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Old World Hantavirus Infection in *Rattus* Species and Risk Management in Urban Neighborhoods of New Orleans, Louisiana

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ABSTRACT: Hantaviruses are lipid-enveloped, tri-segmented RNA viruses belonging to the family *Bunyaviridae*. Hantavirus are divided taxonomically into Old World and New World groups that typically cause hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome, respectively. Each hantavirus is specific to a particular rodent reservoir host. In 1983, an Old World hantavirus similar to Seoul virus was isolated from rats caught in New Orleans, Louisiana (Tchoupitoulas virus). To date, this virus has not been associated with human disease. Since that time, no hantavirus surveillance has been conducted in this geographic area. We sought to determine if Old World hantaviruses still circulate in rodents in New Orleans and, if so, to decrease rat populations in attempts to reduce the risk of human-rodent interaction and the potential for disease transmission. Over a 3-year period, rodents were live-trapped using Sherman and Tomahawk traps. Blood and other tissues were collected and samples tested for the presence of Old World hantaviruses via Reverse transcriptase Polymerase Chain Reaction. Trap sites were identified through selected citizen service requests and routine municipal rodent management activities. Of the 172 roof rats and Norway rats collected, 3.6% tested positive, indicating continued presence of Old World hantaviruses in New Orleans. This study raised awareness of the continued risk of rodent-borne disease in the greater New Orleans area and spawned proactive management strategies on a city-wide basis, including neighborhood surveys, public education and awareness campaigns, and an aggressive rodenticide baiting program in areas with large rodent populations. Continued surveillance and detection of hantaviruses and other rodent-borne pathogens will help preserve the safety and health of New Orleans residents.

KEY WORDS: *Bunyaviridae*, hantavirus, Louisiana, New Orleans, Norway rat, *Rattus norvegicus*, *Rattus rattus*, roof rat, zoonotic disease

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INTRODUCTION

Pathogenic hantaviruses are causative agents for rodent-borne zoonoses that cause moderate to severe disease in humans. There are at least 30 documented hantaviruses in nature, and each is associated with a specific rodent or insectivore reservoir. Transmission is thought to occur via exposure to rodent excreta through inhalation, direct introduction into broken skin or mucous membranes, and/or bites from infected rodents. To date, there is limited evidence to suggest person-to-person transmission is possible (Vitek et al. 1996). Hantavirus disease has many features common to other hemorrhagic fevers including fever,

myalgia, thrombocytopenia, and a hallmark vascular leak syndrome. Unique to hantaviruses infections, however, is a division of pathology that generally correlates with the geographic origin of the infection: viruses that are endemic to the Old World cause a mainly renal syndrome, and those from the New World primarily cause a pulmonary syndrome.

Hantaviruses were first discovered in 1951 during the Korean war, where American doctors documented over 3,000 cases in United Nations troops (Schalmaljohn and Hjelle 1997). Old World hantaviruses, including Hantaan, Dobrava, Saaremaa, Seoul, and Puumala strains, can

cause hemorrhagic fever with renal syndrome (HFRS) (CDC 2012). Symptoms begin suddenly 1 to 2 weeks post-exposure and include intense headaches, back and abdominal pain, fever, chills, nausea, blurred vision, petichial flushing, conjunctival injection, and sometimes a hemorrhagic “rash”. As the disease progresses, patients will experience hypovolemia, vascular leakage, thrombocytopenia, acute shock, and kidney failure (CDC 2012). Current estimates of infections caused by Old World viruses range from 150,000 to 200,000 hospitalizations each year, the majority of which occur in China, Russia, and Korea (Schalmaljohn and Hjelle 1997).

Hantavirus disease in the New World was not described until 1993, when an outbreak of a severe pulmonary disease associated with exposure to the common deer mouse, *Peromyscus maniculatus*, was found responsible for 45 deaths in the Four Corners Region of the United States (Scott 2002). The virus responsible for the outbreak was later named Sin Nombre Virus (SNV). Current estimates of cases of Hantavirus Cardio-Pulmonary Syndrome (HCPS) in the U.S. approach 550 (CDC 2012). Symptoms typically begin 3 days to 6 weeks post-exposure. Initially, patients present with flu-like symptoms including fever, headaches, abdominal pain, and lower back pain. The hallmark feature of HCPS is acute and severe pulmonary edema, which results in impaired breathing, hypoxia, or myocardial depression (MacNeil et al. 2011). Death occurs from shock and cardiac complications, despite medical support (Schalmaljohn and Hjelle 1997, Scott 2002). The Centers for Disease Control and Prevention (CDC) conducted field and epidemiological studies that determined approximately 30% of the deer mice population were positive for SNV (CDC 2012). Since this outbreak, similar cases of HCPS have been reported throughout the U.S. but were determined to be caused by hantaviruses that are genetically distinct from SNV. These include Bayou Virus in Louisiana, Black Creek Canal Virus in Florida, and New York Virus on the East Coast.

Other hantaviruses found in the U.S. are genetically more similar to most Old World hantaviruses, e.g., Prospect Hill Virus was discovered in 1985 but has not been associated with human disease (Lee 1985). Conversely, surveillance data of rodents near port cities, where densities of *Rattus* spp. are historically higher, revealed the prevalence of viruses closely related to Seoul Virus (Childs et al. 1987, LeDuc 1987, LeDuc et al. 1992); however, limited evidence of human exposure or disease came from these studies. Renewed interest in the potential risk for human disease in these areas appeared in 2008, after the first documented HFRS case in the U.S. was confirmed in Maryland (Woods et al. 2009).

New Orleans, Louisiana is one of the United States’ largest and busiest international port cities. Similar to other port cities where Old World hantaviruses have been found, a Seoul virus variant was first documented by T. F. Tsai in 1983. This study surveyed *Rattus* spp. in major port cities across the U.S. (Tsai et al. 1985). In New Orleans, of the 79 rodents tested, 30% were seropositive for a Hantaan-like virus. Morphologically identical to other hantaviruses but serologically distinct by cross-neutralization tests, the new strain was termed Tchoupitoulas Virus (TCHV) and has, to date, not been associated with human

disease (Tsai et al. 1985). Since the time of this study, there has been no work to more carefully examine the risk for hantavirus-related disease in this area.

The objective of this project was to determine if TCHV could again be identified in *Rattus* populations in New Orleans. From summer 2008 to spring 2010, 172 tissue samples were collected from *Rattus rattus* and *R. norvegicus* across the New Orleans area in a collaborative project between the City of New Orleans Department of Mosquito, Termite, & Rodent Control Board and the Tulane University School of Public Health and Tropical Medicine. If TCHV was identified, then the municipal rodent control program would implement management activities that would reduce the potential for human exposure.

MATERIALS AND METHODS

Vector control specialists and public health professionals worked together to achieve trap goals, make environmental assessments, estimate population dynamics, perform necropsies, and conduct laboratory diagnoses. Trap locations were determined based on citizen service requests and/or through routine rodent management operations. Vector control specialists inspected potential sites for study appropriateness by using standard criteria, including 3 or more obvious rodent burrows, consistent rodent sightings by residents, obvious structural damage, and trap security. All trapping and sampling activities followed the published U.S. Department of Health and Human Services and CDC recommendations as described in Mills et al. (1995).

The study was divided into two phases. Phase 1 spanned 12 months and covered 45 nights on which trapping occurred. After the first TCHV-positive rodent was identified, Phase 2 was initiated, consisting of extensive trapping at that location for 39 nights over a 2-month period. Traps were set in a concentric pattern that was expanded each week into areas that met the trapping criteria. We used metal Tomahawk® cage traps (Tomahawk Live Trap LLC, Hazelhurst, WI) baited with a 5-gram ball of peanut butter, lard, and oatmeal. Each trap was numbered and GPS coordinates recorded by using a Garmin® Astro-220 hand-held receiver (Garmin International Inc., Olathe, KS). Traps were set between 2:00 PM and 6:00 PM and picked up between 6:00 AM and 8:00 AM the following morning. Captured specimens were kept in the open air and physically separated from each other in the bed of a pickup truck while being transported to the necropsy site. Personal protective equipment (PPE) worn included dedicated shirts and long pants, closed-toe shoes, N95 respirators, and double latex gloves during trap setting, gathering, and transporting activities.

Necropsies were performed at an isolated, well-ventilated outdoor processing site. The site was set up with two tables, a dedicated clean table that held all clean supplies, and a dedicated necropsy table that was covered with a plastic-lined lab mat. Only the supplies essential to performing dissections were on the necropsy table, which included a sharps container, a 10% bleach in water solution, 90% ethyl alcohol spray bottles, and the utensils. Vector control specialists wore N95 respirators, double layered latex gloves, a long-sleeve cuffed isolation gown, and safety goggles. All PPE remained on for the duration of

necropsies. Sterile long-armed latex gloves were changed between each dissection and removed after each necropsy.

Rodents contained in their cages were placed in air-tight plastic bags and were euthanized using cotton balls soaked in 99.9% isoflurane solution (IsoFlo[®], Abbott Laboratories, Chicago, IL). Cardiac blood was collected by cardiac puncture with a 1-ml sterile hypodermic syringe. The rodent was combed for ectoparasites, which were stored in 90% ethyl alcohol for later identification by a City of New Orleans entomologist. Morphological measurements of the rodents were recorded including species, sex, age, and reproductive status. Lungs, liver, spleen, pancreas, kidney, and tail tip were harvested; all tissue samples were kept on ice during the necropsy and subsequently transported to a -80° F freezer at Tulane University. Reverse transcriptase Polymerase Chain Reaction (RT-PCR) and sequence analysis using previously described methods (Arthur et al. 1992) was carried out on RNA collected from lung tissues (Cross et al. unpubl. data). When virus was detected from lung tissue, then positive amplicons were verified by sequence analysis. Carcasses were stored in formalin in Whirl-Pak[®] collection bags (Nasco, Fort Atkinson, WI).

When implemented correctly, Integrated Pest Management (IPM) is the most effective and sustainable approach for managing urban rodents. IPM is a 5-step process that includes inspection, identification, the establishment of threshold levels, the employment of two or more control measures, and the evaluation of effectiveness (Berman 2002). IPM practices incorporate a realistic and holistic approach to pests. A municipal IPM program begins with an inspection by a vector control specialist of a residential property. Specialists identify the pest, estimate current pest levels, and make recommendations to the homeowner describing best practices based on detailed knowledge of pest biology. Implementation to make structural changes and the purchase of rodenticides and products was the responsibility of the homeowner. Communication between the resident and New Orleans Mosquito, Termite, & Rodent Control staff is paramount to the successful implementation of a well-rounded IPM program. Ongoing support and advice is provided along with rodenticides placed by a certified pesticide applicator. The goal of IPM is to reduce the potential for human health hazards, protect public safety, prevent lost or damaged resources, structures, and properties, prevent pests from spreading into the community, and enhance quality of life (EPA 2012).

RESULTS

The frequency in which a *Rattus* species in New Orleans was likely to occur was determined by calculating the small mammal abundance index (SAI) according to the equation $SAI = (SC \times 100) / (T \times N)$, in which SC is the number of rats captured, T is the number of traps set, and N is the total number of nights that trapping occurred (Tagliapietra et al. 2009). A total of 1,904 traps were set for 84 nights of trapping where 172 rodents collected. There was a 7.6 % frequency in which a rodent would occur in each set trap for the overall project. Our trap success rate was calculated by 172 samples by 1,904 trap night for a 9% success rate.

In total, there were 172 rats captured, including 124 *Rattus norvegicus* and 48 *Rattus rattus* between 2008 and

summer 2010. Lung tissues samples from 6 *R. norvegicus* were sequence positive for TCHV (Cross et al. unpubl. data) 5 were trapped in August 2008 within a 4-block radius alongside a railroad track, while the sixth was taken 6 months later along the railroad on the Mississippi Riverfront in the French Quarter.

The 5 virus-positive rats were taken from a low-to-middle-income neighborhood alongside a major north/south railroad line. The railroad connected to a receiving dock at the east/west junction, which was also the site of the sixth positive sample. The trap site was an empty lot where three cars were being stored for repair. The stored cars contained rodent feces and had obvious gnawing damage to the seats. The lot and adjoining house was inspected and revealed a heavy *R. norvegicus* infestation. The house was a typical New Orleans style raised, wood shotgun. The lot and the house were minimally maintained by the owner, an elderly adult male. He shared his home with an adult female and several children of various ages. A family dog was observed tied up outside and under the house. The dog was outside during the day and we were told by the owner that that pet was let in at night to sleep. The property and home had poor sanitation, extreme clutter, and was poorly maintained. The property owner reported seeing small rodents coming through the window unit.

An inspection of the perimeter of the house revealed a number of conditions that led to and maintained the rodent population: food waste was being thrown under the house; water collected in puddles, buckets, and trash cans that created breeding areas for rat tailed maggots (larvae of the drone fly, *Eristalis tenax*) and mosquitoes (*Culex* spp.); there were several trash cans and an open trailer that contained a large amount of household trash intended for dumping, although the trash was never removed during our Phase 2 trapping activities. Several burrows were observed along the driveway and next to the piers supporting the house. Vector control specialists spoke with the owner of the property several times to request that the conditions be remediated to reduce the rodent population.

An in-depth neighborhood survey was conducted in 2009 in the area where virus-positive rodents were trapped. Several weeks were spent in the area inspecting every house in the neighborhood. The door-to-door campaigns allowed our team to speak directly with as many residents as possible. Residents described issues they had with rodents, both indoors and outdoors. The exterior of each home was inspected for burrows. If burrows were found, they were treated at the time of inspection by using a second-generation anticoagulant rodenticide (Contract Ready-to-Use Place Pacs or Contract All-Weather Box, Bell Labs, Madison, WI) labeled for deep-burrow baiting or contained in tamper-resistant bait stations. If no one was at home, a flyer was left on the resident's door with the city agency's contact information and encouraged citizens to participate in the neighborhood-wide inspections. In addition, all abandoned lots were inspected. Information, both verbal and written regarding IPM best practices for rodent control, was given to each house in the treatment area.

District-wide treatments consisted of treating every storm drain with 6 blocks of rodenticide per drain. Ro-

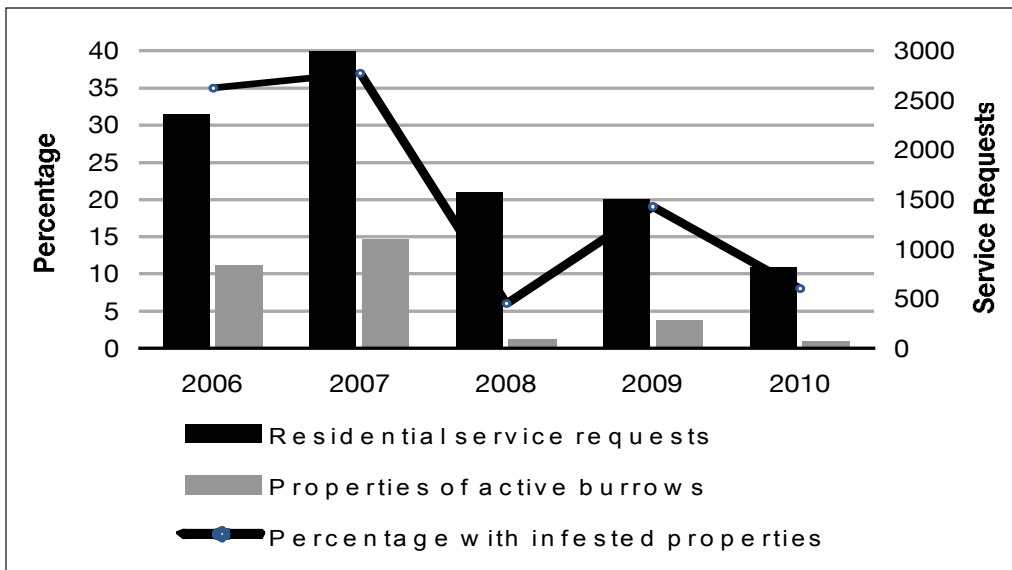


Figure 1. Service requests and active burrow data for Orleans Parish, 2006 through 2010.

were less likely to be disturbed. Citizens would to continue to see rats in the area, but due to agency restrictions inspectors were not allowed to inspect or treated blighted properties where the rats were known to be infesting. In 2009, the residential population rebounded and the number of service request decreased, however, the number of infested properties increased, suggestive that as citizens cleaned up and repaired their properties, active burrows were found and subsequently treated by the city agency (Figure 1).

denticide blocks were hung from metal wires affixed to the drain, leaving the bait to dangle just above the natural waterline in the drain. Between 2006 - 2011, 20 districts received such treatments. During the neighborhood survey of the hanta-positive zone, 89 storm drains were treated with a rodenticide labeled for use in storm drains (Contract Super Size Blox, Bell Labs, Madison, WI) Bait consumption was estimated 30 days later and was given percentage of consumption based on a 10-point scale: 0 = no consumption, 1 = 10% consumption ... 10 = 100% consumption. At the 30th day, an inspection of the drain treated revealed that the bait in 14 of the drains in the hanta-positive zone had at least 50-100% consumption and another 14 had 20-40% consumption. All bait with consumption greater than 50% was replaced at the time of re-inspection. In addition to area-specific treatment, district-wide treatments were conducted in the surrounding neighborhoods.

Our data show that since 2006, number of requests for services decreased from 2,364 requests in 2006 (with 838 residences infested by rats) to 809 requests in 2010 (with 64 residences infested). Several years following Hurricane Katrina (which occurred in late August 2005), well-intended animal welfare groups distributed large amounts of dog and cat food to abandoned properties throughout the City of New Orleans. We know, from an operational standpoint, that this practice greatly contributed to the rat population increase. The situation was not helped by the sheer number of damaged homes and abandoned properties available for rodents to infest. In 2007, our agency began sending inspectors to these sites to remove the pet food. During the same time, people started moving back into the area and clean-up efforts were well on the way. Rat populations started to drastically decrease in 2007 due to the removal of food, water, and harborage that had sustained large populations. By 2008, our agency saw a reduction in the number of service requests, which suggested that rat populated fewer inhabited properties and began to infest blighted properties where their burrows

DISCUSSION

In economically depressed areas, many residents do not have the means to hire pest management professionals. Governmental agencies can provide professional rodent control services such as applying rodenticides according to the label directions, and by providing glue boards, snap traps, and live-catch traps. Assistance and advice needs to be provided throughout the remediation process. In New Orleans, when two or more burrows were found on a property during inspection, vector control specialists re-inspected and treated the burrows again until all signs of activity had ceased. Through this process, citizens began to understand that their own actions such as poor sanitation, clutter, and inadequate home repairs contributed to the presence of the pest.

In urban environments, the best control practices require a partnership between the local government and the citizen in order to conduct a multi-strategy IPM approach. For residents, this is generally a new concept. In order for citizens to buy into the IPM concept, they need to understand that rodents are more than just pests, but that they may carry disease and can adversely affect public health by contaminating food supplies, damaging structures, and causing severe mental distress. The role of the public agency is to educate citizens about best practices and management strategies that are affordable, and to provide cost-effective methods.

Data from this study support the conclusion that TCHV is being maintained in the *R. norvegicus* population in Orleans Parish. The City of New Orleans has a vested interest in the preservation of the health of its citizens. The results of this project have spawned a new incentive to manage and control rodents on a citywide basis, by conducting in-depth neighborhood surveys that include both communication campaigns directed towards citizens and aggressive rodenticide baiting programs. The factors responsible for the decrease in requests and infestations are the success of intensive outreach programs and aggressive district-wide rodenticide treatments.

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