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## Seasonality of Orthohantavirus Seroprevalence in Northwest Arkansas Rodents

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**Seasonality of Orthohantavirus Seroprevalence in Northwest Arkansas Rodents**

An Honors Thesis submitted in partial fulfillment  
of the requirements for Honors Studies in Biological Sciences

By Amy Schexnayder

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Biological Sciences

J. William Fulbright College of Arts and Sciences

University of Arkansas

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## Table of Contents

<b>Introduction.....</b>	<b>5</b>
<b>Methods and Materials.....</b>	<b>8</b>
Study Sites.....	8
Rodent Sampling.....	8
Serology.....	9
Statistical analyses.....	9
<b>Results.....</b>	<b>11</b>
Table 1.....	11
Table 2.....	12
Table 3.....	12
Figure 1.....	13
<b>Discussion.....</b>	<b>14</b>
<b>References.....</b>	<b>17</b>

## **Abstract**

Zoonotic viruses are viruses that can be transmitted from animals to humans. Rodent species are likely to be reservoirs for zoonotic viruses, and particular rodent-borne viruses, such as orthohantaviruses, may greatly threaten human health. Orthohantaviruses are a group of rodent-borne viruses that are at risk for spillover to human populations. Many aspects of orthohantaviruses have been well-researched, yet the seasonality of orthohantaviruses has not yet been thoroughly examined, especially in the southern United States. In this study, we captured 616 rodents trapped over 5953 trap nights across 13 grassland sites in Northwest Arkansas. Rodents were trapped for two consecutive nights every other month from June-November 2020 and April-July 2021 and were screened for antibodies against orthohantaviruses. Seroprevalence was highest in the early summer, followed by autumn, late summer, and late spring. Seasonal fluctuations were largely shaped by rodent life history; seroprevalence was highest early in the year when the population consisted of adults from the previous year, decreased as previous-year adults died and were replaced by juveniles, and increased again as the juvenile population matured. These findings demonstrate the effect of seasonality and host life history on seropositivity of orthohantaviruses and shed light on the prevalence of orthohantaviruses in Arkansas.

## **Introduction**

Zoonotic diseases (zoonoses) threaten human health, as demonstrated by outbreaks of COVID-19, smallpox, and influenza. Prevention of zoonoses is difficult, given their unpredictability, high occurrence, and host generalization, but risk of exposure can be mitigated by understanding the ecology of wildlife systems. For example, many zoonotic diseases fluctuate throughout the year and are especially prevalent in certain seasons (Lal et al., 2012), so human risk of acquiring a zoonotic pathogen can be reduced by determining the seasonality of particular viruses. The seasonality of viruses is dependent on a variety of geographic factors, including latitude, temperature, and climate, making it difficult to draw broad conclusions. Virus seasonality is also largely affected by the population dynamics of its host species.

Rodents are a well-known reservoir hosts of numerous viruses and often play a role in viral transmission to humans (Dahmana et al., 2020). Rodents are found globally, and many species are synanthropic, providing ample opportunity for spillover to humans or other wildlife through urine, feces, and blood (Meerburg et al., 2009). Likewise, rodents reproduce at high rates and maintain large population densities. This is advantageous to virus prevalence, as many viruses follow a density-dependent pattern of transmission, thus virus transmission increases as the population increases and cannot persist below certain density thresholds. Additionally, some viruses cause chronic infections with persistent transmission, allowing them to be maintained in host populations over longer temporal scales.

One important virus group that is commonly maintained and transmitted throughout rodent populations is orthohantaviruses. Orthohantaviruses (order

Bunyavirales, family *Hantaviridae*) are rodent-borne viruses composed of circular, single-stranded enveloped RNA viruses (Payne, 2017). Orthohantaviruses can be zoonotic and are found globally, yet their virulence in humans varies depending on their geographic location. For example, New World viruses, native to the Americas, can cause hantavirus pulmonary syndrome, which has an alarming case fatality rate of 36% (Division of Health Informatics and Surveillance, 2021). Old World viruses, native to most of Europe and Asia, cause more mild infections when transmitted to humans, such as hemorrhagic fever with renal syndrome and its milder form nephropathia epidemica. In rodents however, orthohantaviruses cause asymptomatic infections that are persistent and chronic (Escutenaire & Pastoret, 2000). Rodents that are male, larger, older, or wounded are more likely to be seropositive for hantaviruses (Mills et al., 2007; Hinson et al., 2004). There are also a large number of hosts for hantaviruses, as 97 rodent species have been genetically identified as a host globally (Milholland et al., 2018). Of these, 58 rodent hosts and 20 distinct orthohantaviruses have been discovered in the Americas so far (Mull et al., 2020). Though many factors about orthohantaviruses are well understood, other components such as the seasonality, have not yet been well defined.

Though the seasonality of orthohantaviruses has been commonly researched, most of this research was conducted in Europe and the western United States (Montana, Arizona, Nevada, and Colorado) where the environments are very different from Northwest Arkansas (Madhav et al., 2007; Kuenzi et al., 2001; Calisher et al., 2007; Douglass et al., 2007). Seroprevalence is commonly highest during spring and summer months, correlating with early rodent breeding (Kuenzi et al., 2001; Douglass & Vadell, 2016; Pearce-Duvet et al., 2006). Although higher seroprevalence is generally associated

with warmer months, there are many other factors that affect seropositivity, such as relative humidity, rainfall, food abundance, and predator abundance (Bennett et al., 1999). Orthohantavirus prevalence often follows a pattern of delayed-density dependence, with an increase in population causing a subsequent but delayed increase in infection prevalence (Madhav et al., 2007), though some studies suggest this pattern of transmission may be more complex (Carver et al., 2011).

This study aims to expand our understanding of seasonal trends in orthohantavirus seroprevalence using rodents in Northwest Arkansas grasslands. We expected a higher prevalence of orthohantaviruses during the early breeding season (late spring and early summer) due to higher temperatures and the increased average age of rodents in this season. During the late summer, we expected lower seroprevalence as older rodents began to die off and the population consisted of younger rodents. In autumn, we expected higher seroprevalence again, as younger rodents matured and were exposed to more pathogens.



## Methods and Materials

### Study Sites

Rodents were captured at 13 sites within six prairies throughout Benton and Washington Counties, AR, USA. The 13 sites included three sites at Chesney Prairie, three sites at Woolsey Wet Prairie, one site at Stump's Prairie, two sites at Pea Ridge National Military Park, one site at Wilson Springs Nature Preserve, and three sites at Milo J. Shult Agricultural Research & Extension Center.

### Rodent Sampling

Trapping was conducted for two consecutive nights every other month at each site from June-November 2020 and April-July 2021. Rodents were captured using Sherman live traps (H. B. Sherman Traps, Inc.) set in a series of transects. The traps in each transect were spaced 10 m apart and transects were also spaced a minimum of 10 m apart. Traps were baited with a mixture of millet and black oil sunflower seeds and set at dusk. Traps were checked, and captured rodents were processed the following morning. Initially, all rodents were euthanized for tissue collection except for those classified as species of conservation need by Arkansas Game and Fish Commission (*Reithrodontomys humulis*, *megalotis*, and *montanus*). However, we were unable to euthanize all individuals of abundant species (*Reithrodontomys fulvescens* and *Sigmodon hispidus*) in fall 2020 due to permit limitations.

After trapping, we assessed the species, sex, mass, and reproductive condition of each animal. During processing, a blood sample was collected from most animals via the submandibular vein directly into a microcentrifuge tube and immediately placed on ice. A blood sample was collected from the remainder of the animals via a heart sample

placed in phosphate-buffered saline (PBS) during dissection at a later time. Rodents were euthanized using cervical dislocation and *Sigmodon hispidus* (cotton rats) were anesthetized using isoflurane before euthanization. Euthanized rodents were placed into separate bags and immediately placed on ice. Rodents that were not euthanized were released at their point of capture. Animals were dissected in a biosafety level 2 (BSL-2) hood. Tissue samples were collected aseptically using clean tools and placed in sterilized microcentrifuge tubes. All samples and specimens were stored at -20°C. Animal handling followed the guidelines of University of Arkansas' Institutional Animal Care and Use Committee and the American Society of Mammologists (Sikes et al. 2016).

### **Serology**

Blood samples were tested for antibodies against orthohantaviruses using immunofluorescence assays (IFAs; Kallio-Kokko et al., 2006; Kinnunen et al., 2011; Forbes et al., 2014). Blood samples were diluted with PBS and placed on slides with rodent antigens. Slides were then incubated, followed by several wash cycles. Anti-mouse IgG conjugates and fluorescence were added to the slides and incubated again prior to several additional wash cycles. Slides were examined under a fluorescent microscope.

### **Statistical analyses**

We used a chi-square test of independence to compare seasonal variation in seroprevalence. A Tukey post hoc test was then used to determine the pairwise prevalence of orthohantaviruses among seasons. The seasons were defined as late spring (April/May), early summer (June/July), late summer (August/September), and autumn

(October/November). Each season was sampled once except for early summer, which was sampled in both 2020 and 2021.

## Results

A total of 616 rodents were captured across 5953 trap nights (Tables 1 and 2). Capture success ranged from 0-45% depending on site and season (Table 2). We captured eight different species throughout the study (Table 1), with 2-6 different species at individual sites. The most common species were cotton rats and fulvous harvest mice. A total of 34 individuals were seropositive for orthohantaviruses, consisting of 26 cotton rats, seven prairie voles, and one fulvous harvest mouse (Tables 1 and 2).

The majority of seropositive rodents were caught during early summer (n=16), followed by autumn (n=12), late summer (n=5), and early spring (n=1; Table 2; Fig. 1). Seroprevalence varied among seasons ( $\chi^2=10.38$ ,  $p<0.02$ ). Early summer had higher seroprevalence than late summer ( $\chi^2=9.50$ ,  $p<0.01$ ), but seroprevalence was similar among all other pairwise seasonal comparisons (all  $p>0.05$ ; Table 3).

**Table 1.** Total number of each species captured among all sites.

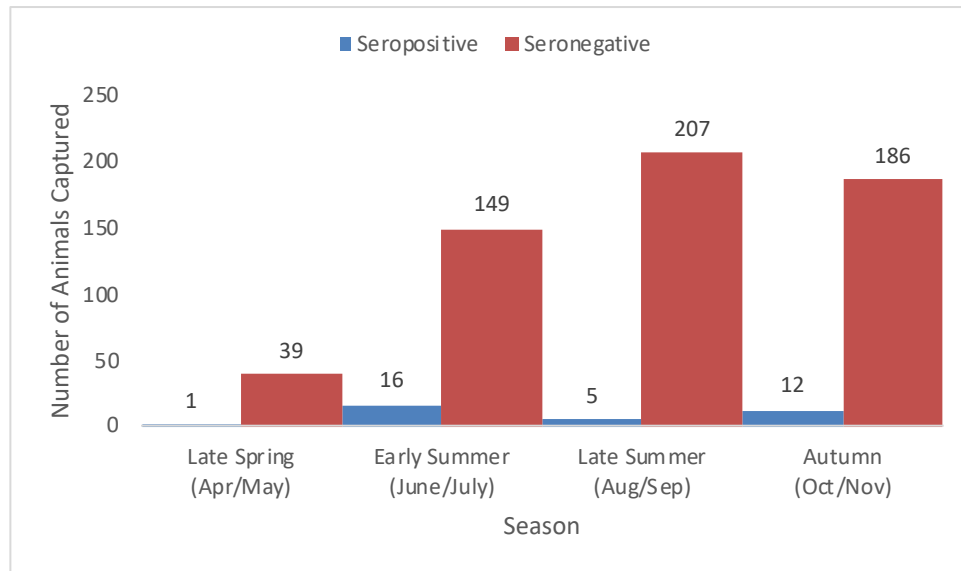
Scientific name	Common name	Captures
<i>Microtus ochrogaster</i>	Prairie vole	47
<i>Microtus pinetorum</i>	Woodland vole	3
<i>Mus musculus</i>	House mouse	2
<i>Peromyscus leucopus</i>	White-footed mouse	51
<i>Peromyscus maniculatus</i>	Deer mouse	50
<i>Reithrodontomys fulvescens</i>	Fulvous harvest mice	123
<i>Reithrodontomys montanus</i>	Plains harvest mouse	2
<i>Sigmodon hispidus</i>	Hispid cotton rat	339
Total		616

**Table 2.** Total number of rodents captured in each season that were seropositive for orthohantaviruses.

Season	Trap Nights	Captures (%)	Seropositive (%)
Late spring	1300	40 (3.1)	1 (2.5)
Early summer	2060	166 (8.1)	16 (9.6)
Late summer	1293	212 (16.3)	5 (2.4)
Autumn	1300	198 (15.2)	12 (6.1)
<b>Total</b>	5953	616	34

**Table 3.** Results of Tukey post-hoc tests for each pairwise seasonal comparison.

Season	X <sup>2</sup>	P-value
Late spring vs. early summer	2.193	0.139
Late spring vs. late summer	0.003	0.957
Late spring vs. autumn	0.817	0.366
Early summer vs late summer	9.500	0.002
Early summer vs. autumn	1.672	0.196
Late summer vs. autumn	3.531	0.060



**Figure 1.** Number of seropositive and seronegative rodents for orthohantaviruses in the 4 different seasons.

## Discussion

We demonstrate that orthohantavirus seroprevalence in rodents fluctuates seasonally, with the highest proportion of seropositive rodents being caught in the early summer at the onset of the breeding season. Though it was not statistically significant, seroprevalence was also relatively high in the autumn as breeding curtailed and populations matured. These seasonal patterns demonstrate the role of host life history in shaping seasonal patterns of orthohantaviruses prevalence.

The high orthohantavirus seroprevalence in the early summer was consistent with our hypothesis, as higher temperatures are also associated with higher seroprevalence (Oliviera et al., 2014; Vadell et al., 2011). The relationship between orthohantavirus seroprevalence and temperature is mediated by rodent breeding, which occurs throughout the summer in temperate regions. Older rodents from the previous year, which have disproportionately higher seroprevalence (Mills et al., 2007) are dying off by the late summer, reducing the number of living infected individuals. Concurrently, younger rodents are being added to the population, increasing the number of uninfected individuals. This combination explains the decrease in seropositivity in the late summer as populations are diluted by young, uninfected individuals and older individuals are removed from populations (Reed et al., 2007; Mills et al., 1999).

The seroprevalence of orthohantaviruses increased in autumn, though overall seroprevalence was too low to determine that this difference was statistically significant. Based on relative proportions in this study, a larger sample size likely would have corrected for this. The increase in seropositivity seen here may have occurred because rodents born in the early summer mature by autumn and have more time to be exposed to

an orthohantavirus and produce antibodies. Maturation leads to increased fighting and mating behaviors, facilitating the spread of orthohantavirus (Hinson et al., 2004; Pearce-Duvel et al., 2006). The mature rodents during this season are the basis for the high seroprevalence that occurs in the following spring.

Our findings were surprising in that seroprevalence in the late spring was lower than seroprevalence in the early summer. Previous studies in Colorado and Montana have found the highest seroprevalence in May, given that rodents are reproducing during the spring season, which can lead to horizontal transmission of orthohantaviruses (Madhav et al., 2007; Calisher et al., 1999). Even in circumstances where the spring season was not associated with high populations, there may still be high antibody prevalence due to high population density in the previous winter (Madhav et al. 2007). Such explanations suggest that we should have found similar seroprevalence in both the late spring and early summer. However, the wet prairie sites where we captured rodents were still partially flooded in the spring, which reduced capture rates during that season and limited our ability to accurately detect seroprevalence (Table 2).

Notably, different locations may have vastly different seasonality, given the variation in geographic factors such as temperature, climate, elevation, and latitude. For example, population size and seroprevalence can be negatively affected by periods of low rainfall (Mills et al., 1999). Likewise, during warmer weather, additional rainfall can have favorable effects on population size, but during colder months, rainfall can have detrimental effects on rodent populations (Luis et al., 2010). Such climatic effects indicate the spatiotemporal variation of seroprevalence, and future studies should seek to



determine the effect of latitude, elevation, and other climatic factors that may affect seasonality among different regions.

Overall, our study provides the first evidence of orthohantaviruses in Arkansas rodents. In addition to identifying the presence of orthohantaviruses in Northwest Arkansas, we also demonstrated the seasonal variation in rodent seroprevalence. As climate change continues to alter wildlife and their habitats, it is especially important to understand seasonal patterns for zoonotic viruses. Such understanding will assist us in mitigating exposure to these pathogens and reducing spillover to humans.

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