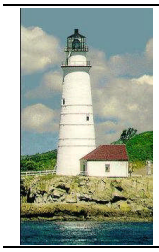

Northeast Branch Newsletter

Number 131 Winter 2008

2008 Programs in Review

Emerging Powassan Encephalitis

The first dinner-meeting of the year, cosponsored by the Northeast Branch-ASM and the Massachusetts Department of Public Health, State Laboratory Institute was held on April 8, 2008 at Cathay Pacific, North Quincy, MA. *Emerging Powassan Encephalitis in New York State and Nearby: Detection with Emerging Technologies* was presented by Susan J. Wong, PhD, Director of Diagnostic Immunology, Division of Infectious Disease, New York Department of Health, Wadsworth Center, Axelrod Institute.

Dr. Wong informed us that Powassan virus (POW), a North American tickborne flavivirus, is part of a newly recognized group of tick-borne encephalitides. It was first isolated in 1958 from a patient with encephalitis who was from a small town, Powassan, in western Ontario. The first patient found to have Powassan in the US was from New Jersey in 1917. Phylogeny of the flaviruses shows that POW's closest relatives include the Central European encephalitis virus that causes thousands of cases of encephalitis annually in Europe. A rarer disease is the Far Eastern encephalitis virus, but these are close enough together that they serologically cross react. Also, POW is far enough away from the mosquito borne West Nile Virus (WNV) that routine assays for the Japanese Encephalitis serocomplex are often negative in sera of patients with tick borne virus infection, and vice versa. POW is the least common cause of arbovirus encephalitis in the US and Canada, while WNV is the most common cause. Like Eastern Equine Encephalitis, however, it has a high mortality rate, reported to be anywhere from 10-40%. There is a high incidence (>50%) of severe neurological sequellae, many patients are compromised and remain in long term care facilities for months following the illness.

(L to R) Northeast Branch President Jeffrey Klinger, PhD, Susan Wong, PhD, and Barbara Werner, PhD

Although POW has been isolated from both ticks and mosquitoes, it is primarily vectored by the woodchuck tick *Ixodes cookei* that preferentially feeds on groundhogs and woodchucks, but also feeds on muscarids as

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Inside This Issue

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Council Meeting Schedule, 2008-2009

Council Meetings this year will continue to be held at the State Laboratory Institute in Jamaica Plain. Members and all interested microbiologists and scientists are welcome to attend. Please notify Irene George at (508) 785-0126 in advance. The next Council Meetings are scheduled for March 11 and April 28, 2009.

Membership News

Dues reminders were sent in late 2008 by email and by postal service to members who did not provide an email address. Membership forms may be found on the NEB website or you may join the both the ASM and the Northeast Branch online through the ASM eStore. Please make the necessary corrections to your demographics and return to the Treasurer or email changes to: NEBranch-ASM@comcast.net. Please check mailing labels as they reflect existing information. Although membership in the national branch automatically makes you a member of the local branch in some organizations, this is NOT the case in the ASM. *To be both a National Member and a NEB member, you have to join each individually.* The Northeast Branch currently has 274 members.

Visit the NEB Web Site!!

The NEB has established a home page on the World Wide Web where all current events and the Newsletter are available. ASM has also established a Branch Meetings page. Visit us via the ASM Home Page or directly at:
http:/www.asm.org/branch/brNoE/ index.shtml

2008-09 Council Elections

Congratulations to the following NEB members whose terms began July 2008. James Kirby, MD was elected President-Elect (one year), Irene George was elected Secretary (three-year term), and Gail Begley was elected Local Councilor (two-year term). We are looking forward to exciting programs this year!

FUTURE MEETINGS

Local Programs:

Announcements of Local Meetings will be mailed to the membership and posted on our website at:
http:/www.asm.org/branch/brNoE/ index.shtml

National Meetings:

May 17-21, 2009
109th ASM General Meeting, Philadelphia, PA
Discounted Registration Deadline: March 20, 2009

May 28-31, 2009
ASM Undergraduate Conference for Undergraduate Educators
Colorado State University, Ft. Collins, CO

September 12-15, 2009
49th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA

For National ASM Meetings information contact:
ASM Meetings, 1752 N Street, NW,
Washington, DC 20036-2940
Tel: 202-838-3600, www.asm.org

Emerging Powasson Encephalitis (from pg 1)

skunks, raccoons, other small mammals such as squirrels, and porcupines Attachment of *Ixodes cookei* near burrows carries the greatest risk of infection.

Isolates have been found over a broad geographic range, such as in Ontario, Connecticut, Massachusetts, California, New York, S. Dakota, and W. Virginia. Infections are rare have been found by serologic testing all across Canada, in Maine, Nova Scotia, and as far down as Senora, Mexico. During the past 8-10 years a variant of the Powassan virus, known as the deer-tick virus, has been isolated from *Ixodes scapularis.* This is of greater concern as people have more contact with this tick, as seen in Lyme disease. Using a mouse model, it has been shown that *I. scapularis* can transmit the POW virus in as little as a 15-20 minute attachment to its host.

Dr. Wong showed a map of New York (NY) indicating areas where the organism was isolated from ticks and where there were human cases of POW. Infections occur not only in humans, but domestic animals such as dogs, horses, skunks, foxes etc. In humans there is a seasonal distribution of illness as in some other vector-borne diseases carried by ticks, most cases occur in May-September, with the peak incidence from June-September. From 1958-1990 for a period of about 40 years there were 27 human cases identified (less than 1case /yr), including both the US and Canada. From 1999-2001 there were 4 new cases in New Hampshire and Maine-this was attributed to the increased surveillance activity for viral encephalitis following the outbreak of WNV in North America. Samples from eight people with severe encephalitis that were negative for WNV were sent to the Centers for Disease Control (CDC) for additional testing. These samples were found to be positive for POW by an ELISA capture assay. From 2004-2005, after Dr. Wong developed an assay for POW, two cases were identified in NY state, one each year. This year to date there were six cases of tickborne flavivirus identified acquired in NY and one imported case. This is six times the national average in one state alone!

Dr. Wong described the symptoms of POW disease. In humans there is 8-34 day incubation period, with early symptoms of sore throat, headache, and disorientation, sleepiness, followed by encephalitis, vomiting, respiratory distress, convulsions, prolonged fever, semiconsciousness, and sometimes paralysis. Sequellae are severe headache, memory loss, paralysis and loss of muscle control. Tissue pathology in fatal cases shows necrosis of basal ganglia and brain stem, very severe demyelinization, perivascular infiltrate and cytoplasmic viral inclusions. This disease causes a very significant economic burden; individuals usually spend 9-10 days in a hospital, then an average of six months in rehabilitation.

There are two distinct genetic lineages of POW. Lineage 1 is the “classical” Canadian and

New York strains that show a somewhat conserved sequence. Lineage 2 is the deer tick virus, a more recently described variant of POW that is carried by *Ixodes scapularis* and was first described in 1997. The year 2001 might be called the year of POW phylogenetic studies; three major scientific papers concluded that the older virus is actually more conserved while the deer tick virus has more variation and more epidemic potential.

Prof. Gregory Reppucci and Students from North Shore Community College

Serodiagnosis of these infections had previously been done by IgM capture ELISA at CDC. The envelope protein of latent viruses such as WNV is the immunodominant protein, and antibodies to the envelope protein within a serogroup are crossreactive. However sera from POW patients are often seronegative in assays for other flaviruses such as assays for WNV and St. Louis encephalitis virus; therefore one needs a specific assay for POW. If a test for WNV was negative, you probably won't know whether the patient has POW or deer tick virus.

Dr. Wong showed a slide of the flavivirus genome. Most essays for WNV and St. Louis encephalitis are based on the envelope protein of WNV. Approximately four to five years ago Dr. Wong developed her own multiplex assay on the Luminex 200 Laser Flow Cytometer where she took the dominant WNV envelope protein and covalently linked it to a polystyrene plastic particle, then performed a suspension phase microspheric assay. Her assay, however, suffered from the same lack of specificity as the CDC's assay, because the WNV envelope protein cross-reacts with the Japanese encephalitis serotypes. Approximately a year later while one of her colleagues was performing enzyme studies trying to find potential drug targets to treat WNV infections he purified non-structural proteins 3 and 5. She thought it would be interesting to see whether the immune response to these WNV enzymes was more specific than the immune response to the envelope proteins. She then set up an assay and discovered that the antibodies to the nonstructural protein replicase were very specific for WNV and did not cross react with the Japanese encephalitis group. In this assay, if the patient was positive to the envelope, and positive to protein 5, this was WNV infection, while if it was positive to the envelope and negative to protein 5, it was an unknown flavivirus infection.

With that as a background, her colleague Greg Abel cloned and expressed the envelope protein of the deer tick virus. Therefore approximately two years ago, Dr. Wong put the envelope protein of the deer tick virus on a different plastic particle and set up a multiplex assay, where she looked at antibody responses to both WNV antigen and to deer tick virus at exactly the same time. This was done because Dr. Wong believes there are significant disadvantages of using ELISA serology in diagnostic laboratories. One is not able to control the orientation of the adsorption of antigen to plastic plates, sometimes resulting in epitopes being hidden, denatured, or not exposed. Moreover, since sometimes you are not working with purified recombinant proteins but with sonicated or cultured material, you may therefore have a lack of specificity in terms of the antibody binding to that material. Often in ELISA assays, the highest thing that you can measure is an optical density 3.0 but your sample may have a higher optical density and you will not know that unless you do serial dilutions on your initial serum sample. The area over which you get a linear response to ELISA it is very short. She then showed slides demonstrating these problems.

With Luminex technology you are using the excitation of a dual laser light source on a

fluorochrome having a high molar extinction coefficient thus enabling an analytical testing range of almost 4 logs and providing an assay with a much greater linear range of response.
In a classical sandwich type assay, the antigens (or antibodies) with which you are working are covalently linked, usually by their amino terminus, to the surface of a fluorochrome labeled polystyrene microsphere. The antigen containing microsphere binds the patient's viral antibody and excess antibodies can be washed away. A secondary antibody, containing a different fluorochrome target is now attached to the viral antibody. The first laser excites the internal fluorochromes imbedded in the microsphere, which serves to identify which antigen-coated bead you are looking at; the second laser operating at a different wavelength excites the reporter fluorochrome on the on the secondary antibody. The level or intensity of fluorescence observed is proportional to the amount of viral antibody present in the patient's serum.

Dr. Wong is now routinely using this technology which provides her with greater sensitivity and specificity than possible with ELISA assays. The instrument's foot print takes up as much bench space as in ELISA plate reader and washer, and once the instrumentation is in-house, your own assays can be developed inexpensively. In addition, multiplexing provides the ability to measure antibody response of up to ten different antibodies in one Luminex microfilter plate well, without having to run ten different parallel ELISA assays. Dr. Wong has multiplexed up to twenty different antigens in one well, and now can look for POW and WNV at the same time.

The first patient identified in New York State with this new assay was in Westchester County in 2005. This was an 83 year old male, a frequent golfer who lived in a rural area. He was admitted to the emergency room in August with fever, headache, altered mental status, stiff neck and muscle weakness. The admitting diagnosis was encephalitis or meningitis, with WNV suspected. A spinal tap was culture negative for bacteria and viruses; his serum was nonreactive to antibodies for EEE, WEE, La Cross and St. Louis, he had not traveled out of the country, thus eliminating dengue and Japanese encephalitis. Two sera were negative for WNV by IgM assay and he was negative to WNV non-structural protein 5 by Luminex technology; this did not look like a current case of WNV. He did however, have moderate to high positive results with the Luminex assay to WNV envelope protein, with a rise in the antibodies. Now it looked like he had a current flavivirus infection. In an IgM version of the Luminex assay he showed a very high seroconversion in IgM to the deer tick virus envelope protein, going from negative to a titer of 1:23. When serum was sent for viral utilization studies, they were negative to WNV, negative to St. Louis, and showed seroconversion with a greater than four fold rise to deer tick virus but even a higher title to the classical lineage of POW. This was the first patient identified with POW in NY State in about 20 years. Patient follow-up showed mild to moderate changes, but he did improve and was discharged to a nursing home. However, he and is still unable to resume normal activities.

Dr. Wong's laboratory receives 300-400 sera received annually for WNV testing. After identifying this patient in 2005, she went back and looked at sera from 2004. The second patient identified was a 91 year old female admitted to the emergency room with a high fever and three day history of headache and stiff neck. The admitting diagnosis at a local hospital was pneumonia and sepsis. She had a history of cardiac problems and obstructive pulmonary disease; she had never had yellow fever or encephalitis vaccine. An EEG suggested encephalopathy and MRI suggested atrophy of aging. CSF suggested viral encephalitis but a battery of tests for bacteria, viruses and fungi were negative. Neurologic diagnostic impressions were acute delirium related to a toxic metabolic encephalopathy with a fever of unknown origin and sepsis. She was discharged to a long term care facility and died shortly thereafter. IgM was negative for WNV, borderline positive to the envelope protein of WNV and negative to the replicase of WNV. She had some flavivirus reactivity but this ruled out WNV. However, she had the highest patient negative ratio the laboratory had ever seen in the Luminex assay for antibodies to the deer tick virus envelope protein. Removal of IgG and

testing with anti-IgM conjugates showed a very significant titer of IgM antibodies to the deer tick envelope protein. Additional testing allowed us to determine whether this was due to tick borne virus or POW. The neutralizing titers to POW were >40,000 whereas to the deer tick virus there was a four fold rise in titer. This again shows the classical lineage of POW. Therefore in 2004 and 2005 NY had seen two POW cases, which was more than the national average of one case per year. The use of Luminex technology shows the disease may not be as rare as thought.

In 2006 two cases of POW were found. A five year old boy residing a rural farm had gone woodchuck hunting with relatives and although he was not known to have touched a dead woodchuck himself, his dog possibly had. On August 6, the boy presented with fever, headache and weakness. He was hospitalized on August 31 and was thought to have WNV. He was IgM negative for WNV, borderline negative in his first sample to WNV envelope protein by Luminex but seroconverted to positive in the second sample, and was negative to WNV replicase. This profile again suggested a flavivirus infection however, his antibody level was reactive in both first and second samples at a high positive level to the deer tick virus envelope protein. There was a four fold rise in neutralizing antibody to POW and negative reaction to WNV.

Dr. Wong's laboratory had also identified the first case of deer-tick virus in the U.S. in humans but the Canadians beat them in reporting this. A case of POW encephalitis was reported in the Canadian Medical Association Journal in 1999, but the virus had not been sequenced. About a year later they sequenced the virus from brain tissue and in 2001, during a publication of this, reported almost as an after thought, that the virus in their previous publication was not POW but deer tick virus. This was the first fatal case of *Ixodes scapularis* deer tick virus reported as POW in North America (US). It alerted investigators that deer tick virus could be potentially epidemic.

In mid summer 2007 a sixty-two year old male with a four day history of fever and fatigue presented with neurologic symptoms and weakness. He was active outdoors in a wooded area and rode horse back. He had a past medical history of leukemia and was treated with antibiotics for presumed infection; he was taken quickly to a hospital in a major medical center. Cultures were negative for virus. He died after seventeen days in the hospital and autopsy was performed three days later. He did have encephalitis which involved the brainstem and cerebellum and spinal cord. The pathologists were convinced this was WNV and the laboratory therefore received brain tissue for testing. A panel for twelve different viruses was negative; it was not WNV. It was concluded that this was either deer tick virus or POW. The genome showed sequences 95% similar to deer tick virus and POW. Additional primers were designed, and this was determined to be the first fatal North American case of deer tick virus encephalitis.

The utility of multiplex assays allows you to identify not only rare infections in this country but also travel associated infections said Dr. Wong. Last summer, a fifteen year old schoolgirl from New York City went to China on a school trip. She arrived on June 11, in good health, and June 23 went hiking to the Great Wall and somewhere acquired bug bites on her legs. On July 4 she had fever and diarrhea and was treated with antibiotics on an outpatient basis; by 7/6 she had fever and encephalopathy. Spinal fluid testing by PCR and serology were negative for herpes and Japanese Encephalitis viruses. She was treated with other drugs and transferred to a major medical center. She was now essentially nonresponsive, with high fever, effusion, Bell's palsy, seizures and a blind stare. All cultures were negative. On 7/18 she was evacuated to a NYC major medical center. CSF was negative for bacteria and viruses; additional testing showed it looked like acute disseminated encephalomyelitis following an unknown infection. She was treated with immunoglobulin and sent to an inpatient rehabilitation facility; she could not eat, talk or use her arms. Attending physicians suspected Japanese Encephalitis Virus that was acquired in China, and sent paired sera to Wadsworth. An IgM assay to WNV was negative in the 1st serum, CSF was indeterminate, and antibody to WNV replicase enzymes was absent. It appeared to be a flavivirus, most likely tick-borne encephalitis,

that had not previously been recognized in travelers to Asia. Samples were sent to both Fort Collins and CDC and both of agreed that this was tick-borne flavivirus (the first imported case in the US).

Dr. Wong added that the coated microspheres used in her Luminex assays are stable, inexpensive, of high quality and last for months. The testing proceeds rapidly while ELISA assays take lots of time. She added that single sera are not good; paired sera collected three-four weeks apart are best. You must look for both IgG and IgM antibodies; and not rely on single sera or PCR alone – use both, and timing is important. She added that POW has been an orphan until now but seems to be appearing more frequently than expected.

(L to R) Priscilla Neves, CFSP, Pat Kludt, MPH and Linda Han, MD from MDPH

Foodborne Illness Surveillance: *Listeria*

The second dinner-lecture of the year, co-sponsored by the Massachusetts Department of Public Health (MDPH), State Laboratory Institute and Northeast Branch was held on May 6, 2008 at the Venezia Waterfront Restaurant in Dorchester, MA. A *Case Study in Foodborne Illness Surveillance: Listeria* was presented Patricia Kludt, MPH, Epidemiology and Immunization Program Coordinator for the Department of Public Health, Linda Han, MD, Director of the Microbiology Division at the State Laboratory Institute, and Priscilla J. Neves, RS, CFSP, MEd, Director of the Food Protection Program. They each described their department's role in the investigation of the *Listeria* contamination in milk products from Whittier Farms Dairy, Shrewsbury, MA. This outbreak was only the third listeriosis outbreak involving pasteurized milk in the country.

Pat Kludt described how data and information is obtained by MDPH. She showed a list of numerous places from which foodborne illness surveillance obtains data, however, they rely primarily on two sources: notifiable diseases and laboratory specimens. Notifiable diseases indicate what diseases should be investigated and laboratory specimens provide answers. She also defined passive versus active surveillance. Passive surveillance is "reporter initiated" (laboratory reports and hospital reports), making it the least expensive form of surveillance. In these instances MDPH requests that institutions and others report what is happening to them. Active surveillance is MDPH initiated. This is done for certain diseases or situations/conditions where comprehensive information is needed as quickly as possible; for example when antibiotic resistance data is desired, or isolates from an outbreak need to be sent to the State Laboratory. This method uses more resources and can only be used selectively, as not every disease requires public health surveillance. It also requires an onsite investigation by an epidemiologist.

Ms Kludt then described National Notifiable Disease Surveillance Program and showed a list of diseases, dangerous to the public health, for which reporting is mandated by federal and state laws/regulations. She went on to describe the following reporting pathway for getting the information to the Centers for Disease Control (CDC) in Atlanta, GA: healthcare providers report to local health departments (BOHs), laboratories and BOHs report to the State Health Department, which in turn reports to the CDC. Much of this reporting is now done electronically.

Linda Han, MD explained how the MDPH Laboratory identifies *Listeria* in a public health

setting. First it is identified by the official FDA Bacteriological Analytical Method (BAM Method), and then its genome is subtyped by PFGE. This culture identification method of *Listeria* in food requires several consecutive incubations in several media, followed by confirmation and speciation of suspect colonies. She showed a slide of an LPM selective agar plate with esculin on which *Listeria* appears as a black colony with a black halo. The FDA BAM procedures require a number of tests be performed for confirmation and speciation of *Listeria*, i.e., catalase testing, Gram stain, hemolysis testing, motility testing, CAMP testing, and carbohydrate fermentation. The two major points she wished to make is that (1) identification of *Listeria* from food samples is a lot more complicated than its identification clinical specimens, because in clinical specimens *Listeria monocytogenes* is the only organism of interest; however in food, there may be multiple *Listeria* species. Second, two of the confirmation tests require seven day incubations, a long time to wait if there are potentially contaminated products on a shelf.

Dr. Hahn showed slides of a typical umbrella-shape obtained on motility testing of *Listeria*, and a slide of the CAMP test, in which a vertical streak of *Staphylococcus aureus* and *Rhodococcus equi* are made parallel to each other on opposite sides of a sheep blood agar plate; isolates from patient/food samples are streaked horizontally in between but at right angles to the vertical streaks of *S. aureus* and *R. equi*. After a 24 hour incubation a zone of enhanced hemolysis will be seen near the *S. aureus* if *L. monocytogenes* is present, whereas there will be enhanced hemolysis near *R. equi* for *Listeria ivanovii*. The test is technically challenging, as is interpretation can be difficult.

The FDA BAM procedures do allow you to replace these two 7-day incubations tests with a rapid commercial test kit. The State Laboratory uses the MicroID *Listeria* Test kit, a commercial assay of which there are several versions. Basically this is a strip with different ampoules filled with reagents to which your test organisms are added (a glorified carbohydrate fermentation panel with extra biochemical tests added). Testing is rapid, incubation takes only 24 hours, the colors are read, worksheets are printed, and this in combination with the CAMP and sheep blood agar hemolysis test gives enough information to rule out or confirm *Listeria monocytogenes*. The test however, is very expensive.

Moving on to subtyping, Dr. Han showed a diagram and slides of pulse field gel electrophoresis (PFGE), which is done at the State Laboratory on *Listeria* and other organisms. A suspension of bacteria is lysed to release their DNA; these are cut with restriction enzymes that are specific to specific nucleotide sequences, to produce DNA fragments of length. An aliquot of these fragments is spotted onto an agarose gel, and an electrical gradient applied. The fragments are separated by size into specific banding "fingerprint" patterns which can be compared. In this way, both patient samples and potentially contaminated food samples can be run together and then compared to determine if the same genomic strain is present in both and hence establishing a link.

Since it is unlikely that during a national outbreak all of the being tested come into a single laboratory the CDC has established the PulseNet System. PulseNet is a national (and international) molecular subtyping network, whereby all of the laboratory members (all of the states, many large cities, and many countries use the same standardized protocols, which allows for interlaboratory comparisons of PFGE patterns, enabling the identification of foodborne outbreaks on a national and international level. All data is shared between partners via messages posted to a secure web port. Therefore using standard methods with computer analysis of each gel lane, matches are sought and epidemiological clusters anywhere in the world can be observed.

There are three major applications of PFGE in the public health setting noted Dr. Han. The first application is the ability to find common sources of illness in multiple people (ex. A common source outbreak among infants with MRSA skin infection in a neonatal unit). A second application is to link illnesses with food or environmental sources (ex. Fifth graders ill with *Salmonella* were linked to their dissection of owl pellets without wearing gloves; PFGE also exonerated the classroom turtle!). A third application is the ability to identify links

between sporadic cases of illness even when there is no clinically apparent outbreak going on (ex. Several years ago 70 cases of *Salmonella schwarzengrund* were identified over a one-year period. As the cases were spread out over time nothing clinically apparent was occurring. Intensive efforts of the epidemiology program and PFGE linked the illnesses to shopping at live bird markets).

Dr. Han concluded by pointing out that one thing to keep in mind when interpreting PFGE information is that different organisms have different degrees of variability in PFGE patterns. For example, 35% of MA isolates of *Bordetella pertussis*, the causative agent of whooping cough, have the same pattern; therefore two people with the same pattern may or may not be linked. On the other hand, PFGE is highly discriminatory for *L. monocytogenes*. In MA, most *L. monocytogenes* PFGE patterns are unique. Therefore, two identical PFGE patterns within 120 days of each other raise a flag that something suspicious is occurring.

Pat Kludt provided some background information on *L. monocytogenes.* Since the period from exposure to symptoms is variable *(*a few days to more than three weeks), the taking of food histories is very difficult. Symptoms of illness caused by *L. monocytogenes* include fever, fatigue, nausea, diarrhea, meningitis, and miscarriage. Foods generally implicated are soft cheeses, cold cuts, and hot dogs. High risk groups include pregnant females, the elderly, and the immunocompromised.

MA averages 25 to 35 cases of listeriosis per year; 2007 was no different, with 33 confirmed cases. Twenty-two isolates were submitted for PFGE testing by laboratories statewide. Only four had patterns that matched the outbreak strains; the remaining eighteen had different patterns than the outbreaks strain and different patterns from each other. PFGE could not be done in 11 cases because the isolates were never sent to the State Laboratory. Currently it is only requested, but not required, that all *Listeria* isolates be submitted to the State Laboratory. However, regulations are now pending that will require that all *Listeria* isolates and certain other bacterial isolates be sent to the State Laboratory. This is particularly important in outbreaks such as this because much time would be saved if all the isolates involved were available.

Ms. Kludt showed a slide of the timeline for the outbreak. Nothing striking was observed from June–December; isolates were seen all over the calendar and all over the state. This was similar to other years. On November 20 the laboratory reported that isolates from three patients had matching PFGE patterns using two restriction enzymes, which was quite unusual and warranted further investigation. The cases were posted to the National PulseNet database to see if there might be a national outbreak of some type. There was none, and at first, the June case was not thought to be related to the October case because of the time gap (>120 days). On June 19, 2007 a laboratory report for Case #1, a 78 year old male with underlying health issues was received. The isolate from blood arrived the next day at the State Laboratory. On October 12, 2007 an isolate from blood were received at the State Laboratory for Case #2, a 75 year old male with underlying health issues. On November 5, 2007 lab report for Case #3 was received; an isolate arrived two days later. This was a 34 year old mother-baby pair, the baby was stillborn; clinical specimens from baby and mother were received. A local health agent reported Case #4 on November 27, 2007. This was an 87 year old male with underlying health issues. A hospital infection control practitioner spoke with the patient and had obtained a food history. The patient mentioned that he had purchased unpasteurized cider and pasteurized coffee-flavored milk from a local farm stand. Epidemiology then notified the Food Protection Program that worked with the local health agent to have these opened food samples from the patient's refrigerator submitted to the Food Laboratory for testing (this is not always done Ms Kludt pointed out, due to the large number of specimens that would need to be tested, but the interest was primarily in the unpasteurized apple cider).

On December 18, 2007, the laboratory reported *Listeria monocytogenes* in the opened container coffee-flavored milk from the home of Case #4. This was very unexpected, and resulted in the Dept. of Food Protection collecting both

raw pasteurized milk products from the implicated dairy for testing.

On December 21, the Pulse Field Laboratory confirmed that the four clinical isolates were the same as the milk isolate. The investigation now had to proceed with litigation as a possibility. For example, did the milk become contaminated before or after it was opened?

On December 21, epidemiologists started reviewing the other cases with the same PFGE pattern and tried to obtain food histories (pasteurized milk is not a high risk food for listeriosis). They discovered the following: Case #1: used home delivered skim milk for 50 years from a seemingly unrelated dairy; upon investigation it was discovered that the dairy in question actually did produce the milk; the other dairy repackaged milk purchased from them and sold it under their own name. Case #2: drank soy milk but had received nine transfusions the prior year. Transmission by transfusion is undocumented but theoretically possible, and although some of the donors were from MA it was it was nearly impossible to link donors to this case. Case #3: the mother with the stillborn baby was never interviewed. Case #4: submitted the first positive samples.

On December 27 the laboratory reported a presumptive positive result for *Listeria* species in one of the unopened coffee-flavored milk containers collected from the Whittier Farms Dairy on December 19. As a result of these findings, the Food Protection Program contacted the dairy who voluntarily agreed to suspend operations and recall food products.

Epidemiologists still wondered if any cases had been overlooked, because not all patient isolates were submitted for PFGE They interviewed eleven (2007) cases for whom no PFGE was done. They discovered a 31 year old female with listeriosis who presented on September 5 with fever and delivered a preterm healthy infant. Placental and mother's blood cultures were positive for *L. monocytogenes;* no isolate was submitted to the State Laboratory by the hospital laboratory. This patient reported drinking 2% whole milk from the Whittier Farms Dairy throughout her pregnancy; she became Case #5.

To summarize the outbreak, three elderly men died, a 34 year old female had a stillborn baby at 37 weeks gestation, and a 31 year old female delivered a premature but healthy baby. *L. monocytogenes* grow out of 8 milks and an environmental swab that was taken from the floor drain near a homogenizer unit; the PFGE patterns all matched. Ms Kludt again stressed the importance of laboratories submitting bacterial isolates to the State Laboratory. This outbreak could have gone on indefinitely, perhaps undiscovered, for a long while, had not some isolates been submitted to the State Laboratory.

Priscilla Neves from the Department of Food Protection (FPP) then spoke on the environmental investigation of the dairy. She has been with the FPP for over 20 years and added that she finds food outbreaks "fascinating" as they provide an opportunity to look at the whole system, to figure out where/what in the system failed. Was it the regulations and policies, the training, the inability to do ineffective enforcement? The FPP tries to look at all of factors in order to improve the level of food safety for consumers. She is always amazed at the sophistication of the science that supports their work.

The Dept. of Food Protection has been part of the Working Group on Foodborne Illness Control since 1986, when it was formed, collaborating closely with the both the laboratory and the epidemiologists. This has become an excellent model, now used nationally, for collaboration between departments (epidemiology, laboratory and environmental). The FPP is also a regulatory agency, with law enforcement responsibilities. When an outbreak occurs they can order embargoes, disposals, exclusions, restrictions of food employees, mandate employee testing and can close establishments. They also collect food samples, patient specimens, examine practices of concern, and look at employee and facility compliance with existing regulations. They assess all situations and check food items at every point of contact from preparation to consumption. Due to the complexity of the food supply system, other agencies may be included in the investigations, such as local boards of health, MDPH, US Dept. of Agriculture, the Food and Drug Administration, etc. FPP relies on science from the laboratory and

epidemiological data to make decisions to protect the food chain and the public health.

The most important aspect of the environmental investigation carried out by the FPP is risk analysis based on Hazard Analysis of Critical Control Points (HACCP). This approach uses a risk based assessment to identify potential sources of contamination or any other hazards. It considers whether the facility is in compliance with the existing regulations, determines if the facility is properly monitoring conditions, taking corrective actions when indicate and verifying and documenting all work. When it was discovered that this unusual outbreak, with four deaths was related to the dairy, the entire dairy "environment" needed to be investigated to determine if it should be allowed to continue to operate.

Recently, there has been a significant increase in the number of *Listeria monocytogenes* recalls noted Ms Neves. Industry takes recalls, with or without actual outbreaks very seriously because of the costs involved, and has increased their *Listeria* environmental monitoring programs significantly. If there are recalls, especially if illness is involved, the recalls are usually voluntary and corrective actions are taken by the industry. Outbreaks are often associated with a production breakdown, as seen in ready-to-eat foods. In food, pervasive strains survive freezing, grow under refrigeration temperature, and can grow in pH about 4.9. They are difficult to eradicate from a food production plant and can persist for many years.

In recent years, the laboratory's capacity and ability to detect *Listeria* in food has improved. The organism is ubiquitous and is found in soil, water, sewage, decaying vegetation, and raw agriculture commodities, as well as in humans and domestic animals. It can be found in a seafood, meat, and poultry, processing aids such as brine solutions, ice, compressed air, food, food-contact surfaces and food non-contact surfaces such as floors and walls and condenser units. There appears to be a pattern with *Listeria* food outbreaks and associated risk factors. They tend to occur in an older, not well designed and poorly maintained plant. Once *Listeria* is introduced into the environment it is difficult to get rid of. Hygienic training, particularly hygienic practices, i.e., how a plant is cleaned and sanitized is important. New personnel, who are not well trained, can be a risk factor for a particular lot of product. Sanitation controls in the plants are important, as is the flow of a product in the plants and the refrigeration shelf life from the plant to the consumer shelf at home. Another risk factor is whether food is being sold to a vulnerable population and clientele, such as the elderly or pregnant, who are more susceptible to *Listeria*.

Ms Neves then reviewed the outbreak from regulatory agency's perspective. The initial corrective action was based on two "open" food samples from a patient's refrigerator collected on 11/29 by the BOH.

- 12/18: the sample of coffee milk showed a presumptive *Listeria* result.
- 12/19: the FPP collected 11 "closed" milk and six environmental swabs at the dairy (it would be very difficult to take regulatory action on an open sample because the sample is compromised and it would be difficult to prove that the consumer didn't contaminate it). This also alerted the dairy owner to have his recall procedure in place. All firms are currently required to be able to inform the FPP, in terms of distribution, how they will notify all their customers, and how the product would be returned to them if necessary, etc.
- Before Christmas, one of the "closed" specimens collected on 12/19 was reported as yielding an isolate resembling *Listeria* and this again was surprising, as there have only been three *Listeria* outbreaks associated with pasteurized milk in the past 30 years in the United States (two were in MA).
- 12/26: the FPP collected 79 environmental samples at the dairy. Due to the presumptive positive for *Listeria* on the closed unopened sample the firm was requested to suspend their operation.
- 12/27: the firm requested to voluntarily suspend their operation based on this presumptive positive report of *Listeria*

from the "closed" sample and to initiate a Class One recall.

- 12/28: the FPP issued the first of the three consumer advisories

"So how did this happen?" asked Ms Neves. The Milk Sanitization Program is one of the oldest in the country and has very strict policies. The best dairy practices are based on the *Pasteurized Milk Ordinance (PMO)*, which is similar to a federal model code for Grade A milk and milk products. Firms can participate in the Interstate Milk Shippers (IMS) Program and be certified if they undergo all the testing program requirements. Although the Shrewsbury firm was small it was part of the IMS program, which does inspections four to six times annually. The Program inspects pasteurization and other equipment and collects numerous samples for testing, but apparently something was missed.

The dairy in question was a small fifty year old facility with six employees, about 6000 square foot in size and a first generation manufacturer. They had an excellent compliance history with PMO requirements and a good reputation. The facility had some issues in regard to design, similar to other older producer-plants in MA. They received milk from their farm located elsewhere by tanks and received milk supplemented with milk from other farms.

The state is the primary investigatory agency in outbreaks like this. The environmental investigation was initiated December 26, 2007 and took four days. State, federal and local agencies checked pasteurization records, which were in order. Post-pasteurization processing, pasteurized products and storage tanks were investigated. Investigators looked at and inside the milk filling pipes. More than 100 samples of milk and environmental swabs were collected.

Ms Neves showed some slides taken at the facility during the investigation and explained the following potential hazards observed.

(a) Pumps convey pasteurized milk to tanks which are located close to the floor. Anything on the gaskets can be sucked into the pumps.
(b) Repairs at the plant were done piecemeal, not all at once.
(c) The floor drain next to the homogenizer came back culture positive for *Listeria monocytogenes*.
(d) The milk bottle washer unit was in poor condition and was swabbed. Filth swabbed in the area that touched the lips of bottles as they came out was culture negative but *Listeria* sp. was found under a shield that covered the clean bottles as they came out. This was another point of potential contamination
(e) Next to the bottle washer was an open window with a screen. There was no controlled ventilation, as you would see in larger plants.
(f) The filler station for plastic bottles had water marks on it from the ceiling dripping onto it for some time because the roof leaked. The plastic bottle filler had no protection. In larger plants, fillers are protected by plexiglas to minimize contamination from the wet environment, since cleaning is usually carried out with hoses resulting in considerable splashing.
(g) The glass bottle rotary filler was also unprotected.
(h) A roof drain pipe over the plastic container rotary filler showed signs of rust and water leakage.
(i) Visibly dirty plastic milk storage crates were brought into the filling area where finished exposed product was present and would have been cleaned manually (which is allowed in small plants). Larger plants would use a crate cleaner and sanitize crates before allowing entry into an area where there was a milk product.

Food outbreaks usually involve retail food facilities, not food manufacturing facilities. The same PFGE pattern was found in a "closed" milk sample, a patient specimen, and the environmental drain sample. It was an open and shut case but the patient was lost.

"What are the lessons learned from this outbreak?" Ms Neves asked. One of the problems was that this plant did not have any type of *Listeria* environmental monitoring program. Resources are tight for some of the smaller plants, and they can't afford it, but neither can they afford to shut down. There is

no requirement currently in the PMO for pathogen testing for *Listeria* or for any milk pathogens. Testing is done for standard plate counts, coliforms, raw milk, and somatic cell counts. FDA was very interested in this outbreak, because they realized the situation was not uncommon. One of their own investigators had given this plant an A rating in September. The current focus is primarily on pasteurization, not post-processing problems. Other industries such as the seafood industry and large milk facilities such Hood, have done a good job with *Listeria* control; small firms are not able to keep up. We need to look at changes in testing requirements and be consistent.

Plan reviews in such facilities are of utmost importance and are very timely. This type of milk facility would not pass inspection if starting out today. A *Listeria* monitoring program would be a requirement for all ready-to-eat foods. However, this plant had been a dairy for 50 years and in compliance. The owner decided that he could not afford to go through remediation in order to reopen the facility and closed the plant in April 2008.

The FPP is currently working with small producers doing presentations and workshops to reach out to small producer dairies. They also collaborate with the Dept. of Agriculture Resources that has an innovation grant for small producers to help upgrade their plants and equipment. Hopefully we can learn from this outbreak.

Pat Kludt concluded the session by telling us that the outbreak was very interesting from the laboratory perspective because it illustrates several important points. First is how the Public Health Laboratory responds to a sudden surge in specimen submissions, second is that PFGE is a powerful public health tool, and third is that our clinical laboratory partners play a critical role in public health surveillance efforts.

Regarding surge capacity, between the two week period of December 19, 2007 and January 2, 2008, fifty-four milk and food specimens were received in the Food Lab. These included a range of milks with different fat content in different flavors, various juices, etc. In addition, eighty-one environmental swabs and specimens were received having various interesting descriptors. Normally serum or stool is received, not environmental swabs and the number of specimens processed annually by the Food Laboratory is approximately 100. To handle this surge involved coordination on the part of the laboratory supervisor, who recruited bacteriologists from different areas to help in the effort. The Food Emergency Response Network Lab, and PFGE lab staff helped and the in-house Media Laboratory supplied much broth and media during the outbreak. Assistance was recruited from the Enteric, Reference and Dairy Labs as needed. The entire floor was essentially involved in this outbreak. Records were set as to the number of specimens received and the heaviest food sample received was a 48 lb drum of coffee dairy syrup.

PFGE is a powerful public health tool and is highly discriminatory for *Listeria* Ms Kludt added*;* the laboratory suspected a link between the first two matching isolates even though they were separated by more than120 days. The outbreak may not even have been detected without PFGE, and currently foodborne outbreaks are increasingly being identified by PFGE. Finally, this outbreak also illustrates the critical role of laboratory partners without whose efforts the outbreak could not have been identified. Clinical laboratory partners in this outbreak were Worcester Medical Center, University of MA Memorial Medical Center, St. Vincent Hospital, and Milford Regional Medical Center. Equally important laboratory partners are many other hospitals that routinely submit specimens to the State Laboratory and have contributed to the identification of other outbreaks and to other public health surveillance efforts.

Ms Kludt showed the most recent listing of isolates and specimens that are requested to be submitted the State Laboratory, adding that many specimens have stringent and cumbersome packaging and shipping requirements. Although it may be cumbersome to submit these specimens, she added, they do serve a critical public health function and translate directly into preventable illnesses and deaths. She thanked all laboratories who submit specimens for surveillance purposes and encouraged them to continue.

Food and Agriculture Safety

SA David Cudmore, FBI (L) and NEB President Jeff Klinger (R) with students from Johnson & Wales

The third dinner-meeting of the year, co-sponsored by the NEB and the William A. Hinton State Laboratory Institute, Massachusetts Department of Public Health, was held on September 10, 2008 at FINZ Seafood and Restaurant, Dedham, MA. Special Agent (SA) David Cudmore from the Federal Bureau of Investigation serves as the FBI Kansas City Office Weapons of Mass Destruction Coordinator and Investigator on the Joint Terrorism Task force. SA Cudmore, who has extensive knowledge and experience in the FBI's response involving agriculture and food related threats, spoke on *Food and Agriculture Safety*.

SA Cudmore initially stressed that the threat of bioterrorism in the United States is very high and that both international and domestic threats exist. He went on to define a weapon of mass destruction (WMD). He explained that anyone in possession of a WMD can be arrested but that intelligence or evidence indicating that they were going to use it for a nonpeaceful purpose must also exist. A WMD is characterized in a Federal Statute called Title 18, section 23332, subsection A. (1) It is basically a bomb or missile, an explosive device, defined as having a certain amount of charge, i.e. 4 oz of propellant charge or ¼ oz of an explosive incendiary charge. It can cause serious bodily injury or death. For example, in the U.S., a person with a pipe bomb in their car can be arrested, but intelligence is needed to show they were going to use it to cause bodily/property harm. (2) A WMD can also be a poison gas (such as chlorine gas in wastewater treatment plants), or any weapon involving a disease organism (that can cause economic loss), such as smallpox, tularemia, botulism, or plague. Foot & mouth disease for example can affect and kill off our beef supply and may not hurt people but can economic and social devastation. (3) It can also be radioactive material (as in laboratories in colleges and universities) that releases radiation at a level dangerous to human life; low or high levels can exist. Gamma sources such as cobalt 60 and cesium 130 are dangerous and usually shielded but cannot be seen or smelled and exposure for several hours or a day can cause serious bodily injury and death. (4) The Patriot Act in 2002 described nonconventional WMD such as planes used in the 9/11/01 terrorist attack; or backpacks with explosives or items that can cause serious bodily injury to humans, property and livestock.

SA Cudmore defined terrorism as the calculated use of violence to obtain goals that are either ideological or political in nature, and usually involves a symbolic event. The central characteristic of a terrorist criminal act is to create a psychological impact that instills fear into everyone, i.e. September 11, 2000 and the anthrax mailings in September 2001. The FBI response between 9/11/01 and 11/19/01 included calls about anthrax, white powder, bomb, threats, etc. Phone responses alone totaled an overwhelming 45,731; each had to be checked out by an agent. A credible threat typically involves a package or envelope with suspicious markings or indicators such as unexplained powder, liquid or stain, questionable or potential materials or an articulated threat.

When interviewing a true “first line terrorist” arrested in Pakistan in Sept 2002, SA Cudmore realized that the true objectives and goals of an international first line terrorist were to 1) to destroy the western (American) way of life; 2) to embarrass their opposition, i.e., the US, Europe, intelligence agencies, police departments, etc., but especially to embarrass the chief elected officials who instill confidence

in the public; and 3) to create a psychological impact, i.e. to instill fear.

If we contrast the Islamic vs. American way of life we can better understand the reasons for fighting this battle in the current manner he said. He compared and contrasted the population, technology, and economics of warfare and culture of both ways of life. There are twenty countries which are mostly Muslim. The US has about 300 million people; the combined twenty Muslim countries have 1.2 billion people. Their outlook on life is totally different than ours, especially regarding technology, water, food, etc. We have a very outlook in life and much to look forward to, while the average citizen in many Muslim countries has little to look forward to. The technological advances we have are far beyond the reach of Muslim countries. In warfare economics, we have the biggest military might in the world and since they can't compete with us on the battlefield, they must fight another way, thus the guerilla warfare and self-detonations, something against which it is difficult to defend. SA Cudmore indicated that homicide and suicide bombs in the U.S are imminent according to intelligence. Regarding culture, in the US religion and government are separate (and many religions exist) while these are one in Middle East countries; their schools are in the mosques. If their teacher is militant in nature, a child will learn an anti-western attitude. However, the majority of Muslims are very religious, peaceful people and exceptional citizens. The FBI estimates that only about 0.8% of the 1.2 billion Muslims are extreme Islamic fundamentalists and cause problems; these are the people to be concerned about.

We will continue to see an increase in WMD used by individuals who don't want us in countries such as Afghanistan; men and women in uniform will be visible targets for potential attacks although these have decreased somewhat currently. Hoaxes, such as that with anthrax, still continue and packages are constantly screened. The Dept. of Homeland Security (the FBI and law enforcement) carefully scrutinizes targets based on past history and current intelligence and investigations. It uses worst case scenarios to plan future protective actions. Potential targets are locations or events where high numbers of people gather, such as at football games and concerts. The central characteristic is to instill fear, especially through the media. Threats are not usually directed against individual private citizens but at the media or government officials. High-level targets overseas include transportation (trains, busses). Domestic terrorism threats are directed at abortion clinics, nuclear power plants, military installations, major corporations, etc.

There is much agricultural vulnerability that could be a problem in the US, said SA Cudmore. An attack in this area will be an economic assault on our national security and infrastructure; early detection and effective intervention is critical. There could be threats to livestock and any other area of the food supply. Our ability to produce safe and inexpensive food drives the American standard of living. Excellent protection systems for food already exist because of regulatory processing by the Department of Agriculture and Food and Drug Administration, the Department of Homeland Security and obviously by law enforcement. Food is suspected to be high on the infrastructure list to be attacked because of its vulnerabilities, and because of information obtained from the interrogations of first line terrorists and intelligence. For example, Boston's FBI office was at the forefront during the Richard Reid incident. Reid was an El Qaeda operative imprisoned for life for attempting to detonate a shoe bomb aboard a commercial airplane flying from France to Miami. SA Cudmore told how Reid was initially detained in France due to unusual behavior, allowed to fly carrying matches, then captured by stewardesses and passengers; the plane was diverted to Boston. His colleague in England, who had identical shoes, and was supposed to have been on another flight simultaneously, was captured three months later. The shoes contained a highly explosive material which is more sensitive than nitroglycerin but mixing it with the plasticizer made it less sensitive. Reid probably didn't know that the material would deteriorate over a thirty-sixty day period of time and would have exploded if he had continued to wear the shoes. What was key from this investigation was his laptop that contained data bases with listings of biological

and chemical threat weapon agents, some of the areas in which they could be used, the most effective ways to use them in the US and Europe, and where to obtain them. We therefore know that terrorists have contemplated to attack our agriculture. There have been three international symposia on agroterrorism to date with well over thirty countries present. Attending scientists, government officials and law enforcement need to work together and share information to prevent intentional contamination of our food supply.

Occasionally outbreaks are accidently found, such as with those recently occurring with tomatoes and hot peppers from Mexico. The Bioterrorism Act of 2002 requires produce processors and distributors keep track of where food comes from and where it goes, restaurants and farms are not required to do so. We are definitely vulnerable in regard to food and need to keep up public health surveillance. Research is ongoing to develop new methods of food protection, such as electronics to monitor health of cattle, and the recent approval of ionizing radiation to help decontaminate fresh spinach and lettuce. The FBI enlists the aid of the FDA and USDA in such investigations.

SA Cudmore went on to compare and contrast international versus domestic investigations. He explained that the best way to prevent an attack on this infrastructure is to actually hear the plans, and the best method to do that is to wiretap. Since 9/11, the FBI has had ten times the number of intelligence cases as before and works with other agencies such as the NHA, CIA, military intelligence, etc. The way information is analyzed and disseminated is critical.

He described how a domestic wiretap (Title III) differs from an international wiretap (FISA or Foreign Intelligence Surveillance Act). Title III is a wiretap on a domestic citizen. If a business phone, home phone, fax, e-mail, cell phone, etc. is to be tapped, all investigative means such as phone records, bank records, trash records, witnesses, etc. must be exhausted because of the constitutional fourth amendment (a reasonable expectation of privacy). Generally this type of wiretap request is denied by a judge. However, once authorization to wiretap is received, if concrete criminal activity is not shown in ten days, the wiretap can be shut down. If criminal activity is found the wiretap may be continued up to 30 days. All wiretap information is sensitive, restrictive, and even confidential; minimization rules state that matters unrelated to criminal activity cannot be recorded and are not allowed as evidence. Wiretaps on an incident involving international matters (foreign citizens, someone with contacts or family overseas, someone who visits other countries or makes calls outside the country, etc.) are a threat to international security. With FISA, 90 days are allowed to complete an international wiretap and everything said may be recorded.

Other intelligence methods are also used in investigations. Undercover operations for example, are extremely successful in domestic terrorism, whereas in the international arena they are difficult to do as relationships between people there are extremely close. The internet and other means need to be used.

SA Cudmore then outlined what might potentially happen in case of an attack.
First would be a law enforcement response, and would involve fire departments, emergency medical service personnel, highway patrol and local law enforcement. The Department of Homeland Security is responsible for training and equipping these groups. Second would be the state responders with a public health response; including state departments of public health at the state and local levels and biological, chemical and radiological levels. These people are needed on the scene and can best diagnose the problem. This involves Health and Human Services (HHS) and the Centers for Disease Control (CDC). Last is the major federal response. The overall response will be slower as they need to gather intelligence and need time for investigation; they would query national and international databases. This group includes FBI agents and an intelligence task force.

SA Cudmore then showed a diagram of FBI relationships with various agencies involved in a threat assessment process at a threat scene. Appropriate protective actions are taken based on what is occurring. The FBIHQ/WMDOU (Weapons of Mass Destruction Operations Unit) is a national command post and is involved in

the threat assessment process 24/7; they can recruit anyone worldwide for expertise in an area. The Unit is comprised of thirty-five different agency representatives that are on call as experts from their agencies. The CDC may be involved in investigations of suspected smallpox, anthrax and other bioterrorism agents that are on the FBI Critical List and other agencies from HHS may be called. The Department of Defense has expertise with explosives, Homeland Security/FEMA deals with natural occurring consequence items as hurricanes snowstorms, etc.; the USDA/FDA deals with food/agriculture; HHS/CDC are involved with public health and bioterrorism; the Department of Energy with radioactive materials, and the Environmental Protection Agency has a chemical spill hotline. There is a local to state to federal structure everywhere.

Also available are HAZMATERIAL response teams out of Quantico, VA, that augment the HAZMAT response teams. These are scientists, firemen, with a law enforcement HAZMAT background. The teams have mobile laboratory capabilities and within four to six hours can be anywhere in the United States. The National Center for the Analysis of Violent Crimes (NCAVC) helps the FBI profile subjects and groups while CIRG (Crisis Incident Response Group) includes SWAT teams, hostage negotiators, and command post resources; they can set up a joint operation and joint information Center if needed.

Responses to bioattacks and chemical attacks differ said Agent Cudmore. With biological weapons, many people with the same/similar symptoms are seen in a physician's office or in a hospital. There is a delayed onset as the agent is a natural agent that replicates; some agents are contagious. With chemical agents, respiratory problems are seen in those exposed and there is a rapid onset of symptoms. Agents are man-made, do not replicate, and are not contagious. Investigation of the anthrax attack that occurred in 2001, for example, took seven years to complete. Microbial forensics traced the anthrax strain involved to an army flask at USAMRID. Dr. Bruce Ivins committed suicide when he learned he was about to be arrested for the incident.

One theory as to why the U.S. has not been attacked yet is that if you look at the countries from which immigrants come to the US (from England, Spain, Malaysia, Europe etc.) we are all immigrants or their decedents. The new immigrants trust US government more than that of the country they left. In addition, innovations such as neighborhood watch, etc. help neighbors watch out for their homes and neighborhood.

SA Cudmore believes that our approach to addressing the terrorism threat will be a long-term project of the highest priority and we need the highest degree of vigilance and dedication from all of us. Success will depend in part on the strength of our partnership.

Tales of the Crypt(ics): Lateral Gene Transfer by "Defective" Prophages

The fall program, co-sponsored by the Northeast Branch American Society for Microbiology and the William A. Hinton State Laboratory Institute, Massachusetts Department of Public Health, was presented on Tuesday, October 7th, 2008 at the Hawthorne Hotel, Salem Massachusetts.

Tour of historic Salem Common

The evening began with a guided walking tour of the historic Salem Common (photo above) lead by Michael Coleman, President,

Salem Common Neighborhood Association a Salem native, and Northeast Branch Past-President Garry Greer. The Salem Common is part of the Salem Common Historic District and its approximately 9.5 acres of land have been central to the civic life of Salem for four centuries. The area is now a landscaped park used for recreation and public events, surrounded by a mostly residential neighborhood.

After a brief reception and dinner-buffet, Dr. Thad S. Stanton spoke on *"Tales of the Crypt(ics): Lateral Gene Transfer by 'Defective' Prophages"*. Dr. Stanton, a former ASM Foundation Speaker, is the Research.

(L to R) Garry Greer, State Laboratory Training and Distance Learning Coordinator and Michael Coleman, President, Salem Common Neighborhood Association

Leader of the Pre-Harvest Food Safety & Enteric Diseases Research Unit at the National Animal Disease Center (NADC) in Ames, Iowa The NADC is the premier research institute within the USDA for studying diseases of large animals. The Unit investigates the interactions of enteropathogenic and non-pathogenic bacteria with each other, with the animal host, and with the farm environment. The Unit then generates strategies to reduce the prevalence of human foodborne pathogens, animal pathogens, and antibiotic resistant bacteria in livestock. Dr. Stanton's specific interests are directed towards antibiotics and antibiotic resistance in swine.

Dr. Stanton first spoke of the "New Phage Age" and the bacteriophage impact on nature. Phages, he said were used in the 1950s and 1960s as tools to study genetics of bacteria. Today we look at them as direct participants in evolution, pathogenesis, and significantly contributing to microbial ecosystems. He went on to define three functional types of bacteriophage, lytic (in which bacterial cells are lysed and destroyed after immediate replication of the phage), lysogenic (in which the viral genome integrates with host DNA and later replicates along with it or may become established as a plasmid), and cryptic phages. Bacteriophages have significant impact on nature and the earth's carbon cycle. They contribute significantly to the biomass and genetic diversity (an estimated 10^{31} of bacteriophage particles) of the earth's biosphere; even more impressive is the great variety of bacteriophages that exist. In the human ecosystem alone, the bacteriophage content of feces is about 1000 phage genotypes per 500 gm of human feces. Lytic bacteriophages directly influence microbial ecosystems by attacks on certain bacterial populations and their explosive release of progeny.

The ocean, one of the densest natural sources of phages, contains incredible numbers of particles, i.e. ten million particles/mL of water. These estimates are provided by genomic analysis. . About one-third of cyanobacteria, which fix carbon in the earth's carbon cycle, are constantly under attack by bacteriophage. During their lytic cycle, phages are released into the environment, and during this phase, a "grazing food chain", they return approximately one-fourth of the carbon cycle (biomass) to the ocean. Thus, phages play an important part in our carbon cycle. This also serves to maintain bacterial diversity, for as bacterial concentrations increase, they become more susceptible to phage attacks. For example, it was found in Bangladesh that the concentrations of bacteriophage populations in the water (when cholera epidemics occur) are a major influence on the levels of the two *Vibrio cholera* serotypes present, 01 and 0139. There is an inverse

Mary Gilchrist, PhD, Director, Bureau of Laboratory Sciences, MDPH, Thad Stanton, PhD, and Betsy Szymczak, MS, Director, Laboratory Response and Communications, MDPH

correlation between the number of bacteriophage present in the water and the levels of each serotype present. Where do the phages come from? Non-cholera serogroups in the waters were found to release bacteriophage that attack disease-causing organisms. The competition occurring between the bacteria therefore can affect human activities and determine disease cycles. Recently, the food industry has started using phages. A "cocktail" of" of six different lytic *Listeria monocytogenes* - specific bacteriophages was approved by the United States Food and Drug Administration for use on ready to eat meat and poultry products

In the lysogenic phage cycle, the bacteriophage genome is incorporated into the host genome and remains there until the bacterium divides or replicates; the phage genome (prophage) then also replicates. In nature, the phage sometimes, for reasons unknown, starts a lytic cycle, which then destroys the bacterium. One of the highly impressive discoveries of genome sequencing projects said Dr. Stanton, is the high incidence of recognizable prophage genes present in the bacteria. For example, several years ago Dr. Sherwood Casjens found that 10-20% of the *Streptococcus pyogenes* genome is occupied by prophage. Since the phage genome may be only ½ % of 1% of the size of the bacterial genome, there may be as many as 20 different prophages inserted into this genome.

What does the prophage do in the bacterial genomes? Looking at various strains of *E coli*, each strain has a prophage that "customizes" the bacterial cells and gives them characteristics other bacteria of the same species don't have, e.g. toxigenic 0157:H7. Why do bacteria tolerate such a molecular parasite that is essentially a time bomb? If a prophage is in a genome, identical prophage can't destroy the cell. The prophage creates a repressor that shuts itself and other prophage off. The prophage can also convey immunity to superinfection by unrelated bacteriophages, by changing the surface properties of their host cell, prohibiting other phages from attaching. In addition, Bacteriophage frequently carry morons (moron=more DNA on) as an extra, non-phage gene within a prophage genome that can be carried between different strains of bacteria (previously called lysogenic conversion). It is a "hitchhiker" which can sometimes create pathogens, i.e. products of moron genes include extracellular toxins such as diphtheria toxin and *Clostridium botulinum* neurotoxin, and antibiotic resistance genes as in *Burkholderia cepacia,* which can transfer resistance to three drugs via bacteriophage BcP15 to *Shigella flexneri*. He emphasized that bacteriophages can jump across genes and transfer drug resistance from one bacterial genus or species to another.

Although cryptic phages were recognized many years ago, said Dr. Stanton, only recently did we begin to appreciate their diversity and numbers. These are "defective", or "partial prophages", that don't confer immunity to super infection, and were once described as "phage on the way out", or "genetic debris". Genome sequencing projects however, have suggested that frequently the prophage content in a bacterial genome is cryptic and that bacterial cells contain numerous cryptic bacteriophage-like genes and gene clusters.

Dr. Stanton's final discussion, "Ascent from the Crypt: VSH-1" involved his research. During efforts to develop genetic tools for the swine intestinal pathogen *Brachyspira hyodysenteriae*, a project that started several years ago, his team uncovered a cryptic, prophage-like element, which they named VSH-1. *B. hyodysenteriae* is a spirochete, and obligate anaerobe (aerotolerant), nutritionally fastidious, and is the agent of swine dysentery;

it has also been isolated from captive rheas. During routine molecular work, when mitomycin C was added to cultures of the organism, the turbidity started to decrease after 4 hours, indicating that a bacteriophage was present. Microscopically, small phages of uniform size, with a 45nm size head and structurally simple straight 64x9 nm tail were observed on the outside of the lysed bacterial cells. The group was stunned when they observed circular nucleic acid inside these phages. More impressive was that when using restrictive enzymes, and comparing digested VSH-1 DNA with the host *B. hyodysenteriae* DNA, they were not distinguishable. VSH-1 particles were scattered throughout the entire *B. hyodysenteriae* genome, thus suggesting that VSH-1 contained host chromosomal DNA. Additional research indicated that VSH-1 packages 7.5-kb fragments of host chromosomal DNA. This was the first time this phenomenon was observed. (J. Bact., Jan. 1997, V179, 323-329).

The next question was can VSH-1 transfer genes between bacteria? The answer was yes, a surprising relationship between prophage transfer and antibiotics resistance was observed. VSH-1 could package 7.5-kb fragments of host chromosomal DNA (an unusual but not unique phenomenon) and transfer it to other bacterial strains! Non-infectious VSH-1 was able to transfer genes between *B. hyodysenteriae* cells and new drug resistant *B. hyodysenteriae* cells. The gene transfer was able to be inhibited by adding purified antisera made to VSH particles to the cells. Dr. Stanton (for the first time publicly) then displayed a slide of the entire genome of VSH-1 which they had sequenced. Additional research showed that mitomycin C induces VSH-1 to affect the gene transfer. VSH-1 has a divided genome that spans 36 KB of the *B. hyodysenteriae* genome, it's no wonder VSH-1 doesn't even form plaques. Dr. Stanton went on to explain that bacteriophage and viruses don't have split genomes, but cryptic phages such as VSH-1 do. It actually is a "defective" prophage but is an "effective" gene transfer system and doesn't need to have its genes together. Dr. Stanton's team then looked at what might induce VSH-1. While oxygen and other chemicals don't stimulate it, environmental inducers as mytomicin C, metronidazole and carbadox do, as do macrolides such as lincosamide, and streptogramin R. And many of these drugs are frequently used in treating and preventing disease in humans and animals. For example, carbadox also induces *Salmonella* phage, *Shigella dysenteriae* phage, and *E. coli* phage. Similar gene transfer agents such as VSH-1 have been reported in a variety of different bacterial genera worldwide, in rickettsia, brucella, etc. and appear to be able to transfer genes from one bacterial chromosome to another. Fragments of phage are present in the bacterial genomes and seem to have appeared early in the evolution of the proteobacteria and still persist. How they function in these species and genera still needs to be studied.

(L to R) James Kirby, MD, President-Elect, NEB; Jeffrey Klinger, PhD, President, NEB and Thad Stanton, PhD

Dr. Stanton summarized the lecture by saying that traditional bacteriophage are now known to have increased applications and effects on microbial ecosystems. Bacteria frequently contain prophages that can contribute specific virulence determinants and other characteristics to bacteria in which they are present. The discovery of a type of a cryptic phage known as a gene transfer agent (such as VSH-1) shows that we should not be overlooking fragments of DNA present in bacterial genomes. He ended with a slide from the New Phage Biology Conference: *Our knowledge of the*

bacteriophage world is an inch wide and a mile deep. Our knowledge is just an inkling of what we know.

After the formal part of his lecture, Dr. Stanton held a short trivial pursuit "Know Your Witches" contest". He showed images from various witch movies or witch theme TV shows and asked the audience to write down answers as to the name of the movie, actor, witch, etc. Although several people did fairly well, the conclusion was that many of us just don't watch enough (witch) movies and TV shows!

Stephen G. Jenkins, PhD and Jeffrey Klinger, PhD, President, NEB

Impact of Pediatric Pneumococcal Vaccines on Disease and Antibiotic Resistance

The final lecture in the 2008 Dinner-Lecture Series, co-sponsored by the NEB-ASM and the William A. Hinton State Laboratory Institute, Massachusetts Department of Public Health, was presented on Wednesday, November 5, 2008 at the Aegean Restaurant in Framingham. Stephen G. Jenkins, Ph.D. of the New York-Presbyterian Hospital-Weill Cornell Medical Center spoke on *"Impact of Pediatric Pneumococcal Vaccines of Disease and Antibiotic Resistance."*

Dr. Jenkins began his lecture by saying that he wanted to describe the impact of the pneumococcal vaccine on the development of antimicrobial resistance. S*treptococcus pneumoniae* (or pneumococcus) is a true pathogen, and is the most important bacterial cause of community acquired respiratory tract infections. It causes the most morbidity and mortality of any bacterial pathogen in the community respiratory setting.

Studies show that *S. pneumoniae* causes from 20 to 60% of community acquired pneumonia; and is the most common cause of death due to pneumonia, worldwide, in children less than five years old. It is an important pathogen in pediatrics, causing 15 to 25% of acute bacterial exacerbations of chronic bronchitis, and 20 to 43% of acute bacterial rhinosinusitis.

Resistance rates have exploded in the past 20 years; it has only been since about 1980 that we had penicillin resistant or intermediate pneumococci. Resistance rates differ worldwide; from 17% to 36% of pneumococci fall into the penicillin resistant category. Resistance is even higher for the macrolides. Azithromycin is the most widely prescribed oral antibiotic in the U.S. today, resulting in its being the driving factor in the evolution of pneumococcal macrolide resistance. Similar situations are found elsewhere in the world, where resistance rates range from 24 to 40%. Currently in China, 84% of group A streptococcus and pneumococcus are macrolide resistant. Furthermore, 22% of the U.S. strains are multi-drug-resistant, which is defined by the Food and Drug Administration as resistance to two or more classes of antibiotics. The organism therefore is problematic from a number of perspectives.

There are forty serogroups of *S. pneumoniae* which are subdivided into 90 subtypes (serotypes) based on their polysaccharide capsules. Some serogroups only have one member i.e., serogroup 14 has only one member, serotype 14, and serogroup 6 has serotype 6A and 6B. There are certain pneumococci that historically have been much more pathogenic and at one time or other were called epidemic in nature because they appeared to cause more disease. The most virulent was type 3, which appears very mucoid and watery on an agar

plate, as it has a thick capsule that allows it to evade polymorphonuclear leukocytes that might otherwise destroy it. Other serotypes that are historically associated with anti-microbial resistance, particularly in children, are 6, 9, 14, 19, and 23.

A 23-valent polysaccharide vaccine has proven to be of value in adults and high risk groups for several years now. Although it has not reduced the rate of pneumonia it has reduced the rate of mortality caused by pneumonia. This vaccine is not effective in young children because their immune system is not mature enough to respond to polysaccharide antigens. The polysaccharide antigens are made more antigenic by conjugation with protein; the resulting complex is capable of stimulating an immune response. Thus conjugated vaccines are used in pediatrics, while the simple polysaccharide vaccines are used in adults.

Multiple pediatric vaccines are under development since the first heptavalent vaccine (PCV7), containing types 4, 6B, 9B, 14, 18 C, 19 F and 23F. These serotypes were included in the vaccine because these strains caused the most disease in pediatrics and had the highest rates of penicillin resistance. Currently, the following vaccines are being developed: 9-valent vaccine that has the same seven strains of the heptavalent vaccine plus 1 and 5; an 11-valent vaccine that will have the same strains as the 9-valent vaccine plus 3 and 7F, and a 13-valent vaccine in which one of the serotypes, 19A, has become the most common cause of pneumococcal disease in children, and was rarely isolated before the vaccine.

The 7-valent vaccine was introduced in the United States in February 2000 and is licensed for children up to five years of age. The general recommendation is that it be used in all children less than two years old. An article this week in the MMWR from CDC discussed the worldwide implementation of vaccination programs with this vaccine. Such programs are currently prescribed in the U.S., Western Europe, Australia, New Zealand, etc., countries which have the least problem with serious pneumococcal disease in children. Third world countries, by comparison, have virtually no pneumococcal vaccine coverage in children; many countries simply can't afford them. The major challenge facing us, said Dr. Jenkins, is to develop a program that can move these vaccines into areas where they are most needed. After the vaccine was marketed, the developed countries witnessed a decline of 77% in invasive pneumococcal disease in children less than two years, with a resulting 39% decrease in hospital admissions for pneumonia among children less than two years of age. The vaccine is tremendously effective!

It was hoped that two of the serotypes (6B and 19F) present in the 7-valent vaccine would also protect against other serotypes in the serogroup. Although cross reacting antibodies to 6B and 6A and 19 F and 19A were observed in the laboratory, these were found to be "non-protective" antibodies; hence the need to add serotypes 6A and 19A to the newer vaccines.

An article published in several years ago in the Journal of Infectious Disease showed the impact of a 9-valent vaccine and pneumococcal carriage in 242 patients who received it. Only about 18% of the patients were shown to carry pneumococcus after vaccination as compared to 36% in the control group. The vaccine had prevented carriage of pneumococcus and was protective in preventing disease. However, if you look at the carriage of strains that were not in the vaccine, it was higher in the group that received the vaccine. These children were being colonized by strains not in the vaccine, especially type 19A; this was a hint that 19A may become problematic in the future. (Note, during the winter months, approximately one third of us will carry pneumococcus in our upper airways, however to prevent carriage you need to prevent disease.)

Another study looked at the 7-valent vaccine. The vaccine efficacy for otitis media was significant. Not only were children being protected against invasive pneumococcal disease, but fortuitous was a drop in pneumococcal otitis media. Pneumococcus was once the most common cause of otitis media in the U.S; it is now *Haemophilus influenza.* In the pre-penicillin era the most common cause of otitis media was beta hemolytic group A streptococcus that caused a more serious otitis media than pneumococcus and could result in mastoiditis, brain abscesses and death. There was some concern that this might return as the

primary cause with the introduction of pneumococcal vaccines but that has not happened to date. There was a 34% drop in otitis media in children with the introduction of the vaccine. There was a 50% drop in otitis media caused by those serotypes in the vaccine; but there was a 34% increase in the serotypes not in the vaccine, an 11% increase in *Haemophilus influenza*, and even a slight increase in *Moraxella catarrhalis*. Another study with a different vaccine gave similar results. Dr. Jenkins emphasized that that when speaking of protecting children with this vaccine from otitis media, it only works if the child receives two doses of the vaccine before two years old.

Another study looked at the invasive pneumococcal disease rate in children less than five years old from 1998 to 2008, and as to whether there were differences in various age groups in terms of invasive pneumococcal disease such as meningitis, bacteremia, septic arthritis, and empyema, etc. There was a dramatic 77% drop in invasive disease in children less than 1 year old after the introduction of the heptavalent vaccine; an 83% drop in children one year of age; a 64% drop in those two years of age; a 60% drop in those three years of age; and a 48% drop in those four years of age. Although it did not protect against otitis in these age groups, it definitely did its job in protecting against invasive pneumococcal disease.

An early CDC surveillance program looked at the effect of the vaccine by serotype. The study looked at baseline data in 2000 and then in 2003, looking at cases per 100,000 population. It showed that roughly, there was a 96% drop in invasive pneumococcal disease rate in children less than two years old (this was later changed to 77%) and a 67% drop in vaccine related strains. Even at this early stage serotype 19A was emerging; there was a 49% increase in invasive disease caused by serotype 19A and a 58% increase in other non-vaccine types of *S. pneumoniae*.

The pneumococcus is the most efficient of all organisms in its ability to pick up extraneous DNA from the milieu said Dr. Jenkins. For example, if an organism dies the pneumococcus can pluck up that DNA and incorporate some of it into its own DNA. In that manner it became resistant to the beta-lactams, the penicillin binding proteins, the modified PPPs, and 2X, which actually came from viridans streptococci. It has now been shown by DNA sequencing that 19A is actually 19F, the vaccine strain, which was the most resistant of all pneumococcus. Under pressure of the vaccine 19F picked up DNA from serotype 19A and changed its capsule. It contains the same resistance genes, but now has a capsule that is resistant to the vaccine; the bug has actually changed its cloak!

There are also differences in colonizing and invasive pneumococcal disease strains in various parts of the world. The vaccine would have covered about 90% of the invasive strains in the Western World when it came to market in 2000, where as it would cover less than 50% of the invasive strains in Asia.

The PROTEKT Program (a large surveillance program) looked at the first three years following the release of the vaccine. Vaccine serotypes and invasive pneumococcal disease all went down as serotypes not in the vaccine went up, particularly serotype 19A. The Program annually collects thousands of pneumococcal isolates in the U.S. and worldwide. Looking just at the U.S. data for the pediatric age group, 75% of blood culture isolates were in the vaccine after the first year, but by third year, only 34% of blood isolates were in the vaccine and by the fourth year only 23% were covered. The same phenomenon occurred among non-blood isolates, which went from 61% coverage to 27% coverage. The types of pneumococcus seen were changing. The same data sorted by age instead of by infection, show the same statistics; a drop in the strains covered by the vaccine and an increase in the strains not in the vaccine. The one thing not seen was a difference in region; every part of the U.S. had the same pattern, and the same rates of resistance.

Levofloxacin is widely used for respiratory infections, but resistance of the pneumococci to fluoroquinolones remains less than 1% in the United States. However, there have been some interesting clusters of infections caused by fluoroquinolone resistant pneumococcus. In Hong Kong, the rate rose to nearly 20% several years ago. This was found to be due to crowded

conditions, the resistant clone was being spread from person to person; a similar cluster recently occurred in Salem, Massachusetts in two large nursing homes.

A study of all of the pneumococcal types recovered from children in the first, third, fourth and fifth years after the introduction of the vaccine showed that the percent of pneumococci recovered from children dropped for all vaccine strains. Recovery of serotype 19 F, before the vaccine was 17.5% and by the fifth year the recovery rate was down to about 8%. At the same time, there was an annual rise in the proportion of nonvaccine strains.

A disturbing trend observed is that if you look at multi-drug-resistant *S. pneumoniae* at years one, two, etc. there is a much higher rate among the vaccine strains than there was before the vaccine came on the market. Multi-drug-resistant rates also increased in the serotypes not represented in the vaccine; this was up to 32% by the fourth year.

Dr. Jenkins briefly outlined the mechanisms of macrolide resistance which occur with drugs most commonly prescribed for respiratory infections, such as clarithromycin, azithromycin, and erythromycin, which is less commonly used now. There are two major mechanisms of macrolide resistance. The first is efflux; as the macrolide enters the bacterial cell, the cell pumps it out. The gene that codes for this efflux pump is the *mef* (macrolide efflux) gene. The second method of resistance is methylation of the ribosome. When the organism has the *erm* (erythromycin ribosomal methylase) gene, a methyl group is placed on the binding site of the 23S ribosomal RNA, and the drug now cannot bind. The MICs of these organisms to the macrolides are incredibly high.

One thing Dr. Jenkins found extremely interesting was that from the year 2000 to 2005, the proportion of pneumococcus that have both mechanisms of resistance have risen from about 10% of the strains to about 20%; resistance is highest in serotype 19A. The rates of resistance are particularly high in the pediatric population, where the vaccines are being used. Currently, 46% of strains isolated are 19A, with 19 F coming down slowly. Another interesting feature about the 19A strains is that they have over time picked up additional antibiotic resistance mechanisms. One that has been particularly bothersome is amoxicillin-clavulinic acid resistance; this resistance rate is an incredibly high 38%. The first year after the vaccine was introduced, MICs were probably between one and two mcg/mL; now 69% of the organisms have an MIC of 8. There appears to be a steady evolution of higher MICs.

An older study from 2002 looked at the serotype distribution from nineteen pneumococcal studies in children that were published from 1928 until the present. For some unknown reason the “epidemic” serotypes disappeared during this period, while there was an increase in the serotypes that were not typically considered epidemic. Another interesting observation (Keith Klugman’s work) was an increase in the pediatric vaccine serotypes and decrease in the invasive serotypes during the same time frame. We were seeing a decrease in the epidemic serotypes and an increase in the serotypes that were covered by the vaccine. This was part of the rationale for the development of the vaccine.

Dr. Jenkins then described an interesting study regarding pneumococcal carriage. Children love to share things, he said, i.e., toys, secretions and pneumococcus. A 2004 study looked at families and pneumococci that were not associated with invasive disease. On examining a parent, a child and its sibling, they found if carriage of pneumococcus occurred, each person had a different serotype. If they were not strains that were prone to cause disease, family members did not share the strains. But if one person in the family had a strain that caused invasive disease, many of the household members were then colonized with the invasive strain. Therefore there is a big difference with the type of pneumococcus that can colonize individuals. This all leads to protection, as children love to share their pneumococci along with everything else. A fortuitous effect from using the vaccine in children resulted in a dramatic decrease in invasive pneumococcal disease in adults, which was totally unexpected. In people over 65 years of age there was a 31% decrease in invasive pneumococcal disease after the advent of the heptavalent vaccine. A 20% decrease in ages 40 to 64 occurred, and a 41% decrease occurred

between 20 and 39 years of age, even though the rates are much lower because these people normally don't get pneumococcal disease. Therefore the “herd effect” has been real in the 65 and older age group; a 65% drop in invasive disease caused by the serotypes in the vaccine occurred, and there was a slight increase of 13% in the serotypes that were not in the vaccine. The same thing occurred in the adults and children; we had a herd immunity protecting the adults by protecting the children. A similar shift in serotypes was seen in the elderly as was seen in pediatrics, a drop in the vaccine strains and an increase in the nonvaccine strains.

In 2004 Klugman’s group also looked at gender as a risk factor for antibiotic resistance and did a multivariate analysis of risk factors in women. The following risk factors appeared: were the women infected with the pediatric serotype, was it a penicillin resistant strain, were they HIV-positive? They were much more prone to pneumococcal disease if they had HIV underlying infection, and if they were over 40 they were more likely to have bacterial resistance. Another report from CDC looked at HIV and the impact of the vaccine introduction. A drop in vaccine strains and an increase in strains not in the vaccine were again seen in patients with HIV and AIDS, the same as in patients that did not have HIV.

Dr. Jenkins then gave the following excellent summary of his talk:

1. The percent prevalence of isolates covered by PCV7 vaccine has decreased dramatically since its introduction. In the 0-2 age group it went from 69% to 23% and in the 3-14 year old age group it went from 65% to 26%. Blood isolates decreased from 74 to 23%, while non-blood isolates from 61 to 27%; the limitation is that you can't correlate this with disease prevalence only with carriage.
2. ST19F has not decreased in proportion to other vaccine serotypes and of all the serotypes in the vaccine, 19F still remains fairly common.
3. ST19A has dramatically increased from 5 to 26% in the 0-2 age group and has evolved as the most important antibiotic resistant pneumococcus.
4. Serotypes 3, 11, 15 and 35B are other major serotypes in which there has been an increase in prevalence.
5. There have been large increases in penicillin resistant *S. pneumoniae*; macrolide and multi-drug-resistance are seen in the nonvaccine strains. There has only been a small reduction in resistance in the pediatric isolates and there has been no reduction in macrolide resistance, which has increased since the introduction of the vaccine.
6. The percent prevalence of macrolide resistance isolate with both *erm* and *mef* has increased dramatically, from 10.7% to 32%; in the 0-2 age group; almost all of these are serotype 19F or 19A.
7. The prevalence of 19A has increased and is now greater than 19F; all the *erm*B and *mef*A isolates in these studies are multi-drug-resistant.
8. The heptavalent vaccine has been very successful in reducing the prevalence of infection caused by those serotypes including systemic infection and otitis media; protection has inadvertently spilled over into adults as a result of herd immunity.
9. ST19A has evolved as the most important antibiotic resistant pneumococcus in the United States.
10. The 13-valent vaccine, which will be out in about ten months will have 19A in it, and with its introduction we hope to see a drop in that serotype.

Infectious Disease Surveillance: A Team Approach

This active surveillance conference was held at the Hoagland-Pincus Conference Center in Shrewsbury, MA on November 6, 2008, and was sponsored by the Massachusetts Department of Public Health (MDPH), Bureau of Communicable Disease Control, Division of Epidemiology & Immunization and the

Northeast Branch. The full-day program was designed for clinical microbiologists, infection control practitioners, epidemiologists and infectious disease specialists.

Melissa Cumming, MS and Tracy Stiles, MS from the William A. Hinton State Laboratory Institute, MDPH spoke on *Post-Pasteurization Contamination of Milk by Listeria monocytogenes in Massachusetts*. They emphasized the importance of molecular typing (PFGE) and the extensive teamwork necessary in detecting foodborne illness. Stephen G. Jenkins, PhD, from the Weill Cornell School of Medicine, New York spoke on *The Laboratory, Action, Clinical Impact, Therapeutic Alternatives and Infection Control Methods Related to Klebsiella pneumoniae Carbapenemase (KPC)-Producing Organisms.* He described their epidemiology, problems, treatment, mechanisms of resistance, and CSLI recommendations for susceptibility testing of these organisms. Conference attendees were then able to participate in two break-out sessions. *Infection Control: Massachusetts Hospital Associated Infections Program Update--- Prevention and Reporting Activities* was moderated by Eileen McHale, RN, BSN, Roberta Bernstein and Laurie Kunches, ANP, PhD. Nellie Dumas, BS, Associate Director Bacteriology Laboratory at the New York State Department of Health in Albany, New York was moderator of *Microbiology: Biosafety Considerations for the Laboratory: a CAP LPS , Teachable Moment.* Stephen I. Pelton, M.D., Director of the Section of Pediatric Infectious Disease at the Boston Medical Center, presented *Lessons Learned from Seven Years of Immunization with PCV7 in Massachusetts Children.* He described a six-year study that attempted to identify demographic features associated with invasive pneumococcal disease in children <18 years in the PCV7 era and direct and indirect effects of the vaccine. This was followed by a discussion of *21st Century Imperatives for 21st Century Threats* by Mary Gilchrist, PhD, Director, Bureau of Laboratory Sciences and Alfred DeMaria, Jr., M.D., Director, Bureau of Communicable Disease Control and State Epidemiologist, both from the William A. Hinton State Laboratory Institute, MDPH.

Hospital Response to Chemical Emergencies

This program was designed for healthcare professionals such as emergency room staff, emergency preparedness coordinators and laboratory staff who may provide patient care or services during a public health emergency, and was presented at four different locations in Massachusetts. On June 25, 2008 in Worcester, MA, June 30, 2008 in Canton, MA, September 23, 2008 in Winchester, MA and on November 18, 2008 in Boston. The program gave a comprehensive overview of response roles during a suspected chemical exposure event and included such topics as a review the agents of chemical terrorism, appropriate specimen collection protocols and shipping procedures for blood and urine samples, "chain-of-custody" requirements, etc. A reference manual was provided for all participants.

Faculty included CPT David DiGregorio, PA-C, MPAS Physician Assistant, 1st Civil Support Team, Massachusetts National Guard; Michael A. Feeney, RPh, JD, CHO, Emergency Response/Indoor Air Quality Program; and Jennifer Jenner, PhD, Coordinator, Chemical Terrorism Response Laboratory, SLI, MDPH.

The programs were sponsored by the Massachusetts Department of Public Health, MDPH, and the Northeast Branch.

NEB Scholarships for Undergraduate Educators

The NEB has always been enthusiastic in their support for educational activities relating to microbiology. This spring, the tradition was continued by offering to support a number of secondary or undergraduate educators to attend the 15th Annual Conference for Undergraduate Educators" which was held at Endicott College Beverly, MA prior to the June General Meeting

of the ASM in Boston. The Branch provided up to $500 per person to partially defray expenses associated with meeting attendance. Educators were asked to send a brief description of their involvement in microbiology education and how they would benefit by attending this meeting.

The six recipients of the scholarships were Gail Begley, Northeastern University; Ellen Fynan, Worcester State College; Anne Hanson, University of Maine; Amy Sprenkle, Salem State College; Paulette Howarth, Bristol Community College and Gregory Reppucci, North Shore Community College.

Science Fair Winners

The NEB annually donates an award of $100 to each of five MA regional fairs and the VT science fair, and $200 to the MA Science Fair. Following are some of this year's winners of the NEB awards and their projects. Congratulations again to the students for their outstanding work. We would like to thank Council members Greg Reppucci, Paulette Howarth, and other NEB members for volunteering to judge at these fairs.

Recipients of this year's science fair awards were:

Region 2: Worcester Regional Science and Engineering Fair: *Bitter is Better: The Medicinal Properties of Chicory* by Clara Wool and Tracy Snyder from the Bromfield School, Harvard, MA.

Region 3: Bristol Community College-Rensselaer Polytechnic Institute Regional Science Fair: *Can Essential Oils Inhibit Bacteria From Developing a Resistance to Antibacterial Agents?* Erin E. Caron, grade 12, age 18, Taunton High School, Taunton, MA.

Region 5: The South Shore Regional Science Fair had two winners: Marco Catipovic, grade 10, age 16, of Falmouth Academy, Woods Hole, MA with *Selection for Tolerance to Low pH in Azotobacter chromococcum,* and Tenley McKee, grade 9, age 15, from North Quincy High School, Quincy, MA with *E. coli Inhibition with Juice*.

We were unable to obtain the names of the winners at the Region 4 Somerville Science Fair, Region 6 Boston Regional Science Fair and the VT State Science and Mathematics Fair.

Deceased Members:
Joseph J. Cooney, Past President, Northeast Branch

Joseph J. Cooney died in a Boston Hospital after a brief 17 February 2008, age 74. Joe was an internationally respected researcher in environmental microbiology and in bioremediation of pollutants.

Cooney was a Fellow of the academy of Microbiology and served as President of the Ohio Branch of ASM from 1971-1973 and as President of the Northeast Branch from 1991-1992. He was an ASM National Lecturer in 1999-2001. He served on the Editorial Board of *Applied and Environmental Microbiology.* He was also very active in the Society for Industrial Microbiology (SIM). He retired as Editor-in-Chief of *Journal of Industrial Microbiology and Biotechnology* in July 2007 after more than 13 years. Cooney played in many other roles within the SIM, including President 1992 to 1993, and 3/16 of the Selman A. Waksman Outstanding Educator Award in 1998 and the Charles A. Porter Award in 2000. He was elected to Fellowship in the American Association for the Advancement of Science and was Fulbright Scholar in Ireland in 1989.

Recently, Cooney retired to the position of Emeritus Professor at UMass-Boston, to a horizontal house near Plymouth, MA, overlooking a pond more suitable in size for swimming than for sailing. His numerous colleagues, friends, and family will miss his warmth, his humor, his wisdom and his advice.

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Boston Light

NORTHEAST BRANCH
AMERICAN SOCIETY FOR MICROBIOLOGY

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Has your membership expired? ↑