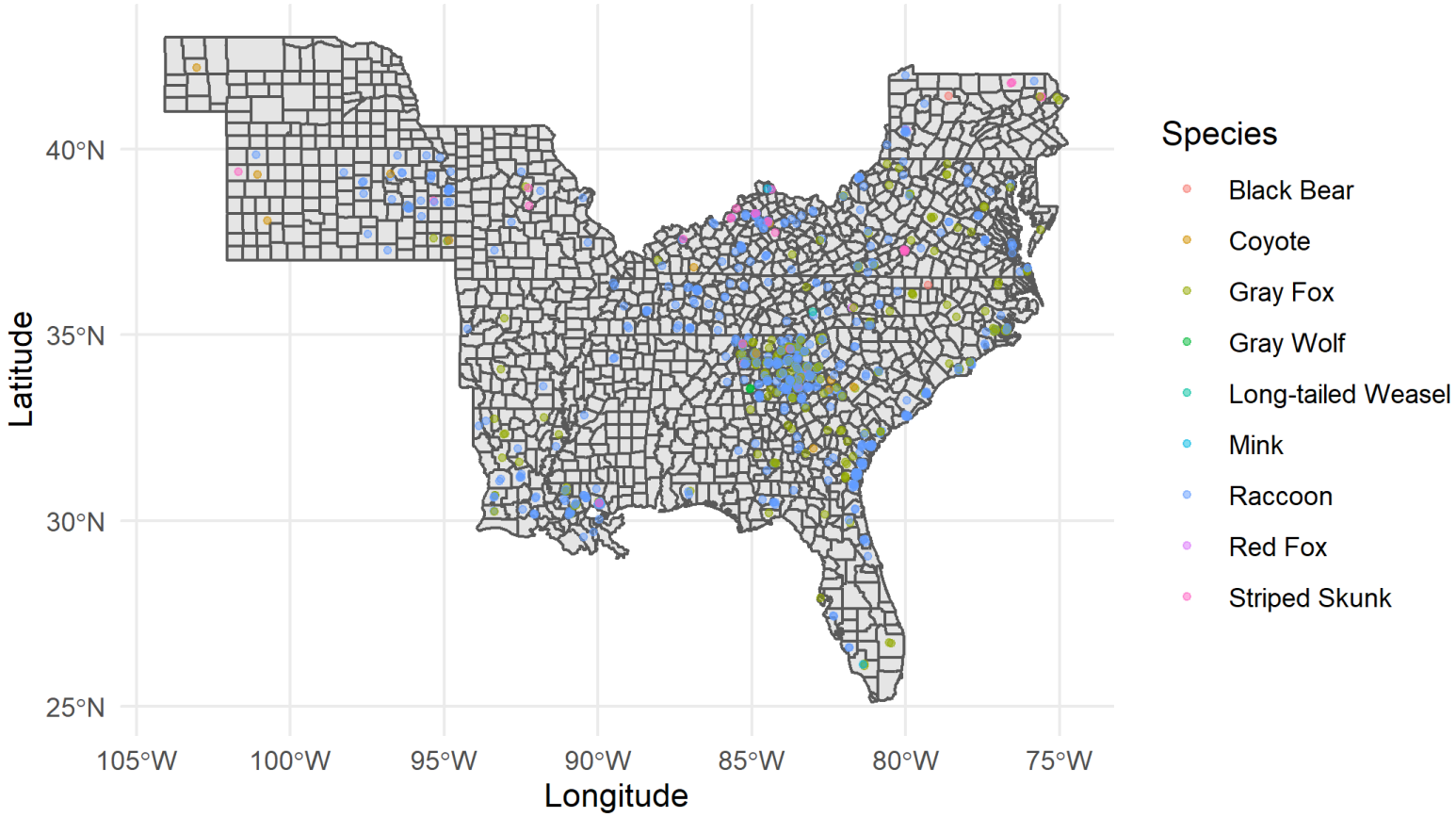
In both predictive models, the test data, which used the more recent data to test the accuracy of the model, had a lower error than the model training data. This can be explained by the variability of the case submission data over time. For example, the year-to-year variation in distemper case numbers among raccoons and gray foxes in Georgia was higher prior to 1996. The training data ran from April 1975-July 2011, so the model had to contend with more variability in case numbers than for the test data (August 2011-December 2019), which probably reduced accuracy of the output. The higher RMSE in

the raccoon prediction model may be explained by the larger degree of variability in submitted raccoon case numbers throughout the study.

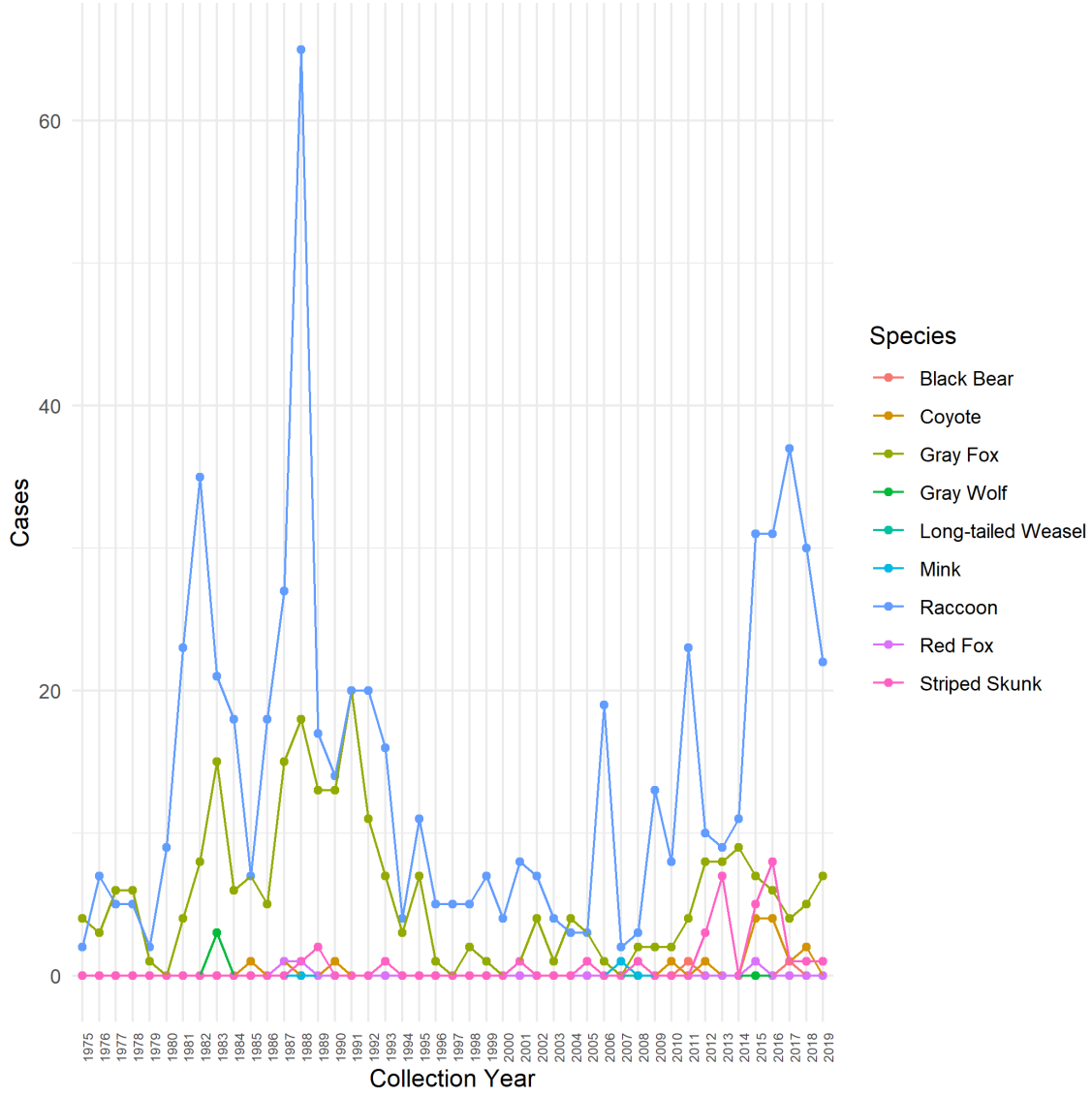
In conclusion, this analysis suggests that CDV infection is widely distributed in the southeastern US; it was diagnosed in nine different carnivore species from 17 states, with raccoon and gray foxes being most commonly diagnosed with canine distemper. The other species diagnosed were generally from the same or adjacent counties to those with raccoon or gray fox cases. Within the most represented state, Georgia, further analysis indicated distinct temporal and spatial patterns of CDV cases in raccoons and gray foxes, with cases more likely to occur during the breeding season. Spatially there was clustering of cases of both species within the same areas, with cases tending to be focused in more suburban areas. Our results also suggested that numbers of gray fox and raccoon cases can be predicted using data on past cases in foxes and raccoons. Among wild carnivore species, raccoons are easiest to capture and sample, and thus may serve as a useful predictor for other, less tractable species.

Ultimately, there were sufficient potential relationships suggested from this passively collected data to support recommendation of targeted active surveillance, including comprehensive sampling of wild carnivores over time and space to try to elucidate spillover trends between species, particularly in suburban areas. Data from both active and passive surveillance systems can also inform predictive models (Gras et al. 2018), which may aid in management decisions that help to reduce disease incidence, and facilitate molecular epidemiological studies, which can uncover more in-depth relationships between the different host species (Packer et al. 1999, Kamath 2020, Piewbang et al. 2020), as well as clarify the directionality of cross species transmission

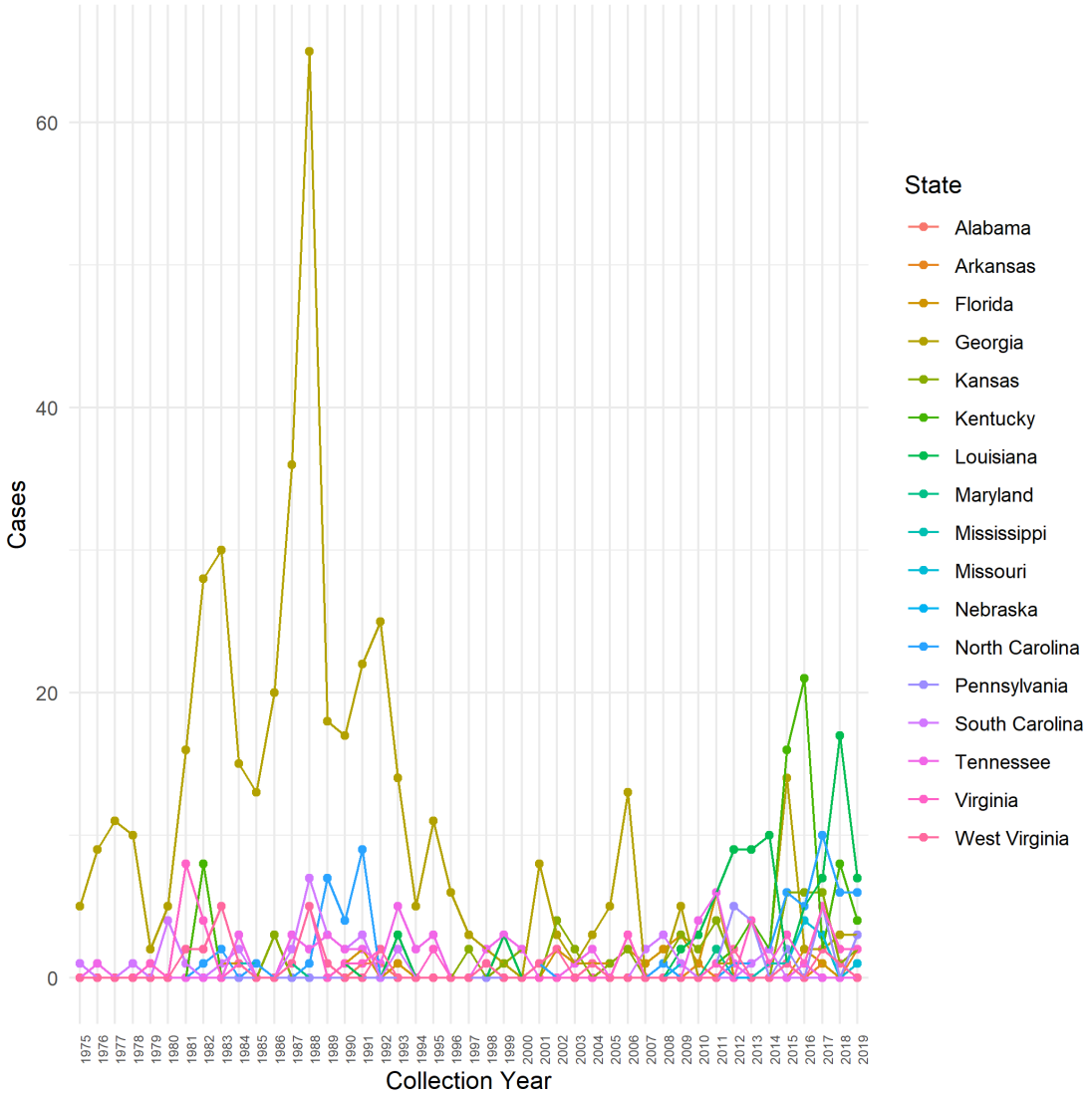
between different host species (Weckworth et al. 2020). This could also inform decision making regarding vaccination (Viana et al. 2015) and other strategies aimed at reducing transmission among both domestic and wild animals.



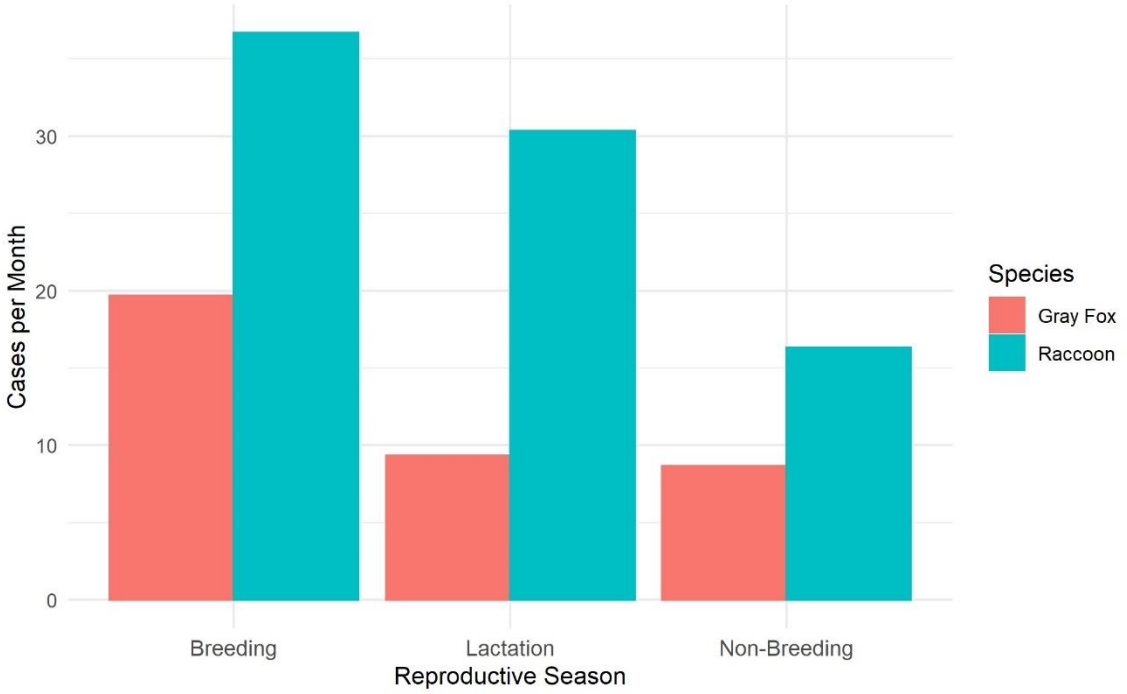
Supplementary Figure 4.1: Locations of wild animals diagnosed with canine distemper that were submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019. Species key: raccoon (Procyon lotor), gray fox (Urocyon cinereoargenteus), striped skunk (Mephitis mephitis), coyote (Canis latrans), red fox (Vulpes vulpes), gray wolf (Canis lupus), black bear (Ursus americanus), mink (Mustela vison), long-tailed weasel (Mustela frenata).



Supplementary Figure 4.2: Total number of canine distemper diagnosed in each species per year that were submitted to the Southeastern Cooperative Wildlife Disease Study, 1975- 2019. Species key: raccoon (Procyon lotor), gray fox (Urocyon cinereoargenteus), striped skunk (Mephitis mephitis), coyote (Canis latrans), red fox (Vulpes vulpes), gray wolf (Canis lupus), black bear (Ursus americanus), mink (Mustela vison), long-tailed weasel (Mustela frenata).



Supplementary Figure 4.3: Total number of canine distemper cases diagnosed in each state per year that were submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019.



Supplementary Figure 4.4: Total number of raccoon and gray foxes diagnosed with canine distemper per month, from Georgia, in each part of the species reproductive cycle, submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019. *The breeding season was defined as the period from January to March, and the period of lactation was defined as April to June. The remainder of the year was designated as the non-breeding season.*

Model Number	Model	Predictors	AIC
1	Fit_1GF	Gray_Fox_Cases_lag1,2 + Gray_Fox_Error_lag1	822.6792
2	Fit_2GF	Gray_Fox_Cases_lag1,2 + Gray_Fox_Error_lag1 + Raccoon_Cases_lag0	810.8488
3	Fit_3GF	Gray_Fox_Cases_lag1,2 + Gray_Fox_Error_lag1 + Raccoon_Cases_lag0,1	812.8090
4	Fit_4GF	Gray_Fox_Cases_lag1,2 + Gray_Fox_Error_lag1 + Raccoon_Cases_lag0,1,2	814.7624
5	Fit_5GF	Gray_Fox_Cases_lag1,2 + Gray_Fox_Error_lag1 + Raccoon_Cases_lag0,1,2,3	816.0758
6	Fit_6GF	Gray_Fox_Cases_lag1,2 + Gray_Fox_Error_lag1 + Raccoon_Cases_lag0,1,2,3,4	815.1845
7	Fit_7GF	Gray_Fox_Cases_lag1,2 + Gray_Fox_Error_lag1 + Raccoon_Cases_lag0,1,2,3,4,5	817.0028
8	Fit_8GF	Gray_Fox_Cases_lag1,2 + Gray_Fox_Error_lag1 + Raccoon_Cases_lag0,1,2,3,4,5,6	818.4572
9	Fit_9GF	Gray_Fox_Cases_lag1,2 + Gray_Fox_Error_lag1 + Raccoon_Cases_lag0,1,2,3,4,5,6,7	815.6756
10	Fit_10GF	Gray_Fox_Cases_lag1,2 + Gray_Fox_Error_lag1 + Raccoon_Cases_lag0,1,2,3,4,5,6,7,8	815.5924
11	Fit_11GF	Gray_Fox_Cases_lag1 + Raccoon_Cases_lag0,1,2,3,4,5,6,7,8,9	820.3420
12	Fit_12GF	Gray_Fox_Cases_lag1,2 + Gray_Fox_Error_lag1 + Raccoon_Cases_lag0,1,2,3,4,5,6,7,8,9,10	819.3913
13	Fit_13GF	Gray_Fox_Cases_lag1 + Raccoon_Cases_lag0,1,2,3,4,5,6,7,8,9,10,11	821.1584
14	Fit_14GF	Gray_Fox_Cases_lag1,2 + Gray_Fox_Error_lag1 + Raccoon_Cases_lag0,1,2,3,4,5,6,7,8,9,10,11,12	816.0878
0	Null	Null Model	867.1008

Supplementary Table 4.1: Summary table of the AIC for the auto-regressive integrated moving average models tested for predicting canine distemper cases in raccoons in Georgia, using the numbers of raccoons and gray foxes diagnosed with canine distemper per month, which were submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019. *The lag numbers correspond to the number of months in the past; with 0 being the present month, 1 being the previous month, 2 being two months previous and so on.*

Model Number	Model	Predictors	AIC
1	Fit_1R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1	1317.628
2	Fit_2R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0	1309.358
3	Fit_3R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0,1	1308.566
4	Fit_4R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0,1,2	1297.329
5	Fit_5R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0,1,2,3	1299.113
6	Fit_6R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0,1,2,3,4	1301.110
7	Fit_7R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0,1,2,3,4,5	1302.347
8	Fit_8R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0,1,2,3,4,5,6	1299.008
9	Fit_9R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0,1,2,3,4,5,6,7	1300.957
10	Fit_10R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0,1,2,3,4,5,6,7,8	1302.537
11	Fit_11R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0,1,2,3,4,5,6,7,8,9	1302.087
12	Fit_12R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0,1,2,3,4,5,6,7,8,9,10	1304.045
13	Fit_13R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0,1,2,3,4,5,6,7,8,9,10,11	1305.509
14	Fit_14R	Raccoon_Cases_lag_1 + Raccoon_Error_lag_1 + Gray_Fox_Cases_lag_0,1,2,3,4,5,6,7,8,9,10,11,12	1305.149
0	Null	Null Model	1430.343

Supplementary Table 4.2: Summary table of the AIC for the auto-regressive integrated moving average models tested for predicting canine distemper cases in gray foxes in Georgia, using the numbers of raccoons and gray foxes diagnosed with canine distemper per month, which were submitted to the Southeastern Cooperative Wildlife Disease Study, 1975-2019. *The lag numbers correspond to the number of months in the past; with 0 being the present month, 1 being the previous month, 2 being two months previous and so on.*

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CHAPTER 5

CANINE DISTEMPER VIRUS PHYLOGENETIC STRUCTURE AND ECOLOGICAL CORRELATES OF INFECTION IN MESOCARNIVORES ACROSS ANTHROPOGENIC LAND USE GRADIENTS

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To be submitted to mSphere

Abstract

Human land use change has a significant impact on infectious disease dynamics at the wildlife-domestic-human interface by changing the spatial distribution, behavior, density, and population dynamics of wildlife. Canine distemper virus (CDV) is a significant cause of morbidity and mortality in a range of wildlife and domestic species, yet the dynamics of CDV infection within multi-host systems, such as carnivore communities, remain unclear. The southeastern United States provides a multi-host system for CDV that harbors many potential host species. Given the propensity of CDV to infect synanthropic mesocarnivores, it is important to investigate if land use features affect CDV infection patterns in wildlife. Here, we investigated the phylogenetic structure and spatial patterns of CDV infection in wild mesocarnivores in the southeastern United States using carcasses (N=270) submitted to the Southeastern Cooperative Wildlife Disease Study from January 2019 to December 2022, and by sequencing the CDV H-gene of CDV-positive animals (N=31). Additionally, we developed a statistical model to identify ecological and land use variables, which may increase the risk of CDV in these species. There was significant genetic diversity in CDV H-gene sequences among wild mesocarnivores in the southeastern United States that exhibited geographic separation into groups east and west of the Mississippi River. Furthermore, a generalized linear model for wild mesocarnivores diagnosed (postmortem) with canine distemper showed that surface imperviousness and precipitation were significant positive explanatory variables, whereas elevation and being in the juvenile age class showed a significant negative association with the likelihood of an animal being CDV positive.

The results of this study may have important implications for surveillance and conservation efforts. By identifying areas of intense human development as being at the highest risk for disease, it may be possible to focus surveillance efforts in these areas, allowing outbreaks to be identified earlier, and potentially preventing cross species transmission of CDV.

INTRODUCTION

Anthropogenic land use has a significant impact on the population structure and dynamics of infectious diseases at the wildlife-domestic-human interface (Bradley and Altizer 2007, Patz et al. 2008, Gottdenker et al. 2014, Plowright et al. 2021). Proposed mechanisms by which anthropogenic land use (e.g., deforestation, silviculture, agricultural activities, urbanization, suburbanization) can alter structure and spread of infectious disease within and between wildlife populations, domestic animals and humans include altered host density, behavioral changes (including contact within and between species and movement of hosts), and impaired host immune function (Slingenbergh et al. 2004, Lambin et al. 2010, Zylberberg et al. 2013, Guo et al. 2019). For directly transmitted pathogens, host density drives contact rates and thus pathogen spread (Tompkins et al. 2011). High densities usually result in increased contact within and between species and individuals and subsequently greater prevalence of infection (Ditchkoff et al. 2006, Almberg et al. 2009). This idea of higher density populations and greater contact rates leading to more disease is of particular importance in synanthropic species that thrive in human disturbed environments where supplemental resource availability, shelter and lack of predation can harbor large populations (Cavallini 1996, Prange et al. 2003, Contesse et al. 2004). Despite this, some studies have suggested that highly urbanized areas may dampen spread of infectious diseases among wildlife due to reduced population sizes (Gras et al. 2018). Thus, it remains unclear how continued urban development affects the dynamics of directly transmitted pathogens in synanthropic species. Therefore, it is crucial to understand the relationships among land use change, the role of human development, and infectious disease transmission in synanthropic

wildlife to develop effective management and conservation strategies to mitigate the negative impacts of disease and protect affected species and their ecosystems.

Canine distemper virus (CDV) belongs to the genus *Morbillivirus*, family Paramyxoviridae, and is a notable study system for investigating land use-infectious disease relationships in multi-host (wildlife and domestic animal) systems. Although morbilliviruses tend to have a narrow host range, CDV goes against this trend by its ability to infect a wide variety of carnivore hosts, and rarely other species groups (Appel et al. 1991, Watson et al. 2020) and has been implicated in severe population declines in multiple species, including the near-extinction of the black-footed ferret (*Mustela nigripes*) in the USA (Williams et al. 1988). It is also an important disease in domestic dogs, and CDV can be transmitted between wildlife and dogs and vice versa (Kapil and Yeary 2011). CDV also has been proposed as a risk to human health, and it is hypothesized that waning population level measles immunity may leave humans susceptible to CDV infection (Martinez-Gutierrez and Ruiz-Saenz 2016). CDV infection is commonly reported in the raccoon (*Procyon lotor*) (Hoff et al. 1974, Cranfield et al. 1984, Roscoe 1993, Lednicky et al. 2004, Renteria-Solis et al. 2014), a synanthropic mesocarnivore that lives in high density and well connected populations in urbanized areas in North America (Smith and Engeman 2002, Hirsch et al. 2013). However, there is an incomplete understanding of CDV infection dynamics within many multi-host systems, such as carnivore communities. The role that different carnivore species play in the maintenance and spread of CDV is not understood, and consequently the targeting of mitigation measures is not well informed.

The southeastern US has a wide variety of potential CDV host species. Preliminary work from postmortem diagnostic data of CDV-infected wild carnivores has demonstrated that CDV is widespread in the southeastern US with at least 9 carnivore species experiencing mortality as a result of infection. In the most commonly infected species, the raccoon and gray fox (*Urocyon cinereoargenteus*), 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 synanthropic mesocarnivores, it is important to investigate whether there are human land use features that affect the likelihood of virus transmission among wildlife.

Here, we investigate the phylogenetic structure and spatial patterns of CDV infection in wild mesocarnivores in the southeastern US from January 2019 to December 2022. The objectives of this study were to:

(1) explore patterns of CDV genetic diversity in wild mesocarnivores in the southeastern US;

(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 ecological factors that may increase the risk of CDV. Specifically, we aimed to investigate how land use influences the likelihood of severe to fatal CDV infection (i.e., diagnosed postmortem) in wild mesocarnivores.

METHODS

The Southeastern Cooperative Wildlife Disease Study (SCWDS; Athens, Georgia, USA) incorporates 17 states, most of which are located in the southeastern US. Here, we used a data set that included the cause of death of 270 mesocarnivores from January 2019 to December 2022. The majority (158) of these animals were diagnosed with canine distemper via postmortem evaluation. The raw data from SCWDS included the variables: state, county, area, coordinates where animal was found (when provided), species, date, sex, age, weight, and diagnoses. Additionally, the land cover data for each location were extracted from raster maps available from the National Land Cover Database (NLCD). The different land cover types are described in supplementary Table 5.1. The classification system used by NLCD is modified from the Anderson Land Cover Classification System (Anderson 1976). Along with elevation data from the *elevatr* package (Hollister 2021), average temperature and precipitation values were accessed from the PRISM database (PRISM Climate Group 2022). 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 the TIGER database (U.S. Census Bureau 2022). The R script for the data collection, cleaning and analysis is available at https://github.com/JJWilson1991/Chapter3_GLM.

The animals submitted were found dead or were found moribund and were subsequently euthanized. If the animal was observed alive, clinical signs often were described and generally included lethargy, lack of fear of humans, remaining in same place for a day or more, visible during daylight hours, and sometimes neurologic signs (e.g., mental dullness, confusion, ataxia, disorientation). Cases of CDV infection were

identified at necropsy by characteristic histopathology (see below) and one or more of the following diagnostic features: CDV antigen detection by fluorescent antibody testing (Fairchild et al. 1971) or immunohistochemistry (Palmer et al. 1990). CDV causes depletion and necrosis of lymphatic tissue, nonsuppurative interstitial pneumonia sometimes with viral syncytia, and often with intranuclear and intracytoplasmic inclusion bodies in respiratory, urinary, and/or gastrointestinal epithelium. Brain lesions can include neuronal degeneration, gliosis, demyelination, perivascular cuffing, leptomeningitis, and inclusion bodies in neurons and glial cells. Lesions vary depending on the stage of the disease and affected organ, and may include mild inflammation in early stages, and severe inflammation and necrosis in later stages. The respiratory tract may show diffuse inflammation, thickening, and hyperplasia of the epithelial cells, and accumulation of inflammatory cells in the lumen of the airways. Nervous system pathology may include inflammation, degeneration, and perivascular cuffing. Gastrointestinal tract may show inflammation, necrosis, and presence of lymphocytes and macrophages in the lumen (Van Moll et al. 1995, Jubb et al. 2012).

Nucleic acid detection/sequencing/analysis

Tissue samples (most often brain, but also lung, liver, spleen) were collected from a subset of 32 known-CDV positive cases for viral RNA extraction. CDV RNA was extracted from tissue 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. The forward and reverse primer pair used to amplify the approximately 1000bp region of H-gene were synthesized based on primer pair 7 in (Riley and Wilkes 2015). 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 microliters 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 were edited and assembled using Geneious© software. This involved taking raw chromatogram results for forward and reverse primer reads and trimming the ends to remove poor quality regions. The error probability limit was set to 0.05 to trim bases with a quality score less than ~13. Next, forward and reverse pairs were assembled using the de novo assembly feature. Finally, a consensus sequence was generated for each pair. Further available H-gene sequences from 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. The full list of isolates from this study are listed in Supplementary Table 5.2.

Statistical analysis

Diagnostic data from SCWDS cases were imported into R Studio (version 2022.12.0+353) (Posit team 2022). A detailed description of data analysis is contained in the scripts within the project repository (https://github.com/JJWilson1991/Chapter3_GLM). All analyses described below were conducted in the R programming environment (version 4.2.0.)(R Core Team 2022). References to packages in this methods section indicate specific packages used within the R programming environment.

Analysis of spatial clustering for all necropsy cases was performed using Ripley's K analysis from the *spatstat* package (Baddeley et al. 2015). This analysis identifies if, and at what spatial scale, spatial point data are more clustered or dispersed compared to a random distribution.

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 (R Core Team 2022). A logistic link function was applied, and a binomial error distribution was assumed.

A positive or negative diagnosis of canine distemper 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. The GLM was then fit to the data using the `glm()` function. The model was fit using maximum likelihood estimation. To select the

best model, different models were fitted and compared based on their AIC (Akaike Information Criterion) (Akaike 1981). The general workflow involved creating a global model involving all the variables at a basic level. The `add1()` function from the *stats* package was then used to test for interacting variables. Interacting variables that made a significant improvement to the AIC were added to the model. Next the `dropterm()` function from the *MASS* package (Venables 2002) was used to evaluate the impact of removing each variable from the model using AIC. Those four variables that had a negative influence on AIC were removed from the model. `influencePlot()` and `outlierTest()` from the *car* package (Fox 2019) were used to test for outliers in the data that may have had a significant impact on the model fit. AIC was again used to evaluate the model improvements. Ultimately, three outliers were removed from the data that had a significant influence on the model. The final model was test for fit with the data and for over fitting by plotting Pearson's residuals and fitted values. The data set was initially split into a training and test set. The predictive ability of the model was then tested on the test data. Finally, the model coefficients and their standard errors were interpreted to understand the relationships between the predictor variables and the response variable. The significance of the predictor variables was determined using p-values.

RESULTS

A total of 270 mesocarnivores underwent postmortem evaluation at SCWDS from January 2019 to December 2022. At necropsy, 158 out of the 270 mesocarnivores (58.5%) were diagnosed with CDV infection (Figure 5.1). There were four host species represented in these data: raccoon, gray fox, striped skunk (*Mephitis mephitis*), and red

fox (*Vulpes vulpes*). These animals originated from 13 different states. The state and species distributions are shown in Table 5.1.

Table 5.1: Summary of necropsy cases by select mesocarnivore by species and state submitted to Southeastern Cooperative Wildlife Disease Study from January 2019 to December 2022

	Gray Fox	Raccoon	Red Fox	Striped Skunk	Total
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
Total	36	182	23	29	270

The entire data set for all necropsy cases, including those diagnosed with canine distemper as well as those from which CDV was not detected, showed spatial clustering with Ripley's K analysis (Figure 5.2). This pattern was most obvious at shorter distances, whereas the plot almost returned to being randomly spatially distributed at greater distances. The distemper cases showed a greater degree of clustering, especially at larger distances compared to the whole dataset. CDV negative cases only had a small degree of clustering at short distances before the graph returned to normal limits.

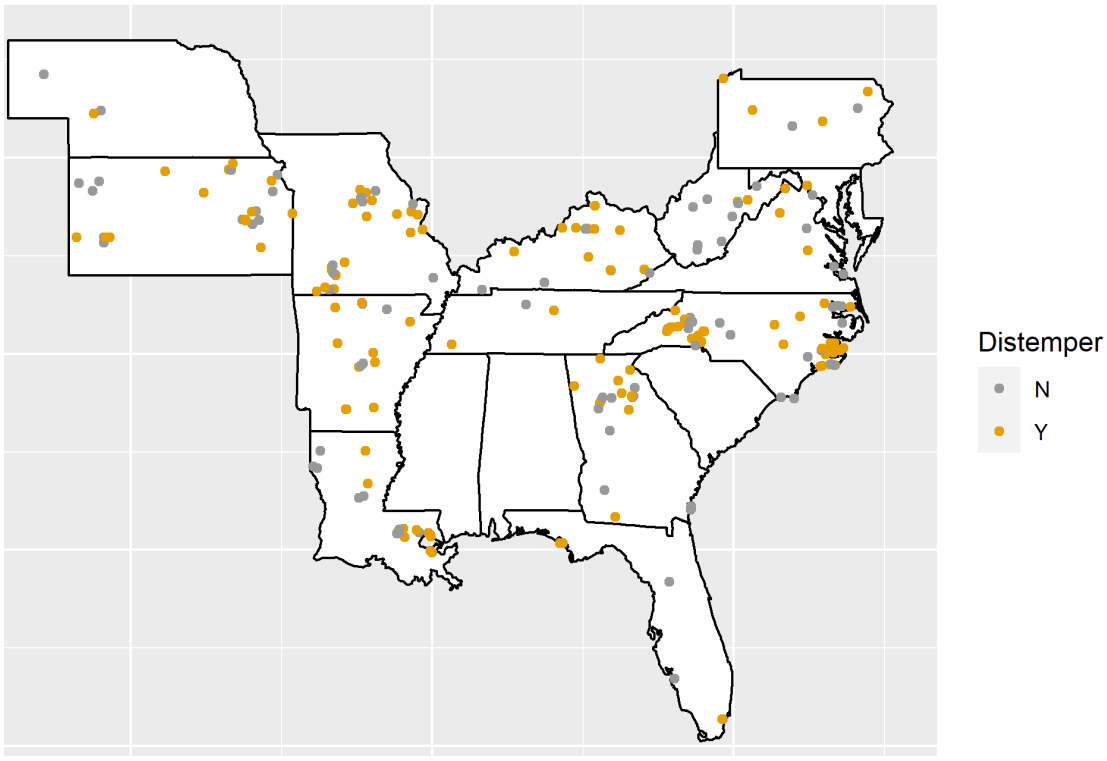

Figure 5.1: Map of Southeastern Cooperative Wildlife Disease Study member states from which necropsy cases of mesocarnivore species were analysed for canine distemper virus infection from January 2019 to December 2022 (n=270). *They are coded according to distemper diagnosis, with green being positive and red negative.*

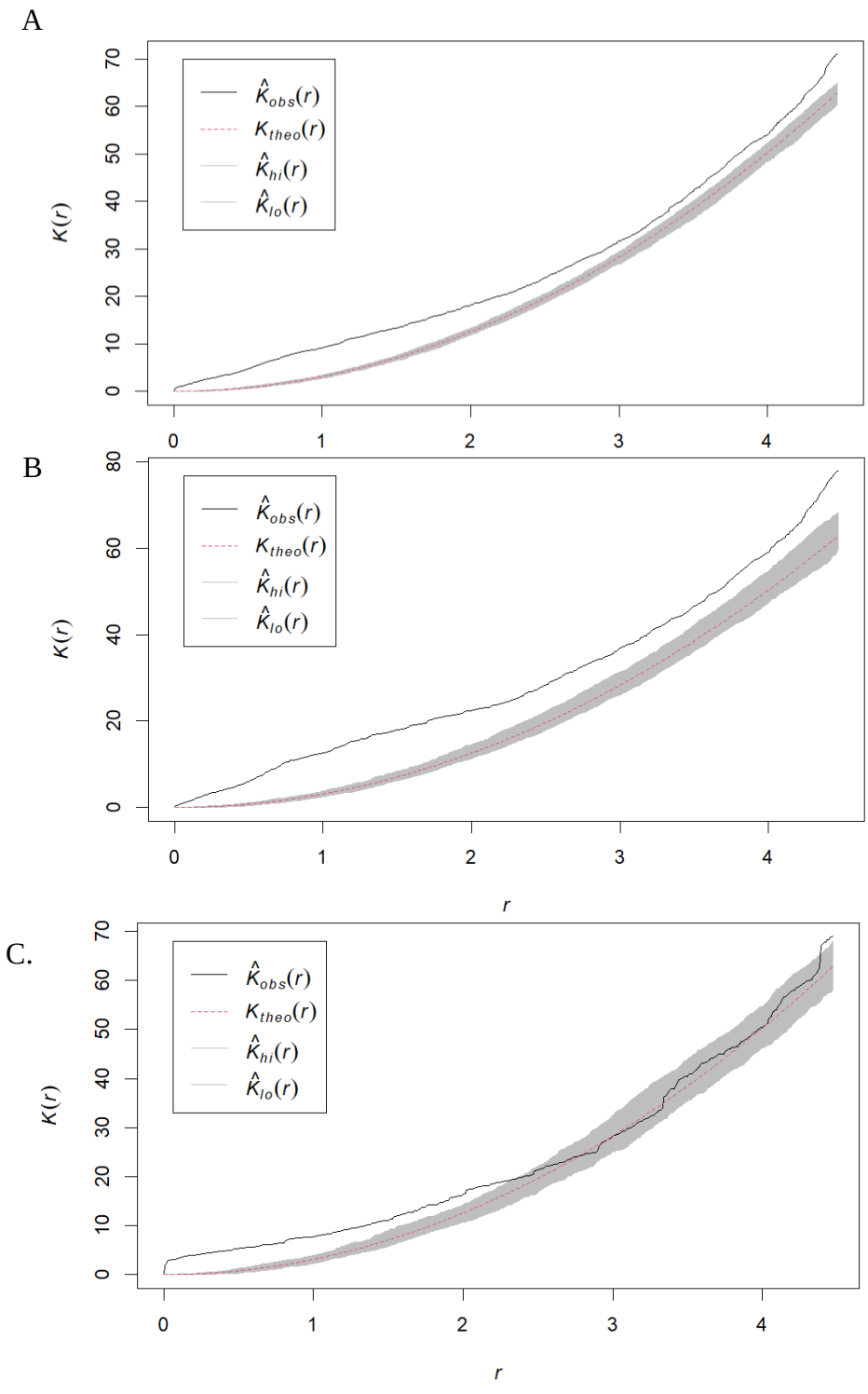

Figure 5.2: Ripley's K analysis of cases of select mesocarnivore species submitted to Southeastern Cooperative Wildlife Disease Study from January 2019 to December 2022. Plot A: Entire data set. Plot B: CDV positive cases. Plot C: CDV negative cases. *r (the x-axis) represents distance from a point and K(r) represents the K*

function.$\widehat{K}_{obs}(r)$ *(solid line) is the Ripley's K statistic for the observed cases.* $K_{theo}(r)$ *(dashed line) is the K statistic for a completely random (Poisson) point process.* $\widehat{K}_{hi}(r)$ *and* $\widehat{K}_{lo}(r)$ *(shading around dashed line) are the upper and lower envelopes for the Poisson simulation.*

CDV Phylogeny

A total of 32 CDV partial H-gene (~1200 bp) isolates from 5 states from this study (GA, FL, NC, MO, AR), in addition to 56 additional sequences downloaded from GenBank, were aligned in Geneious and used to build a phylogenetic tree. This analysis was conducted to understand evolutionary relationships between the CDV isolates found in this study and other available isolates from across the US. After tree inspection, the sequences from this study fall into two clades. 22 of the isolates from eastern states (NC, FL, GA) grouped together in one large cluster, with moderate bootstrap support values of 65.7. Isolates from Missouri and Arkansas (west of the Mississippi River) generally clustered distinctly from this large eastern clade; although one NC and one GA isolate grouped with these more western isolates (Figure 5.3). This western clade again had moderate bootstrap support of 64.6.

Generalized Linear Model of Canine Distemper Diagnosis

The results of the model showed surface imperviousness, precipitation, elevation, age and species to be the most statistically significant ($p<0.01$) explanatory variables in the model. The full results of model analysis for all explanatory variables are shown in supplementary Table 5.4.

The best fitting model from the data is described below:

formula = Distemper ~ Species + Age + month + latitude + knn.dist +
Elevation + Precipitation + Temperature + Imperviousness +
descriptionLandUse + Species:knn.dist + Species:Temperature + lat:Elevation +
Elevation:Imperviousness + Age:month

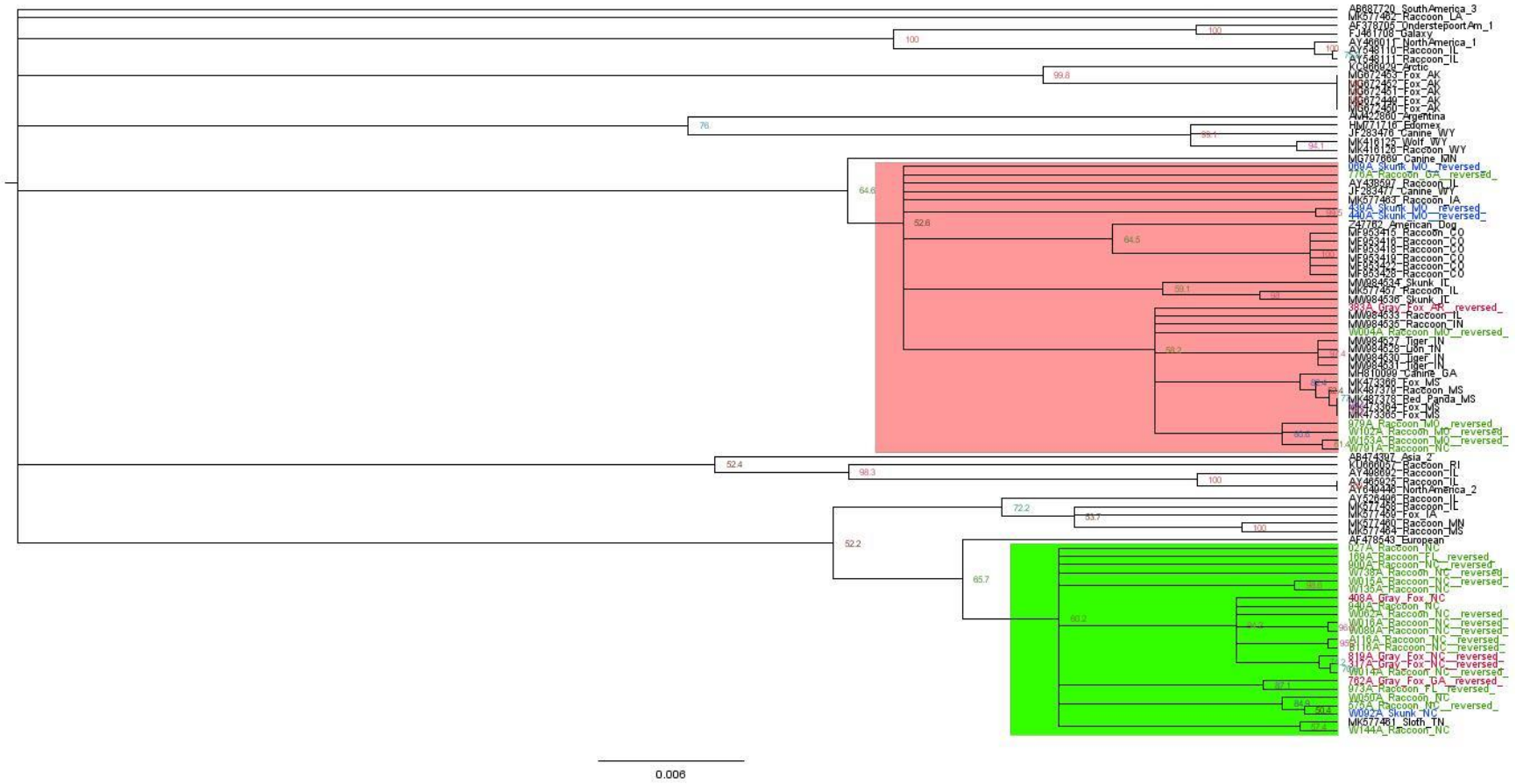

Figure 5.3: Phylogenetic tree for H-gene of canine distemper virus isolates from necropsy cases of mesocarnivore species submitted to the Southeastern Cooperative Wildlife Disease Study from January 2019 to December 2022.
Isolates in coloured text were produced by this study. Those in black text were accessed from Genbank. The green highlighted cluster is the proposed eastern group of viruses isolated in the study from CDV cases in states east of the Mississippi river (NC, GA,FL). With the red shading corresponding to the western group with viruses from AR and MO.

The model improvements throughout the fitting process are summarized in Table 5.2; the full list of models is included in Supplementary Table 3. The statistically significant ($p<0.01$) explanatory variables from the results of the best fitting GLM are summarized in Table 5.3.

Table 5.2: Summary of the improvements to the model metrics throughout the GLM fitting process

Model	Residual Deviance	Residual d.f.	AIC	Deltaic	AIC weight
Global model	179.05	173	237.05	87.46	1.02×10^{-19}
Interactions	101.37	163	179.37	29.78	3.41×10^{-7}
Backwards selection	103.17	168	175.17	25.58	2.79×10^{-6}
Outliers	77.59	165	149.59	-	
Best fit	77.59	165	149.59	-	

Model testing showed a prediction accuracy for the best fit model for distemper cases of 0.61. The model had a precision of 0.56 and a recall of 0.75 with an F1 score of 0.64.

Table 5.3: Summary statistics for significant (p<0.01) explanatory variables for the GLM, ordered by absolute value of the standardized coefficient. *Full summary of the model is in supplementary table 5.4. Interaction terms are those separated by a colon. Note: The glm function in R uses a technique called "dummy coding" to convert categorical variables into a set of binary variables, also known as "indicator variables" or "dummy variables". This is done so that the categorical variable can be included in the model as a predictor. When a categorical variable is used in a model, it is split into one binary variable for each level of the categorical variable, with a value of 1 indicating membership in that level, and a value of 0 indicating non-membership. The summary function then displays each of these binary variables as a separate factor in the output. This allows the user to see the effect of each level of the categorical variable on the response variable.*

Explanatory variable	Estimate	2.50%	97.50%	Std. Error	z value	Pr(>|z|)	Standardized coefficient
SpeciesRaccoon:Temperature	4.296854	2.370153	7.260268	1.182571	3.633485	0.00028	3.978035
SpeciesRaccoon	-73.3049	-124.918	-39.2485	20.9445	-3.49996	0.00047	-3.90249
Elevation:Imperviousness	-0.00027	-0.00044	-0.00013	7.93E-05	-3.34629	0.00082	-3.8565
lat:Elevation	0.004873	0.002497	0.007868	0.00135	3.610439	0.00031	3.720908
Elevation	-0.1585	-0.26241	-0.07577	0.046851	-3.38301	0.00072	-3.55856
Precipitation	0.00533	0.001752	0.009342	0.001914	2.784664	0.00536	3.520709
SpeciesStriped Skunk	-78.5665	-133.088	-41.7943	22.4632	-3.49757	0.00047	-3.4664
SpeciesStriped Skunk:Temperature	4.741659	2.589425	8.075528	1.333282	3.556382	0.00038	3.238648
SpeciesStriped Skunk:knn.dist	-0.00016	-0.00029	-8.4E-05	5.27E-05	-3.03961	0.00237	-3.23276
Imperviousness	0.11912	0.034926	0.213466	0.044956	2.649723	0.00806	2.827399
AgeJuvenile	-8.02765	-13.8458	-2.39898	2.872082	-2.79506	0.00519	-1.82368

DISCUSSION

This study demonstrated significant genetic diversity in CDV H-gene sequences among wild mesocarnivores in the southeastern US that broadly separated into groups east and west of the Mississippi River. Additionally, a generalized linear model for wild mesocarnivores diagnosed with canine distemper showed that surface imperviousness, precipitation and species were significant positive explanatory variables whilst elevation

and juvenile age had a significant negative association with the likelihood of the animal having canine distemper.

The phylogenetic analysis revealed significant diversity in the H gene sequence of CDV isolates, which appeared to form regional clusters. This observation is supported in the literature that describes significant genetic diversity in CDV isolates from wild carnivores in North America (Anis et al. 2020) and virus lineages formed regional clusters (Anis et al. 2018). In our study, the phylogenetic analysis of CDV isolates west of the Mississippi River showed a very distinct cluster from those from wild mesocarnivores in states east of the Mississippi River. The vast majority of isolates from NC, GA and FL clustered closely within the phylogenetic tree. Isolates from more western states, AR and MO, clustered separately from these eastern isolates and in a less clearly defined group. This suggests that all the eastern isolates are closely related and given that the states of GA, FL and NC are in close geographic proximity, may have originated from a large regional outbreak. Secondly, the Mississippi River forms a much more difficult barrier for the virus to traverse than smaller rivers. This is consistent with virus transmission, as it is not transmitted by vectors, birds or airborne transmission, so would need to be brought across the river by an infected mammal. This may have resulted in distinct strains evolving on each side of the river. The Mississippi River as a barrier to disease dispersal in wildlife has been demonstrated for rabies (Kuzmina et al. 2013). However, based on our study and others, it is not an impenetrable barrier, as there was one GA isolate and one NC isolate that clustered with isolates from MO. This suggests that at some point in time, these viruses spread across the river, whether through

an infected wild mammal swimming or using a bridge, inside a truck, or perhaps via an infected dog or even via a fomite.

The Ripley's K analysis showed more spatial clustering of canine distemper cases compared to the negative cases particularly at larger distances. This follows the ecology of the disease as it is a directly transmitted pathogen so an outbreak spreads and is maintained through an in-contact, susceptible population, resulting in case clustering. There is generally some spatial autocorrelation in the data at shorter distances, which is to be expected given the passive nature of the sample collection. In addition, citizens reporting and/or biologists submitting cases likely have a radius within which they operate with a gap distance until the next person, resulting in this clustering pattern.

Surface imperviousness is a significant explanatory variable in the model with a positive relationship between increased imperviousness and likelihood of being diagnosed with canine distemper. This is the most important result of the GLM in light of study objectives, suggesting that land use plays a role in the disease ecology of distemper. In general, higher surface imperviousness corresponds to areas of greater human development (Sutton et al. 2009). There are several reasons why these areas result in a higher likelihood of CDV in wild mesocarnivores. Urban areas often possess abundant resources for anthropophilic species, such as the raccoon (Bozek et al. 2007). These resources may be less prone to seasonal fluctuations and include supplemental food sources (e.g., household waste, supplemental outdoor feeding of cats or wildlife) as well as shelter. As a result, urban and suburban areas are capable of supporting much greater raccoon population densities (Prange et al. 2003) than more natural habitats. 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 or deliberate food placed for feral cats or other wildlife results in two factors that are of importance in disease transmission; migration of individuals into the area and exceptionally high intra- and interspecific contact rates (Becker et al. 2015). Contact rates play a vitally important role in disease transmission with higher population density resulting in greater contact rates and consequently greater rates of pathogen transmission (Hu et al. 2013). One study showed that higher population density in response to resource availability resulted in higher parasite richness and increased prevalence of the zoonotic nematode *Baylisascaris procyonis* in raccoons (Wright and Gompper 2005). A study by Hwang et al. revealed higher prevalence of severe fever with thrombocytopenia syndrome virus antibodies in urban dwelling feral cats than in their rural counterparts (Hwang et al. 2017). Canine distemper cases are more prevalent in urban and suburban counties than in rural counties in parts of the US, which support a much lower population density of raccoons (Taylor et al. 2021). Finally, the question of how the quality of more urbanized diets (Schulte-Hostedde et al. 2018) affects the immune response of these individuals and whether this may also result in increased pathogen shedding and disease susceptibility. Theoretical studies also have shown that resource provisioning can have significant effects on pathogen prevalence in urban environments (Becker and Hall 2014). Prevalence of directly transmitted parasites was shown to respond more strongly to provisioning, which has direct consequences on out study system, as CDV is a directly transmitted pathogen (Erazo et al. 2022). 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, as CDV spillover from dogs into wildlife is often reported (Gowtage-Sequeira et al. 2009, Bianco et al. 2020).

The model also revealed a significant interaction between imperviousness and elevation, with a negative relationship between these factors and CDV infection. This is likely because impervious surfaces at high elevation correspond to stony surfaces in more natural vs. developed areas. This implies that as a single variable, elevation is negatively associated with CDV infection (i.e., wild mesocarnivores at higher elevations are less likely to contract CDV infection). As previously discussed, this is a density dependent disease with higher population densities producing larger outbreaks and being more able to sustain outbreaks. At higher elevations, population densities of mesocarnivores tend to be significantly lower for a number of reasons, such as less suitable habitat, harsher environmental conditions (e.g., temperature), and less food availability (Slate et al. 2020). Increased precipitation also had a significant positive association with CDV infection. This can be explained from both the standpoint of the virus and the host. Higher rainfall may increase humidity within the study area, which in turn may facilitate aerosol transmission, as with CDV (Lowen et al. 2007). Additionally, increased humidity may prolong survival of the virus on fomites, allowing transmission to new susceptibles (Boone and Gerba 2007). From the host behavioral standpoint, increased precipitation may lead to increased denning behaviors, which could result in increased CDV transmission.

While species was significantly associated with distemper diagnosis in our model, the size of the error in these cases warrants caution in this interpretation and a larger data set is needed to test this hypothesis. Because our data were subdivided by species, the

sample sizes were small for foxes and skunks (N<40). Animal age class, specifically juvenile, showed a negative association with CDV infection. This may be due to maternal antibodies lessening susceptibility to disease (Junge et al. 2007), or increased likelihood that this young age group would be killed by other means (e.g., vehicular trauma), which may skew the data.

The results of our 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 earlier identification of outbreaks. From the standpoint of developing a future surveillance system, while the more specific landcover data did improve the model, as a whole the individual landcover types were not significant explanatory factors. Perhaps for a streamlined system of risk evaluation, the imperviousness data would be more prudent to use. It may even be possible in these urban areas to instigate a citizen 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 that threaten more vulnerable mammal species.

Limitations

The limitations of this study mostly apply to sampling biases. The samples were collected passively through submissions to SCWDS by state wildlife agencies and federal government partners (e.g., U.S. Fish & Wildlife Service), mostly across the southeastern US. This is dependent on a number of factors; a dead or ill animal being reported to the authorities (often by concerned members of the public) or being seen by them and a willingness to submit for necropsy, undoubtedly excluding large numbers of affected

animals that are either not reported, or not seen (e.g., in more remote or natural habitats). This also leads to a number of potential areas of sampling bias, with more populated areas and areas with state/national parks and wildlife refuges 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 similarly from a clinical standpoint. As such, cases commonly are assessed for both viruses (i.e., CDV and rabies virus), with occasional documentation of coinfection (Jardine et al. 2018). Additionally, the raster (i.e., land cover) data from NLCD is from 2019, which is before most of our samples were collected; however, the landcover is unlikely to have changed much in this timeframe, particularly with the economic impact of the COVID-19 pandemic, the effects of this time lag are likely to be minimal.

Finally, whilst the GLM and phylogenetic tree may indicate some potential relationships between land use and CDV in the case of the GLM, and geographically in the case of the tree, neither model had very strong statistical support, both in the 0.60-0.70 range. Testing of these hypotheses with a larger and more comprehensive dataset would be an important next step in attempting to reveal more about these potential relationships and developing models with stronger support.

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 shows that there is significant CDV genetic diversity in the region, particularly divided by the

Mississippi River. Secondly, human land use may play an important role in the disease ecology of this virus, with wild carnivores in areas of intense human development at higher risk for CDV infection. Land use change is a complex challenge 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 (Wilkes 2023), particularly regarding diseases in synanthropic species also can play a crucial role in dynamics of wildlife disease in urban environments.

Acknowledgments

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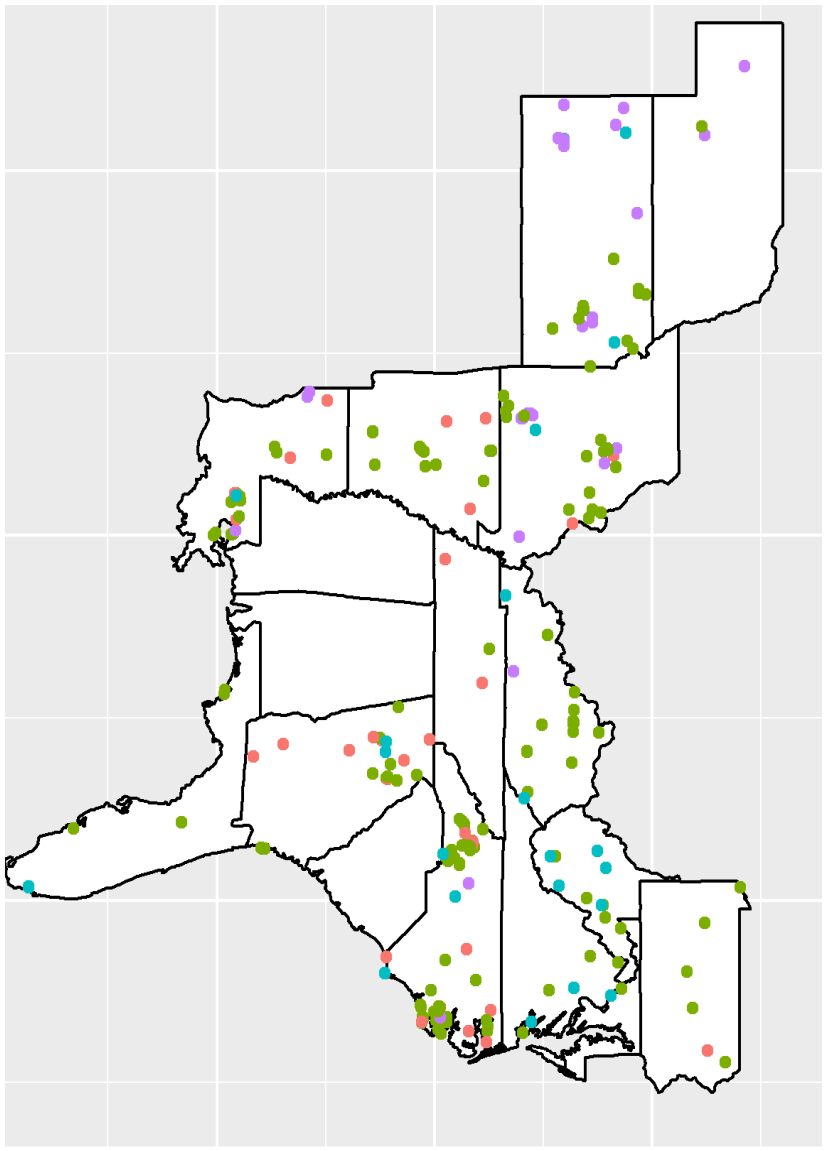
SUPPLEMENTARY MATERIALS



Supplementary Figure 5.1: Map of distribution of mesocarnivore necropsy cases submitted to the Southeastern Cooperative Wildlife Disease Study from January 2019 to December 2022, coded by species.

National Land Cover Database Class Legend and Description

Class\ Value	Classification Description
Water	
11	**Open Water**- areas of open water, generally with less than 25% cover of vegetation or soil.
12	**Perennial Ice/Snow**- areas characterized by a perennial cover of ice and/or snow, generally greater than 25% of total cover.
Developed	
21	**Developed, Open Space**- areas with a mixture of some constructed materials, but mostly vegetation in the form of lawn grasses. Impervious surfaces account for less than 20% of total cover. These areas most commonly include large-lot single-family housing units, parks, golf courses, and vegetation planted in developed settings for recreation, erosion control, or aesthetic purposes.
22	**Developed, Low Intensity**- areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 20% to 49% percent of total cover. These areas most commonly include single-family housing units.
23	**Developed, Medium Intensity** -areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 50% to 79% of the total cover. These areas most commonly include single-family housing units.
24	**Developed High Intensity**-highly developed areas where people reside or work in high numbers. Examples include apartment complexes, row houses and commercial/industrial. Impervious surfaces account for 80% to 100% of the total cover.
Barren	
31	**Barren Land (Rock/Sand/Clay)** - areas of bedrock, desert pavement, scarps, talus, slides, volcanic material, glacial debris, sand dunes, strip mines, gravel pits and other accumulations of earthen material. Generally, vegetation accounts for less than 15% of total cover.
Forest	
41	**Deciduous Forest**- areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. More than 75% of the tree species shed foliage simultaneously in response to seasonal change.
42	**Evergreen Forest**- areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. More than 75% of the tree species maintain their leaves all year. Canopy is never without green foliage.
43	**Mixed Forest**- areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. Neither deciduous nor evergreen species are greater than 75% of total tree cover.

Shrubland	
51	**Dwarf Scrub**- Alaska only areas dominated by shrubs less than 20 centimeters tall with shrub canopy typically greater than 20% of total vegetation. This type is often co-associated with grasses, sedges, herbs, and non-vascular vegetation.
52	**Shrub/Scrub**- areas dominated by shrubs; less than 5 meters tall with shrub canopy typically greater than 20% of total vegetation. This class includes true shrubs, young trees in an early successional stage or trees stunted from environmental conditions.
Herbaceous	
71	**Grassland/Herbaceous**- areas dominated by gramanoid or herbaceous vegetation, generally greater than 80% of total vegetation. These areas are not subject to intensive management such as tilling, but can be utilized for grazing.
72	**Sedge/Herbaceous**- Alaska only areas dominated by sedges and forbs, generally greater than 80% of total vegetation. This type can occur with significant other grasses or other grass like plants, and includes sedge tundra, and sedge tussock tundra.
73	**Lichens**- Alaska only areas dominated by fruticose or foliose lichens generally greater than 80% of total vegetation.
74	**Moss**- Alaska only areas dominated by mosses, generally greater than 80% of total vegetation.
Planted/Cultivated	
81	**Pasture/Hay**-areas of grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops, typically on a perennial cycle. Pasture/hay vegetation accounts for greater than 20% of total vegetation.
82	**Cultivated Crops** -areas used for the production of annual crops, such as corn, soybeans, vegetables, tobacco, and cotton, and also perennial woody crops such as orchards and vineyards. Crop vegetation accounts for greater than 20% of total vegetation. This class also includes all land being actively tilled.
Wetlands	
90	**Woody Wetlands**- areas where forest or shrubland vegetation accounts for greater than 20% of vegetative cover and the soil or substrate is periodically saturated with or covered with water.
95	**Emergent Herbaceous Wetlands**- Areas where perennial herbaceous vegetation accounts for greater than 80% of vegetative cover and the soil or substrate is periodically saturated with or covered with water.

Supplementary Table 5.1: National Land Cover Database Class Legend and Description. *Detailed descriptions of landcover classes designated to each pixel of the national land cover database for the United States. Original source: https://www.mrlc.gov/data/legends/national-land-cover-database-class-legend-and-description*

Supplementary Table 5.2: List of CDV partial H-gene isolates generated in wild mesocarnivores diagnosed with canine distemper at the Southeastern Cooperative Wildlife Disease Study from January 2019 to December 2022 and their sequence quality metrics.

Name	% GC	% HQ	% Identical Sites	% LQ	% MQ	% Pairwise Identity	At least Q20	At least Q30	At least Q40	Mean Coverage	Sequence Length
027A_Raccoon_NC	44.00%	79.20%	99.80%	9.30%	11.50%	99.80%	90.70%	83.44%	79.19%	1.707965	1129
069A_Skunk_MO	41.90%	92.70%	95.30%	2.00%	5.40%	95.40%	98.04%	96.16%	92.69%	1.268032	1121
169A_Raccoon_FL	43.90%	95.00%	99.10%	1.70%	3.30%	99.10%	98.31%	96.17%	95.01%	1.902852	1122
317A_Gray_Fox_NC	43.70%	92.80%	99.60%	3.20%	4.00%	99.60%	96.81%	94.85%	92.81%	1.631766	1127
383A_Gray_Fox_AR	42.50%	95.10%	99.10%	1.60%	3.30%	99.10%	98.40%	96.62%	95.11%	1.911922	1124
408A_Gray_Fox_NC	43.90%	94.20%	99.80%	1.70%	4.10%	99.80%	98.32%	96.45%	94.24%	1.757092	1128
439A_Skunk_MO	42.90%	90.10%	95.20%	1.30%	8.60%	95.30%	98.74%	94.97%	90.13%	1.298923	1114
440A_Skunk_MO	42.70%	93.90%	99.60%	1.20%	4.90%	99.60%	98.84%	96.17%	93.94%	1.654189	1122
575A_Raccoon_NC	43.40%	96.20%	99.20%	1.00%	2.80%	99.20%	99.02%	97.32%	96.25%	1.780161	1119
762A_Gray_Fox_GA	44.00%	95.70%	99.50%	1.40%	2.80%	99.50%	98.58%	97.33%	95.73%	1.9065	1123
776A_Raccoon_GA	42.10%	94.60%	96.10%	1.50%	3.90%	96.10%	98.49%	96.98%	94.58%	1.452529	1125
819A_Gray_Fox_NC	44.50%	89.90%	92.80%	1.60%	8.50%	93.30%	98.35%	94.42%	89.85%	1.178245	1094
900A_Raccoon_NC	43.50%	92.60%	96.60%	3.20%	4.20%	96.70%	96.79%	94.21%	92.61%	1.577265	1123
940A_Raccoon_NC	43.30%	92.50%	98.60%	3.60%	3.90%	98.70%	96.38%	94.44%	92.50%	1.383936	1133
973A_Raccoon_FL	43.50%	96.40%	98.90%	1.30%	2.20%	98.90%	98.66%	97.41%	96.42%	1.740608	1118
979A_Raccoon_MO	41.90%	95.50%	99.00%	1.60%	2.90%	99.00%	98.39%	96.60%	95.53%	1.438283	1118
A116A_Raccoon_NC	43.60%	94.00%	95.60%	1.90%	4.10%	95.70%	98.13%	95.91%	94.05%	1.618037	1126
B116A_Raccoon_NC	43.80%	95.20%	97.70%	1.50%	3.30%	97.70%	98.49%	96.81%	95.21%	1.579646	1128
W004A_Raccoon_MO	42.40%	96.00%	98.10%	1.50%	2.50%	98.10%	98.49%	97.24%	96.00%	1.963523	1124
W014A_Raccoon_NC	43.90%	92.80%	98.10%	2.70%	4.40%	98.10%	97.26%	94.87%	92.83%	1.552608	1130
W015A_Raccoon_NC	44.00%	94.60%	97.10%	1.50%	3.90%	97.20%	98.48%	96.70%	94.55%	1.528571	1120
W016A_Raccoon_NC	43.50%	96.60%	99.50%	1.30%	2.00%	99.60%	98.67%	97.42%	96.62%	1.949288	1124
W050A_Raccoon_NC	43.80%	82.70%	99.90%	9.80%	7.50%	99.90%	90.18%	86.31%	82.70%	1.772072	1110
W062A_Raccoon_NC	43.50%	95.80%	98.80%	1.00%	3.20%	98.80%	99.02%	97.42%	95.82%	1.811388	1124
W089A_Raccoon_NC	43.60%	97.40%	98.10%	1.50%	1.10%	98.10%	98.49%	98.04%	97.42%	1.943111	1125

W092A_Skunk_NC	43.60%	94.80%	100.00%	1.40%	3.80%	100.00%	98.57%	97.23%	94.82%	1.719392	1119
W102A_Raccoon_MO	42.00%	95.90%	97.10%	1.20%	2.90%	97.20%	98.84%	97.24%	95.91%	1.696	1125
W135A_Raccoon_NC	44.10%	93.50%	99.50%	3.00%	3.50%	99.50%	96.97%	95.10%	93.49%	1.842246	1122
W144A_Raccoon_NC	43.40%	85.30%	98.10%	5.10%	9.50%	98.10%	94.87%	90.29%	85.34%	1.802336	1112
W153A_Raccoon_MO	42.20%	95.60%	96.20%	1.20%	3.10%	96.20%	98.75%	97.06%	95.64%	1.820285	1123
W738A_Raccoon_NC	44.00%	93.20%	98.80%	2.20%	4.60%	98.80%	97.80%	94.63%	93.22%	1.45022	1135
W791A_Raccoon_NC	42.20%	92.20%	99.80%	3.10%	4.60%	99.80%	96.88%	93.94%	92.25%	1.786477	1122

Supplementary Table 5.3: Full list of models and summary statistics included in GLM fitting process

	Residual Deviance	Residual d.f	AIC	deltaAIC	AIC weight
Global Model	179.05	173	237.05	87.46	1.02×10^{-19}
Interactions					
+Species:knn.dist	155.84	170	219.84	70.25	5.56×10^{-16}
+Species:Temperature	136.42	167	206.42	56.83	4.57×10^{-13}
+Latitude:Elevation	123.28	166	195.28	45.69	1.20×10^{-10}
+Elevation:Imperviousness	110.59	165	184.6	35.01	2.50×10^{-8}
+Age:Month	101.37	163	179.26	29.78	3.41×10^{-7}
Backwards selection					
-Longitude	101.48	164	177.48	27.89	8.79×10^{-7}
-Sex	101.95	167	175.95	26.36	1.89×10^{-6}
-Distance to water	103.17	168	175.17	25.58	2.79×10^{-6}
Remove outlying data (x3)	77.59	165	149.59	-	-
Best Fit Model	77.59	165	149.59	-	-

Supplementary Table 5.4: Full list of explanatory variables from best fit model with summary statistics ranked in order of p-value from most to least significant.
Note: The glm function in R uses a technique called "dummy coding" to convert categorical variables into a set of binary variables, also known as "indicator variables" or "dummy variables". This is done so that the categorical variable can be included in the model as a predictor. When a categorical variable is used in a model, it is split into one binary variable for each level of the categorical variable, with a value of 1 indicating membership in that level, and a value of 0 indicating non-membership. The summary function then displays each of these binary variables as a separate factor in the output. This allows the user to see the effect of each level of the categorical variable on the response variable.

	Estimate	2.50%	97.50%	Std. Error	z value	Pr(>|z|)	std_coef
SpeciesRaccoon:Temperature	4.296854	2.370153	7.260268	1.182571	3.633485	0.00028	3.978035
SpeciesRaccoon	-73.3049	-124.918	-39.2485	20.9445	-3.49996	0.000465	-3.90249
Elevation:Imperviousness	-0.00027	-0.00044	-0.00013	7.93E-05	-3.34629	0.000819	-3.8565
lat:Elevation	0.004873	0.002497	0.007868	0.00135	3.610439	0.000306	3.720908
Elevation	-0.1585	-0.26241	-0.07577	0.046851	-3.38301	0.000717	-3.55856
Precipitation	0.00533	0.001752	0.009342	0.001914	2.784664	0.005358	3.520709
SpeciesStriped Skunk	-78.5665	-133.088	-41.7943	22.4632	-3.49757	0.00047	-3.4664
SpeciesStriped Skunk:Temperature	4.741659	2.589425	8.075528	1.333282	3.556382	0.000376	3.238648
SpeciesStriped Skunk:knn.dist	-0.00016	-0.00029	-8.4E-05	5.27E-05	-3.03961	0.002369	-3.23276
Imperviousness	0.11912	0.034926	0.213466	0.044956	2.649723	0.008056	2.827399
Temperature	-2.76154	-5.86262	-0.57162	1.287085	-2.14558	0.031907	-2.48958
descriptionDeveloped, Medium Intensity	-6.04146	-11.8222	-0.78923	2.778377	-2.17446	0.029671	-2.48655
descriptionDeveloped, Low Intensity	-3.66313	-7.44737	-0.11401	1.847468	-1.98279	0.047391	-2.45429
AgeJuvenile	-8.02765	-13.8458	-2.39898	2.872082	-2.79506	0.005189	-1.82368
AgeJuvenile:month	0.856084	0.162105	1.579651	0.356134	2.403827	0.016224	1.737953
Month	-0.11836	-0.318	0.073871	0.098538	-1.20115	0.229691	-1.67541
descriptionMixed Forest	16.69947	4.56977	39.28099	10.46531	1.595698	0.110556	1.557538
Lat	0.569104	-0.41372	1.642417	0.517027	1.100724	0.271017	1.41443
SpeciesRaccoon:knn.dist	-2.6E-05	-8.3E-05	3.73E-05	2.93E-05	-0.87108	0.383713	-1.1827
descriptionEvergreen Forest	-3.95826	-10.2537	1.268413	3.050739	-1.29748	0.194467	-1.12189
descriptionDeveloped, Open Space	-0.69567	-3.31331	1.886063	1.303719	-0.53361	0.593614	-0.56971
(Intercept)	21.30329	-49.1478	98.13981	36.93669	0.576751	0.564107	0.520499
SpeciesRed Fox	-102.014	-103.629	-100.372	87.06704	-1.17167	0.24133	-0.51378
descriptionDeveloped, High Intensity	-3.15043	-11.5119	4.865918	4.13097	-0.76264	0.445679	-0.51183

SpeciesRed Fox:Temperature	4.956784	4.836163	5.076222	6.420792	0.77199	0.440121	0.467403
descriptionDeciduous Forest	-0.41913	-3.55563	2.766624	1.584585	-0.2645	0.791393	-0.34728
descriptionPasture/Hay	12.49099	0.790716	6.343992	17.43858	0.716285	0.473816	0.183449
AgeSubadult:month	-3.2532	-9.24064	-0.15731	3.943849	-0.82488	0.409441	-0.15699
AgeSubadult	32.36474	1.312872	97.0145	43.01389	0.752425	0.451795	0.147526
descriptionWoody Wetlands	-3.24403	-8.40186	1.318706	2.400264	-1.35153	0.176525	0.143006
knn.dist	-1.1E-06	-6.4E-05	5.47E-05	2.89E-05	-0.03921	0.968719	0.121688
SpeciesRed Fox:knn.dist	1.67E-06	-1E-06	4.39E-06	0.000139	0.012031	0.990401	-0.07871
descriptionGrassland/Herbaceous	-24.3637	-1154.67	176.2917	3956.198	-0.00616	0.995086	-0.00206
descriptionOpen Water	15.08734	-376.435	NA	2796.828	0.005394	0.995696	0.001376
descriptionScrub/Shrub	17.26584	-781.423	NA	3956.181	0.004364	0.996518	0.001112
descriptionEmergent Herbaceous Wetlands	-15.1971	NA	782.3874	3956.181	-0.00384	0.996935	-0.00103

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CHAPTER 6

CONCLUSIONS

The devastation caused by the COVID-19 pandemic illustrates the threat posed by cross species transmission of RNA viruses. Multihost pathogens and interspecies pathogen transmission are complex fields of study as demonstrated by the scope of this dissertation. Understanding the dynamics of these pathogens and the roles played by humans can help alleviate some of the risks posed. Our research has advanced the understanding of the ecological and evolutionary dynamics and risks factors and the role of pathogenesis in cross species viral transmission, with particular emphasis on canine distemper in wildlife.

The synthesis of cross species transmission events and pathogenesis raised two main points. Firstly, the analysis suggests that distinct patterns can be observed in the pathology data related to virus-host interaction, particularly in spillover events involving humans. In particular, viruses that cross species barriers tend to exhibit common pathogenic traits that are often associated with specific host types, for example, respiratory tropic viruses being transmitted between humans and other primates. These trends underscore the need for deeper research into the cellular and molecular pathology of cross species transmission events, particularly in under-studied wildlife, and for greater integration of this approach with disease ecology and epidemiology. Secondly, the study emphasizes the insufficient availability of precise and comprehensive virus-host pathogen interaction data in multiple host species, particularly at the cellular and

subcellular levels. It underscores the importance of increased collaborations and concentrated efforts among the scientific community to identify receptors and pathogenic pathways that may signal spillover risk. By shedding light on the potential role of pathogenesis in cross species transmission, this research may enable the incorporation of such data into predictive frameworks of spillover risk.

The study on viral epizootics demonstrated that the highest transmissibility and genetic diversity occur at different stages of the epizootic and are not synchronized with the peak prevalence of infection. This finding is significant because it indicates that the highest risk of a cross species transmission event occurs at different times during the epizootic, with high transmissibility in the early stages being a risk factor for closely related species, and high genetic diversity in the later stages being a risk factor for less closely related species. Moreover, the study suggests that viral populations may adopt a bistable strategy, wherein they either evolve into a moderately transmissible population with a slower epizootic or become maximally transmissible with a very rapid epizootic, depending on certain conditions.

The case study of CDV in wildlife indicates that CDV infection is widely prevalent in the southeastern US, as it has been diagnosed in nine different carnivore species across 17 states. The most commonly diagnosed species with canine distemper were raccoon and gray fox, with other diagnosed species generally found in the same or adjacent counties as those with raccoon or gray fox cases. Further analysis in the most represented state, Georgia, revealed distinct temporal and spatial patterns of CDV cases in raccoons and gray foxes, with a higher likelihood of cases occurring during the breeding season. Spatially, there were clusters of cases for both species in the same

suburban areas. The study also showed that past cases in foxes and raccoons can predict the number of gray fox and raccoon cases. Raccoons, being easier to capture and sample, may serve as a useful predictor for less accessible species among wild carnivores. The final chapter draws two main conclusions. Firstly, it presents additional evidence of the widespread occurrence of CDV infection in wild mesocarnivores in the southeastern US and highlights the significant genetic diversity of CDV in the region, especially on either side of the Mississippi River. Secondly, the study suggests that human land use may be a crucial factor in the disease ecology of CDV, with wild carnivores in areas of high human development facing a greater risk of CDV infection and disease. Ultimately, there were sufficient potential relationships suggested from the passively collected data used in these two studies to support the recommendation of targeted active surveillance, including comprehensive sampling of wild carnivores over time and space to elucidate infection trends among species, particularly in urban and suburban areas.

The results presented here provide another piece to the puzzle that is cross species pathogen transmission and stimulate future research questions.

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