Summer of 2012 Marked by Extreme Heat, Drought and Record West Nile Virus Activity

By Mark Glazier

Even though the Indiana State Department of Health Virology Lab detected its first West Nile virus (WNV) positive mosquito pool the last week of May, nearly six weeks earlier than in 2011, it would still have been difficult to foresee the number of positives that would be detected over the next several months. While the mild winter and exceptionally warm early spring were probably responsible for the much earlier appearance of WNV in Indiana this year, the record number of WNV positive mosquito pools were at least partially due to the extreme heat and drought of early summer.

In 2012, the lab tested over 137,000 mosquitoes, divided into nearly 2000 pools, collected from all 92 counties in the state. West Nile virus was confirmed in 576 mosquito pools, with 91 of 92 counties having mosquitoes test positive for WNV this year (see Centers for Disease Control & Prevention Arbonet map). In 2011, only 112 pools tested positive for WNV by the ISDH Lab, with positives pools collected from 34 counties.

Not surprisingly, the dramatic increase in WNV positive mosquito pools translated to a similar increase in human WNV cases. There were 70 cases of human WNV disease with seven deaths in Indiana in 2012. In 2011, there were only 10 probable or confirmed cases of WNV with one death. Although Indiana saw a significant increase in human cases of WNV, other states were hit even harder.

As of November 14, 2012, a total of 5,128 cases of WNV disease in people, including 229 deaths, have been reported to CDC. This is the highest number of WNV disease cases reported to the CDC through the second week of November since 2003. Nearly 80% of the cases have been reported from 12 states, with a third of all cases reported from Texas.

If scientists are correct about climate change, we can expect warmer temperatures and more extreme weather conditions throughout the year. How these changes will affect disease transmission, particularly those viral pathogens like WNV and influenza that have typically been more seasonal, remains to be seen. Now that the 2012 WNV season is officially over, the lab will take a well deserved breather and begin preparations for whatever may come in 2013.
A Puzzling Worm – a Case Study
By Brent Barrett

What is it?

A hospital submitted a worm to our public health laboratory for identification. It was removed during a colonoscopy from a 31 year old male. Further patient medical history was not provided. Macroscopic (gross) observation showed a worm that measured about 20mm long and one end appeared to be broken off, suggesting a longer overall length.

Initial thoughts were that the worm was an adult *Ascaris lumbricoides* or *Enterobius vermicularis*, since these are common and large enough to see with an unaided eye. But after viewing under the stereoscope, these options were ruled out. After taking several digital images, the worm was cut open and revealed uncharacteristically clear but morphologically perfect shaped eggs.

Based on the egg morphology, the specimen was identified as the nematode (roundworm) *Trichuris trichiura*, also called the human whipworm. The submitted specimen was a female with a missing anterior “whip like” end.

*Trichuris trichiura* eggs are 50-55 micrometers by 20-25 micrometers. They are barrel-shaped, thick-shelled and possess a pair of polar “plugs” at each end. The eggs are unembryonated when passed in stool.

Right: Eggs of *T. trichiura* in an unstained wet mount
Adult males of *Trichuris trichiura* are 30-45 millimeters long, with a coiled posterior end. Adult females are 35-50 millimeters with a straight posterior end. Both sexes have a long, whip-like anterior end. Adults usually reside in the large intestine, cecum and appendix of the host.

Left: Adult male *T. trichiura* removed during a colonoscopy (not the same specimen). The image shows a complete “whip worm.”

The unembryonated eggs are passed with the stool (1). In the soil, the eggs develop into a 2-cell stage (2), an advanced cleavage stage (3), and then they embryonate (4); eggs become infective in 15 to 30 days. After ingestion (soil-contaminated hands or food), the eggs hatch in the small intestine, and release larvae (5) that mature and establish themselves as adults in the colon (6). The adult worms (approximately 4 cm in length) live in the cecum and ascending colon. The adult worms are fixed in that location, with the anterior portions threaded into the mucosa. The females begin to oviposit 60 to 70 days after infection. Female worms in the cecum shed between 3,000 and 20,000 eggs per day. The life span of the adults is about 1 year.
Staff members from the ISDH Laboratories hosted a booth at the 2\textsuperscript{nd} Annual Celebrate Science Indiana event on Saturday, October 6, 2012. This event was held at the Indiana State Fairgrounds and welcomed families, educators, and children to learn the importance of science education and to take part in hands-on activities from many different exhibitors. Approximately 1,000 children and their parents visited the ISDH booth during the event to explore the world of public health.

Members of the food microbiology laboratory were available to demonstrate how testing is performed on food to rule-out Salmonella. They also explained the presence of beneficial bacteria in certain foods along with the importance of washing fruits and vegetables before eating them.

Other hands-on activities included the Pulse Field Gel Electrophoresis (PFGE) matching game. Contestants could compete against a staff member in a race to complete the PFGE matches. This was only after participants learned about how PFGE is used to identify strains of bacteria and how that data determines outbreak linkages.
The microbiology staff had a little fun with fomites as well. After a small experiment in the laboratory, an interesting and interactive lesson was created. “Fun with Fomites” illustrated where bacteria may be lurking on common items such as door knobs, shoes, and staplers.

Children and parents alike were able to discover a different world through the microscope when they learned about gram stain techniques to identify bacteria. They were also able to see a head louse and flea...up close.

Glo Germ™ drew the biggest crowd in the booth as children were fascinated to actually see the simulated bacteria on their hands. The Glo Germ™ was used to expose the “germs” under UV light. Then, the children were asked to wash their hands and return to see if they washed all the “bacteria” away. They were amazed at the difference they could see after a good hand washing! Those with good washing skills were deemed “Super Soapers”.

This event was a huge success for our laboratory team which included volunteers: Kara Hames, Jyl Madlem, Liz Church, Zach Beals, Engra Castiglione, Valrie Westmoreland, Mark Glazier, Phil Zillinger, Jackie Surma, Nicole Simpson, Kiran Khurana, and Hesham Elgaali. Preparations will soon begin for next year’s event.
A Visit from an Accrediting Body
By Phil Zillinger

If anything will bring fear into a laboratory, it is an on-site assessment from an accrediting body. In my case, it was an assessment of our environmental lead analysis activity by the AIHA Laboratory Accreditation Programs. Our laboratory is accredited for the analysis of paint chips, dust wipes and soil samples from homes where a child has been found to have a high blood lead level. To get ready for the evaluation, you review and make any needed corrections to the quality manual, the standard operating procedures, the sample handling process, the reporting process, maintenance and review of instrument logbooks, expiration dates of standards and reagents, staff qualifications, any new requirements of the accrediting body since the last assessment, calibration status of any instruments or support equipment (weights, balances and pipettes) and calibration status of fume hoods. And, if that isn’t enough, you ensure that the laboratory and work areas are clean and free of clutter.

Each assessor comes from a different background, so you never know what to expect. Some assessors are focused on performance of the analyses; others on the laboratory’s quality system and how it functions; others on how the analysis and quality control data is reported and monitored; and, still others on a combination of all the previously mentioned areas. This year’s auditor started by observing the performance of the analysis of samples. Then, he looked at the standards used for calibration and quality control. There is a new AIHA requirement that reference material providers be accredited. Fortunately, they allow standards that were purchased before March 30, 2012 to be used until the container is empty. Then, he looked at how the quality control samples were evaluated for trends in their values. Doing this would allow the laboratory to detect a bias in the data and let us take preventive action before an out-of-control situation occurs. Next, he looked at document control and record retention. Currently, we use Microsoft SharePoint for our document control, but we did not reference this in the quality manual. We maintained a listing of the records we maintain, but did not include how long the records are retained and, in some cases, what is done with them after the retention date. Finally, he looked at training records. We had records for the testing procedures for all of the analysts, but did not have records for training and demonstration of proficiency for the use of pipettes.

Each of the issues identified in the assessment gets noted as a deficiency. Deficiencies are items that are contrary to the standards of the program and require identification of the cause, what action was taken to address the deficiency and what action will taken to ensure the deficiency will not occur again. Also, the assessor can make recommendations for items that are not deficiencies, but allow the laboratory to improve. Laboratories can implement the recommendations, consider them for future implementation or decide to take no action.

So, what have we learned from this experience? We are not perfect; we were cited for several deficiencies. Addressing these deficiencies make us better than what we were. The auditor also made several suggestions. Implementing the suggestions also make us better. The goal of any laboratory is quality improvement. However scary, an audit is just another step in the quality improvement process.