



# THE LABYRINTH

Indiana State Department of Health Laboratories Newsletter

## Jamestown Canyon and Powassan – Two Arboviral Diseases of Growing Epidemiological Concern in the Northeastern and Midwestern United States (Part 2)

By Erica Vecchio,  
Microbiologist



In the past, many arboviruses have often “disappeared” after causing limited initial outbreaks in human populations, with little evidence of having been an issue until their next resurgence. Recurrences of these diseases have generally been unexpected and unpredictable, with large periods of time (often decades) passing between known episodes. In recent years, however, many arboviral pathogens appear to be returning more frequently and expanding in range. According to the World Health Organization (WHO), 17% of global disease is now caused by vector-borne pathogens [1]. Although much of the world’s focus has been upon mosquito-borne arboviral diseases such as malaria, dengue, Zika, and West Nile, ticks also serve as vectors of pathogens. The greatest number of vector-borne infections in the U.S. is attributed to ticks. They are capable of transmitting bacteria, protozoans, and/or viruses to human populations. Notable tick-borne diseases include babesiosis (protozoal), Lyme (bacterial), Rocky Mountain spotted fever (bacterial), and tick-borne encephalitis (viral) [2].

Ticks are found throughout the world, but most arboviral diseases are endemic to specific areas of the globe. Northern temperate regions such as the United States are the particular locales of tick-borne encephalitis viruses [3]. In the U.S., tick-borne arboviruses and tick-borne diseases in general are considerable public health concerns because of the diversity of pathogens carried by ticks and the number of cases that occur nationally. Additionally, associated disease rates have been steadily increasing in recent decades [4].

In the past 20 years, Powassan virus (POWV) disease has received attention as an emerging or returning arbovirus with human cases concentrated in the northeastern and midwestern U.S. POWV is a member of the family Flaviviridae, *Flavivirus* genus, which includes diseases found all over the world and spread by both mosquitoes and ticks [5, 6]. Of the tick-borne encephalitis flaviviruses, POWV is the only species found in North America [6-9]. Reported human cases of POWV disease have increased substantially, particularly in the U.S., over the past two decades. From 1958 to 1998, 27 total instances of POWV disease from eastern Canada and the northeastern United States were reported [1, 9, 10]. By contrast, 108 infections were reported to the Centers for Disease Control and Prevention (CDC) from 1999 to 2016 in the U.S. [10-12]. The CDC also received reports of 34 cases in 2017 and 21 in 2018 nationally; numbers from 2019 are not yet available [13, 14].

States including Connecticut, Indiana, Maine, Massachusetts, Michigan, Minnesota, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Pennsylvania, Rhode Island, Vermont, and Wisconsin have notified the CDC of residents with the virus [10, 12-15]. Human cases in the U.S. were initially found primarily in the Northeast. In the 2000s, the Midwest (Michigan, Minnesota, Wisconsin) began reporting its first infections [10, 16]. Patients from the three states located outside of the Northeast and Midwest (Indiana, North Carolina, North Dakota) acquired the disease from either blood transfusion or travel to an area with a history of the virus.

POWV was isolated from *Dermacentor andersoni* ticks in Colorado in 1952 [9], but POWV disease was not recognized as a human pathogen until later. In 1958, CNS tissue samples containing the virus were collected in Powassan, Ontario, during the autopsy of a 5-year-old child who died from encephalitis [17, 18]. (continued)



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### Our Mission:

The Indiana State Department of Health Laboratories partners with other public health agencies to provide timely and accurate information needed for surveillance and outbreak investigations to protect and improve Hoosier health.

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## Jamestown Canyon and Powassan Arbovirus (*continued from page 1*)

The first case of POWV disease in a U.S. citizen was diagnosed in New Jersey in 1970 [11, 19]. In 1996, deer tick virus (DTV), a subtype of POWV, was discovered in *Ixodes* ticks in Massachusetts and Connecticut; the first human encephalitis case, also in New England, was reported the following year [1, 8, 20]. POWV neuroinvasive disease became reportable to the CDC in 2001 and POWV non-neuroinvasive disease in 2004 [11].

There are two genotypes of POWV that are capable of producing human disease - POWV Lineage I (POW-L1) and POWV Lineage II (DTV). Due to their antigenic similarity, the two cannot be distinguished through serological testing alone, and genotyping is necessary for definitive diagnosis of viral infection [6, 11, 16, 20-22]. There is more concern about POWV Lineage II spreading to humans due to the tick species involved in transmission. Five species of ticks have been found to carry POWV in North America: *Dermacentor andersoni*, *Ixodes cookei*, *Ixodes marxi*, *Ixodes scapularis*, and *Ixodes spinipalpus* [21].

POW-LI is historically believed to circulate in two major enzootic cycles in which the virus is maintained in particular mammalian hosts. In the first, *I. cookei* feed upon groundhogs and/or skunks; in the second, *I. marxi* bite squirrels. Both tick species are nidicolous (found primarily in the nests or burrows of their hosts) and generally host-specific. As such, people are very rarely bitten and infected. There is currently some doubt as to whether POW-LI is actually maintained in these cycles due to the age of the studies, the lack of data on efficacy of virus transmission by these species, and the evidence of host preference in *I. cookei* and *I. marxi* [4, 9, 11, 21].

DTV is maintained in an enzootic transmission cycle between *Ixodes scapularis* and white-footed mice (*Peromyscus leucopus*). Although *I. scapularis* ticks most frequently feed upon white-footed mice [4, 8], they will aggressively bite other potential hosts, including humans. They are therefore regarded as the primary vector of POWV/DTV to humans [22]. These ticks also display questing behavior, in which all life stages actively seek organisms for blood meals. Consequently, various possible hosts, including people, have a higher probability of encountering infected individuals [4]. After hatching, each stage of the tick must obtain a blood meal from an available host. Although nymphs and adults are the stages that most successfully bite and obtain blood meals, nymphs are suspected of the most transmissions of DTV [20]. Unlike nymphs in the Southeast, those in the Northeast and Midwest ascend leaf litter to quest, thus increasing encounters with humans in these regions [4].

The tick species has enlarged its range in the past two decades, likely due to a number of factors including reforestation of farmland, increasing populations of host species such as deer and mice, and climate change. Distribution has expanded most in the midwestern and northeastern U.S., which may partially account for the higher volume of reported cases in these regions. Overall warmer temperatures have also led to increased levels of human activity outside, thus elevating exposure risk to infected ticks [4, 6, 23].

Ticks may become infected with the virus through a variety of means. In transstadial transmission, the virus is maintained within the tick as it molts into another stage of life. Some species are capable of passing the virus to their offspring through vertical (transovarian) transmission. This strategy helps perpetuate the virus through overwintering in eggs. In horizontal transmission, POWV is received through a blood meal from viremic small mammals. Larger mammals and humans are dead-end hosts. Therefore, the virus is not spread by coughing, sneezing, or skin contact. Transmission in humans may also occur through blood transfusions.

DTV is very efficiently transmitted to vertebrate hosts by *I. scapularis*; transference to previously uninfected individuals may occur within 15 minutes after vector attachment [4, 6, 16, 21, 23, 24]. Tick saliva likely plays some role in increasing transmission time of DTV by shielding the virus and affecting immune response at the site of insertion [6, 25]. Additionally, *I. scapularis* is the primary vector of seven pathogens to humans, including DTV [4, 22, 24, 26]. As such, coinfections with other diseases such as Lyme are possible and may increase the severity of disease [9, 22, 27, 28].

The majority of people infected with Powassan are asymptomatic. Symptoms appear in others between 8 and 34 days and initially mimic a common cold. These signs may include fever, headache, nausea and vomiting, weakness, sore throat, and muscle and joint aches. As the disease progresses, more severe features rapidly develop due to the onset of encephalitis or meningitis: confusion/ disorientation, difficulty speaking, respiratory distress, loss of coordination, seizures, and paresis or paralysis. The mortality rate is 10-15% in patients who develop severe cases of the disease. Additionally, about 50-70% of survivors develop long-term health issues such as recurring headaches, muscle weakness, loss of muscle mass, memory problems, and focal paralysis. Extensive monitoring and rehabilitation are often required post-hospitalization [6, 9, 15, 16, 22].

POWV infections are most commonly reported from late spring through early summer, but cases have been observed in almost every month [11]. All ages and both genders have become infected with the disease, but more cases have been reported in those aged 50 or older, and victims are more commonly male. This may be due to a variety of factors, including greater exposure to ticks, less use of preventative methods, and underlying health issues that increase the risk of neuroinvasive complications after infection with the virus [11].

Arboviral testing is recommended during the diagnostic process in cases with acute meningitis or encephalitis infections when the patient has had possible exposure to ticks in POWV endemic areas. Serum or CSF may be tested for POWV antibodies by qualitative IgM capture ELISA at some health department laboratories or the CDC. The CDC utilizes a POWV-specific IgM ELISA. Positive antibody results are confirmed by PRNT of serum, also at state health laboratories or the CDC. Positive molecular testing (RT-PCR) of serum, CSF, and tissue samples may also confirm POWV infection [15]. The TBD-Serochip, which will simultaneously test for IgG and IgM antibodies to eight tick-borne diseases including Powassan, is currently in development [29].

As with JCV, only supportive care is available since no effective protocol for treatment is currently in place. This generally includes respiratory support, administration of intravenous fluids, and management of cerebral edema. High-dose corticosteroid treatment may also play a crucial role in management of hospitalized cases [9, 15]. No vaccine exists for POWV disease, and prevention is the best deterrent for the virus. This is best accomplished through minimizing exposure to ticks in POWV endemic areas. Long pants and shirts should be worn, and tick repellants with DEET or permethrin should be utilized if tick exposure is anticipated. Additionally, ticks should be frequently searched for and removed from the body, and clothing should be placed in the dryer to kill any remaining ticks.

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**Jamestown Canyon and Powassan Arbovirus (continued from page 3)**

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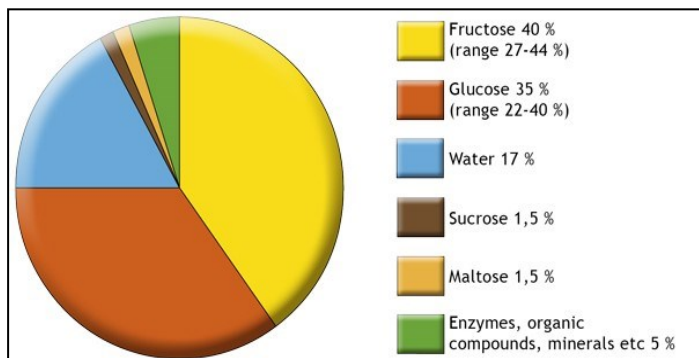
## What Is in Your Honey?

By Mary Hagerman,  
Chemistry Division Director

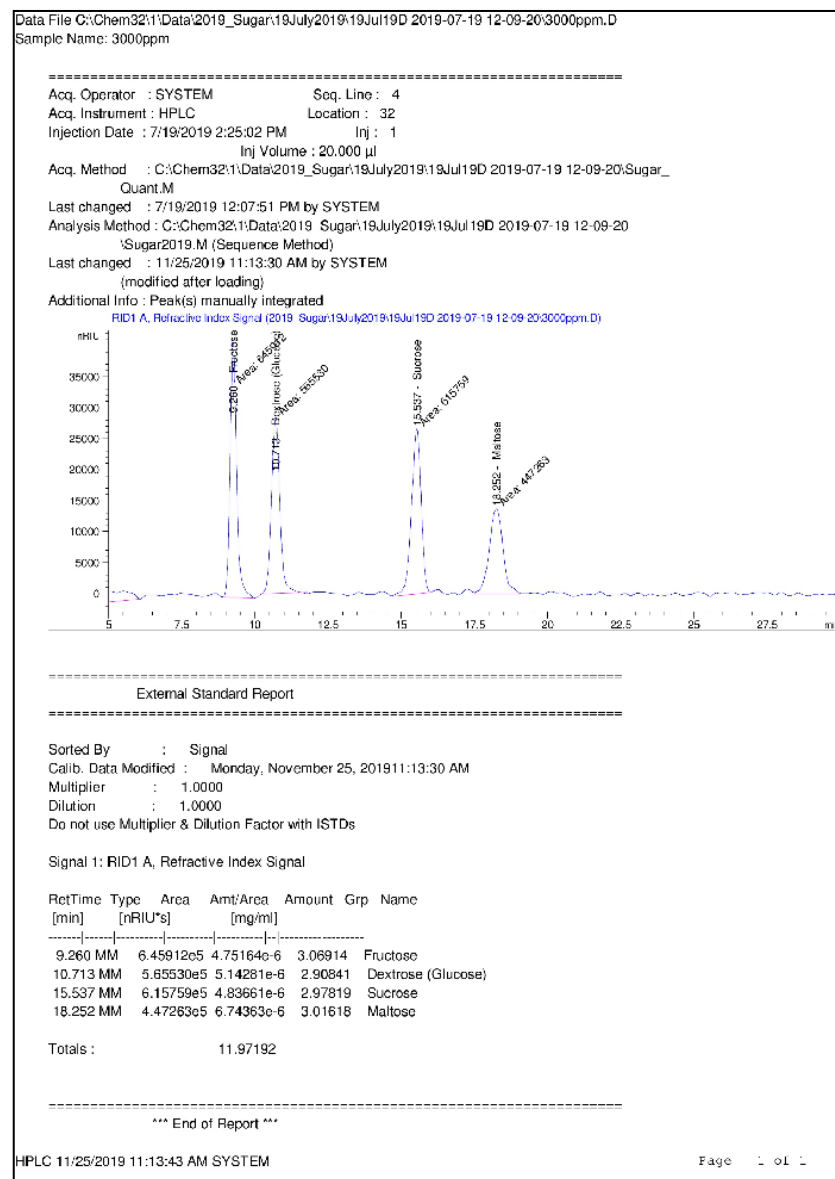


Honey consumption has grown significantly during the last few decades due to its high nutritional value (Figure 1) and unique flavor. The price of natural bee honey is much higher than other sweeteners, making it susceptible to adulteration with cheaper cane or corn sweeteners.

The ISDH Food Protection Division became aware of a situation in which a honey operator was suspected of adulterating honey with corn syrup, resulting in potential



(above) Figure 1: Honey nutrition  
(left) Figure 2: Sugar standard



economic fraud. ISDH's Food Chemistry Laboratory was contacted and asked if it would be possible to analyze honey samples for sugar composition. Food Chemistry's copy of the AOAC International's Official Methods of Analysis contained such a method, and we also had a copy of PerkinElmer's technical note for this purpose, but the lab had not analyzed any such sample in the past. We had the necessary reagents and sugar standards in stock for analysis.

ISDH chemist Manto Gulati performed a quantitative analysis of sugar standards using the AOAC method and technical note to ensure the honey sample could be analyzed for sugar composition. Commercially available samples of honey were analyzed and contained FDA-approved compositions of sugar, showing that the procedure worked as intended. The results exhibited very good retention time repeatability, as well as excellent linearity over the tested concentration ranges.

After the lab informed Food Protection of their readiness to accept samples, sample collection was scheduled for the first week of July 2019. The sample was collected and received by the lab on July 17.

(continued on next page)



**The New Carbapenemase on the Block: IMP-27**  
**By Cassandra Champion, Anna Hasche-Kluender, Adam Green**  
**Assay Development**

For much of the past decade, it seems all eyes have been on carbapenemase-producing carbapenem-resistant organisms, colloquially referred to as “super-bugs.” In Indiana, this usually means organisms whose resistance is caused by the KPC carbapenemase, a member of a group of enzymes commonly called the “Big Five” carbapenemases, which include KPC, NDM, VIM, IMP, and OXA-48-like genes. In recent years, however, the number of non-KPC carbapenemases has been on the rise—specifically NDM, VIM, and OXA-48-like. Yet one member of the group, IMP, has remained silent. Until now, anyway.

In 2018, the Indiana State Department of Health Laboratories (ISDHL) began to see an increase in the number of isolates that tested PCR negative for the “Big Five” yet showed phenotypic carbapenemase production. From June 2018 to May 2019, five isolates in the *Proteus*, *Morganella*, and *Providencia* genera tested negative by PCR at ISDHL but positive for IMP at either the Midwest Regional Laboratory or the Centers for Disease Control and Prevention (CDC). So what caused these false negative PCR results? Was the assay no longer working properly? To answer that question, we needed a little history.

In 2015, ISDH validated an in-house developed PCR for the detection of IMP, VIM, and OXA-48-like carbapenemase genes to complement the CDC PCR assay for the detection of KPC and NDM-1. One major challenge in designing a PCR for tracking drug resistance genes is that, over time, genes mutate and create gene variants. In an ideal world, a PCR would detect all known gene variants, but in practice this is very difficult to accomplish. During validation of the in-house developed PCR, ISDHL noted that two gene variants for IMP were not detectable: IMP-14 and IMP-27. At the time, this limitation was deemed acceptable; IMP was very rare in the United States and ISDHL had a secondary phenotypic test in place to catch carbapenemase producers that were not detected by PCR. The good news is that this algorithm worked, and phenotypic testing caught the five false negative PCR results. However, it was still unknown if the IMP markers were one of the two known variants that our PCR would not detect or if there was a larger problem.

A review of the scientific literature revealed that IMP-27 is the most common variant among the *Providencia* and *Proteus* genera, leading to a hypothesis that the false negatives resulted from poor assay sensitivity to the gene variant present in the isolates. In an effort to address this, the Assay Development team began validating a PCR for the detection of VIM, IMP, and OXA-48-like carbapenemases, which was released by the CDC AR Lab Network. Shortly after, however, performance problems with the detection of the IMP-14 variant were discovered. By comparing whole genome sequencing data from NCBI to the primer sequence for one of the IMP reverse primers, three mismatches were found. When enough mismatches occur between primers and their target sequence, it becomes difficult for the primer to bind correctly, and this interferes with amplification. To correct this, the Assay Development team modified one of the IMP reverse primers to include mixed base pairs at each of the identified mismatches (Figure 1).

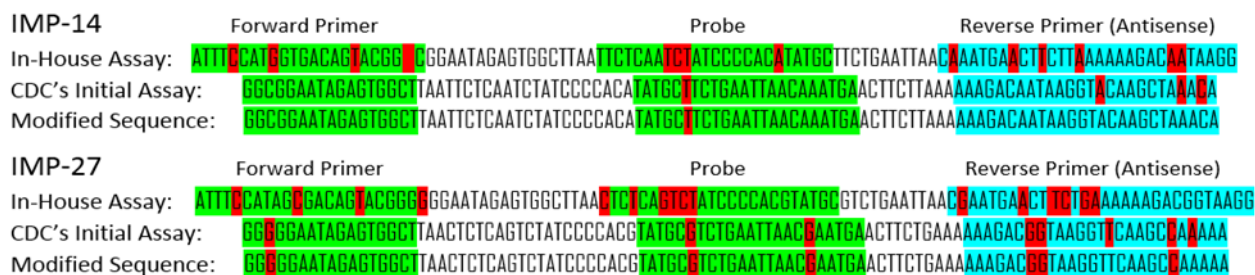


Figure 1: Comparison of IMP Reverse Primer 2 to the IMP-14 and IMP-27 Gene Sequences

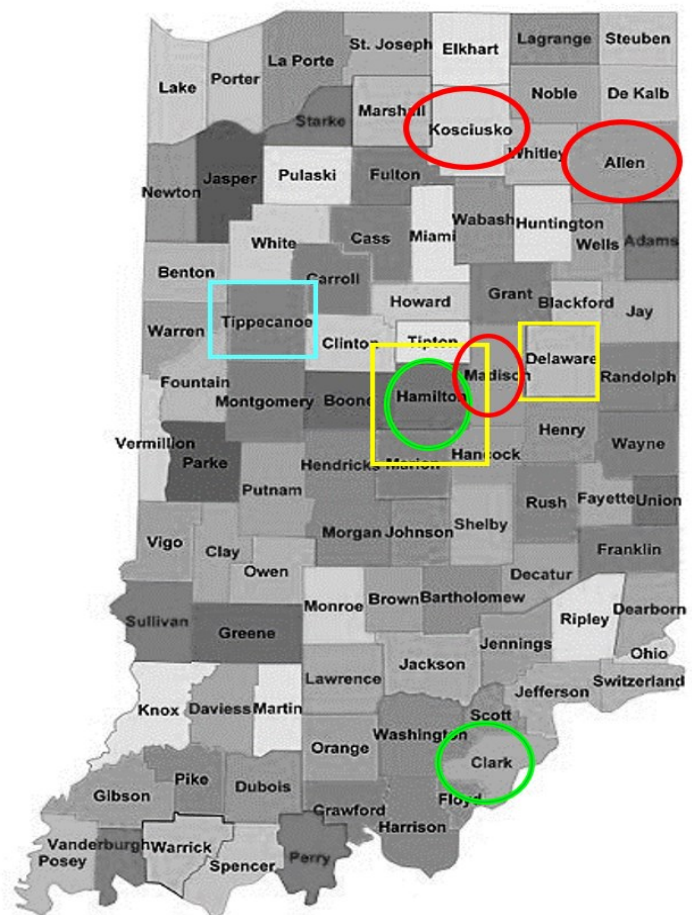
## The New Carbapenemase on the Block: IMP-27 (Continued)

These mixed base pairs allow the assay to maintain specificity while binding more than one type of nucleotide (A and T, for example) at the site of the mismatches. This modification aids primer binding at the target sequence. This change allowed ISDHL to now detect the two problematic IMP variants from the previous assay. But, did this mean that the new assay is capable of detecting the IMP gene present in the false negative isolates? The five false negative isolates were retested using the new assay and were detected, indicating that the false negative results were due to lack of sensitivity for the variant found in those isolates. Between August and December 2019 another four IMP positive isolates have been detected, all of which fell in the *Providencia* and *Proteus* genera, and all were detected by the new assay.

Given that IMP had previously not been seen in Indiana, the identification of nine IMP isolates was certainly enough to raise eyebrows. Could these isolates constitute an outbreak, or had they occurred independently? To investigate this, ISDHL sought to discriminate the variant of IMP gene expressed in each isolate and to assess the overall genetic similarity of the eight *Providencia* and *Proteus* organisms through the use of whole genome sequencing (WGS). Unlike PCR, which requires specific primers to indicate the presence of targeted genes, WGS sequences and compiles fragments of DNA to reconstruct the entire genome. This allows for gene detection at the variant level and enables us to detect the number of nucleotide differences between organisms (single nucleotide polymorphisms (SNPs)). WGS is a powerful tool in outbreak analysis, as it can be used to determine whether one particular gene-variant is spreading through Indiana, and if isolates are genetically related.

In January 2019, ISDHL validated the use of WGS on clinical isolates to detect resistance and construct SNP trees to indicate isolate relatedness. This IMP analysis has been the first “real-world” WGS investigation since its validation and, after sequencing each isolate, ISDHL was able to determine whether the eight *Providencia* and *Proteus* IMPs were part of an outbreak. The genomes of each isolate were run against a database of known antibiotic resistance gene variants, and all isolates showed one gene variant with a 100% match. The match was the same across all isolates: IMP-27. The hypothesis that a lack of PCR sensitivity caused the initial false negatives was correct. The IMP-27 gene was initially missed due to the lack of sensitivity of the original ISDHL PCR to detect IMP-27.

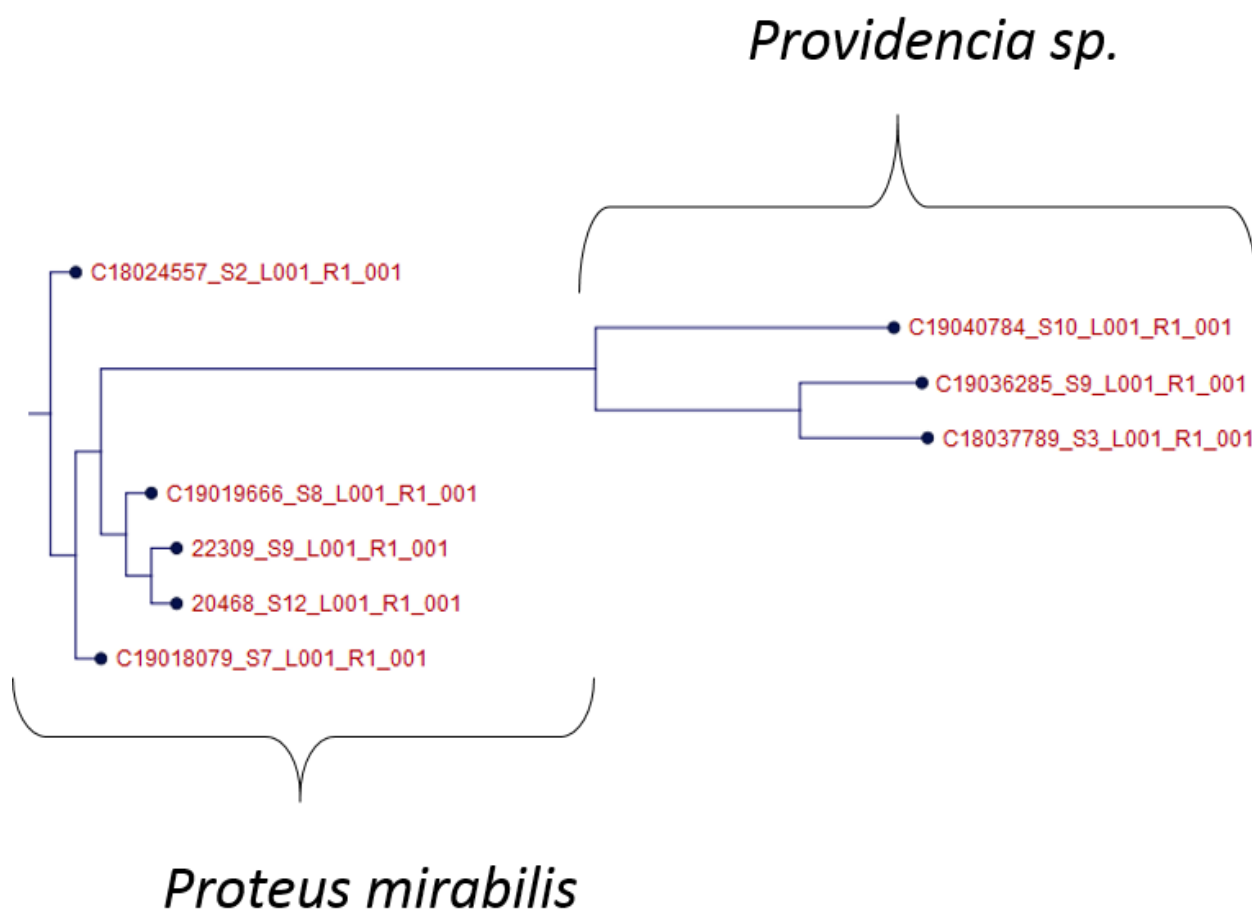
But, did the detection of this IMP-27 variant across all isolates confirm an outbreak? To answer this question, the isolates of the *Providencia* and *Proteus* genera were compared using a SNP tree, and known epidemiological information on isolates—such as location and date—were considered (Figure 2). Two main clades formed the SNP tree, one containing the *Providencia* isolates and one containing the *Proteus* organisms. The two similar isolates both were *Proteus*; however, they were submitted by sites that considered linked from an epidemiological perspective.



Square-*Providencia* isolate; Circle-*Proteus* isolate. Colors denote that isolates occurred within the same month.



From this analysis, ISDHL determined the IMP isolates did not constitute an outbreak but, rather, the emergence of a new carbapenemase variant in Indiana. Fortunately, the Assay Development team’s improved PCR has demonstrated its sensitivity to IMP-27 so that this emerging gene variant can be detected as part of normal testing procedures. The appearance of this new gene variant in Indiana illustrates the ever-changing world of antimicrobial resistance. With our updated PCR and the use of WGS, ISDHL is better equipped to monitor for emerging resistance and outbreak analysis.



## **3 Divisions, 2 Women, 1 Mission: Healthy Hoosier Children**

*By Jyl Madlem and Victoria Konstantinidis*

Did you know children receiving Medicaid benefits are required to have a blood lead test performed at age one and again at age two? However, according to the 2018 Childhood Lead Surveillance Report, only 21% of Hoosier children ages 1 and 2 who are receiving Medicaid benefits had a blood lead test performed in 2018. And while the total number of tests has increased, the number of Medicaid-eligible children tested at federally required ages has not. Children younger than 6 years old are more susceptible to the dangers posed by environmental lead because their brains are still developing.



### **Three ISDH Divisions: Laboratory, WIC, Lead and Healthy Homes**

This year the State Laboratory; the Women, Infant, and Children (WIC) Program; and the Lead and Healthy Homes Divisions of the Indiana State Department of Health (ISDH) have partnered together for the sake of protecting Hoosier children. The development of the WIC Pilot Program allows for specified WIC personnel to collect blood lead specimens on children who would already be having a point-of-care hemoglobin test.

This also provides better compliance with federal requirements for Medicaid-eligible children to be tested for lead at appropriate ages while minimizing additional workload for the WIC staff since they would already be performing fingersticks on these children.

Pilot Program coordinator Victoria Konstantinidis, who developed the informational presentation for the WIC agency coordinators, shipped collection supplies for the 154 WIC clinics through their respective agency coordinators at the launch of the pilot in July 2019. She is also primarily responsible for all data entry into the laboratory information system (LimsNet), calling parents/guardians with results, and answering questions from all 154 clinic sites. She was granted some additional help in October when Vicki Kendall joined the team, due to specimen volume increase.



### **Two Women: Victoria Konstantinidis and Jyl Madlem**

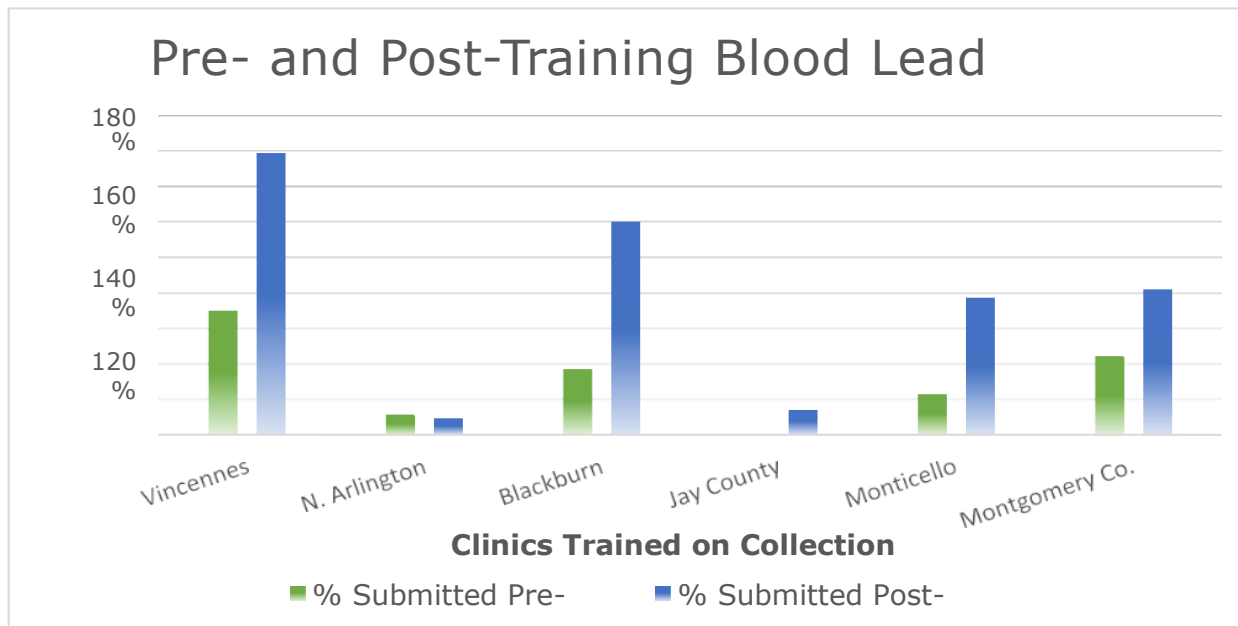
Once collection issues began to arise, agency coordinators and their staff were really interested in how to properly collect these specimens. Victoria and Laboratory Program Advisor Jyl Madlem visited those sites requesting additional help with specific training techniques. Clinics visited thus far include Vincennes (Knox County), North Arlington (Marion County), Blackburn (Marion County), Jay County, Monticello (White County), Peru (Miami County), Wabash County, and Crawfordsville (Montgomery County), with more clinics scheduled in 2020.

Jyl presented an abbreviated specimen collection portion of the Blood Lead/Case Management training course, while Victoria answered programmatic-specific questions. Reference materials and contact information were also provided to the clinics.

Of the clinics trained, the percentage of blood lead specimens collected prior to the training averaged 31% of the children who were tested for hemoglobin at those clinics. Since being properly trained on blood lead specimen collection, there has been an average overall increase of 114% in the number of children for whom blood lead specimens have been collected.

An unexpected benefit was noted when we learned some clinics have been collecting lead specimens independent of hemoglobin testing. There is a two-fold advantage to this practice; parents/guardians now asking for the test indicates growing interest in having their children tested, and this additional testing improves compliance with federal requirements to have Medicaid-eligible children tested at age 1 and 2.

The primary goal of the pilot was to perform blood lead testing on 100% of the children who would already be having a fingerstick hemoglobin test. The data indicate (Chart 1) improvement in blood lead screening, with as much as 160% of the children being screened for hemoglobin. Furthermore, two clinics had individual increases of >200% in the number of lead specimens collected after being trained and a third with a >100% increase since being properly trained.



**Chart 1.** Specimen submissions have improved for nearly all clinics receiving sampling training. Attendees are welcoming, inquisitive, and grateful for the personalized training sessions.



**One ISDH Common Goal: Protecting Indiana’s Children**

Interagency division cooperation at the state level delivering grass roots outreach has produced better than expected results. More children are being tested than before. Indiana’s children are being protected from the harmful effects of lead by having them tested in a timely fashion and acting accordingly when results dictate. The ISDH Laboratories’ Outreach and Training Team is dedicated to building those bridges between commissions and divisions, uniting our agency for the health of all Hoosiers.

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