Evaluating the Prevalence of Tick-Borne Viruses Circulating in Virginia Using a One-Health Approach

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Abstract

Ticks are hematophagous ectoparasites capable of transmitting various pathogens, including bacteria, protozoa, and viruses, to vertebrates. In the United States, tick-borne pathogens are responsible for around 95% of arthropod-borne diseases. Lyme disease is the most common tick-borne illness. However, emerging tick-borne viruses such as Bourbon virus (BRBV), Powassan virus (POWV), and Heartland virus (HRTV) can cause more severe health problems, including death and neurological abnormalities. The reports of molecular detection of viral RNA in field-collected ticks and serological evidence in a pilot study of wildlife species suggest the presence of these emerging viruses in Virginia. The presence poses a serious health threat, but the extent of their presence or circulation in Virginia is unknown. The objectives of the research are (1) to determine the evidence of circulation of POWV, HRTV, and BRBV in Virginia through serological assessment of domestic and wild animals in Virginia and (2) estimate transmission parameters and the basic reproduction number underlying tick-borne virus distribution and prevalence via a mathematical model. Here, we discuss the known literature relevant to tick-borne virus emergence; we assessed the presence of specific neutralizing antibodies against POWV, HRTV, and BRBV in wildlife and livestock sera collected from different health planning regions in Virginia. We used a susceptible-infected-susceptible (SIS) ordinary differential equation model to estimate transmission parameters that best describe the disease dynamics of emerging tick-borne viruses in Virginia. In our study, wildlife sera were seropositive against POWV (18%), BRBV (8%), and HRTV (5%). A wide range of different wildlife species were shown to be exposed to each virus examined. Livestock are also exposed to tick-borne viruses, with seroprevalences of 1%, 1.2% and 8% detected in cattle for POWV, BRBV and HRTV, respectively. We estimated the transmission rate and basic reproduction number to be 1.57 and 0.645 respectively. In conclusion,

there is a widespread circulation of tick-borne viruses in western and northern Virginia within diverse species of animal populations.

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General Audience Abstract

Ticks are blood-sucking ectoparasites that can transmit various pathogens, including bacteria, protozoa, and viruses, to humans and other vertebrates. In the United States, tick-borne pathogens are responsible for about 95% of all arthropod-borne disease cases. Lyme disease is the most common tick-borne illness. However, emerging tick-borne viruses such as Bourbon virus (BRBV), Powassan virus (POWV), and Heartland virus (HRTV), can cause more severe health problems, including potentially death or neurological abnormalities. The reports of molecular detection of viral RNA in field-collected ticks and serological evidence in a pilot study of wildlife species suggest the presence of these emerging viruses in Virginia. However, there is a lack of knowledge on the extent of their circulation. Firstly, this study aims to determine the evidence of circulation of POWV, HRTV, and BRBV in Virginia through serological assessment of domestic and wild animals. Secondly, this study aims to estimate transmission parameters and calculate the basic reproduction number of emerging tick-borne viruses. Evidence of prior infection against all three tick-borne viruses was detected in both wild and domestic animal species from the five Virginia health planning regions, with most samples in the study coming from southwestern and northwestern regions. In conclusion, there is a circulation of tick-borne viruses in Virginia, which is a potential threat to the public health.

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Chapter 1

Literature Review of Emerging Tick-Borne Viruses in Virginia

1.0. General Introduction

Emerging infectious diseases (EID) are newly identified infectious diseases in a population or previously known infectious diseases that are rapidly increasing in incidence or geographic range (McArthur, 2019; Morens et al., 2004). EID pose a serious health concern worldwide and when they spread from one geographical region to another, there can be severe consequences on human and animal health. Drivers of pathogen emergence include human and animal transportation, land-use change, drug-resistance, and microbial adaptation (Chala & Hamde, 2021; Jones et al., 2008; McArthur, 2019; Morens et al., 2004). Despite improved preventive approaches in endemic and susceptible areas, the emergence of infectious diseases remains largely unpredictable. Prominent EID in recent years include Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS), coronavirus disease (COVID-19), Ebola virus disease, Lassa fever, Rift valley fever, Crimean Congo Hemorrhagic fever, Nipah fever, Zika virus disease (Chala & Hamde, 2021; Jones et al., 2008; Morens et al., 2004; Otte & Pica-Ciamarra, 2021). Most EID are zoonotic, primarily caused by pathogens of animal origins and transmitted either directly between animal and human, or indirectly via ingestion of pathogen-contaminated food, water, or bite from an arthropod vector (Jones et al., 2008; Otte & Pica-Ciamarra, 2021). Given this, the emergence of novel zoonotic pathogens encourages the adoption of different methods to reduce the overall disease burden, such as the One-Health approach.

The One-Health approach aims to understand underlying factors from different disciplines integrating human, animal, and environmental health to provide epidemiological and ecological knowledge about emerging diseases including vector-borne disease (Leifels et al., 2022). Globally, vector-borne diseases (VBD) constitute a major health threat responsible for more than 700,000 deaths annually (Breedlove, 2022). In the United States (US), VBD are responsible for

significant morbidity and mortality (Beard et al., 2019). Over the last two decades, the number of human cases has increased due to the geographical expansion of competent arthropod vectors and the detection of new pathogens. For example, a total of 642,602 human cases of 16 different VBD diseases, caused by bacteria, viruses, or parasites transmitted through the bites of mosquitoes, ticks, or fleas, were reported to the Centers for Disease Control and Prevention (CDC) between 2004 - 2016. Specifically, nine VBD were reported for the first time from the US and US territories during the same period. Moreover, tick-borne diseases more than doubled in the 13 years and represented 77% of all vector-borne disease reported. Lyme disease accounted for 82% of all tick-borne cases, with increases also observed in other tick-borne diseases such as babesiosis, anaplasmosis, spotted fever rickettsioses and ehrlichiosis (Rosenberg et al., 2018; Rodino et al., 2020; Chala & Hamde, 2021).

New or re-emerging mosquito-borne pathogens such as Zika virus, chikungunya virus, and dengue virus have been reported in a series of travel-related cases in the US. However, autochthonous transmission currently remains rare (Rosenberg et al., 2018). Whether the increase in VBD statistics are due to better reporting or a true increase in incidence, this increment of VBD in the US poses a burden on the nation's healthcare (Madison-Antenucci et al., 2020; Tsao et al., 2021; Diuk-Wasser et al., 2021). Efforts to control VBD in the US has been frustrated by lack of licensed vaccines, no specific therapy and vector range expansion (Beard et al., 2019).

Despite the presence of pathogen competent mosquitoes in the US such as *Aedes aegypti*, ticks represent primary importance as an arthropod vector (Eisen et al., 2017; Rosenberg et al., 2018), being responsible for 77%-97% of annually reported vector-borne disease cases (Eisen et al., 2017) with Lyme disease (*caused by Borrelia burgdorferi*) being the most prevalent (Nelson et al., 2015; Rochlin & Toledo, 2020). Notable ticks of public health concern in the US are *Amblyomma americanum*, *A. maculatum*, *Dermacentor andersoni*, *D. occidentalis, D. variabilis*, *Ixodes scapularis*, *I. pacificus* and *Rhipicephalus sanguineus sensu lato* (Eisen et al., 2017).

Apart from favorable abiotic conditions, the distribution and abundance of ticks within a geographic area are greatly influenced by the availability of suitable hosts (Tsao et al., 2021). Ticks feed on a wide range of vertebrate hosts, once for each different stage of their life cycle. Hosts include mammals of all sizes, which are consequently continuously exposed to ticks and tick-borne pathogens (Mlera & Bloom, 2018). While some tick species have specific host preferences, others are generalists that feed opportunistically on diverse vertebrate species throughout their life cycle. Due to the parasitic relationship between ticks and their host, the composition of a wildlife community can impact the species of ticks and associated tick-borne pathogens prevalent in a community (Tsao et al., 2021). For example, *I. scapularis* (the blacklegged tick) and *A. americanum* (the lone star tick) are often found in areas with high deer populations (Paddock & Childs, 2003; Paddock & Yabsley, 2007). In the absence of preferred host access, ticks may utilize a variety of other mammalian hosts.

Host-seeking behaviors promote tick survival and expansion in new areas, as was reported in Eastern Virginia, where many medium-sized mammals served as primary hosts to emerging *A. maculatum* (Paddock & Goddard, 2015). A wide range of mammals served as hosts to a tick that is recently invasive to the US, *Haemaphysalis longicornis*, in areas with low livestock densities (Nadolny & Gaff, 2018; Tufts et al., 2019; US Department of Agriculture, 2023). Aside from serving as a blood-source, vertebrate hosts play a role in the transmission of tick-borne pathogens from an infected tick to an uninfected tick, either by acting as a reservoir host (systemic transmission) or facilitating co-feeding (non-systemic transmission). As a reservoir host, a vertebrate host usually maintains systemic infection (viremia) and serve as an infection source for naïve ticks feeding on them (Ashford, 2003). On the other hand, a vertebrate host could also provide a suitable environment for pathogen transmission between an infected and an uninfected tick feeding in close proximity of each other on the same host (non-viremic) (Labuda et al., 1993, Gern & Rais, 1996). In general, ticks and tick-borne disease control remains a big challenge in the US.

Ticks as a vector are capable of transmitting various classes of pathogens to vertebrate hosts, including bacteria, protozoa, and viruses (Eisen et al., 2017; Rosenberg et al., 2018). Viruses transmitted by ticks are known as tick-borne viruses (TBVs), and these viruses are members of several families, including *Asfarviridae*, *Flaviviridae*, *Reoviridae*, *Orthomyxoviridae*, *Rhabdoviridae*, *Nyamiviridae*, *Peribunyaviridae*, *Phenuiviridae*, and *Nairoviridae* (Bartíková et al., 2017; Brackney & Armstrong, 2016; Kazimírová et al., 2017; Shi et al., 2018). There are around 160 named tick-borne viruses, all of which are RNA viruses, with the exception of African swine fever virus (*Asfarviridae*) (Bartíková et al., 2017; Brackney & Armstrong, 2016; Madison-Antenucci et al., 2020). Most of these viruses have no public health significance, with only about 25% of TBVs are associated with animal or human disease (Bartíková et al., 2017; Kazimírová et al., 2017). The efficiency of ticks as a vector of viruses has been attributed to their extended lifespan, consistent blood-feeding habits across all life stages, lengthy feeding periods, digestion of blood within midgut cells, and ability to maintain viral infection once acquired (Sonenshine et al., 2014, Nutall & Labuda, 2003).

This chapter continues by examining the existing research on emerging tick-borne viruses, with a focus on the Commonwealth of Virginia, USA. The attention is on three specific viruses: Heartland virus (HRTV), Bourbon virus (BRBV), and Powassan virus (POWV). Firstly, an overview of these viruses was discussed including their taxonomy, epidemiology, vector, and host associations. Then, methods of serological surveillance and what is currently known about tick-borne viruses in Virginia is mentioned. Lastly, this chapter addresses gaps in current research about the prevalence of these viruses in Virginia (indicated in Figure 1).

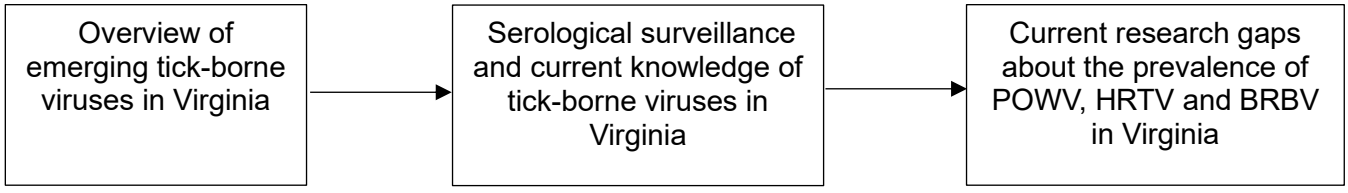

Figure 1: A flowchart showing progression of this literature review.

2.0. Powassan Virus (POWV)

2.1. Taxonomy, Virology and General Description of Powassan virus

Powassan virus (POWV) is an emerging tick-borne flavivirus (family *Flaviviridae*) that causes severe neurological damage and death (Ebel, 2010; Hermance & Thangamani, 2017; Kazimírová et al., 2017). The virus was first isolated in 1958, in Powassan, Ontario, Canada, from the brain of a five-year-old boy who died of encephalitis (McLean & Donohue, 1959). In the neighboring US, the first human case was reported in 1970 (in New Jersey; Goldfield et al., 1973). Currently, while rare, POWV is endemic to the northeastern and midwestern US and cases increasing (Hassett & Thangamani, 2021).

Other genera of *Flaviviridae* are *Pestivirus*, *Hepacivirus*, and *Pegivirus* (Simmonds et al., 2017). All members of *Flaviviridae* are positive-sense RNA viruses, characterized by unique morphological structures but different antigenic properties (Kemenesi & Bányai, 2019; Pierson & Diamond, 2020). The genus *Flavivirus* includes many virus species such as Zika virus, Yellow fever virus, dengue virus, and tick-borne encephalitis virus (Fatmi et al., 2017; Kemenesi & Bányai, 2019; Pierson & Diamond, 2020). The flavivirus virion is a small, spherical viral particle that consists of single-stranded, positive sense RNA of an average length of 11kb that encodes a single open reading frame (Hermance & Thangamani, 2017; Kemenesi & Bányai, 2019; Pierson & Diamond, 2020; Saeedi & Geiss, 2013). The 5’ end of the genome has a type 1 cap structure and possesses three structural protein: the capsid (C) protein which makes up the nucleocapsid, the pre-membrane (prM), which stabilizes the E protein in the immature virion, and the envelope (E) glycoprotein which mediates virus entry during replication ((Hermance & Thangamani, 2017; Kemenesi & Bányai, 2019; Pierson & Diamond, 2020; Rey et al., 1995).

The 3’ end of the genome possesses seven non-structural proteins and terminates in a stable-loop structure due to the absence of a poly (A) tail (Kemenesi & Bányai, 2019; Saeedi & Geiss, 2013). The non-structural proteins participate in protein processing and as effectors against host antiviral defense (Kemenesi & Bányai, 2019; Saeedi & Geiss, 2013). After successful

viral entry and replication, flavivirus have affinity to invade neural tissues resulting in neurological disease manifestation in severe cases, with long-term neurological sequelae in survivors.

2.2. Epidemiology, Vector, and Host Associations of Powassan Virus

Since the first detection of POWV in Canada, multiple human cases of POWV have been reported in the US, Canada, and Russia (Hassett & Thangamani, 2021). All human age groups are susceptible to POWV infection, however, it is more common in males and tend to be fatal in individual over the age of 50 years (Krul-Lucal et al., 2018; Hassett & Thangamani, 2021). Most reported cases in the US are from the northeastern states and the Great Lakes region. This geographical range coincide with areas where *Ixodes* spp. ticks are commonly found. Disease incidence is believed to peak from May to November when the tick vector is active. Recent increase in disease incidence in the US compared to the last two decades has been attributed to improved surveillance, improved diagnosis, geographical expansion of competent vectors, and increased human exposure (Fatmi et al., 2017). Despite being rare, POWV infection represents a higher public health risk than many other tick-borne diseases in the US because it has more serious symptoms, along with an 10% average case fatality rate; notably this virus could be transmitted within a short time (15min) followingtick attachment on a host. However, this is not the case for other tick-borne pathogens. For instance, *Borrelia burgdorferi* requires at least 24-48 hours after tick attachment to be transmitted to a host. (Fatmi et al., 2017, Hassett & Thangamani, 2021). The short pathogen transmission period of POWV results from tick salivary gland factor and the virus's presence in the salivary glands before tick-host attachment and blood acquisition, as opposed to other tick-borne pathogens that are usually summoned from the mid-gut.

POWV has two genetically distinct lineages or genotypes that are maintained in separate transmission cycles (Hermance & Thangamani, 2017). The prototype strain and POWV lineage I is thought to be maintained between *I. cookei* ticks and groundhog/skunk (Ebel, 2010; Main et al., 1979). POWV lineage II or deer tick virus (DTV) (Ebel, 2010; Telford et al., 1997) was first

identified in New England, isolated from *I. scapularis* approximately two decades after discovery of lineage I (Telford et al., 1997). Serologically, both lineages are indistinguishable (Beasley et al., 2001).

Both *I. scapularis* and *I. cookei* ticks have the potential to cause virus spillover to humans (Hassett & Thangamani, 2021) but based on host preference and geographical distribution, *I. scapularis* is a major vector for POWV transmission to humans than and *I. cookei*. The former is a generalist tick, feeding on a wide variety of vertebrates and humans, and has a broader geographical distribution while the latter is host specific, localized, and less exposed to humans (Ebel, 2010). Aside from *Ixodes* ticks, POWV has also been isolated from field-collected *D. variabilis* (Hart et al., 2023; Thomas et al., 1960). Spillover to non-*Ixodes* spp. tick species may arise via co-feeding due to ecological and host overlap between *Ixodes* and non-*Ixodes* tick species. Earlier research demonstrated that non-*Ixodes* human-biting ticks such as *A. americanum, H. longicornis*, and *D. variabilis*, are capable of vertical and horizontal transmission of POWV (Sharma et al., 2021). Nevertheless, natural evidence to support the vector competencies of non-*Ixodes* ticks in the transmission of POWV is yet to be adequately provided.

The coexistence of multiple pathogens in a tick vector can also pose an increased threat. Co-infected pathogens may synergistically suppress the host immune status or improve susceptibility or replication of other pathogens in ticks. For example, previous research has shown that infection with *Borrelia burgdorferi* (the etiological agent of Lyme disease) increases the replication and dissemination of coinfecting POWV in *I. scapularis* (Hassett & Thangamani, 2021). In the US, *I. scapularis* is associated with other human pathogens, but the influence of these pathogens on POWV replication and dissemination is yet to be studied.

3.0. Bourbon virus (BRBV)

3.1. Taxonomy, Virology and General Description of Bourbon virus

Another emerging tick-borne virus in the US is Bourbon virus (BRBV). This virus is the first member of genus *Thogotovirus* (family *Orthomyxoviridae*) in the US capable of causing human disease and death (Hao et al., 2022). The virion of BRBV has a segmented, enveloped, single-stranded RNA genome of around 10 kb in size, displaying pleomorphic (filamentous and spherical) morphology with numerous surface projections (King et al., 2018; Kosoy et al., 2015). The viral genome is made up of six negative strands RNA segments of varying nucleotide sizes which encode the glycoprotein (GP), matrix protein (M), nucleoprotein (NP), and three polymerase subunits (PB1, PB2, and PA). The polymerase units make up the RNA-dependent polymerase (RdRP) which is vital for its viral replication. The NP encloses the genome, and the M protein links the viral envelope with the virus core while the GP participates in virus attachment and fusion (Hao et al., 2022; King et al., 2018).

The only known human pathogenic tick-borne *Thogotoviruses* are BRBV, Thogoto virus, and Dhori virus (Lledó et al., 2020; Roe et al., 2023). Based on genomic sequences, *Thogotovirus* can be divided into Thogoto-like and Dhori-like thogotoviruses (Bussetti et al., 2012; Lambert et al., 2015). Members of the same group share antigenic and cross-reactivity properties. While BRBV is phylogenetically related to Dhori-like thogotoviruses, whose members cross-react (Fuchs et al., 2022; Hao et al., 2022), no other pathogenic Dhori-like thogotoviruses, such as Batken virus and Dhori virus, have been reported in the US.

3.2. Epidemiology, Vector, and Host Associations of Bourbon Virus

Generally, a wide gap exists in understanding BRBV disease pathogenesis, epidemiology, including transmission routes, potential vectors, and reservoir hosts (Hao et al., 2022; Roe et al., 2023). The human disease incidence is currently low, but given the seriousness of the virus deserves adequate research consideration to understand these gaps (Kosoy et al., 2015; Roe et

al., 2023), especially since the virus is capable of morbidity and mortality. In total, only five human cases, which resulted in two fatalities, have been reported from Kansas, Missouri, and Oklahoma in the US. To date, no human case has been reported outside these areas. The index fatal case involved an adult male in Kansas. Subsequent epidemiological surveillance and vector competency studies revealed *A. americanum* as the main vector for BRBV (Savage et al., 2013; Godsey et al., 2021). The second fatal case was reported from Missouri in 2017. Both patients had similar history of tick exposure prior to hospitalization (Hao et al., 2022) and clinical symptoms such as leukopenia, lymphopenia, thrombocytopenia, and high levels of aspartate aminotransferase and alanine aminotransferase (Roe et al., 2023). Currently, like for many tick-borne viruses, there is no specific treatment or vaccine for BRBV infections in humans. Although unique, our understanding of BRBV circulation in Virginia is limited. Additionally, knowledge of the virus's reservoir host and natural maintenance is not clear.

4.0. Heartland virus (HRTV)

4.1. Taxonomy, Virology and General Description of Heartland virus

In 2009, the centers for disease control and prevention (CDC) isolated a new virus from acute samples of two northwestern Missouri farmers, later described as Heartland virus (HRTV) (Brault et al., 2018; McMullan et al., 2012). Based on recent reclassification, HRTV belongs to the genus *Bandavirus* (family *Phenuiviridae).* The HRTV genome is enveloped, negative-sense, single-stranded tripartite RNA which consists of Large (L), medium (M), and small (S) segments which encode for the RNA-dependent RNA polymerase, two envelope glycoproteins (Gn and Gc) and nucleocapsid (N) protein respectively (Elliott & Brennan, 2014; Yoshimatsu & Arikawa, 2012; Zhu et al., 2017).

Phylogenetic studies have revealed four tick-borne *Bandavirus* lineages or groups based on genetic or serological similarities, namely: the severe fever with thrombocytopenia syndrome (SFTS) group, Bhanja group, Kaisodi group, and Uukuniemi group (Matsuno et al., 2018). HRTV

and severe fever with thrombocytopenia syndrome virus (SFTSV) are related novel tick-borne viruses within the SFTS serogroup; SFTSV occurrence is within Asia (Brault et al., 2018). The Bhanja group contains previously described North American phleboviruses, the lone star virus (LSV) and the Sunday canyon virus (SCV). However, there is a clear antigenic distinction between HRTV and the North American phleboviruses (Brault et al., 2018).

Just like BRBV, HRTV is also transmitted by *A. americanum*. According to CDC, as of November 2022, over 60 cases of HRTV disease have been reported in several states across the US's Midwest, Northeast, and South regions. This suggests that the virus has spread over a wide geographical area. Most reported cases displayed fever, nausea, muscle pain, joint pain and weakness, leukopenia, and thrombocytopenia. Currently, there is no specific therapy or vaccine for the treatment or prevention of HRTV. Previous infections have been managed based on supportive care involving antipyretics and analgesics.

4.2. Epidemiology, Vector, and Host Associations of Heartland Virus

Field surveillance in Missouri following the discovery of HRTV identified the viral RNA in ten pools of host-seeking nymphs of *A. americanum*, of which nine of the pools were collected from index patients' residences (Savage et al., 2013). Experimental studies supported *A. americanum* as the primary vector of HRTV, as it can transmit the virus both transstadially and horizontally (Newman et al., 2020; Savage et al., 2013; Tuten et al., 2020; Godsey et al., 2021). *A. americanum* is known to feed on a wide range of medium and large-sized vertebrate hosts and is prevalent across the eastern US (Kennedy & Marshall, 2021). There is serological evidence of widespread exposure of vertebrate hosts to HRTV, mainly in areas where human cases or viral molecular detection have occurred (Riemersma & Komar, 2015; Bosco-Lauth et al., 2015). However, little is known about HRTV circulation in Virginia. In general, there is a dearth of knowledge also about the reservoir host and how the virus is maintained in nature. Conducting serological surveillance is one methodology that can help understand vertebrate host exposure and the geographical extent

of the virus (as described in the next section). It is important to determine the prevalence of HRTV in the eastern US, where the tick vector is widespread, but the circulation of the virus is less well known.

5.0. Methods of Serological Surveillance

The One Health approach finds significant application in the surveillance of viruses that may be transmitted or maintained by different vertebrate hosts. These hosts can be investigated for evidence of arbovirus spread or exposure using molecular and serological techniques. Arbovirus surveillance primarily relies on serological techniques, as most viruses have a short replication period (Hermance & Thangamani, 2017). However, interpreting serological results can be difficult due to antibody cross-reactivity within the same serogroup and long-lasting antibodies from previous infections (Piantadosi & Kanjilal, 2020). Serological surveillance involves detection of antibodies, which are specialized proteins produced by the immune system in response to pathogen exposure.

In arbovirus surveillance, Immunoglobulin G or neutralizing antibodies (NAb) are of interest. The presence of a NAb is an essential marker in establishing previous exposure to a virus. This information is useful for understanding how a particular pathogen is circulating in a population (Hermance & Thangamani, 2017). Plaque reduction neutralization test (PRNT) is considered a gold-stand technique in serosurveillance as it can quantify neutralizing antibodies, is highly specific, and is unaffected by the history of prior infections. Other possible techniques include enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA). However, these tests have less specificity, subjective interpretation, and more cross-reactivity issues that may complicate arbovirus surveillance when compared to PRNT in vertebrate host surveillance (Hermance & Thangamani, 2017).

5.1. Serological Surveillance of Emerging Tick-Borne Viruses in the United States

Surveillance of ticks and vertebrate hosts has been important in monitoring the circulation of emerging arboviruses in the US. The first human cases of HRTV and BRBV in the central and eastern US, triggered active surveillance in the vicinity of human patient residencies (Savage et al., 2013; Savage et al., 2017). After identification of the tick vectors, the viruses were presumed to be maintained in nature through vertebrate hosts. Bosco-Lauth *et al.* (2015) conducted serological surveillance using PRNT methodology, to ascertain evidence of vertebrate host exposure to HRTV in animals living near the index human-case residences in Missouri. HRTV-NAb were detected in various mammals, including raccoons, horses, white-tail deer, domestic dogs, and Virginia opossums, in the proximity of the two index human cases of HRTV (Bosco-Lauth *et al.* 2015). This finding revealed potential wildlife reservoir hosts which might be involved in maintaining the virus in an enzootic circle. Following human reports of HRTV in Oklahoma and Tennessee, serological investigation was also conducted to ascertain whether the distribution of HRTV overlapped with the range of *A. americanum* in the US (Riesmersma and Komar, 2015). Multiple wildlife species including white-tailed deer and raccoon from 19 central and eastern states, including Virginia were surveyed in the investigation and the results revealed presence of seropositive animals in 13 states. However, no seropositive individuals were detected in Virginia during that study (n=37 tested). In addition, serological investigation conducted in New York detected HRTV seroprevalence of 10% in white-tailed deer (Dupuis et al.,2021).

Consequently, increase in the number of POWV human cases in recent years have required the understanding of its transmission. White-tailed deer are known to be parasitized by blacklegged ticks. Nofchissey et al. (2013) conducted a study to investigate the exposure of white-tailed deer to POWV. The researchers analyzed white-tailed deer serum samples collected from Connecticut, Maine, and Vermont between 1979 - 2010 for NAb against POWV. The study found evidence of POWV exposure in samples from all the three states. In another study by Dupuis et al. (2013), evidence of vertebrate exposure to POWV in New York was discovered. The

researchers screened 739 vertebrate hosts for POWV NAb using the PRNT. The results showed that woodchucks (4/6 tested), one opossum (1/6), and birds (4/727) had NAb against the virus. In Alaska and New Mexico, there have been reports of serologic evidence indicating the circulation of POWV among wild small-to-medium-sized mammals. The investigation involved approximately 503 animals from Alaska and New Mexico, and their serum samples were tested using a strip-immunoblot assay with recombinant DTV envelope glycoprotein. The study revealed the presence of DTV-reactive antibodies in the serum samples of northern red-backed voles (14/243), southern red-backed voles (6/89), deer mice (2/33), and pinon mice (2/9) (Deardorff et al., 2013).

Although evidence in Virginia is still to be defined, serological surveillance has also been carried out in Missouri and North Carolina to determine the exposure of vertebrate species to BRBV in areas where BRBV RNA has been detected in ticks and areas where human cases have been confirmed. In Missouri, 301 birds and mammals were screened for BRBV NAb using the PRNT. BRBV-NAb was detected in domestic dogs (2/13), eastern cottontails (2/9), a horse (1/24), raccoons (31/62), and white-tailed deer (12/14) (Jackson et al., 2019). In North Carolina, evidence of BRBV circulation was also reported, as NAb was detected in white-tailed deer (18/32) (Komar et al., 2020). All these outcomes demonstrated the presence of these three tick-borne viruses in the US and their broad geographical range. However, there is a need to ascertain their prevalence in Virginia.

6.0. Current Knowledge About Tick-Borne Viruses in Virginia

Evidence suggestive that emerging tick-borne viruses have reached Virginia include the following: a reported human case of each of POWV and HRTV, laboratory confirmation of POWV, HRTV, and BRBV in ticks, and detection of NAb in vertebrate hosts. This supports the hypothesis that these emerging viruses are present and circulating in the Commonwealth of Virginia. However, there is a limited knowledge on their prevalence and exposure range in Virginia. The little that is known is primarily on the southwestern region of the state. Cumbie *et al.* (2021)

reported the presence of POWV RNA in *I. scapularis* (one nymph and one adult male) collected from Floyd County. In another study, Cumbie et al. (2022) reported the detection of BRBV RNA in field-collected *A. americanum* and a tick species not previously associated with BRBV before, the invasive *Haemaphysalis longicornis*. For the latter species, the BRBV RNA was detected in one nymph from Patrick County, one nymph from Staunton city, one larval pool and one adult female from Wythe County. One BRBV viral RNA-positive *A. americanum* nymphal pool was detected from Patrick County. Cumbie et al. (2022) also detected BRBV-NAb in white tailed deer and groundhogs in western Virginia (Cumbie et al., 2022). In addition, HRTV RNA have been detected in field collected *A. americanum* in Western Virginia (Eastwood; unpublished/personal correspondence). Nevertheless, these findings suggest that these viruses are present and might be circulating in Virginia, although there have been no substantive human cases in the state. There is a need to provide evidence of circulation of these viruses in vertebrate host in Virginia.

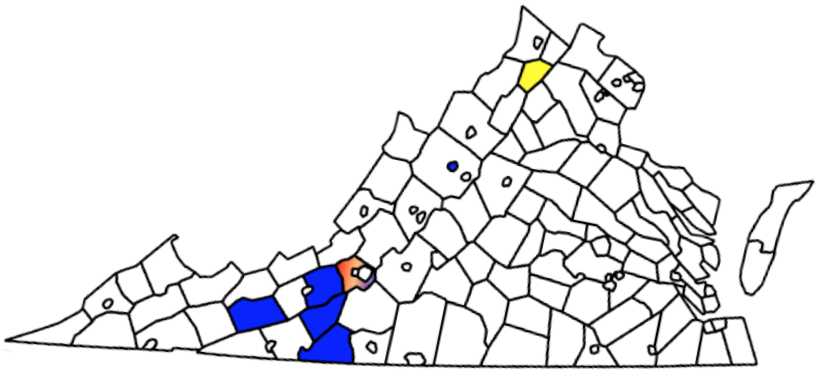

Figure 2: Current known distribution of tick-borne viruses in Virginia. Blue = BRBV (evidence in wildlife or ticks), Yellow = HRTV, Mixed color = POWV+HRTV+BRBV.

6.1. Current Research Gaps & Conclusion

Despite the presence of suggested tick vectors in Virginia, molecular evidence in tick species, and one human case of each of POWV and HRTV, there is currently little understanding of POWV, HRTV, and BRBV in Virginia, and the extent of their circulation. Cumbie et al. (2022) reported the presence of specific NAb against BRBV in several wildlife species of southern

Virginia, however this was a small sample size; furthermore, there are no reports of vertebrate species exposure to HRTV and POWV in Virginia. Therefore, there is a need to understand the distribution and prevalence of all these emerging viruses in Virginia.

Emerging tick-borne viruses pose a significant threat to public health, potentially resulting in human morbidity and mortality. Currently, there is a gap in knowledge regarding the ecology of POWV, HRTV, and BRBV in the Commonwealth of Virginia. The associated tick vectors (predominantly *A. americanum*, *I. scapularis*) persist in expanding populations in Virginia, and been found infected, suggesting an arthropod vector risk, however, wider distribution and exposure rates remain to be determined. The emergence of new pathogens is difficult to predict, but understanding current geographical distribution helps in preparedness for disease prevention. Effective surveillance measures can help measure the risk of new pathogen emergence. Serological surveillance of vertebrate hosts is useful in monitoring the geographical spread and circulation of these pathogens in the environment and is recommended for assessing transmission of these three emerging tick-borne viruses in Virginia.

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Chapter 2

Evaluating the Prevalence of Tick-borne Viruses; Bourbon, Heartland, and Powassan Viruses Circulating in Virginia Using a One-Health Approach

1.0. Introduction

Ticks (families *Argasidae* and *Ixodidae*) are blood-feeding ectoparasites responsible for many vector-borne disease cases worldwide (Rochlin and Toledo, 2020), as they can transmit a variety of pathogens to vertebrate hosts, including bacteria, protozoa, and viruses (Eisen et al., 2017; Rosenberg et al., 2018). Tick-borne pathogens are currently responsible for about 95% of all vector-borne diseases in the United States (US) (Rosenberg et al., 2018). Tick-borne diseases threaten human and animal lives and have a significant economic impact due to the costs associated with their control and treatment (Rochlin and Toledo, 2020). Lyme disease, caused by the bacterium *Borrelia burgdorferi*, is the most prevalent and well-known tick-borne illness in the US (Nelson et al., 2015; Rochlin & Toledo, 2020). In the US alone, the cost of overall patient care for Lyme disease is estimated to be around 1 billion dollars annually (Adrion et al., 2015). Aside from Lyme disease however, babesiosis, ehrlichiosis, anaplasmosis, and Rocky Mountain spotted fever are further expanding tick-borne illnesses in the US, and in addition, tick-borne viruses rpresente an emerging threat to public health. Factors promoting the incidence of tick-borne diseases include expanding tick ranges, climate change, lack of vaccines for endemic tick-borne diseases, and emergence of novel tick-borne pathogens (Beard et al., 2019). In addition, invasive or new tick species are being detected in novel territories with expanding distribution (Eisen et al. 2017).

Despite efforts to address the economic and health impact of tick-borne diseases, there has been a significant rise in the occurrence of these diseases over the past two decades (Kobayashi, 2018). Emerging tick-borne viruses are a rare agent but can pose more severe threat to human health that non-viral agents, with no specific therapy or vaccine available, and consequences of infection including death or ongoing neurological abnormalities (Ebel, 2010;

Kosoy et al., 2015; McMullan et al., 2012). In the US, three emergent tick-borne viruses in particular, Bourbon virus (BRBV), Powassan virus (POWV), and Heartland virus (HRTV), have been identified in ticks as well an occurrence of human cases from multiple regions, highlighting the potential threat of these viruses to public health.

Powassan virus (POWV) is a rare but fatal neurotropic, tick-borne flavivirus (family *Flaviviridae*) (Ebel, 2010). It is the only member of the tick-borne encephalitis serogroup in North America (Kemenesi & Bányai, 2019). There are two serologically indistinguishable lineages of POWV. Lineage I (POWV-I) is primarily associated with *Ixodes cookei* (the groundhog tick*)* and *I. marxi* (the squirrel tick) (Lange et al., 2022). Lineage II (POWV-II) or deer tick virus (DTV) is associated with *I. scapularis* (the blacklegged tick) (Hassett & Thangamani, 2021). Since *I. scapularis* is a human-biting tick and *I. marxi* or *I. cookei* feed infrequently on humans, human-exposure to POWV is generally associated with *I. scapularis*, whose distribution extends predominantly into the eastern states of the US including Virginia (Ebel, 2010; Ebel & Kramer, 2004). Most human cases of POWV have historically been reported in the central or northeastern regions of the country, despite the tick vector's distribution extending over a further geographical region. In Virginia, *I. scapularis* is present (Eisen et al., 2016), yet little is known about the extent of POWV distribution. However, molecular detection of POWV RNA in *I. scapularis* ticks collected in Virginia is documented (Cumbie et al., 2021), and a confirmed human case suspected to have occurred in Virginia in 2009, together provide evidence that POWV is present and circulating in Virginia.

A second tick-borne virus in the US is Heartland virus (HRTV). HRTV is a novel tick-borne bandavirus (family *Phenuiviridae*) (Zhu et al., 2017). It was first isolated and identified in two separate human cases involving Missouri farmers in 2009 (Zhu et al., 2017). HRTV infections are characterized by fever, leukopenia, and thrombocytopenia. Genetically, HRTV is closely related to a serious tick-borne virus described outside the US - severe fever with thrombocytopenia syndrome virus (SFTSV), which causes mortalities and morbidities in Asia (Matsuno et al., 2018).

HRTV is believed to be transmitted by *Amblyomma americanum* (the lone star tick) to human (Savage et al., 2013). Since the first index case in 2009, over 60 new clinical cases of HRTV have been reported mostly in the South and Midwest states of the US according to the Centers of Disease Control and prevention (CDC). On the other hand, no confirmed cases have been reported in Virginia and little is known about the distribution of HRTV in the state.

A third emerging tick-borne virus in the US is Bourbon virus (BRBV). BRBV is a tick-borne thogotovirus (family *Orthomyxoviridae*) which was first detected in a blood sample collected in a fatal case involving an adult male (over 50 years) resident of Bourbon County, Kansas, US, in 2014 (Savage et al., 2017). Human infection is characterized by fever, leukopenia, and thrombocytopenia (King et al., 2018; Kosoy et al., 2015). BRBV is the first thogotovirus to be identified in the US with the ability to cause disease and death in humans (Hao et al., 2022; Kosoy et al., 2015). BRBV uses *A. americanum* as its arthropod vector (Savage et al., 2017). However, despite sharing the same tick vector with HRTV and having a wide distribution, fewer, only five, human cases of BRBV, with two fatalities, have been reported so far in the US. Cumbie et al. (2022) identified the presence of BRBV RNA in *A. americanum* and *Haemaphysalis longicornis* ticks collected in several western counties of Virginia. They also detected neutralizing antibodies against BRBV in wildlife species located in the same region. Despite this, there remains a lack of clear understanding regarding the distribution of the virus throughout Virginia.

In general, most human cases of BRBV, HRTV, and POWV have been restricted to the geographical range of their primary tick vectors (*A. americanum* or *I. scapularis*), thus in Eastern and Midwestern part of the US where the tick vectors are prevalent. Animals that share similar ecological habitat in these endemic regions are usually exposed (Dupuis et al., 2013; Bosco-Lauth et. al., 2015; Jackson et al., 2019) and may thus contribute to the transmission of BRBV, HRTV and POWV as a reservoir or amplifier to human, a dead-end host. Tick-borne viruses are believed to be maintained in an enzootic cycle between diverse small to medium-sized vertebrate host species and competent tick vectors (Kazimírová et al., 2017), albeit that localized (tick co-

feeding) transmission may occur on a host; without the host acting as a reservoir. To effectively monitor the spread and ascertain the current distribution and prevalence of POWV, HRTV, and BRBV in the US, it is necessary to identify previous exposure in vertebrate species through serological assessment. Serological surveillance is one methodology that can be used to monitor arboviruses within known arboviral ecology. For instance, *I. scapularis* and *A. americanum* ticks feed on vertebrate hosts, which might play a role or be impacted in HRTV, POWV, and BRBV transmission dynamics. Serological techniques have been used in endemic areas to assess wild and domestic animals for possible viral exposure (neutralizing antibodies) and pathogen distribution. Neutralizing antibodies (NAbs) counter viral infections by binding to pathogen antigens and impeding their interaction with host cells, effectively reducing or halting the infection. NAbs are an integral part of the adaptive immune system's humoral response and are produced by both infections and vaccinations against infections. (Klasse, 2014; Li et al., 2022). NAbs against BRBV, POWV, and HRTV have been detected in both wild (white-tailed deer, raccoons, groundhogs, red squirrels) and domestic animals (dogs, horses) in different parts of the US, notably in areas where human cases of these three tick-borne viruses were reported (Bosco-Lauth et al., 2015; Dupuis et al., 2023; Jackson et al., 2019). However, this current study describes the first comprehensive attempt to examine livestock and wildlife species in Virginia for exposure to POWV, HRTV, and POWV.

While there are existing measures to prevent tick bites and monitor arboviral diseases, understanding transmission parameters, such as transmission rate, can offer valuable insights into the spread of arboviruses beyond what serological surveillance alone can provide (Lou & Jianhong, 2017). Since presently, there is a lack on the understanding of rate of exposure of vertebrate host to emerging tick-borne viruses in Virginia, using an appropriate model, we can estimate the transmission rate to predict risk of exposure. Also, the basic reproduction number (R_o) can be used to identify factors useful in controlling the spread of tick-borne viruses in Virginia using the next generation approach (Van den Driessche & Watmough, 2002).

Our research aims to use this model to understand the rate of transmission and the basic reproduction number of emerging tick-borne viruses among vertebrate host populations in Virginia. This information is critical for public health officials and researchers in determining the best control strategies for tick-borne viruses. By understanding the rate of exposure in wildlife, we can better understand the ecology of and how these three viruses circulate in new regions.

2.0. Materials and Methods

2.1. Sample Collection

Both passive and active surveillance techniques were utilized to collect blood samples from small and medium-sized mammals across Virginia health planning regions (HPRs; Figure 1) from July 2020 to November 2022. In passive surveillance, blood samples were collected from (1) the body cavity of deer brought to Virginia department of wildlife resources (DWR) check stations for chronic wasting disease surveillance in Virginia (2021 and 2022), (2) wildlife patients submitted to rehabilitation centers in northern and western Virginia, and (3) recently killed animal carcasses (roadkill samples) and hunter donations. Active surveillance involved small mammal trapping targeting white-footed mouse (*Peromyscus leucopus*) using seed-baited modified fitch traps and Sherman live traps, in Montgomery (MO), Rockbridge (RO), Fauquier (FA), Warren (WR), Patrick (PA), and Floyd (FL) counties (indicated within Figure 1). A total of 357 traps were set for an average of three nights per month, resulting in a total trap time of 456 hours. A blood sample was taken from captured target species; briefly, a sterile 5.0 mm lancet was used to obtain about 5 - 40uL of blood via submandibular venipuncture, transferred into capillary tubes (DWR Scientific Collection Permit #069872, IACUC#20-197). After sample collection, each captive was marked using ear tags, observed for 10 minutes for signs of stress or shock, and released in the same capture location.

The blood samples were transported to the laboratory at Virginia Tech on ice. Sera were separated from the blood sample by centrifugation at 5000 rpm for 6 min. The resulting sera were

transferred to a sterile micro-centrifuge tube. A total of 817 wildlife serum samples were collected for this study across all five HPRs in Virginia (indicated in Figure 2). For cattle sampling, a passive surveillance method was used with 500 serum samples provided from Dr. Lahmers' archive collection held at the Department of Biomedical Science and Pathology in the College of Veterinary Medicine at Virginia Tech. The cattle samples were from various breeds and animal ages, and were collected from both local auctions and private owners across all HPRs in Virginia (indicated in Figure 2) between 2019 and 2022.

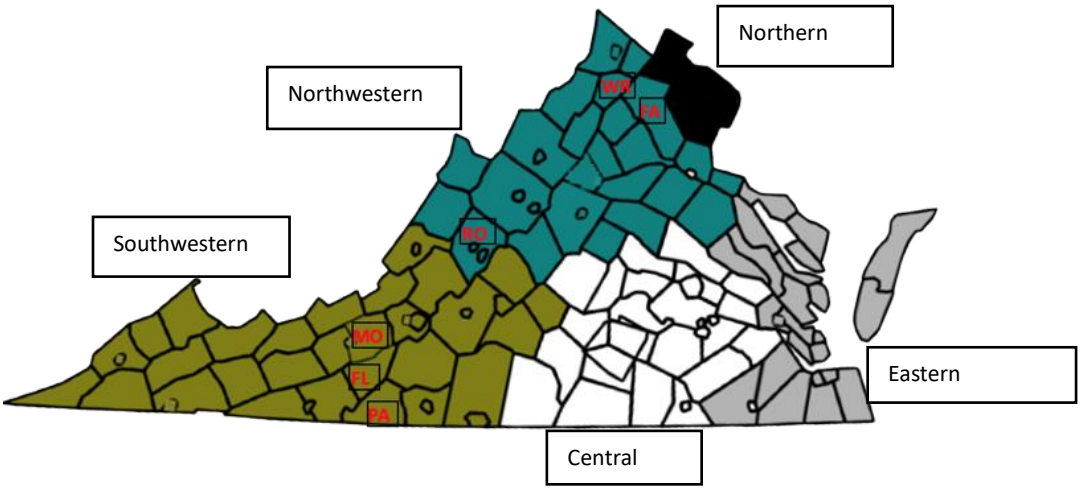


Figure 1: Map of Virginia showing the five health planning regions, Montgomery (MO), Rockbridge (RO), Fauquier (FA), Warren (WR), Patrick (PA), and Floyd (FL) counties. The six marked counties are areas of active surveillance.

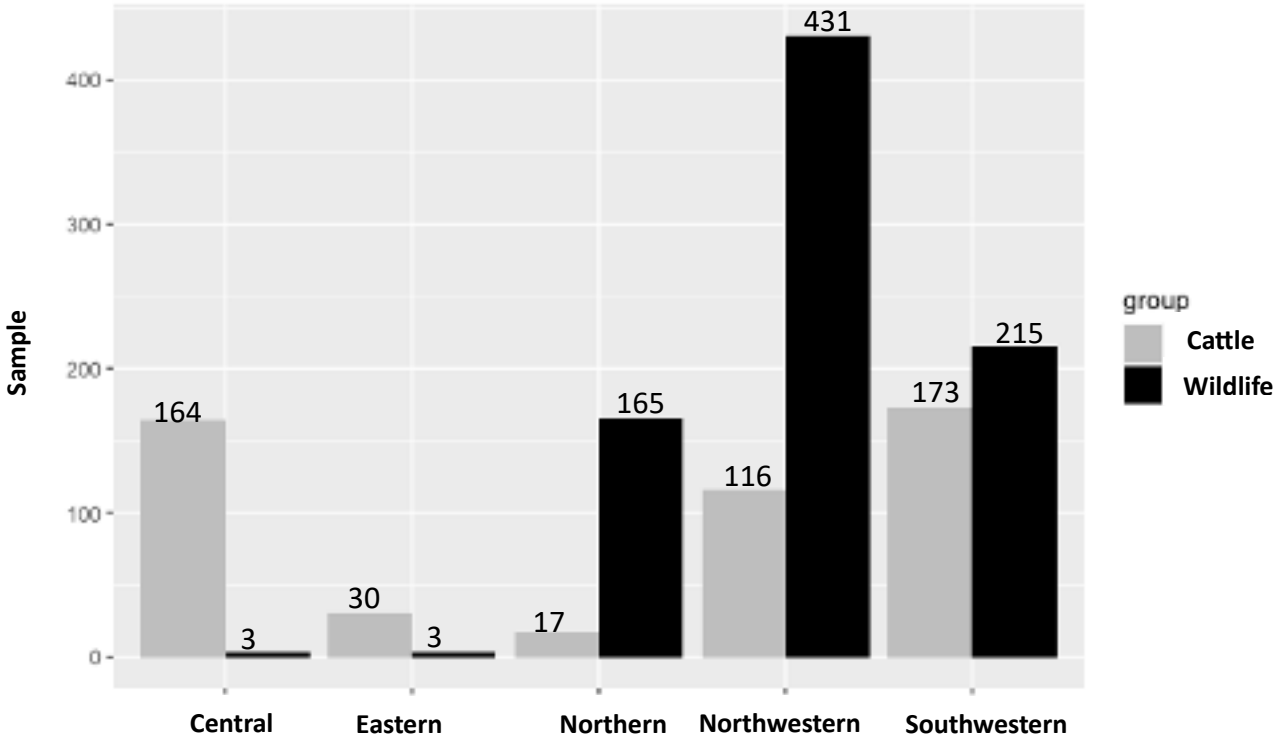


Figure 2: Total number of wildlife and cattle serum samples collected and their geographical location within Virginia.

2.2. Serological Assessment

Before serological screening, aliquots of all sera samples were heated at 56°C for 30-60 minutes to inactivate cell growth inhibitors in culture, and then diluted 1:20 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2% fetal bovine serum (FBS), 1% penicillin-streptomycin and 0.037% sodium bicarbonate. Plaque reduction neutralization test (PRNT) was used to screen all sera samples for the presence of NAb against HRTV, BRBV, and POWV, as described by Eastwood *et al*. (2017). Briefly, the diluted sera were challenged against a virus suspension at a working concentration of 800 PFU/ml of either HRTV (MO-4 strain, BEI Resources; propagated and original titer at 3 x 10^7 PFU/ml), BRBV (Original strain, BEI Resources; 2 x 10^8 PFU/ml), POWV (POWV-WNV chimeric, kindly provided by Greg Ebel, Colorado State University; 5.5 x 10^4 PFU/ml) . Seropositive samples demonstrating at least an 80% reduction in plaque formation were confirmed by serial 2-fold dilution to establish the minimum antibody neutralization titer (end-

point titer). Only seropositive samples with antibody titers ≥ 40 were considered seropositive. Rabbit antisera (1:10 dilution) (kindly provided by the Centers for Disease Control and Prevention) was used as a positive serum control, while DMEM and a no-template control were used as negative controls for the assays.

2.3. Statistical Analysis – Seroprevalence rates and their corresponding confidence intervals (CIs) were calculated using the RStudio software (R Core Team, 2021). The statistical significance of the test results was assessed using hypothesis testing. This analysis utilized a 95% confidence interval for all positive test results.

2.4. Mathematical Modeling - Numerical simulations were performed using the software MATLAB and RStudio to create an SIS epidemic model with the following assumptions: (1) Animals can continuously become infected through exposure to infected ticks, and the infection does not provide immunity. Infected individuals can return to the susceptible state after they have cleared the infection (2) Susceptible hosts can experience repeat or recurring infections (3) The overall population is not constant and is influenced by introducing new hosts and natural or disease-induced deaths. The model only considers animal host populations and is divided into two compartments: Susceptible and Infectious hosts. Since we do not have any data on tick abundance, we exclude them from the model. The flowchart represents the progression of disease dynamics in the host population, with arrows indicating the transitions between compartments. New animal hosts are recruited into the susceptible population at a rate of Λ^h, which is the host recruitment rate. Susceptible animal hosts become infected when bitten by an infected tick, at a rate expressed as $\beta^h \frac{S^h}{N^h} I^h$, where β^h is the transmission rate from vector to host, I^h is the number of infected hosts, S^h is the number of susceptible hosts, and N^h is the total animal host population. Infected animals recover at a rate of $\gamma^h I^h$, where γ^h is the host per capita

recovery rate. The mortality rates for susceptible and infected populations are expressed as $\mu^h S^h$ and $\mu^h I^h$, respectively, where μ^h is the host death rate. To construct this model (1) the model was parameterized using data (Table 2) from the literature and represented by the following ordinary differential equations:

$$\frac{dS^h}{dt} = \Lambda^h - \beta^h \frac{S^h}{N^h} I^h - \mu^h S^h + \gamma^h I^h$$

$$\frac{dI^h}{dt} = \beta \frac{S^h}{N^h} I^h - (\gamma^h + \mu^h) I^h \quad (1)$$

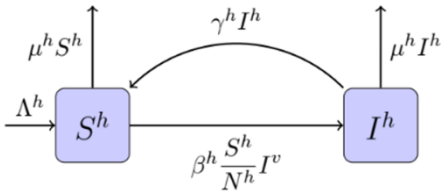


Figure 3: Flow diagram of SIS vector-borne disease dynamics considering only animal host.

Table 1: Definition of model parameters represented in (1)

State Variables and Parameters	Description
S^h	Number of susceptible hosts
I^h	Number of infected hosts
β^h	Transmission rate from vector to host
Λ^h	Host recruitment rate
γ^h	Host per capita recovery rate
μ^h	Host death rate

Table 2: Initial parameters.

Parameter	Value	Source
Transmission rate from vector to host	1.4151	Estimated
Host recruitment rate	1.0422	Estimated
Host per capita recovery rate	1.1104	Estimated
Host death rate	0.1	Estimated

Also, (2) the model was fed with seroprevalence data obtained from cattle and wildlife samples over 36 and 32 months, respectively. Seroprevalence data is a measure of the proportion of cattle

and wildlife hosts that have been exposed to HRTV, POWV, and BRBV at a given time. However, the seroprevalence was cumulated and combined for all viruses at each time point due to limited data points available for each virus. The time (t) was measured in months. Based on this seroprevalence data, the following transmission parameters: host death rate, host per capita recovery rate, host recruitment rate, and transmission rate from vector to host were estimated at different model (a) Cattle Only (b) Wildlife Only (c) Cattle and Wildlife combined at 32 Months (d) Cattle and Wildlife combined at 43 Months. (3) A least squares method was used to determine the "best" parameters for the model given the seroprevalence data.

The optimization algorithm used to estimate the parameters β^h, Λ^h, γ^h, and μ^h focuses on minimizing the least square equation (LSE):

$$LSE(t,p) = \sum_{i=1}^{n} |y(t_i,p) - data(t_i,p)|^2. \qquad (2)$$

In other words, the algorithm searches for the set of parameters p that yields the smallest value for the function $LSE(t,p)$. A small value for equation (2) will inform us how well our model output $y(t_i,p)$ does compared to the data set $data(t_i,p)$. We start the procedure by initializing values for the parameters β^h, Λ^h, γ^h, and μ^h from Table 2. Since our model describes an epidemiological system, we define a lower bound for the parameter values to prevent the optimization algorithm from generating negative parameter values. We used MATLAB's function *fminsearchbnd* which is a nonlinear programming solver that finds the minimum value for $LSE(t,p)$. Also, we did this fitting procedure in RStudio using a similar function referred to as *optim*. After the scheme concluded, we used the estimated parameters to generate plots of the model output with respect to the data set.

3.0 Results

3.1. Wildlife and Livestock Serology

Of the 811 wildlife samples tested for POWV NAb, 146 (18%) samples were seropositive, while 61 out of 770 tested samples (8%) were seropositive against BRBV. For HRTV, 38 out of 766 tested samples (5%) were seropositive (Table 3). A total of 500 cattle serum samples were screened for HRTV, BRBV, and POWV NAb. Out of the total samples, 40 (8%) samples were found to have neutralizing antibodies against HRTV, six (1.2%) samples showed NAb against BRBV, and five (1%) samples had Nab against POWV (Table 4). The serotiter for all seropositive samples ranged between 1:40 to 1:≥320. In both cattle and wildlife, there are significance difference between the tick-borne viruses with a p-value less than 0.05.

Table 3: Seroprevalence of POWV, BRBV, and HRTV in wildlife serum samples.

Tick-Borne Virus	Number tested	Confirmed Seropositive Number (%; 95% CI)	Serotiter
Powassan virus	811	146 (18; 15-21)	1:40 - ≥ 1:320
Bourbon virus	770	61 (8; 5.6-10)	1:40 - ≥ 1:320
Heartland virus	766	38 (5; 3.4-6.5)	1:40 - ≥ 1:320

Table 4: Seroprevalence of POWV, BRBV and HRTV in cattle serum.

Tick-Borne Virus	Number Tested	Confirmed Seropositive (%; 95% CI)
Powassan virus	500	5 (1; 0.1-1.9)
Bourbon virus	500	6 (1.2; 0.2-2.2)
Heartland virus	500	40 (8; 5.6-10)

Table 5 and 6 displays the number of seropositive samples from each Virginia HPRs. Concerning POWV, the southwestern region had the highest number of seropositive wildlife samples (n=114). However, four out of five seropositive livestock samples were found exclusively in the southwestern region. As for BRBV, the northwestern region had the highest number of

seropositive wildlife samples (n=47), and it also recorded three seropositive livestock samples in the northwestern region. In the case of HRTV, the northwestern region had the most seropositive wildlife samples (n=20), while the central region of Virginia had the highest number of seropositive livestock samples (n=24).

Table 5: Total number of seropositive wildlife samples from each Virginia HPRs

Health Planning Region	**Number individuals tested**	**Number POWV Seropositive Samples (%; 95% CI)**
Northwestern	429	24 (5.6; 3.4-7.8)
Northern	164	7 (4.3; 1.2-7.4)
Southwestern	212	114 (54; 47-60)
Central	3	0 (0; 0-0)
Eastern	3	1 (33; 0-87)
Health Planning Region	**Number individuals tested**	**Number BRBV Seropositive Samples (%; 95% CI)**
Northwestern	423	47 (11; 8.1-14.1)
Northern	165	6 (3; 0.4-5.6)
Southwestern	176	8 (4.5; 1.5-7.6)
Central	3	0 (0;0-0)
Eastern	3	0(0;0-0)
Health Planning Region	**Number individuals tested**	**Number HRTV seropositive samples (%; 95% CI)**
Northwestern	422	20 (4.7; 2.7-6.8)
Northern	164	3 (1.8; 0-3.9)
Southwestern	174	13 (7.5; 3.6-11.4)
Central	3	1(0; 0-87)
Eastern	3	1 (0; 0-87)

Table 6: Total number of TBV-seropositive livestock samples from each Virginia HPR

Health Planning Region	Number Tested	Number of POWV Seropositive Samples (%; 95% CI)	Number of BRBV Seropositive Samples (%; 95% CI)	Number of HRTV Seropositive Samples (%; 95% CI)
Northwestern	116	1 (0.9; 0-2.5)	3 (2.6; 0-5.5)	9 (7.6; 2.9-13)
Northern	17	0 (0; 0-0)	0 (0;0-0)	1 (5.9; 0-17)
Southwestern	173	4 (2.3; 0.1-4.6)	0 (0;0-0)	4 (2.3; 0.1-4.6)
Central	164	0 (0; 0-0)	2 (1.2; 0-2.9)	24 (15; 9.2-20)
Eastern	30	0 (0; 0-0)	1 (3.3; 0-9.9	2 (6.7; 0-16)
Total	500	5	6	40

Neutralizing antibodies against POWV, BRBV and HRTV were detected in 15 distinct wildlife species, as shown in Table 7. Among these were white-tailed deer, raccoon, American black bear, eastern cottontail, red fox, Virginia opossum, great horned owl, American toad, chipmunk, grey fox, eastern red bat, American beaver, skunk, groundhog, and North American river otter. These seropositive wildlife animals are distributed throughout the five Virginia health regions (indicated in Figure 4**).**

Table 7: Wildlife animal with specific neutralizing antibodies against POWV, BRBV and HRTV

Wildlife Species	Number Tested (POWV seropositive)	POWV Seroprevalence (%)
White-tailed deer (*Odocoileus virginianus*)	255 (127)	49.8
Raccoon (*Procyon lotor*)	111 (3)	2.7
American black bear (*Ursus americanus*)	5 (1)	20
Eastern cottontail (*Sylvilagus floridanus*)	67(1)	1.5
Red fox (*Vulpes vulpes*)	40 (1)	2.5
Virginia opossum (*Didelphis virginiana*)	86(8)	9.3
Great horned owl (*Bubo virginianus*)	4(1)	25
American toad (*Anaxyrus americanus*)	1(1)	100
Chipmunk (*Tamias striatus*)	1(1)	100
Grey fox (*Urocyon cinereoargenteus*)	1(1)	100
Eastern red bat (*Lasiurus borealis*)	2(1)	50

Wildlife Species	Number Tested (BRBV seropositive)	BRBV Seroprevalence (%)
White-tailed deer (*Odocoileus virginianus*)	249(33)	13.3
Red fox (*Vulpes vulpes*)	40(5)	12.5
American black bear (*Ursus americanus*)	4(1)	25
American beaver (Castor canadensis)	2(2)	100
Skunk (*Mephitis mephitis*)	14(1)	7.1
Eastern cottontail (*Sylvilagus floridanus*)	68(3)	4.4
Groundhog (*Marmota monax*)	3(2)	66.7
Virginia opossum (*Didelphis virginiana*)	63(1)	1.6
Raccoon (*Procyon lotor*)	112(13)	11.6
Wildlife Species	**Number Tested (HRTV seropositive)**	**HRTV Seroprevalence (%)**
White-tailed deer (*Odocoileus virginianus*)	247(21)	8.5
Racoon (*Procyon lotor*)	112(12)	10.7
American black bear (*Ursus americanus*)	6(2)	33.3
American beaver (Castor canadensis)	2(1)	50
Skunk (*Mephitis mephitis*)	14(1)	7.1
North American river otter (*Lontra canadensis*)	1(1)	100

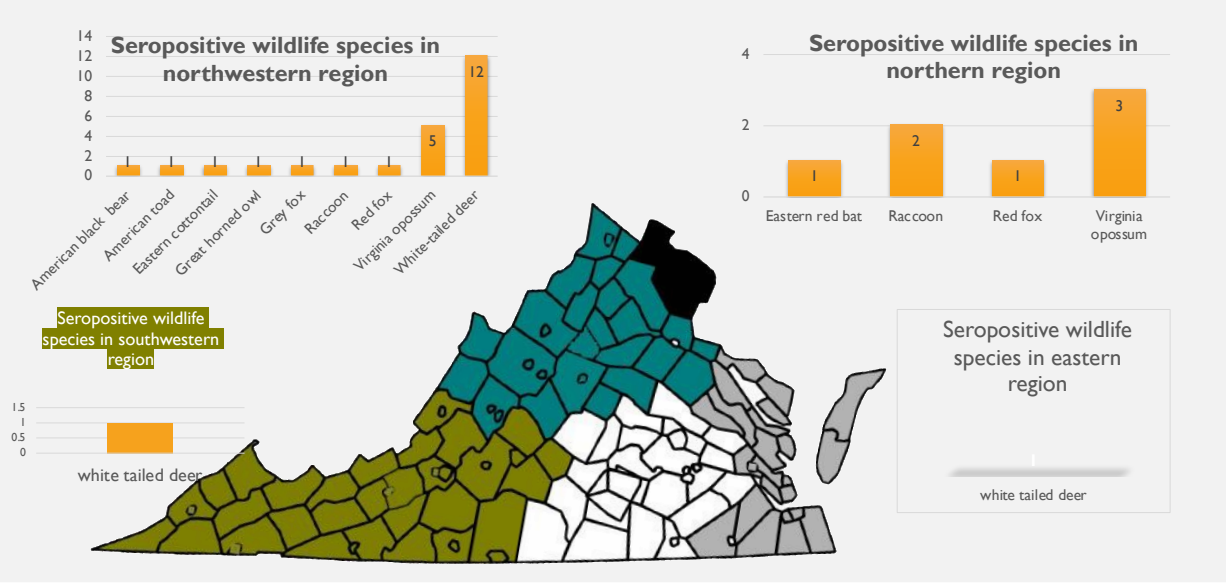


Figure 4a. Map of Virginia showing distribution of POWV seropositive wildlife samples.

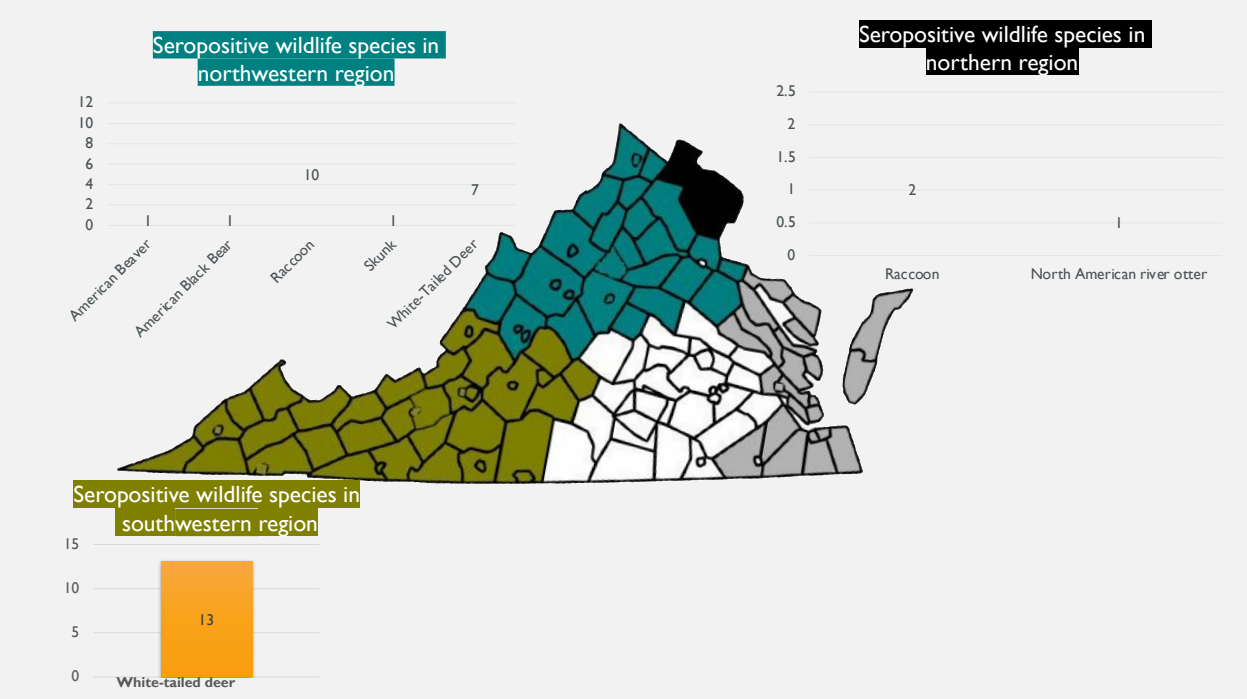


Figure 4b: Map of Virginia showing distribution of HRTV seropositive wildlife samples.

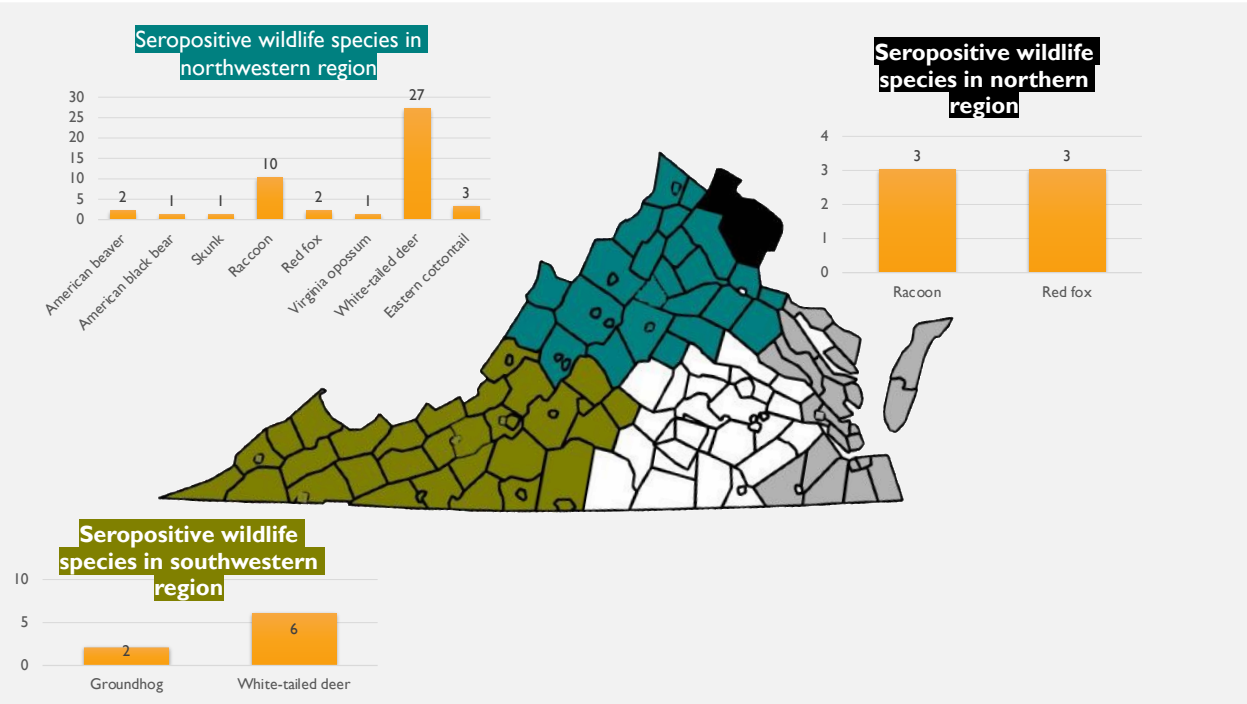


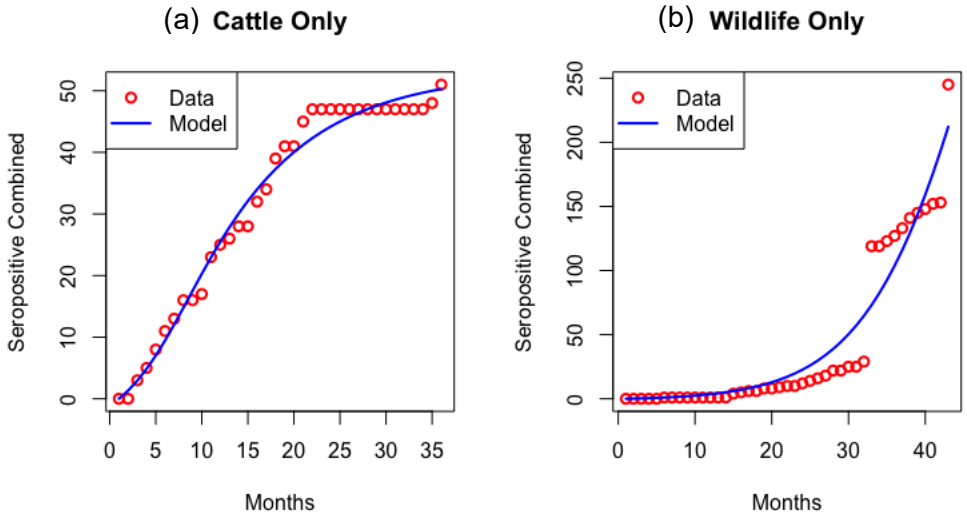
Figure 4c: Map of Virginia showing distribution of BRBV seropositive wildlife samples.

3.2. Mathematical Modeling: We calculated the parameter set for each model that best fit the seroprevalence data. We observed that most of the estimated parameters from each model varied greatly from one model to another. The table below illustrate each seroprevalence data's estimated parameters and reproduction numbers.

Table 8: Estimated parameters and the reproduction number for each model

Seroprevalence data (Months)	Host death rate	Host recruitment rate	Transmission rate from vector to host	Host per capita recovery rate	Reproduction number
Cattle Only (36)	0.10	0.0	1.25	0.90	0
Wildlife (43)	0	13.08	0.14	0.0	**Inf**
Cattle and Wildlife Combined (32)	0.07	0.57	1.57	1.33	0.645
Cattle and Wildlife Combined (43)	0	60.71	1.14	1.07	64.77

The plot of seroprevalence data of cattle for 36 months showed an overall increasing trend in viral exposure over time but stagnant from 20 months to 36 months. Similarly, the plot for wildlife for 43 months also showed an increasing trend but a sharp jump from 32 months to 43 months. However, the combined seroprevalence data set for cattle and wildlife samples over 32 months, appeared to describe the model's behavior best (indicated in Figure 5).



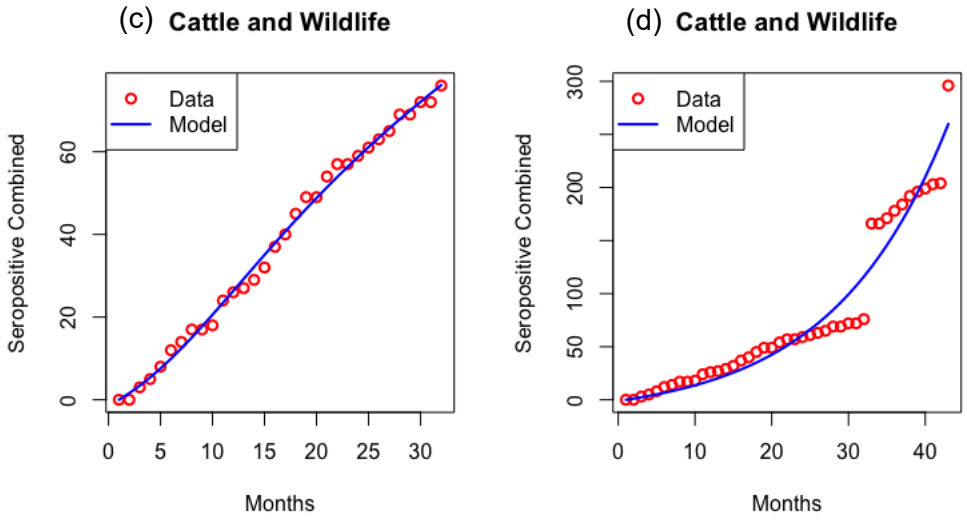


Figure 5: Model fitting for seroprevalence data (a) Cattle Only, (b) Wildlife Only, (c) Cattle and Wildlife for 32 months, (d) Cattle and Wildlife for 43 months

The basic reproduction number for each model is defined by:

$$R_0 = \frac{\beta^h \Lambda^h}{(\mu^h + \delta^h)} \qquad (3)$$

Equation (3) is an important epidemiological and mathematical threshold that provides valuable information about the number of secondary cases one infectious individual produces in a fully susceptible population. In addition, if R_0 is less than 1, the infectious disease will die out given that there are not enough secondary cases being produced to sustain an outbreak. In the contrary if R_0 is greater than 1, we will have an outbreak. Another use for computing the basic reproduction number is the insight we gain by altering certain parameters to observe the impact it has on R_0. For example, if targeted control measures were implemented to reduce transmission, how much of an influence does that have on the probability of an outbreak occurring?

4.0. Discussion and Conclusion

We examined livestock and wildlife to determine the prevalence of tick-borne viruses circulating in Virginia. Our results provide evidence that all three tick-borne viruses under focus are circulating in Virginia, with exposure to BRBV, POWV, and HRTV observed in species of wild animals and as well as in domestic cattle from multiple HPRs in Virginia. These investigations reveal the first detection of NAb against HRTV and POWV in vertebrates. To understand the dynamics of these emerging tick-borne viruses, our model analyzed the seroprevalence result or data and estimated the transmission parameters, including the transmission rate and the basic reproduction number. These parameters are valuable in enhancing our understanding of these emerging tick-borne viruses.

This study detected NAb against HRTV in wildlife species from all HPRs. Similarly, NAb against POWV were detected in all HPRs except for the central region, indicating a widespread exposure in Virginia. These findings have important implications for public health and highlight the need for continuous tick-borne virus surveillance to identify disease risk areas. Earlier, there was a report of NAb against BRBV in white-tailed deer and groundhogs in the southwestern region of Virginia (Cumbie et al., 2022). Our current study supports that finding by revealing serological evidence of BRBV exposure in white-tailed deer, raccoon, red fox, American black bear, American beaver, skunk, eastern cottontail, groundhog, and Virginia opossum. Our findings also identified new areas of BRBV circulation in Virginia by reporting the detection of NAb against the virus in the northwestern and northern regions. These findings support the hypothesis that these emerging tick-borne viruses are circulating in Virginia.

Detection of NAb only indicate previous viral exposure and not current viral presence in the host. Nevertheless, detecting such antibodies in multiple animal species across all Virginia HPRs suggests a widespread circulation and geographical expansion of POWV, HRTV, and BRBV in Virginia. This study recorded species variation in exposure to these three tick-borne viruses. For POWV, our research found a seroprevalence of 49.8% (n = 255) among the white-

tailed deer, which is higher than the seroprevalence of any other vertebrate species in this study. This finding further reinforces the notion that white-tailed deer are hugely exposed to POWV as also reported by Nofchiessy et al. (2013) that white-tailed deer may play a role in transmitting POWV. The high exposure may be due to a strong association between white-tailed deer and adult *I. scapularis* ticks. Nevertheless, this high POWV exposure is concerning, given Virginia's enormous white-tailed deer population. This could contribute to POWV geographical range expansion and increase the risk to human health in Virginia.

Another interesting discovery is the detection of NAb against POWV in a bird, the great horned owl. Even though the great horned owl is residential and not migratory, birds generally have the potential to serve as host for different stages of tick development and transport ticks to new areas. This may increase pathogen range and serve as a source of infection to naïve immature stages of ticks. This discovery is in line with Dupuis et al. (2013), who detected Nab against POWV in each of the northern cardinal (*Cardinalis cardinalis*), gray catbird (*Dumetella carolinensis*), and Eastern towhee (*Pipilo erythrophthalamus*) and veery (*Catharus fuscescens*) while sampling avian hosts in New York state. However, the specific role of birds in POWV transmission is not clear. Although *I. scapularis* is known to feed on birds, their exposure may not always be through the primary tick vector. Therefore, it is critical to intensify tick surveillance efforts to identify non-primary tick vectors that may contribute to the transmission and geographical expansion of the pathogen in Virginia. Moreover, our research reported POWV exposure in new wildlife species, such as eastern cottontail and American black bear, thus expanding the list of potential sentinel candidates that could be useful in predicting epidemics. However, further research is necessary to fully incriminate these species.

Considering HRTV, previous studies by Riemersma & Komar (2015) and Bosco et al. (2015) have identified raccoons and white-tailed deer as key species likely to contribute to HRTV transmission. Our study supports this hypothesis in terms of exposure of these species to the virus and found a higher seroprevalence in white-tailed deer than in raccoons, as reported by

Riemersma & Komar (2015) and Bosco et al. (2015). The higher seroprevalence is likely because the tick vector, *A. americanum*, feeds more frequently on deer than on raccoons (Mock et al., 2001). In addition to raccoons and white-tailed deer, other wildlife species such as the American black bear, skunk, American beaver, and North American river otters were also exposed to this bandavirus. These findings expanded the vertebrate host range exposed to HRTV and highlighted the potential for further transmission among wildlife. However, additional surveillance is needed to understand better the virus ecology and its potential impact on host animal health.

For BRBV, a wide range of wildlife species were similarly seropositive for BRBV. These findings confirmed earlier revelation of white-tailed deer, groundhogs, and raccoon exposure to BRBV in Virginia (Cumbie et al., 2022). In support of serosurveys done in Missouri and North Carolina (Jackson et al., 2019; Komar et al., 2020), white-tailed deer and raccoons remain the most exposed wildlife species to BRBV. They may be useful as wildlife sentinel candidates in tracking pathogen spread since all life-stages of *A. americanum* feed on deer. Nevertheless, serological investigation must be continued to identify possible exposure in other species. The red fox, American black bear, American beaver, skunk, and Virginia opossum are other wildlife species exposed to BRBV in this study. The diverse exposure of vertebrate host to BRBV highlights the potential spread and potential impact of the pathogen in Virginia.

For livestock assessment, this study has reported the first evidence of livestock exposure to three tick-borne viruses in Virginia, indicating a potential risk to the livestock industry, especially with the recent detection of BRBV in *H. longicornis*, a tick species associated with transmission of a newly detected pathogen, the *Theileria orientalis* Ikeda genotype in Virginia, which has already caused a significant threat to Virginia's cattle industry (Oakes et al. 2019, Cumbie et al. 2022). Although the pathogenicity of these viruses has not been established in livestock, they are nevertheless being exposed to these viruses as revealed here. Livestock potentially poses a greater health risk to humans as a potential viral amplifier due to close association to human and human habitat compared to wildlife and humans often transport livestock over long distances for

commercial purposes. it is essential to investigate the potential impact of these viruses on livestock health and reproduction, as well as their capacity to play a role in the transmission of these viruses. Surprisingly, there was a significantly higher HRTV seroprevalence compared to the other two tick-borne viruses in livestock, with a p-value less than 0.05 the majority (24 out of 40) of seropositive samples coming from the central region. The reason for this significant difference is unclear, but it may be due to low sampling from other regions. In Virginia, more serological surveillance studies are needed to determine exposure in other livestock species besides cattle. With the continued expansion of competent tick vectors, understanding the potential risks to animal and human health is essential for effective prevention and control strategies. In addition, there is a need to investigate the rate of exposure to HRTV in wildlife around the central region to gain a better understanding of prevalence of exposure in that area.

In comparison, the high tick-borne virus seroprevalence in wildlife and low exposure seroprevalence in cattle highlights the variation in tick access to free-living animals in an uncontrolled natural environment versus livestock confined to a controlled or limited environment. This outcome also supports the hypothesis that ticks feed more on wildlife animals than on domestic ones. Other authors have reported low livestock or domestic animal exposure to these emerging tick-borne viruses. For instance, Jackson et al. (2019) found a BRBV seroprevalence of 4% (n=24) and 15% (n=13) in horses and dogs, respectively, compared to 86% (n=14) in deer and 50% (n=62) in racoons. Similarly, Bosco-Lauth et al. (2015) reported a seroprevalence of 42.6% (n=68) and 14.3% (n=14) for HRTV in northern raccoons and white-tailed deer, respectively, compared to 7.7% in dogs (n=13). So far, wildlife seems to be frequently exposed to POWV, HRTV and BRBV. In contrast, livestock are frequently treated with antiparasitic medication which could affect results; they also tend to be confined to a limited area of grassland, reducing their exposure to tick infestation. This study suggests that wildlife animals should be the focus of tick-borne pathogen surveillance as they are exposed at higher rates, and “may” contribute to the geographical expansion and maintenance of tick-borne diseases in the environment.

Both wildlife and livestock hosts from the western region of Virginia, specifically the southwest and northwest, tend to be more exposed to HRTV, POWV, and BRBV than other regions of the Commonwealth. The reason for this is unknown and should be investigated by means of tick-surveillance in those regions. However, wildlife movements are uncontrolled, and to some degree, exposure might have occurred elsewhere. Nevertheless, more wildlife species in the study have relatively small home-ranges, thus it is most likely that an infection was acquired in the nearby region to where they were sampled. Animals in central and eastern regions might also be exposed to these viruses but are not being detected due to lower sampling. To address this gap, serological surveillance must be continued in those regions to reveal evidence of POWV, HRTV, and BRBV circulation in central and eastern health planning regions.

Bosco et al. (2016) conducted a host susceptibility test, which showed that not all exposed vertebrates developed detectable antibody responses. This finding raises important questions about detecting neutralizing antibodies in exposed vertebrates. It suggests that some vertebrates without neutralizing antibodies may still have been exposed to the virus or could result to a lower seroprevalence. Despite its inability to identify potential exposed but non-seropositive hosts, the plaque reduction neutralization test (PRNT) remains the gold standard for arbovirus surveillance. To address this limitation, a susceptible host test is needed to assess the viremic potential of key animals' species. This will help to exclude some potential candidates and identify species with viremic possibilities that may serve as sentinels for monitoring the spread of emerging viruses.

To understand more about the dynamics of these emerging viruses, our mathematical model evaluated four different data sets or scenarios to capture the model's behavior. There was an increasing trend in exposure for all the data sets, but some have fluctuations that make capturing the model behaviors difficult. For the cattle-alone data set, there seems to be a stagnation in seropositive from month 22 data point. In contrast, for the wildlife-alone data set, there was a sharp jump in seropositive due to high seropositive samples within a sample collection period after month 32 data point. This makes the fitting hard and unable to predict the model's

behavior accurately. As a result of this, both data sets were combined, but it was difficult to fit the model past 32 months. We, therefore, adopted the combined data set to 32 months, given that this gives the best fit and makes the most biological sense.

The highlight of the parameter estimation is the transmission rate and the basic reproduction number, which are 1.57 and 0.645. A basic reproduction number lesser than one is interpreted as the inability of these viruses to spread extensively in the vertebrate population and may later die out. Even though transmission is still occurring, there is no explosion or epidemic in the population, and this also supports the idea that these viruses are emerging. The findings from this study can be adopted to inform control measures for controlling the spread of these viruses in Virginia. For instance, if the transmission rate is lowered by acaricide application which decreases tick population, this could lead to a lower transmission rate and, in turn, lower the basic reproduction number.

However, this model is not reactive and can be improved by introducing factors like environmental conditions that may influence tick and host behaviors. Additionally, this model can be enhanced by including the vector transmission dynamics depending on whether we had information on vector abundance, vector transmission rate, and vector morality rate. Furthermore, sensitivity analysis can be conducted to know how other factors influence the basic reproduction number.

In summary, this study has provided crucial evidence in support of widespread circulation of HRTV, POWV, and BRBV in Virginia. The detection of serological evidence in a wide range of vertebrate species has significantly expanded the understanding of the vertebrate host range of tick-borne viruses and their distribution in Virginia. It has also provided insight into the transmission dynamics of these emerging tick-borne viruses. The estimated parameters can be used as a basis to spread of tick-borne viruses and disease incidence in humans. Future studies should focus on understanding these transmission dynamics and introducing more parameters that may influence transmission. Furthermore, the study highlights the need for vector and host

surveillance to better understand the ecology of these viruses, and their potential to act as amplifiers or reservoir hosts. The understanding is essential for developing effective strategies to monitor the risk of these pathogens in Virginia where they may pose a public health risk.

5.0. References

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