



## THE LABYRINTH

Indiana State Department of Health Laboratories Newsletter



### A Case of OXA-48 Detected in Indiana, Carbapenem-Resistant *Enterobacteriaceae* (CRE)

By Kelly Tippmann, ISDH Microbiologist



Plates growing bacteria in the presence of discs containing various antibiotics. The left plate is susceptible to the antibiotics on the discs while the right plate has a CRE that is resistant to all of the antibiotics.

(Image source: CDC)

Antibiotic resistant bacteria are a rapidly growing threat to public health, as most members of the public health community are aware. However, the ease in which clinicians can fight infections with antibiotics is at an end due to the shrinking list of effective antibiotics in their arsenal. Clinicians and researchers using advanced technology have the ability to develop new antibiotics, but their

bacterial adversaries have the advantage of billions of years of adapting to and surviving in hazardous conditions and toxic chemicals. The current strategy must not only be the development of new antibiotics, but also the surveillance and prevention of resistant bacteria.

Carbapenem-resistant *Enterobacteriaceae* (CRE) are resistant to carbapenems, broad spectrum  $\beta$ -lactam antibiotics considered the last line of defense against serious infections. Some CRE are resistant to most other antibiotics as well. *Enterobacteriaceae* are a family of hardy, naturally occurring intestinal bacteria that includes organisms such as *E. coli* and *Klebsiella pneumoniae*. CRE infections most often occur in hospitalized or long-term care patients on ventilators, catheters, or long courses of antibiotic treatments (1). Due to the ease of international travel and medical tourism, the spread of CRE has proven to be a global issue (2). The rapid spread of resistant genes can also be attributed to the ease by which these elements can be transferred on plasmids from one cell to another.

In an effort to curb the spread of CRE, the ISDH Reference Bacteriology Laboratory is helping ISDH establish a CRE surveillance program. CRE have various mechanisms by which they destroy carbapenems. The Reference Laboratory performs real-time PCR molecular testing to detect *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-beta-lactamase-1 (NDM-1) containing isolates, the two common mechanisms. All isolates that test negative for both KPC and NDM-1 by PCR undergo further testing for the other mechanisms of resistance. Since starting testing in 2013, the reference lab has tested 185 CRE isolates, 127 of which tested positive for KPC, 2 isolates from the same patient tested positive for Oxacillinase-48 (OXA-48), and no isolates have tested positive for NDM-1.

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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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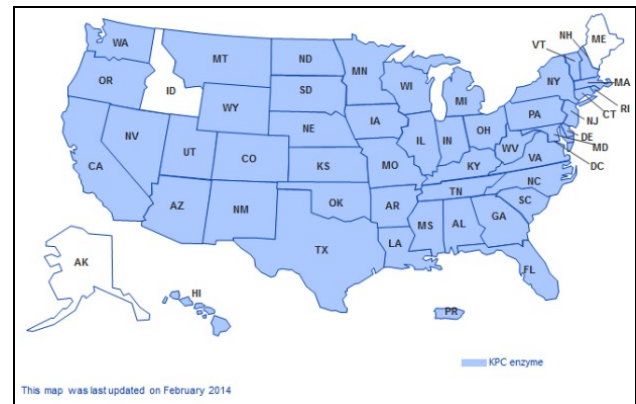
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### ***Klebsiella pneumoniae* carbapenemase (KPC)**

KPC is a protein which hydrolyzes carbapenems as well as other antibiotics including penicillins, cephalosporins, and aztreonam. It has also shown resistance to clinically available  $\beta$ -lactams *in vitro*. KPC was first described in a *K. pneumoniae* isolate recovered in North Carolina in 1996 and is the most common mechanism of carbapenem resistance for *Enterobacteriaceae* in the US. In 2011, a KPC carrying *K. pneumoniae* was the source of an outbreak at a National Institute of Health Clinical Center causing the death of six patients out of the 18 infected (3). The gene coding for the KPC protein has been mapped to a highly conserved transposon, a mobile genetic element which can change its position within the genome. Further contributing to resistance against multiple antibiotics, plasmids carrying the gene for KPC harbor the genes for resistance against fluoroquinolones and aminoglycosides (4).



**Carbapenemase-producing CRE found in United States.**

(Image source: CDC)

### **New Delhi metallo-beta-lactamase-1 (NDM-1)**

NDM-1 is a protein that hydrolyzes  $\beta$ -lactams. It was first identified in Sweden in 2008 in a patient that was hospitalized in India. In 2010, an NDM-1 carrying isolate was first detected in the US (5). Similar to KPC expression, NDM-1 expression is also associated with resistance to multiple antibiotics such as fluoroquinolones and aminoglycosides (4).

### **Oxacillinase-48 (OXA-48)**

OXA-48 hydrolyzes carbapenems as well as penicillins and is resistant to  $\beta$ -lactamase inhibitors. Due to low carbapenemase activity and susceptibility to cephalosporins in some isolates, OXA-48 is the most difficult carbapenemase to identify (6). It was first found in Turkey in 2004 and has recently spread to Europe and the Middle East. The first clinical cases of OXA-48 were at the University of Virginia Medical Center in 2012. Both patients were previously hospitalized in Saudi Arabia and India before their return to the US. The main difference between KPC, NDM-1, and OXA-48 is OXA-48 is associated with a single plasmid, whereas NDM-1 and KPC are associated with a variety of plasmids (5, 7). In November 2013, the ISDH Reference Laboratory received a *K. pneumoniae* isolate for CRE testing. The patient, an Indiana resident, had been hospitalized during a recent trip to India. Real-time PCR showed the isolate was negative for KPC and NDM-1 encoding genes. Through antibiotic susceptibility testing the isolate displayed resistance to each antibiotic tested and a weakly positive Modified Hodge Test, indicating carbapenemase production. The lab forwarded it to the Centers for Disease Control (CDC), and the isolate tested positive for OXA-48. This is the first case of OXA-48 in Indiana.

Bacteria have proven themselves to be successful by surviving millenniums of environmental challenges. Steps toward minimizing the impact of antibiotic resistant organisms include not only the development of new antibiotics, but also public health surveillance and reducing transmission.

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## The Broken Arrow Incident, Indiana 1964

By Jane Smith, ISDH Chemist



In December 1964, a B-58 Hustler bomber was involved in an accident during takeoff. The following is a chronology of the investigation of the accident and its environmental impact, conducted by the United States Air Force (USAF), the Indiana State Department of Health (ISDH; then the Indiana State Board of Health) and the Indiana Department of Environmental Management (IDEM). Parts of the information concerning the 1964 B58 crash still remain classified.

### December 8, 1964

At approximately 11:46 a.m., a Broken Arrow incident (an accident involving radioactive contamination, loss in transit of a nuclear asset with or without its carrying vehicle, or jettisoning of a nuclear weapon or nuclear component) occurred at Bunker Hill Air Force Base (now known as Grissom Air Reserve Base) near Peru, Indiana. At the time of the accident, approximately 7 inches of snow covered the ground, and intermittent snow showers continued in the area. During an Operation Readiness Alert, one of several B-58 Hustler bombers got caught in another plane's jet blast before takeoff and skidded off the icy runway, collapsing the front landing gear of the craft and igniting its external fuel tank. The crew was forced to abandon the aircraft. The pilot and defensive systems operator escaped with minor injuries; however, the navigator was killed when he activated his ejection pod. The B-58 was carrying five nuclear weapons, thought to be four B-43's and either a W-39 or a W-53 in the pod, at the time of the incident. The fire destroyed all five nuclear weapons on the aircraft. The high explosives in the weapons did not detonate but instead melted and burned.



*Left to right: B-43, W-53, and W-39 nuclear weapons*

Air Force notes from December 8 stated that Dr. Henry Briggs and Hal Stocks from the Indiana Board of Health were at the scene of the accident until 8:00 p.m. However, during a 1996 interview of Dr. Briggs and Mr. Stocks, both men stated they drove six hours through the ice and snowstorm to reach Bunker Hill Air Force Base, only to be turned away at the gate.

### December 9, 1964

The Air Force conducted weapon recovery operations. Four of the five weapons were recovered and moved to a storage igloo for packaging. When personnel attempted to recover the fifth weapon from beneath the aircraft, exposure to the air caused the weapon to burst into flames, suspending recovery operations for the remainder of the day.

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**December 10, 1964**

The fifth weapon was still burning. In an effort to extinguish the fire, a trench 6 feet x 8 feet x 12 inches was dug approximately 150 to 175 feet away from the accident site, and the weapon was placed inside and covered with sand. The remaining wreckage from the aircraft was turned over to base personnel for removal later that night.

**December 11, 1964**

The B-58 aircraft debris was moved to an area enclosed with four strands of barbed wire and posted with radiation signs. The concrete and dirt from the crash scene were monitored, and no radiation levels exceeding 4.5 milliroentgens/hour (mR/hr) were measured. The natural radiation background in this area of Indiana is about 6 microroentgens/hour ( $\mu$ R/hr).

**December 15, 1964**

The burnt remains of all the weapons were packaged in a steel box that contained a fire-brick liner. The box was then covered with a layer of dry sand, and radiation tests were performed on the material. All results were negative. The containerized weapons were then shipped to Atomic Energy Commission contractors (some reports say Department of Energy contractors) in Tennessee and Texas. All shipments were received by Dec. 22 and disposed of properly.

**Radiation Monitoring**

Radiation monitoring of the accident area had begun when the equipment was set in place on December 8. Continuous monitoring of the area from a distance of 2000 feet continued until 10:00am the following day. No alpha activity was observed above the method detection limit (MDL) of 0.04 picocuries per cubic meter ( $\text{pCi}/\text{m}^3$ ) of air and 5 picocuries per liter ( $\text{pCi}/\text{L}$ ) of water.

**1991 Congressional Inquiry**

At the request of Indiana Senators Dan Coats and Richard Lugar, the USAF prepared a report "concerning the environmental clean-up and general facts concerning 'a' nuclear mishap at Grissom Air Force Base (AFB), Indiana." According to the report, "in 1991, the barbed wire and radiation sign around the burial site no longer existed and there is no record of when they were removed."

**ISDH and IDEM INVESTIGATION**

On March 26, 1996, more than 32 years after the incident, ISDH Radiological Health staff conducted surveys of the former accident site at the request of the IDEM. A hot spot was discovered in the vicinity of where the trench was thought to have been, and soil samples were collected by the ISDH. This area would later be the focal point of the accident site remediation efforts performed from August 1 to September 4, 2000. Although the USAF denied that any enriched uranium ever escaped during the accident, soil samples analyzed by both the ISDH Radiochemistry Laboratory and the United States Environmental Protection Agency (USEPA) National Laboratory in Las Vegas, Nevada, contained U-235 and other nuclear weapon isotopes. Normal Indiana soil contains approximately 2 to 3 picoCuries per gram ( $\text{pCi}/\text{g}$ ) of

uranium-238; uranium-235 and uranium-234 are not detectable. Following the March 1996 findings, ISDH and IDEM made a formal request to the USEPA in Chicago for field and laboratory technical assistance with a remediation project. Two locations were of interest: the crash site and the area where the B-58 remains were buried.

**1998 and 1999**

Several surveys of both the accident site and the burial site were conducted. During this time, ISDH Radiological Health Section staff worked beside USEPA and USAF health physicists. The USEPA used a Geonics High-Sensitivity Metal Detector, commonly known as an EM61, from the United States Geological Survey to locate the burial site. The burial site of the B-58 was located in an area that already required remediation. The B-58 crash site and the B-58 burial site were remediated in two separate events.

**Crash Site Remediation-August and September 2000**

An area of 300 square meters was remediated, with excavation volumes estimated at 105 cubic meters of soil. The residual depleted uranium on the site was estimated at 1.3 pCi/g, based on final soil samples. These findings showed the site met unrestricted public use criteria as recommended by the USEPA.

**Burial Site Remediation-October and November 2000**



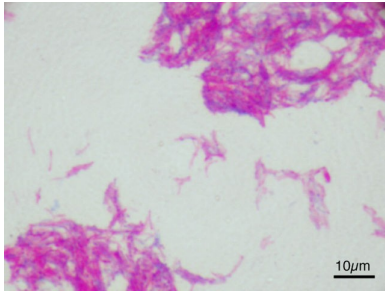
Remediation was divided into four phases: excavating the B-58 wreckage and surrounding soil, surveying the excavation pit to ensure the remaining soil was uncontaminated, backfilling of the excavated pit, and characterizing in detail all excavated debris and soil for signs of contamination post-excitation. The entire worksite was fenced and labeled with "CAUTION-Radiation Area" signs. Only one point of entry and egress into the worksite existed. All survey instruments were located at the entry and egress point inside the fence to prevent any contamination from leaving the restricted area of the worksite. Items that created additional disposal problems at the burial site included the ejection seats, 55 and 30 gallon drums, and petroleum contaminated liquids present in the excavation pit.

The Air Force reported the following disposal activities: all debris and soils contaminated with highly enriched uranium (HEU) were ultimately disposed of at EnviroCare in Utah, and all other radioactively contaminated debris and soil were disposed of at Waste Control Specialists in Andrews, Texas.



## **Mycobacterium bovis, Bacillus Calmette-Guérin (BCG), Isolated from Spinal Canal Culture**

By Edward Harris, ISDH Microbiologist



*Mycobacterium bovis* (Source: Wikipedia)

The TB lab received an isolate growing on Lowenstein Jensen (LJ) medium from an Indiana hospital for identification. The isolate source was from the lumbar spinal canal of an 80-year-old male. Colonies on the LJ slant displayed a non-pigmented and rough colony morphology. Growth from the slant was extracted for High Performance Liquid Chromatography (HPLC). HPLC is a method used to identify *Mycobacterium* species by analyzing the mycolic acids (long chain fatty acids) present in the cell wall (2). *Mycobacterium* species and a few closely related genera have mycolic acids in their cell walls. The resulting chromatogram provides a “fingerprint” for species identification. Surprisingly, the chromatogram for the isolate tested matched *Mycobacterium bovis*, Bacillus Calmette-Guérin (BCG).

*Mycobacterium bovis* is a member of the *Mycobacterium tuberculosis* complex (MTBC). MTBC consists of a number of species, including *Mycobacterium tuberculosis* (MTB), *Mycobacterium bovis*, *Mycobacterium africanum* and others which are found in animals and rarely infect humans. *Mycobacterium tuberculosis* is the primary cause of tuberculosis (TB) in humans and is transmitted from person to person. *Mycobacterium bovis* is responsible for TB in cattle and some wild animals and can cause human infections, primarily through consumption of unpasteurized dairy products from infected cows. It is relatively uncommon in the United States due to the testing of cattle for *M. bovis* infection and pasteurization of milk. Based on genotyping data, 1.4 % of linked TB cases in the United States are due to *Mycobacterium bovis* (5). *Mycobacterium africanum* causes TB in humans, but it is commonly found only in West African countries.

BCG is an attenuated strain of *Mycobacterium bovis* that is used as a vaccine to prevent TB in humans in countries with a high prevalence of the disease. It is most effective in preventing childhood TB meningitis and disseminated infection; it is less effective in preventing adult pulmonary TB. BCG is also used after transurethral removal of bladder tumors to prevent recurrence of superficial bladder cancer. Typical treatment consists of one dose of BCG per week for six weeks. In the intravesical BCG therapy, BCG is infused into the bladder, in order to produce an immune/inflammatory response, and is effective at reducing recurrence of the tumors.

The Centers for Disease Control (CDC) reported that HPLC is a reliable and reproducible method to separate BCG from MTB and wild type *Mycobacterium bovis* (4). An extract was prepared for polymerase chain reaction (PCR) testing and the result was positive for MTBC. Results were reported to the submitter and susceptibility testing was performed. The organism was susceptible to rifampin, isoniazid and ethambutol, but resistant to pyrazinamide. *M. bovis* is known to be inherently resistant to pyrazinamide (5). For epidemiology purposes, all MTBC isolates in Indiana are sent to the Michigan Department of Community Health for genotyping. Genotyping consists of spacer oligonucleotide typing (spoligotyping) and Mycobacterial interspersed repetitive unit (MIRU) typing. The spoligotype is expressed as a 15 digit octal and the MIRU is expressed as a 12 character designation. The isolate had both a spoligotype and a MIRU matching reported results for BCG (5,8).

A search of the literature revealed that BCG infection of the spine is a rare complication of intravesical BCG treatment for bladder cancer (1,3,6,7). All the published reports occurred in elderly men (mean age 79), most often months to years after BCG therapy (mean 31 months) (7). Patients are treated with antimicrobial therapy and surgery to repair the spine.

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### **Mumps Information**

**By Brian Pope & Jamie Yeadon-Fagbohun, ISDH Microbiologists**



Mumps is a highly contagious disease caused by Mumps virus, an RNA virus belonging to the family Paramyxoviridae. In the last five years, Indiana has documented 7 total cases of mumps, all identified through Polymerase Chain Reaction (PCR). To date, the Virology department at Indiana State Department of Health (ISDH) Labs has utilized PCR to confirm 4 cases of the viral disease statewide in 2014. Mumps is best described by the notable feature of parotitis (swollen salivary glands), which can be accompanied by a low-grade fever, headache, rash, and loss of appetite. In children, mumps rarely causes deadly side effects and is generally considered a mild disease, but for an individual that has already gone through puberty the disease can have more serious consequences. In about 10% of post-puberty males, orchitis (swelling of the testes) can cause some degree of testicular atrophy and, in rare cases, even sterility.

A mumps vaccine was introduced in 1967, but later combined with a vaccine for the prevention of measles and rubella in 1971. The resulting combination vaccine was known as the MMR, or Measles, Mumps, Rubella. In 2005, varicella was added to the vaccine, making it the MMRV, which was cleared for use in the United States that same year. This reduced the number of vaccines a child receives, so the MMRV is commonly given in place of the separate MMR and varicella vaccines. Since the introduction of the vaccine, yearly cases of mumps have dropped by 99%. Over 200,000 cases of mumps were detected per year prior to vaccination; in 2012 only 229 cases were reported in the United States.

The Centers for Disease Control and Prevention (CDC) recommendation for the vaccine is one dose at 12-15 months of age and a second dose at 4-6 years of age. In 2012, 89.7% of the US population had both dosages of the MMR or MMRV vaccine. Of those who have received the vaccine, about 78% produce immunity to the disease after just one dosage with the second dosage producing immunity in about 88% of the vaccinated population. Vaccination of persons born before 1957 is generally believed to be unnecessary because many had the disease as children and have natural immunity. The MMRV is not recommended for persons over the age of 13 at this time. If vaccination is required after 13 then the MMR and Varicella vaccines should be given separately.

The best way to prevent a mumps infection is to ensure proper vaccination. It is important that both doses of the vaccine are administered, with the second dose being given at least three months after the initial dose. According to the CDC in 2012, about 90% of the population of Indiana was properly vaccinated for MMR. Through due diligence of proper vaccination and public awareness of the disease, ISDH hopes to decrease the incidence of mumps cases in Indiana. We believe that public awareness about mumps, including education on the importance of vaccination can help us accomplish this goal!

## Taxonomic Identification of Bats by the Indiana State Department of Health Rabies Lab

By Erica Vecchio, ISDH Microbiologist



Rabies, a zoonosis caused by an RNA virus in the *Lyssavirus* genus, (2, 3) has been documented in over 150 countries on every continent except Antarctica (3, 8). The disease is nearly always fatal without post-exposure prophylaxis, known as PEP (1, 2). Over 55,000 human deaths worldwide are attributed to rabies infection annually, with the majority of victims being children under the age of 15, and transmission occurring primarily through bites or wound exposure to infected saliva from rabid dogs (1, 3, 8). Due to the implementation of rabies control programs, which include widespread vaccination of domestic animals, oral vaccination baiting of wildlife in targeted areas, education of the public and medical health professionals, and utilization of PEP (1, 2, 3), human rabies

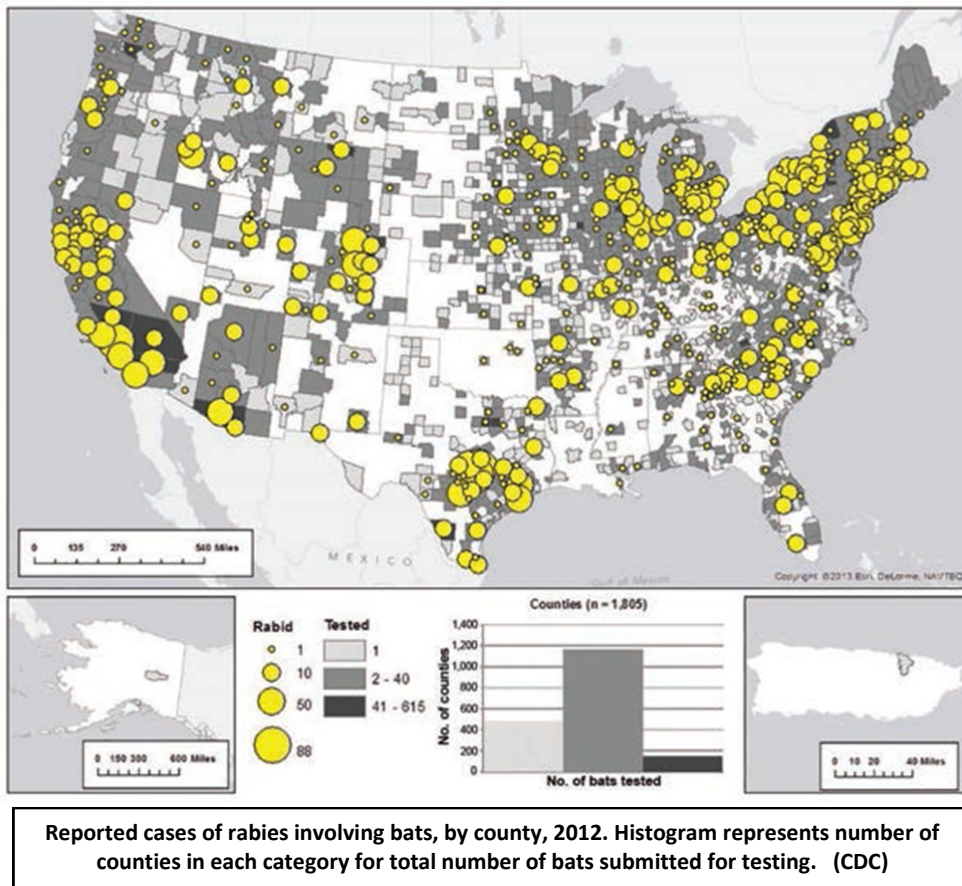
infections in the United States are rare, averaging only confirmed one to two cases yearly (3). Bats are the most common vectors (1, 2, 3, 4, 7, 8). From January 2002 to July 2012, there were 33 reported cases of rabies in people in the United States and Puerto Rico, with 21 of the 24 domestically acquired cases definitely linked to exposure to bats. Most other U.S. cases involved victims traveling to or emigrating from rural areas in high-risk countries where exposure to unvaccinated rabid terrestrial animals had occurred (1, 2). A total of 23,370 bats were tested for rabies nationwide in 2011; 1380 bats of varying species, or approximately 5.9% of those submitted, were positive for the virus (2).

In Indiana, there have been 132 documented human rabies cases since the year 1900; all but two occurred before 1959 (4). In 2006, a 10 year-old girl in Marshall County was diagnosed with rabies, and in 2009, a 43 year-old man in Clark County contracted the virus. Both victims were reportedly bitten by bats but did not receive PEP in the required time-frame and died while hospitalized (1, 2, 4, 5, 6). Since 1965, when the ISDH rabies lab received and confirmed through direct fluorescent antibody (DFA) testing the first positive bat submission in Indiana, bats from 79 of Indiana's 92 counties have been positively identified for rabies in the lab (4, 5, 6, rabies lab). Over 9000 bats of varying species have been tested by ISDH from 1965 until June of 2014, and approximately 632 have been confirmed as rabid (4, 7, rabies lab). This percentage (approximately 5-6%) was similar to the national average in 2011 (5.9%) for positive submissions, but not reflective of the general population of bats, where less than 1% are estimated to be rabid. The bats sent for testing represented a biased sampling of the population as they generally displayed erratic, motor-impaired behavior which made them easier to catch or were already dead as a result of the disease and easily collected for submission (1, 2, 7).

The last terrestrial mammal that tested positive for the disease in Indiana was a skunk in 2004 (4), and bats are currently the most common rabid animal submitted to the ISDH Labs for testing. Both the skunk and bat variants of rabies are endemic to Indiana (4, 7). Other species variants found in the United States (i.e. raccoon, red fox, gray fox) are not currently found in the state, but may be introduced through virus transmission among mammalian populations (1, 2, 4). In terrestrial mammals, the disease is generally confined to a specific geographic region, passed among individuals of the same species in the area, and maintained locally for long periods of time once established. Some interspecific transmission of the virus does occur but generally is neither widespread nor persistent enough to become established among new populations (1, 2).

Positive bat specimens have been identified in Indiana as recently as June 2014 (rabies lab), and every state except for Hawaii has reported rabid bats (1). Individuals from over 30 species of bats in the United States have been positively confirmed as rabid, and more than eight distinct bat variants of the disease, each normally associated with a particular species of bat, have been discovered through genetic testing. Considerably less, however, is understood about how the different bat variants are distributed geographically and how they are transmitted among mammals, than is known about the circulation of terrestrial virus variants. Interspecific transmission, particularly among closely related species of bats, is more common in bats than terrestrial mammals. As noted previously, bats are the most common vector of the virus in human cases. Furthermore, two known instances of bat rabies variants switching hosts from bat reservoirs to other





species have been noted; the first occurred in gray foxes in southern Oregon and northern Arizona, and the second developed in skunks in Flagstaff, Arizona (1,2).

The Centers for Disease Control and Prevention (CDC) requests detailed information on all specimens submitted to labs for rabies testing. This includes species, county, and dates of collection/testing. When possible, identification of the rabies virus variant present in infected animals has also been performed by laboratories and forwarded to the CDC. This information is being used in part to monitor existing reservoirs of disease and to identify areas with potential host switching events. Accurate identification of bat species will contribute to the understanding of rabies virus variants and to the control

of the disease in areas where new reservoirs may be developing (2). The ISDH Labs currently identifies all submitted bats to the species level then sends them to Indiana State University for use in ongoing university research projects.

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## ISDH Laboratories Conduct a Series of Trainings for Indiana First Responders

By Shelley Matheson & Jyl Madlem, ISDH Program Directors

The Indiana State Department of Health (ISDH) Laboratories, in conjunction with the Marion County Public Health Department (MCPHD), the 53<sup>rd</sup> Civil Support Team, and the Federal Bureau of Investigation (FBI), has conducted a series of trainings for Indiana First Responders this year. These trainings, entitled “A Training for First Responders: Biothreat Environmental Sample Collection for ISDH Laboratories”, are intended for Indiana Hazardous Materials (Hazmat) teams and postal inspectors, who might respond to a potential credible threat involving suspicious substances that may contain biological agents. The goal of these trainings is to provide first responders with the necessary tools to properly collect and package suspicious substances for biothreat testing using the ISDH Biothreat Environmental Sample Collection (BTESC) Kit. The trainings include a didactic portion and a hands-on portion. Mark Glazier (Division Director, Public Health Preparedness, Laboratory Outreach and Logistics at ISDH Laboratories) provides an overview of the Laboratory Response Network (LRN), a description of the contents of the kits, and information regarding collection and submission of samples to the attendees. Jeff Larmore, Supervisor, Hazardous Materials at MCPHD, then discusses screening guidelines, communication procedures, and chain of custody. During the hands-on portion, members of the 53<sup>rd</sup> Civil Support Team demonstrate how to collect samples using the ISDH Laboratories BTESC Kit.

Thus far, trainings have been held in five preparedness districts in Indiana, as well as during the 2014 Annual Indiana Drug Enforcement Agency (IDEA) Weapons of Mass Destruction (WMD) Conference. The ISDH Outreach and Training Team plans to take this training to all preparedness districts throughout Indiana by the end of 2015 and will continue to provide it as needed or as requested. During the trainings already conducted, more than sixty-five first responders were trained on how to properly collect and package suspicious substances for biothreat testing using the ISDH Laboratories BTESC Kit. The number of attendees per course is detailed in Figure 1 below.

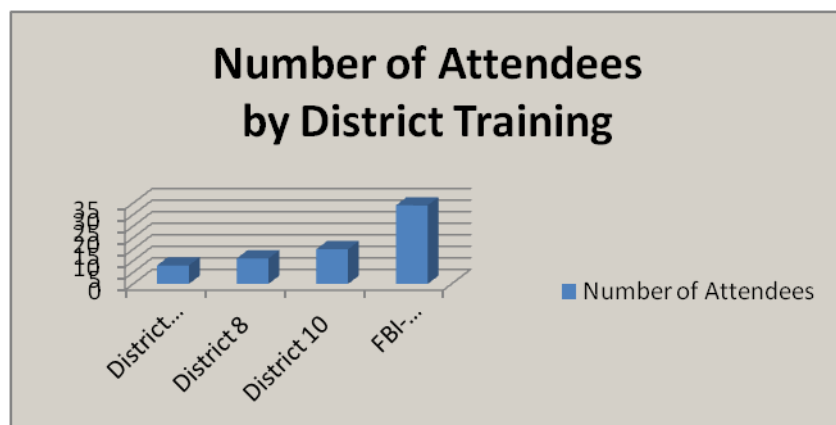


Figure 1: Number of Attendees by District

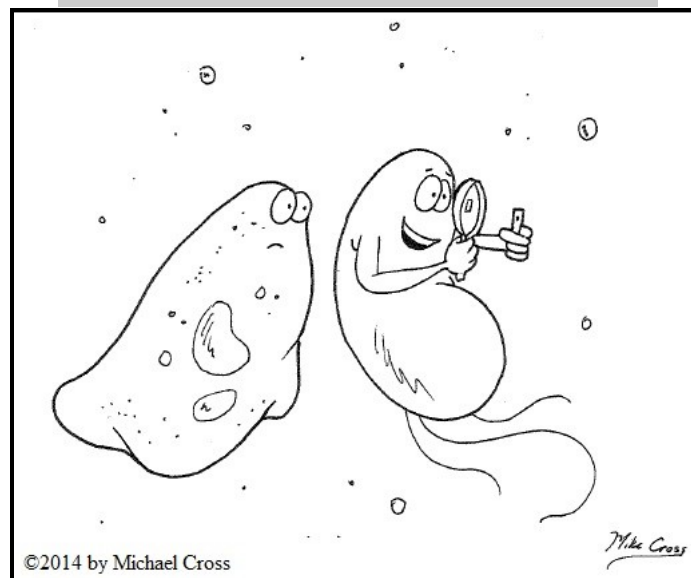
Information gathered from course evaluations indicated all of the attendees either agreed or strongly agreed that the training was well-structured and organized; hands-on exercises were plausible and realistic; presentations were helpful; presenters were knowledgeable about the material; and participation was appropriate for those who attended. The response on course evaluations were positive. “Excellent training!”, “I am very new to this and found this entire training very informative and interesting”, “Great class”, and “Good training, great refresher on sampling techniques!” were the comments received. Special Agent Bruce Guider, FBI WMD Coordinator for the State of Indiana said, “This training is spot-on to what is needed and is second-to-none in the nation. I shared this training with FBI headquarters in Quantico, Virginia, and they want to share it with all WMD Coordinators in the nation. This training is exactly what every state in the nation needs.”

As a follow-up to these trainings, the ISDH Laboratories will be making BTESC kits for Indiana's first responders on an as-needed basis. In addition, take-home materials for attendees include a series of educational materials on how to use the collection kits in the hot, warm, and cold zones on-scene, along with a list of materials included in the kit. This list of materials is included for those units wishing to create kits of their own. In addition, a training video was developed by ISDH for attendees to take with them to instruct second and third-shift first responders who were unable to attend the training. This video is available to first responders upon request.

Those responsible for providing this training are Shelley Matheson, Mark Glazier, Jyl Madlem, and Lori Rector, all with ISDH Laboratories; Jeff Larmore, MCPHD; Bruce Guider, FBI; and CPT Amy Azeez and her staff, the 53<sup>rd</sup> Civil Support Team. If you have any questions regarding first responder training, please contact Shelley Matheson, State Training Coordinator, at 317-921-5890.

**Microtoon**

by Michael Cross



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**“Fascinating little things...those viruses.**

**About The LAByrinth**

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