



Northeast Branch Newsletter

Number 133

Summer 2010

2010 Programs in Review

Pandemic H1N1 Influenza Virus

The first NEB dinner-meeting of 2010 was held on February 23, 2010 at Vinny Testas's of Boston, in Dedham, MA. Edward Balkovic, PhD, Principle Scientist for Microbiology Technical Support at Genzyme Corporation in Framingham, MA spoke on the *Current Status and Understanding of the New Pandemic H1N1 Influenza Virus* and discussed the current pandemic H1N1 virus in humans.

In March of 2009, a new influenza virus (pandemic H1N1 "swine" virus) began circulating in Mexico and from there spread rapidly around the world. Dr. Balkovic gave an overview of the flu virus and gave a history of past flu pandemics, of how the virus changed over time and of H1N1 in humans. He also described what is available for treatment and prevention, and gave possibilities as to what changes can occur to the virus in the future.

Dr. Balkovic showed an electron photomicrograph of the influenza virus, pointing out the outer membrane proteins which both play a key role in infectivity and virulence, and can immunologically protect against reinfection. This envelope virus is somewhat unique in that its genome is made up of eight separate RNA segments, each segment coding for at least one of the viral proteins. Thus when we speak of recombinant proteins, we actually mean a reassortment of the proteins, not a recombination. New viruses originate when multiple viruses get into the same cell, all their RNA segments mix, and a number of new viruses originate that have a different mix of the various genetic segments. The outer membrane surface contains hemagglutinin and neuraminidase, with hemagglutinin being the more important protein and exceeding by far the amount of neuraminidase on the outer surface.

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Edward Balkovic, Ph.D.

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**NORTHEAST BRANCH-ASM OFFICERS
and STANDING COMMITTEE CHAIRS**
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NEB Council Meetings

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 Meeting is scheduled for September 22, 2010.

Membership Notes

Dues reminders will be sent in late 2010 by e-mail. Members who did not provide an e-mail address will receive correspondence by postal service. 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 dues to the Treasurer. Emeritus members need to reply if they wish to remain on the mailing list. Changes only may be e-mailed to: NEBranch-ASM@comcast.net. Please check mailing labels on postal correspondence 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 224 members.

Visit the NEB Web Site!!

The NEB has a home page on the World Wide Web where all current events and the latest, as well as past issues of our Newsletter are available. ASM also has a Branch Meetings page. Visit us via the ASM Home Page or directly at:
<http://www.asm.org/branch/brNoE/index.shtml>

2010-11 Council Elections

Congratulations to the following NEB members whose terms began July 2010. James Kirby, MD was re-elected President (one year), and Patricia Kludt was elected Treasurer (three-year term).

FUTURE PROGRAMS

Local Programs:

Announcements of Local Meetings are posted on our website at:
<http://www.asm.org/branch/brNoE/index.shtml>

September 30, 2010

What Does Patient Safety have to do with the Laboratory?

Speaker: Nancy L. Manchester, C.S.H.A., L.H.C., Director of Regulatory Readiness, South Coast Hospital Group.

Location: Dinner-Meeting at the Johnson & Wales Inn, Seekonk, MA

Sponsored by: Northeast Branch-ASM and the American Society for Clinical Laboratory Science, Central New England (ASCLS:CNE).

October 21, 2010

Bioweapons, Emerging Infectious Diseases and the Role of the National Emerging Infectious Disease Laboratories (NEIDL)

Speaker: Robert B. Corley, Ph.D., Associate Provost for Research, Associate Director, National Emerging Infectious Diseases Laboratories

Location: Dinner-Meeting at Sagra Ristorante, Dedham, MA.

Sponsored by: The Northeast Section, American Society for Clinical Chemistry and the Northeast Branch-ASM.

November 3, 2010

Preventing Healthcare-Associated Infections: Whose Problem Is It?

Audience: This program is designed for microbiology supervisors, infection preventionists, physicians and epidemiologists. It is designed to address the roles of clinical practitioners, laboratory personnel and government entities in tracking and preventing healthcare-associated infections.

Location: Half-day program at the Cranwell Resort Spa and Golf Club, Lenox, MA

Sponsored by: The Massachusetts Department of Public Health, Bureau of Infectious Disease Prevention, Response and Services, Division of Epidemiology & Immunization and the Northeast Branch-ASM

Contacts for Local Programs: Irene George at NEBranch-ASM@comcast.net or Gail Begley at g.begley@neu.edu

November 9-10, 2010

45th Annual Region I Meeting of the Eastern New York, Northeast, Connecticut Valley and New York City Branches of the American Society for Microbiology.

Hosted by the Eastern New York Branch.

Location: Albany Marriott, 189 Wolf Road, NY. Additional Information pending

National Meetings:

September 12-15, 2010

50th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). Boston, MA. See: www.icaac.org

May 21-24, 2010

111th ASM General Meeting, New Orleans, LA. See: generalmeeting@asmusa.org.

For additional information on ASM Meetings and Conferences please contact: (Tel) 202-942-9248, meetingsinfo@asmusa.org

Pandemic H1N1 Influenza (Continued)

Hemagglutinin allows viral attachment to cells and antibody against it confers immunity. Neuraminidase is a unique protein, but plays a less important role. It is actually an enzyme that dissolves sialic (neuraminic) acid which most cells have on their surface, and which is part of the virus receptor. We may wonder why the virus has a protein that dissolves its own receptors. Host mucous contains a large amount of sialic acid, so when the virus gets into the respiratory tract, neuraminidase almost "eats" its way through the mucus to liquefy it, and allows the virus to attach to lung cells. Also, as the virus buds out of the cells, the progeny have their own sialic acid on them, so theoretically the virus will start clumping together. The neuraminidase allows for the cleavage of the sialic acid off the virions so the virus particles separate and spread more easily throughout the body. There are currently about 15-16 viral hemagglutinins in nature; only H1, H2 and H3 have produced epidemics in humans. There are certain viruses prevalent in pigs and horses, but the reservoir for these hemagglutinins, and thus

Pandemic H1N1 Influenza (Continued)

for all flu viruses, is primarily the wild avian species. The viruses don't cause illness in the birds because they have co-existed for a long time. Virus spreads both through their respiratory and digestive tracts into the feces, getting into water and spreading to water fowl.

Dr. Balkovic then described human flu outbreaks that occurred over the last century. The original flu we worried about was Spanish flu, the 1918 H1 pandemic. The identity of the causative virus was unknown then as the flu virus itself was not isolated until 1933. Serologic studies were able to link viruses isolated in the 1930's (now known to be H1N1) from swine and humans to the 1918 pandemic as the 1918 sera were able to neutralize virus isolated from pigs and humans.

The original H1 hemagglutinin from the 1918-1919 Spanish flu was prevalent through 1957. A new virus, H2, appeared in 1957-1967, H2N2 virus-Asian Flu. This was prevalent for about 10 years and then we saw the appearance of H3, Hong Kong-like flu in 1968; this is still prevalent and causing yearly outbreaks. The Russian flu virus was seen in 1977, when H1 virus reappeared. This was hard to understand, because there was abundant immunity in the population from when H1 was previously present. It closely resembled the 1957 virus genetically and was originally called Russian flu. Reexamination of some of the studies showed that the virus probably originated in China. It was believed that the Chinese were experimenting with live virus vaccines at that time and that this was actually a vaccine virus that maintained enough virulence to start spreading in the population.

The seasonal outbreaks that we have been experiencing until recently have been a mixture of either H1 or H3 influenza A viruses. There is an influenza B virus that has only one hemagglutinin type and tends primarily to cause disease in children.

Looking at the last century, we know new viruses always appear, and when they enter a community where there is no immunity to a particular hemagglutinin, disease appears. The question now is when the next major hemagglutinin change may occur and present a

What Not to Do to Stay Healthy!

(Balkovic)



“new” virus, for which no immunity exists in the population and result in a pandemic.

When a new virus appears it is called “antigenic shift”. Major changes occur, for example, when both a human virus and bird virus infect a pig at the same time, and then recombine. After genetic reassortment in the cells, we now have a virus with new hemagglutinin and new neuraminidase that can replicate in humans; there is little immunity and an epidemic can occur. Historically, such genetic reassortment occurred in the Far East, especially in China where large number of people and animals such as pigs and chickens live in close proximity.

However viruses just don't come and go - they stay around for a long time explained Dr. Balkovic. The H3 virus has been around since 1968. This happens because the flu virus not only has the ability to make these major shifts (reassortments), but it also mutates. The flu hemagglutinin can not only change, it can also “drift”; antigenic “drift” is an accumulation of small changes (such as point mutations) over time. Therefore if you're infected with flu one year, and build antibodies against that strain, you may be immune to that strain the following year. The third-year, the virus changes a bit, and you still may have some protective antibodies. The virus can pick up point mutations in the hemagglutinin molecule in the non-binding regions and not change its binding site, but when the antibody binding sites change, you eventually start losing antibody (immunity) to

Pandemic H1N1 Influenza (Continued)

that virus. Both hemagglutinin and neuraminidase can accumulate changes so that someone immune to the original strain is not immune to the drifted one, and we have sporadic outbreaks.

The Hong Kong flu (Influenza A H3N2) which occurred from 1968-1996 drifted and had twenty point mutations during that time. With each change a person can be reinfected; five-ten years of changes can lead from an H1N1 to H3N2 flu virus. When the virus runs out of variants, a new virus appears. The virus can be prevalent for a number of years and then slowly drift away, so you may not get the flu for several years. After three or four years there have been sufficient genetic changes that the resulting strain is no longer recognizable by your reactive neutralizing antibodies, hence you can get reinfected, or you may be reinfected and not be as ill, but you can still propagate the virus through the community. The question the scientific community asks is “how much change can there be?” The H1 virus stayed for about twenty years, the H2 for about 10 years, but H3 has been with us since 1968. The thought that the entire population would soon become immune due to a limited number of mutations proved to be incorrect.

We worry about flu pandemics because we constantly fear a 1918-like recurrence. Such a virus is likely come out of the avian pool, H5, H7, H9, H10, to which the entire world’s population is immunologically naive and would have no protective antibody. Dr. Balkovic showed a map indicating the spread of the 1918 flu across America, which took only a couple of months. There was poor transportation, medical knowledge was totally different then, and despite this it spread by coughing, sneezing and aerosols. Compare that with West Nile virus which is spread by mosquitoes and took three-five years to cross from the east to the west coast of the USA. In the U.S. in 1918 there were 20 million cases with a half million fatalities (the US had a population of 100 million at that time); 20-40 million people were estimated to have died worldwide. Half of the world’s population, about 1 billion people, was ill. The other thing that was unique about the 1918 strain is that typically in influenza

pandemics, deaths occurred among the very young and very old; but here there was also a large peak in young healthy adults. Centers for Disease Control scientists were actually able to reconstitute the 1918 virus from frozen samples and reconstruct it genetically. Experiments using infected animals showed that when the virus gets into the cells it replicates in the macrophages and for some unknown reason causes a release of cytokines. Death then is due to a massive cytokine storm, which then causes a huge inflammatory response in the lungs; it’s almost an immunologic death. (NEJM 2005, 252: 1839-1842).

Twenty million people died from H1N1 in 1918, and about 1 million died from the Asian flu (H2N2); both hemagglutinin and neuraminidase had changed. There were about 800,000 excess deaths from the Hong Kong flu (H3N2); some people were protected because protective neuraminidase antibody still remained in the population. There was little mortality with the Russian (H1N1) flu in 1977; primarily people under 20 years old became ill because older people had been previously exposed. Again, we worry about these new pandemics because today a virus can spread worldwide very quickly. We have a much larger population with increased population density, along with a huge difference in the speed of travel. In 1918 travel was primarily by steamship, and we had crowded troop ships during the war. Today you can get on a plane while incubating a disease and fly across the country or even the world and disembark before becoming ill.

“What’s next?” asked Dr. Balkovic. Some people believe drifts in H1 and H3 can go only change so far, therefore reassortment to form a new virus is to be expected, as had occurred in the late 90’s. What also occurred in the late 90s were human cases of viruses with other hemagglutinins present, H5, H7 H9, primarily from the avian species; the great fear in the last couple of years was, and still is, that an analogous bird flu or avian flu will occur again. The belief was that there already has been a circulation of the H5N1 avian virus, however, although it had the ability to infect people, it was not highly infectious and did not spread well. Most of the cases and deaths occurred in people that had very close contact with birds,

Pandemic H1N1 Influenza (Continued)

mostly in the Far East. Human to human spread occurred only among very close contacts; such as a brother, sister, spouse of an ill person. The virus might spread if large doses were present in secretions. The big fear was that there might be a reassortment of H5 that would readily multiply in human cells. That wasn't the case this year with H1N1; if we had a virus that could adapt, we would now have a virus with a different hemagglutinin to which no one had been exposed, and we would have had a high mortality rate. We always think we know what's going to happen, but as microbiologists know, the microbes are smarter than we are.

The H1N1 swine flu outbreak in Mexico appeared in middle-late March 2009. No one had expected this virus and some people had crossed the US border to participate in flu kit clinical trials (MMWR 2009, 6/5/09). The virus was isolated, but could not be identified; a sample was sent to the California state laboratory for forwarding to the Centers for Disease Control (CDC). Recalling how easily a virus can spread, although this epidemic started in March, by May it was seen worldwide (according to World Health Organization statistics). We then went from worrying about an avian flu pandemic to wondering if it would be called swine flu, Mexican flu or pandemic H1N1! WHO categorizes pandemic flu by stages; we were at about stage 2 with the H5N1 avian flu which indicates there are no human cases, whereas with the onset of the H1N1 swine flu we were suddenly in stage 6, where the disease is efficiently spreading and circulating throughout the world.

The flu virus generally follows the weather patterns and tends to spread in the wintertime in whatever part of the world winter is occurring. It appears during our winter (December-March) and migrates into the Southern Hemisphere during our summer. Dr. Balkovic then explained three CDC graphs. One graph (US Outpatient Influenza-like Illness Surveillance Network) showing overall influenza-like illness in the US in 2007-2009 indicates that peaks of illness historically tend to occur between December-February. At the end of 2008 and toward the beginning of 2009 illness peaked

slowly; this was the initial H1N1 flu that started in Mexico. An unusual feature was that the peak occurred during spring (April-June), and had dropped off in the middle of the year. A large peak of illness occurred in late fall-early winter of 2009, and was burning itself out by January, before the usual peak influenza season. Dr. Balkovic indicated that flu is very efficiently spread by students in schools, who then bring it home during vacations. Another CDC graph showed pneumonia and influenza mortality in 122 U.S. cities. The last big peak in deaths occurred in 2007-2008 due to H3N2 Influenza A virus, with not much in 2008-09. Although the disease incidence was quite high, the amount of mortality from pneumonia and influenza was low because most of these cases tended to be in younger people and many older people were immune. A third CDC graph showed influenza-associated pediatric deaths. In 2008-09 there were 132 deaths, while 2009-10 had 260 deaths.

Since this new H1 virus was circulating in swine, it was called swine virus, but where did it originate? The fear again was that a human virus had gotten together with an avian virus in swine and had undergone reassortment, to emerge as a 1918-like H1N1 virus. Hence, it was assumed that it was going to cause 1918-like disease. But when the virus was studied, it was in reality found to be a swine virus that was just now transferring back into people. Prior to the 1918 pandemic, veterinarians had never seen swine flu, but afterwards it began to circulate in pigs. Therefore the belief is that the original 1918 pandemic flu strain entered pigs and began circulating in them sometime after 1918 (1920's to 1930's); the 2009 virus is actually an old virus and not a new reassortment!

Genetic studies show this virus is really a reassortment of four different viruses. It is a mixture of some genes from the classic H1N1 swine plus some genes from a recent influenza H3N2 virus, some internal genes of avian virus, and some from a Eurasian swine flu virus that's a little different than the US swine flu. Therefore the current human H1N1 viruses are a genetic mix from four different viral lineages. Since millions of pigs are raised worldwide, we annually have a large new susceptible population in which such reassortment can occur.

Pandemic H1N1 Influenza (Continued)

The Centers for Disease Control estimated that about 50 million people, 17% of the U.S. population, were infected with the new pandemic H1N1 in 2009 through December 2010. A recently published study from the Pittsburgh Children's Hospital presented data in which a large number serum samples were tested for antibodies against the new flu virus. Extrapolating these results nationally, it is estimated that about 63 million people, or approximately 1/5 of the US population, were infected with H1N1. Twenty-nine percent were children less than 9 years; forty-five percent were age 10 to 19; but only about 5% were in seniors. About 50% people in their 70s and 80s demonstrated antibodies to both 1957 and 1918 viruses, probably as a result of being infected with the flu (or related influenza viruses) repeatedly during their lifetime. Therefore older people may now be protected, or may develop a less serious case of the flu due to this boosted immunity, while people born before 1957 are susceptible. A recent study in the United Kingdom showed similar results, that this flu is really a disease of young people similar to what they saw in 1977 with the Russian flu.

Dr. Balkovic then looked towards the future and what we might expect with this pandemic virus regarding treatment and prevention. Antivirals and vaccines were not available in 1918 therefore we are much better off now. In 2010-2011 the main treatment is still using an antiviral that is a neuraminidase analog. This is a molecule (in Tamiflu and Relenza) that mimics the substrate that is used by the neuraminidase molecule; it clogs the reactive site and neuraminidase can't function. This results in the virus being less effective in chewing through the mucus; it can't get rid of its own sialic acid and tends to stick together. The immune system can take over to control the infection. Resistance to the drugs however, is appearing. Most of the flu viruses today are resistant to Amantadine, a good antiviral previously used for Influenza A. Therefore our only current treatment is the neuraminidase substrate found in Tamiflu and Relenza.

We also have the viral vaccines. (It's little known that Jonas Salk, known for his polio

vaccine, also played key role in developing inactivated influenza vaccine.) Approximately 9-months before the start of each flu season, an advisory committee is convened at the Food and Drug Administration to decide which strains should be included in the influenza vaccine for the upcoming year. The typical seasonal vaccine is trivalent. The 2009-10 vaccine contained two old viruses from 2008-09 vaccine, A/Brisbane/59/2007 H1N1-like virus and A/Brisbane/10/2007 H3N2-like virus, and a new B/Brisbane/60/2008-like virus.

Vaccine strains are picked by looking at which strains are circulating in the southern hemisphere (when our winter is ending, winter is beginning in the southern hemisphere). A vaccine is made that is the closest antigenic match to the virus circulating in the southern hemisphere during their winter and that is expected to be prevalent in the northern hemisphere during our winter. H1N1 appeared after the companies were well into their production of the 2009 seasonal vaccine. They had to finish seasonal vaccine production then rush into producing H1N1 vaccine using the InfluenzaA/California/07/2007(H1N1)-like virus.

Dr. Balkovic showed the vaccine manufacturing timeline that was used at Sanofi Pasteur in 2004 and described the process used at Connaught. Influenza vaccine is still made in embryonated eggs; about 100,000 eggs are used daily, seven days a week, for about five-six months. A virus which has been cultivated in specific cell lines in the laboratory will not reproduce well when grown in eggs, resulting in low hemagglutinin titers such as 1:8-1:32, and is not a good candidate for vaccine production. The virus must therefore be engineered to grow in eggs, which is done employing a high-yield reassortment process. A virus well adapted to growing in eggs (an old PR8 virus from 1933) and the new clinical isolate are grown together in eggs, and then screened for reassortment. We now have the hemagglutinin and neuraminidase of the new virus with good growth in eggs due to the old virus.

The FDA Advisory Committee met on February 22, 2010 and selected the new strains for 2010-11 vaccines. They replaced the old H1A/Brisbane virus with the new pandemic

Pandemic H1N1 Influenza (Continued)

virus H1N1 Influenza A/California/07/2009. They also picked a new H3N2 virus from Perth, Australia, and will keep the old B/Brisbane/62/2008-like virus. The best match for next year's virus is sought after, but the virus circulating next year might be a bit different from those in the vaccine. The more difference there will be, the less protection you will receive from the vaccine; that's why no vaccine is 100% effective!

Dr. Balkovic then described the flu vaccine manufacturing timeline, which is a nearly a year-long production. Generally manufacturers like see at least one vaccine strain picked by January so that the companies can begin work. They might make bulk monovalent vaccines from January-June, prepare formulated vaccine (mixtures of monovalent bulk vaccines) from June-July, and fill and ship vials countrywide/worldwide from July-August; so that vaccination can occur in September-October. In July-December they also will manufacture diphtheria, tetanus, pertussis, yellow fever, and other vaccines.

The current H1N1 swine flu wasn't prevalent until March, the virus was isolated in April, and millions of doses of vaccine were available that season; it's amazing that that was done in embryonated eggs. There was concern as to whether the limited number of eggs needed to produce both seasonal and pandemic vaccine would be available. Fortunately H1N1 came after seasonal production stopped and egg production was continued. There was a bigger problem when the avian flu circulated because it also killed eggs; reverse genetic engineering was used to produce a virus that could grow in eggs and not kill them. Scientists were also working on new vaccines using animal cell culture, insect cell culture, etc. to produce live virus, as well as genetic engineering. A problem with current cell-culture derived vaccines is obtaining a high yields; eggs are always better. Recombinant work is being done with hemagglutinin where high yields can be obtained, such as with insect cell lines. The ideal, said Dr. Balkovic, would be to put a virus into a cell culture, and if there was a pandemic and a low supply of eggs, cell lines could be

employed readily for bulk vaccine production at any company working with cell cultures.

Whole virus gives a good immune response and is used for adults (inactivated whole virus vaccine), but a detergent-disrupted or split product vaccine, where the envelope is disrupted is used for children; it has less immunogenicity but results in fewer side reactions. The problem of lower immunogenicity with split products (subunits) is being addressed by testing adjuvants. However, such products are new and safety data is lacking.

Dr. Balkovic then described a number of possible scenarios for the H1N1 virus next season. The best scenario would be that it looks like the Russian flu. Many people were infected this year, and if it circulates again, there will be a high level of immunity in both the young survivors and the older people. The worst scenario is that it would resemble the 1918 flu. In 1918, the first wave had a high rate of infection but relatively few deaths, as we saw in 2009-10. The 2nd wave in 1918 in humans then had a mutated virus that produced a cytokine response and high mortality. H1N1 virus in 2009-10 has infected many people, and we have six additional months of genetic mixing that can occur to produce a new and more lethal H1N1 virus. Numerous other viruses are circulating worldwide right now also that can enter the scene next year. There some H3N2 viruses, old H1N1 viruses, a pandemic swine H1N1 virus, and some human avian flu cases remaining in some parts of the world. Fortunately these can be tracked due to better surveillance today than in 1918. Another scenario is that the swine virus (or any seasonal H1N1 or H3N2 virus) that spreads well in people but doesn't kill many could combine in a person with the highly lethal H5N1 avian virus that doesn't spread very well. This could potentially produce many new lethal H5 viruses that are highly transmissible and to which people worldwide don't have immunity...and this can look like 1918 again.

We always worry about pandemics, but simply looking at the annual impact of typical seasonal influenza shows how devastating it can be. Nationwide there are about 36,000 deaths, compared with 43,000 auto deaths and about 16,000 murders. There are about 200,000 people

Pandemic H1N1 Influenza (Continued)

hospitalizations and 17-50 million ill people. Massachusetts has about 800 deaths annually with about 3000 hospitalized.

There is the famous quote by Edgar Marcuse, MD. Chairman of the National Vaccine Advisory Committee, 1994-1994: “The pandemic clock is always ticking; we just never know what time it is”. People were worried about H3 and H5, but no one predicted H1N1 would appear, and we just don’t know what is going to happen next.

To receive a copy of this presentation please e-mail ed.balkovic @genzyme.com. Dr. Balkovic also mentioned a book of interest written by Rebecca Skloot, which is currently on the best-seller list. *The Immortal Life of Rebecca Lacks*” describes the life of the African-American woman whose cancer cells were turned into the HeLa cell line that is used in cell culture worldwide.

Career Development Day - Boston Area Student Chapter

The Boston Area Chapter of ASM sponsored its first Annual Career Development Day on Tuesday, March 23, 2010 at the Tufts University Boston Campus. The program included dinner and a career panel discussion. Katie Price, a graduate student at Tufts and current President of the Boston Student Chapter welcomed approximately fifty graduate and post-doctorate students and a group of undergraduates from the University of New Hampshire Student Chapter.

Panelists were Shann Kerner, a partner at the Wilmer Hale law firm, specializing in intellectual property; Gail Begley, professor at Northeastern University; Harvey George, Director of Laboratories at Callaway Labs, specializing in drug testing, David Holzman, Journal Highlights Editor for the ASM magazine *Microbe* and a regular contributor to many other scientific publications; and Julie Schwedock, manager of Microbiology Research & Development at Rapid Micro Biosystems, Inc.

Kathryn Lange, Associate Dean of the



(L to R) Moderator Kathryn Lange, Gail Begley, Harvey George, Shann Kerner, David Holzman, Julie Schwedock

Sackler School at Tufts acted as moderator, and asked the panelists to first introduce themselves, then state the degrees they hold and how they became interested in the area in which they work. The panelists all agreed that they did not go into their current job immediately after graduation but followed various paths/tracks, or explored other careers and then changed to a field in which they were interested.

When asked what factors determined their final choice of career, panelists replied that you should do what you are good at and what you enjoy doing, but always be alert to what comes your way. You might even be accidentally be recruited by a headhunter! The R&D scientist found a single research project boring because you didn’t see the outcome of the overall project. Family matters also influenced her career; both husband and wife decided to stay together in the same city, near family, and where there were lots of opportunities for employment. The journalist specialized for a while in one journal, but now has a broad base of writing and does various things regarding science and medicine. It was mentioned that looking for a job through the grapevine and friends is very important.

When the panelists were asked what a typical day is like in each of their fields, all replied: no day is really typical. The professor has lots of reading and writing, talking to students, preparing assignments, giving lectures, meeting with students and advising them regarding academic programs and post college potential,

Career Development Day (continued)

mentoring, preparing curriculum, supervising undergraduate writing and preparing posters and papers. In the laboratory, quality assurance and quality control are of utmost importance, and need to be reviewed daily; also interacting with the technologists and other staff members is necessary. An attorney might edit patent applications submitted by inventors, meet with management to see what inventions are worth patenting, counsel clients; work with clients, associates, and litigators; do depositions, license technologies etc. The job is quite varied. In writing, you would interview people - primarily by phone, research your writing, and do a lot of thinking. In a research laboratory it is usually an eight hour day, but there is homework. There are meetings with your supervisor and staff to review goals strategies and progress made; also meetings with the CEO and staff, and teleconferences with IP attorneys. You also oversee research associates and as time permits, work in the laboratory on experiments yourself.

The most/least favorite parts of each panelist's jobs were: reading, writing, talking about interesting things and student interaction are important for a college professor; the least favorite tasks are grading and faculty meetings. You can really make a difference and actually change student's lives! Filling out forms and preparing Power Point slides are dislikes in the laboratory. The attorney dislikes billing her time and discussing bills with clients and partners but enjoys seeing good patents accepted and the client doing well with his/her invention. In writing, dislikes are trying to contact people repeatedly and receiving no reply, while talking with people in the scientific fields and putting a good story together are very satisfying. Research is like solving a problem or a puzzle. The R&D scientist enjoys looking at data and analyzing it; enjoys troubleshooting problems and data, solving problems and writing reports. Repetition is boring and dislikes are time-consuming procedure manuals and quality control documents because they can be mind numbing and don't let you "think".

When asked how much independence each panelist has in their position, the professor replied that she likes to work independently and



(L to R) Stephanie Mitchell and Katie Price

not be told what to do daily. You should have control of your job and do it without having your supervisor or boss standing right over you. In law, clients tell you what to do, but an attorney must use "realistic creativity" and determine how to do the job. In writing, independence is variable; you have leeway with some magazines and with others you must adapt to their style. The research scientist dislikes being told how to carry out her experiments and having to plan someone else's work; she also dislikes being told what to do and telling someone else what to do. She prefers to be given a general project in which she can decide how to manage and problem solve.

In conclusion, Dr. Lange asked the panel to give the audience a few final words of advice. These final words of wisdom were: attending meetings and conferences, talking to people, and networking are important; and don't be afraid to make "cold" calls or send "cold" e-mails. Become active in an organization and be flexible in your outlook; look into areas outside of your primary interest. Don't fear additional education; have an open mind. Know yourself and what you like, but don't have preconceived notions. Be true to yourself - don't let someone mold you into what they want you to be!

This event was supported by the Northeast Branch. Gail Begley, Branch Councilor from Northeastern University, recruited the panelists. Assisting with the program were Stephanie Mitchell, a student member of the Boston Chapter and Alex Cowan from the Tuft's Dean's office.

One Health Initiative/ Emerging Infectious Disease

The fourth NEB program of the year was a dinner-lecture held on April 7, 2010 at Sky Restaurant in Norwood, MA. Stanley Maloy, PhD, ASM Branch Lectureships Program Speaker, and Dean, College of Sciences, Center for Microbial Sciences, San Diego State University in San Diego, CA, spoke on the *One Health Initiative/Emerging Infectious Disease*. The One Health Initiative is a national effort by many scientific, medical, and veterinary associations that emphasizes the interrelationship between human, animal, and environmental



(L to R) Stanley Maloy, PhD, ASMBL Speaker and James Kirby, President, Northeast Branch

health. The interrelationship is particularly clear with emerging infectious diseases: most emerging human diseases are acquired from animals, facilitated by a disruption of the natural environment of the animals. Dr. Maloy spoke of emerging infectious diseases, which was the focus that led to thinking about One Health, and their origin. He also emphasized zoonotic diseases, new environmental niches, and described work on an exotoxin gene reservoir. This was tied in with the One Health movement and addressed future challenges we will face.

Dr. Maloy showed a graph of infectious disease mortality in the US 1900-1980, and the drop in mortality that occurred over time. Changes in sanitation, particularly clean water supplies, made a huge impact and resulted in the largest drop in mortality. Vaccines then came

Mycology Workshop

Our third program of 2010, a one-day basic-intermediate mycology workshop on the “Molds and Yeast” was held at Bristol Community College in Fall River, MA on March 26, 2010 and was designed for individuals with a working knowledge of microbiology, but little or no mycology experience. The instructor was Linda Binns, MS, MT(ASCP), Pathology Technical Specialist, Mycology/Mycobacteriology, from Rhode Island Hospital, Providence, RI. Twenty-six workshop attendees participated in lectures, discussion and demonstrations with hands-on training.

The course included lectures, demonstrations, case studies and laboratory exercises related to fungi, including a limited discussion of related infections and disease states. Microscopic preparations were examined for characterization and identification. Topics covered were laboratory methods and safety procedures necessary for working with pathogenic fungi; old and newer techniques for the identification of yeast; the isolation and identification of dermatophytes and black moulds by conidiogenesis, and the isolation and identification of dimorphic and opportunistic fungi from clinical specimens.

The workshop was co-sponsored by the Northeast Branch, American Society for Microbiology, the William A. Hinton, State Laboratory Institute, Massachusetts Department of Public Health, and Bristol Community College. The course was facilitated by Garry R. Greer, BS, State Laboratory Training & Distance Learning Coordinator, W.A. Hinton State Laboratory Institute, Boston, MA, and Paulette Howarth, MS, CLS, Clinical Laboratory Science Department, Bristol Community College, Fall River, MA.

into play, followed by antibiotics. When the mortality became low some people thought it was time to close the book on infectious diseases and focus healthcare elsewhere because it was no longer of any concern. This led to the famous quote by the US Surgeon General,

One Health Initiative (Continued)

William Stuart in 1967: “Time to close the book on infectious diseases, declare the war against pestilence won and shift national resources to such chronic problems such as cancer and heart disease.” But he was wrong in many ways – we had more, not less emerging infectious diseases. This increase didn’t occur immediately, but it took a while. We had new and emerging diseases never anticipated and antibiotic resistance. The annual death rates due to infectious diseases doubled in the last two decades, something that again could not have been anticipated.

Worldwide, infectious diseases also remained the leading cause of death; there were no geographic boundaries. Another thing of which William Stewart was unaware was the important role of microbes in causing chronic human diseases. When he made that statement in 1967, one case of microbes clearly causing chronic human disease was *Streptococcus* and it was associated with rheumatic heart disease. That alone should have led him to realize that there are other chronic disease associations with microbes. We have seen numerous outbreaks of infectious diseases since then. Dr. Maloy showed a global map assembled in 2004 by a group at the National Institutes of Health, and a key impressive observation was the number of infectious diseases caused by parasites, viruses and bacteria, and their distribution worldwide. This therefore has become a global problem; and it wasn’t something people were widely aware of in the mid-1960s.

Where are these emerging infectious diseases coming from? Why weren’t they on the radar screen in the 60s? If one looks at human infections said Dr. Maloy, less than one hundred pathogens are human specific. Many other cases of human disease are caused by organisms that are otherwise commensals or organisms that exist in nature and the environment without causing problems for humans. Many types of *Pseudomonas* are fundamentally environmental bacteria; *Staphylococcus* can fundamentally be thought of as a commensal organism. When you look at the origin of these emerging and reemerging diseases more than 60% come from animals and are caused by zoonotic pathogens.



Fourth from L, Stanley Maloy, PhD;
Third from L, Professor Patricia Overdeep
with students from Johnson & Wales University

One good example of a disease that came from a wildlife reservoir to domestic animals to humans is the Nipah virus. A relationship was recently shown between human disease and the Malaysian fruit bat, which carries the virus. These fruit bats are normally infected in nature and don't cause huge problems for humans. In Malaysia however, pig farms started to be built in areas where there were substantial human populations. Pigs are fond of fruit; therefore fruit trees were grown next to the pig farms. The bats would come into the area also, partake of the fruit, and in the process infect the pigs through their droppings, and the virus is ultimately transmitted to humans.

The impressive thing about these emerging diseases is that the rate is increasing over time said Dr. Maloy. He showed a slide of some of these diseases based upon information from the Centers for Disease Control and derived from material assembled by Lonnie King who was the founding director of the National Center for Zoonotic, Vector-Borne and Enteric Disease (NCZVED) which is a new Center at the Centers for Disease Control. The slide was based upon the idea of the relationship between animal diseases and human diseases. In 1993 there was Hanta virus, the Four Corners outbreak associated with rodents; plague in India and Africa occurred in 1994 and there was concern about it becoming a pandemic transmitted by rats; in 1995 there was Ebola in apes and chimpanzees; in 1996 there was variant CJD-disease, presumably related to mad cow disease; in 1997 H5N1 influenza from fowl; in

One Health Initiative (Continued)

1998 there was Nipah from bats. The diseases can be followed to the 2009 H1N1 influenza outbreak! The list shows a continuous animal association with diseases that seem to be springing up out of nowhere-who knows what the next one on the list will be.

The important point here, said Dr. Maloy, is that in 1967 we didn't see such diseases as have become prevalent only over the last 20 years. You may ask why this is increasing and why are we seeing it now versus a few decades ago. To a very large extent many of these diseases are associated with human impacts upon environment, Dr. Maloy explained. One example of environmental disruption is Lyme disease, attributed to placing human populations in formerly wild areas where the disease has been endemic in deer and deer mice. Changes in human technology using water cooling towers allowed infections with *Legionella*. Changes in human demographics are associated with a number of diseases such as Nipah virus, which in turn is associated with the human demographics of how farms were built. Dengue fever is another example; it is spread by mosquitoes that don't travel very far, but humans have built an infrastructure that allows the mosquitoes to breed in successively more populated areas. Increased travel clearly helped spread SARS rapidly. Changes in agriculture and food distribution are strongly associated with *Salmonella* and *E. coli* 0157:H7 outbreaks.

There are many examples of the disruption of public health. One of importance in Africa currently involves rabies. It is estimated that to control the disease at least 20% of the animals need to be immunized, which is not happening there; many rabid dogs are left dying on the street and spread the disease. The rabies epidemic seems also to be correlated strongly with the AIDS epidemic. Climate change has an association with Chagas disease, a strong association with cholera transmission, and epidemics of plague as well. As you look at these types of cases, the striking thing is the interplay between environment, animals and humans. Disease can be transferred back and forth between humans and animals, with an environment providing a reservoir of these

diseases. Therefore environmental disruption greatly increases the potential for transmission.

If you place humans, the environment, and animals at the corners of a triangle: reservoirs of potential pathogens exist in the environment, disruption of the environment leads to the adaptation of microbes to those new changes, which leads to the evolution of new virulence traits. When you disrupt the environment it provides a mechanism for increased exposure of humans to animals and hence the transmission between animals and humans increases.

One of Dr. Maloy's favorite examples of such interaction is the impact of global warming on Chagas disease (caused by the parasite *Trypanosoma cruzii*), which is the largest cause of heart disease in humans in the world. There is an autoimmune response that affects the aorta and tissues around the heart which results in a serious chronic disease seen later in life. Chagas disease, called South American sleeping sickness, is common in South America, especially Brazil. It is transmitted by an insect vector that lives in wooden or thatched roofs and falls down at night to bite its victim, particularly on the face; hence it is called the "kissing bug". The disease was primarily restricted to warm tropical areas and not seen in North America except in travelers to those areas. The average temperature in Mexico has now increased and the disease is moving from Mexico to the border areas. San Diego now routinely tests for Chagas disease. Related to this, açai berry juice is currently being widely advertised as a potent antioxidant and is being currently imported into the United States for use for example, in smoothies. There have been recent cases in Brazil where oral consumption of the juice was clearly associated with disease. The bugs live in the açai leaves and fall into the bags when berries are gathered. They were then crushed with the berries, but the trypanosomes survived and were detected by molecular tests. Unfortunately we will not know if there will be an impact on heart disease in the US associated with these berries for another 20-30 years. Pasteurization, which would solve this problem, is not used for açai or for raw milk, which may be yet another public health problem. Smaller batches of berries could also be ground up before crushing for use and tested using a very

One Health Initiative (Continued)

sensitive molecular test.

Another example given by Dr. Maloy is the relationship between animal agriculture and Q fever, caused by *Coxiella burnetii* (Science, Jan 15, 2010). There was an increase in Q fever in the Netherlands annually from 2007 to 2009, and it was found again in goats this year. Disease occurs widely in domestic animals such as goats, sheep and cattle; there are abortions but no serious disease, and few die. The disease can be passed from animals to animal and animal to human by urine, feces, and particularly via the placenta after birthing, in which the organisms appear to be extremely robust and survive in the environment for a very long time. People who tend to raise large numbers of these animals tend to ignore Q fever because it's too expensive to do anything about. The disease can be spread to humans by aerosols when birthing and in this particular instance most cases occurred during the birthing season of goats. The veterinarians were aware of what was happening and the medical community was seeing cases of Q fever that had no connection with the veterinary community. Over the past year it became clear that there was a human disease problem associated with this and it resulted in the culling of large populations of goats throughout the Netherlands.

There are also other ways in which human changes in the environment can impact transmission of disease and induce new virulence traits. Many of the sources of recent *Salmonella* outbreaks were the classic reservoirs for the organism, including a wide variety of animal products, poultry, cheese, chocolate, pet turtles and pet treats. Over the last few decades however, changes occurred in the source of *Salmonella* infections, which now became associated with plant products. In the 70s there were alfalfa sprouts, bean sprouts, melons, marijuana, lettuce, onions, peppers, rice, black pepper, and peanut butter. In the sandwich shops, alfalfa sprouts were dipped in a Clorox solution, and the organisms which seemed to have come from the fertilizer used were only on the surface of the plant, and were killed. Recently it was discovered that there are alfalfa sprouts and other plants that even when treated

with Clorox will not eliminate the *Salmonella*, and there are currently numerous plants associated with salmonellosis, including marijuana. Why are we seeing this shift in disease and how the disease is transmitted? Previously, *Salmonella* was able to be eliminated by treating the surface of a plant, but now, it appears that both *E. coli* and *Salmonella typhimurium* seem to have now developed the ability to grow in and reproduce inside plants. Why would the organisms develop this ability? If you look at changes in agriculture and the pressure that is put on plants to grow, the most likely guess is that this is associated with modern agriculture techniques that put new environmental constraints on *E. coli* and *Salmonella*. There are many questions as to how the organisms grow inside plants and how fertilization and watering of plants becomes important. These problems seemed to have developed in Mexico where there are initially high infection rates and high contamination rates; in United States fields, less contamination is seen. Again, these are diseases associated with animals that are transmitted to humans because of changes in the environment.

Dr. Maloy then described a study done in collaboration with a colleague who studies disease in corals which surprisingly, identified a reservoir of virulence genes in nature that we never knew existed. This gene reservoir was discovered by the process of environmental metagenomics; in this case the metagenomics was done on any kind of virus, plant, animal and bacterial, which was isolated from seawater. It is known that seawater contains more than 10^6 viruses per mL. From the DNA sample that was isolated, clone banks from the entire mixture were constructed, and then the DNA sequence of those clones (the entire DNA sample from the environment) was determined. Many viral sequences were found, but that is not surprising; the thing that was really amazing was that a very high hit frequency of exotoxin genes was found. The question then was why many exotoxin genes were found in the marine environment? Exotoxin genes are commonly carried on bacterial viruses or phage. For example *Vibrio cholera*, carries the lysogenic CTXf bacteriophage phage which determines whether the organism will produce cholera toxin

One Health Initiative (Continued)

and produce the disease; botulinum toxin has a few phages and there are different forms; *C. diphtheriae* bacteriophage carries the Tox gene, *Streptococcus pyogenes*, has a toxin associated with scarlet fever, and *E. coli* has the shiga-like STX toxins. All of these exotoxin genes are carried on bacterial viruses and are transmitted between the host bacteria on those viruses. Therefore if we look at viruses, perhaps it isn't surprising that exotoxin genes are found in nature. The group then looked for such genes in other environments in addition to the marine environment, such as in the natural environment. Environmental samples were collected from around the San Diego region; these consisted of water, soil and sediment all over town, from schoolyards, and other presumably pristine areas. Both bacteria and phages were isolated by size fractionation, total DNA was extracted, and using those DNA samples, dot blot, DNA hybridization, and PCR using probes for exotoxin genes were done. If a particular DNA carries an exotoxin gene it can be identified by this particular approach. Many of the places around town had hits with exotoxin genes. The group looked for Shiga toxin, staphylococcal toxin A, diphtheria toxin and cholera toxin. There have been no cases of cholera in San Diego for a long time but a surprisingly high number of samples were positive for cholera toxin genes. We normally think of cholera toxin as being associated with the bacterium *Vibrio cholera* or one of its close relatives and diphtheria toxin associated with *Corynebacterium diphtheriae* or one of its close relatives. The samples that were tested however, did not have the cognate bacteria. Normally phages need to grow inside some type of cell in order to survive and if bacterial viruses presumably grow in some type of bacteria, then what type of bacterium was the phage growing in? Other questions were whether this is a source of phage encoded exotoxin gene, or was it a new virus in the phage or the same virus as in its pathogenic host?

The bacteria from the environmental samples were then grown on agar plates, and replicas of the plates made in order to perform hybridization experiments with exotoxin probes

to see if an exotoxin gene was present. To identify the bacterium, 16S ribosomal sequencing was done. When looking at staphylococcal enterotoxin type a, the exotoxin gene was present in a psychrophilic *Pseudomonas*, which did not grow at 37°C and was clearly an environmental isolate. *Staphylococcus* is gram-positive, and *Pseudomonas* is gram-negative, but it appears that the toxin normally associated with gram-positive bacteria is in a gram-negative bacterium in nature. While the organism is not likely to cause human disease or grow at 37°C, it still remains a reservoir containing an exotoxin gene; and this is only one of a number of virulence genes found. We don't know in many cases what the gene does in a pathogen such as *Staphylococcus*. This indicates that there is presumably a way of transfer between widely different bacteria; much additional work needs to be done in this area.

These experiments revealed to the researchers that there are mechanisms for transfer between widely different bacteria in nature. The conclusion is that there seems to be a gene pool of exotoxin genes in nature that are located on natural viruses that can infect environmental bacteria but presumably upon release from that phage, may infect organisms that cause animal disease or human disease. This is a natural reservoir, an actual pool of virulence genes that serves as raw material for the evolution of new types of pathogens. The environmental organisms, some of which are not normally associated with human disease, serve as a population of potential pathogens. These organisms can provide the raw materials for the evolution of new diseases and it's likely that environmental change in a particular environment provides a selection that would be an advantage for some new type of pathogen to develop. And more importantly, we would have never seen these results 20 years ago or even 10 years ago because tools to perform these experiments were not available. The ability to use molecular approaches, the deep sequencing of metagenomics in particular, provides an insight into the world of what is happening in environment, which was too big and too unknown for us to even ask these kinds of questions years ago.

One Health Initiative (Continued)

The point Dr. Maloy wanted to make is that the environment plays a key role in acting as a reservoir for pathogens, as it is here that the raw material exists for the evolution of new diseases, such as from virulence genes that can be transmitted back and forth between animals and humans. Animals are a major source of new human disease, and we can impact this in many ways, especially by manipulating the environment. Environmental changes may select for new phenotypes that can today be detected via molecular approaches such as metagenomics.

There were primarily three spheres of interest in the past; human health, veterinary health, and environmental factors, and usually people worked only in the sphere of their interest. Rarely did they combine their intellects to understand disease associations. The key aspect of the One Health Initiative is the integration of all three spheres, a collaborative effort of multiple disciplines working to integrate veterinary medicine, human medicine and environmental sciences, with the goal of achieving optimal health in humans, animals and the environment; and most importantly, microbiology is the foundation, the underlying feature that ties all this together.

The One Health Initiative uses the concept of bringing people together, but even more importantly it results in a shift in the paradigm as to how to approach public health. In the past, disease surveillance primarily focused on human disease. When disease was detected there was an investigation, identifying mechanisms of transmission, etc. and then treatment evoked to respond to that disease that had occurred. The One Health paradigm, in contrast, is focused on prediction and upstream information, then intervention. It uses the concept of environmental surveillance, (nature, animals, and human disease) to make predictions about where the potential problems are and how we might deal with them. Based on these predictions, upstream prevention will be used to stop the disease before it becomes a human health problem. For example, if you knew something about the environmental surveillance of Nipah virus you would use this information

to determine where you would grow your fruit trees and plant them relative to your pig farms.

Many groups are interested in this concept, such as the American Wildlife Federation, American Society for Microbiology, American Veterinary Association, American Medical Association and environmental groups worldwide. G-8 leaders met 7/16/2006 in Saint Petersburg and strongly supported the One Health Concept. This is extremely important because of what it says for the future of microbiology. To quote: "A vigorous response to the threat of infectious disease, a leading cause of death worldwide, is essential for global development and the well-being of the world's population". Compare that to William Stuart's statement in 1962! The G-8 leaders argue for the importance of these types of concepts, i.e., that to do this we need to improve international cooperation on disease surveillance which will include coordination between animal and human health communities; we need to build laboratory capacity in places where early detection of diseases can be done; and there needs to be timely sharing of clinical samples and information about disease outbreaks. This has been a huge problem in a number of outbreaks in some parts of the world recently. They argue that we need to intensify scientific research on infectious diseases, and in addition to doing research in the developed countries we need to train more scientists from developing countries and develop international scientific research programs.

However, research in the United States is driven by and dependent upon funding, and there are three distinct sources of funding. Environmental concerns are almost entirely funded by the National Sanitation Foundation (NSF), and animal concerns largely funded by the US Department of Agriculture (USDA) with a few exceptions of mouse studies. Human work is primarily funded by the National Institutes of Health (NIH). There are some efforts to develop bridges between these groups and areas but as it currently stands, they still function independently. If we really are going to approach the issue of One Health, the agencies all need to cooperate with each other where they will share problems and resources to attack problems at the interface.

One Health Initiative (Continued)

Dr. Maloy left a bit of advice for people working in research - think of what type of research you will be doing in the next 10 years. There will be a huge potential impact on biotechnology and the farming industries, particularly in the growing area of molecular diagnostics, and a huge impact on clinical scientists as we begin to think more and more about issues that we were blind to in the past and may have to respond to in the future! One Health will be a big change that has huge implications on human medicine and veterinary medicine funding for science in the near future. He also suggested checking Microbe World on the ASM website, which contains lots of good material. Information on One Health will also be appearing in future issues of the ASM publication *Microbe*.

“Cotton Mather, you damn dog you!” A Look at Smallpox and its Impact on American History

The third dinner-meeting of the year was held on May 13, 2010 at the Old Chapel at Tewksbury Hospital, Tewksbury, MA. Harold (Harry) Sanchez, M.D., Associate Chief of Pathology and Medical Director of Microbiology at the Hospital of Central Connecticut, presented a talk entitled “*Cotton Mather, you damn dog you!*” A Look at *Smallpox and Its Impact on American History*. Dr. Sanchez is also Adjunct Professor in the Department of Biology, Fairfield University and Lecturer in the Physician Assistants Program at Quinnipiac University. He discussed the impact of the disease on the Americas with an emphasis on New England and the impact of public health measures before the WHO Eradication Program.

Dr. Sanchez described smallpox as a disease of civilization; wherever there were large populations of people throughout recorded history there was smallpox. The Europeans brought the disease to the new world and it had a profound impact on the history of this country as well as on the world. He also spoke of how



(L to R) Alfred DeMaria, Jr, MD,
Speaker Harry Sanchez, MD, and James Kirby, PhD,
President, Northeast Branch

complicated the practice of public health is. The idea of wiping out smallpox required an enormous amount of cooperation, coordination and communication. The tools for preventing smallpox have been available since about 1800; therefore having the technology to prevent a disease is only part of the picture.

Dr. Sanchez described the clinical presentation and some of the unique features which make smallpox such a devastating disease. Viral particles can be very efficiently transmitted from person to person by either inhalation or direct contact with fluids. Smallpox is a disease of civilization and it is estimated that since you need a certain critical mass of people to maintain smallpox in the population, hunters and gatherers could not sustain the disease. The virus is usually spread by close contact, whereby millions of viral particles are spread from skin lesions and sores in the mouth and throat. The envelope virus is amazingly hardy, allowing transmission of viable virus by fomites such as clothing and bedding. The incubation period is one-two weeks; the prodrome or preeruptive stage is characterized by fever up to 104F°, chills, headache, backache, nausea, and vomiting, followed by characteristic rashes. There are different types of rashes, some more serious than others. After about 12 days, we see typical flat red vesicles, pustules, then scabs, starting at the face and spreading to the arms, chest, back and legs. Dr. Sanchez showed a number of slides depicting the disease in which all the

Smallpox (Continued)

Smallpox lesions appear to be at approximately the same stage of development. Changes in internal organs can occur, with serious pathology and internal bleeding, depending on the type of disease that develops; 20 to 25% of those with the disease will die; in naïve populations the mortality would even be higher. Survivors are left with serious pock marks and some with blindness, especially children. Autopsies were not usually performed on people who had smallpox, even on royalty such as Louis XV of France, as the disease was greatly feared; it was only after the age of vaccination that autopsies were performed.

In order to approach, contain or treat a disease you need some theory as to how it is caused. Theories of causation were numerous, and included miasmatic (atmospheric changes); humoral (phlegm, bleeding, purging); congenital (digestion); animalcule (tiny particles were responsible). There was also the theory of Divine Retribution, that all epidemics are acts of God and not to be interfered with. The virus was finally observed in 1947-48 when the electron microscope became available.

The earliest definitive evidence of smallpox in antiquity is evidenced by the well-preserved skull of Ramses V who died in 1145 BC. The disease has no animal reservoir and is exclusively a human pathogen. It was believed to have originated somewhere in India or Egypt and made its way to Asia Minor, into Asia and eventually to Europe. There was the Plague of Athens in 430-427 BC which was thought to be smallpox; and in 165 AD the Antonine plague, which was brought to the Roman Empire by troops returning from the Far East, and which killed emperor Marcus Aurelius. Unlike some diseases that target certain sections of the population, smallpox killed everyone. From the Mediterranean basin, smallpox gradually made its way up to Europe.

Smallpox also occurred in the Royal Houses of Europe, killing five reigning monarchs. Elizabeth I of England in 1528 survived but was described after recovering as bald and without eyelashes; the Duke of Mantua died in 1567; Emperor Ferdinand IV of Austria died in 1654. A number of people in the House of Stuart were



Gregory Reppucci (C) with students from North shore Community College

infected as well as the son of King George III. There were many others throughout history, and the disease was feared in all walks of life.

Although the disease migrated to Asia Minor, Asia, and Europe, the Americas were spared. The first people came from Siberia across the land bridge (where the Bering Strait is now) to Alaska, and made their way to North America, then to South America. If they brought smallpox with them their original populations would have been too small to sustain the disease. The Americas became an isolated land mass like Australia and remained free from smallpox until the arrival of European explorers and settlers.

Columbus arrived in 1492, and the first reliably recorded outbreak of smallpox in the Americas occurred in 1507 in Hispaniola (now the Dominican Republic) where Columbus had founded the first European settlement. We now had an instance in which an epidemic disease that is endemic in part of the population collided with a totally immunologically naïve population. The records showed that 20% of the dead were Europeans and 70 to 90% were natives. Settlers from here later brought the disease to Mexico with them in 1520.

In 1521, Hernán Cortéz and the Conquistadors arrived and attacked the Aztecs. They carried smallpox with them, and the Aztecs, along with their Emperor, died in droves. It is argued that Cortez could never have conquered Mexico without the help of smallpox. The same epidemic spread to the Yucatan Peninsula, then to Columbia then to

Smallpox (Continued)

Cusco, Peru and to the Inca Empire. In the same decade, Francisco Pizarro confronted the Incas with a small number of soldiers, but smallpox had sickened the hostage Inca king and many of his people, making the Empire easy to conquer. The epidemic spread into North America through Mexico to the Mississippi and Ohio River Valleys and destroyed many of the Mississippi Valley Indian populations before the European settlers arrived there.

In North America, the Pilgrims landed in New England in 1620 and initially became friends with the Indians; the relationship later became fractious and disagreeable. When smallpox hit the settlers and Indians, Increase Mather, a leading Congregationalist minister in Massachusetts, said that God ended this controversy by sending the disease. At this point in time no one had an idea that it was caused by a virus or how to treat the disease. They believed in divine intervention and used European remedies such as sweat boxes, a variety of emetics, and bleeding. The color red was thought to ward off the disease therefore they dressed in red and painted their rooms red. They prayed to a number of deities one of which was Obaluaye, the Santero god of smallpox, which was transformed in music and popular culture to Babalu, which is what Ricky Ricardo sang on the "I Love Lucy" show. Little could be done to prevent the disease at this point. If someone came into contact with a case they used quarantine and isolated affected patients as best as they could, as on ships. Others, if they could, just left the area. The first really effective means of control was inoculation.

Populations in Africa and Asia had centuries of experience with smallpox and had learned how to produce a less severe disease and less mortality by exposing people to infected material from skin lesions or scabs (variola). The dried material was placed into nostrils, into small cuts in the skin, etc., but sometimes with severe consequences. A very small percentage of variolated persons died of smallpox, and side effects included transmitting other diseases such as tuberculosis or syphilis from the pus of the donor. Variolated persons were also infectious and could occasionally transmit smallpox, thus

triggering epidemics. Variations of variolation were used in China, in Turkey in 1672, and by Martin Lister in 1700.

Lady Mary Wortley Montagu, wife of the English Ambassador to Turkey who was stationed in Constantinople, had been disfigured by smallpox, and wanted her children to be variolated after she observed the practice in the Ottoman Empire in 1717. Dr. Charles Maitland successfully inoculated them in England in 1721, and soon other people also wanted the treatment. The British Royal Family, when the 1721 London epidemic hit, had their children variolated also, but only after an experiment was done on six condemned prisoners who were inoculated and exposed to smallpox; all survived and were pardoned. Dr. Sanchez pointed out that although there was evidence that variolation worked, there was a wide variability from location to location as to how people accepted the practice. For example, news of this had spread all over but it wasn't until 30 years later, in 1755, that the method was used in France.

Outbreaks of smallpox were recorded in Boston as early as 1640. An ill sailor from an English vessel coming from Barbados came ashore into Boston and started the 1721 epidemic, ending in 110 deaths. Cotton Mather (1633-1728), a leading Congregationalist pastor like his father, Increase Mather, had an interest in medicine. His African slave had a scar and spoke of "variola" with material from a smallpox scab, a common African folk-practice that would protect against the disease. Mather convinced a healer, Zabdiel Boylston, to try this out on his son and two slaves, with successful results. A violent controversy started and mobs harassed Boylston and tried to kill Mather. William Douglass, the only physician in Boston with a European medical degree, was against this dangerous practice, calling it tantamount to murder, and it was banned. Boylston was reprimanded by a Board of Selectmen but continued to inoculate about 250 people. At the height and fury of the epidemic and the opposition to variolation, a hand grenade was thrown through Cotton Mather's window on which was a note which read: "Cotton Mather, you damn dog you; I'll inoculate you with this, with a pox to you." Boylston and Mather

Smallpox (Continued)

performed one of the first analyses of disease rates, using a mathematical approach to show that this method worked to protect against the disease. They showed that the smallpox case fatality was over 20% while the case fatality for variolation was about 2.5%. Their paper was published and circulated in Europe, and was one of the first times that American medicine began to influence European medicine.

Another footnote to the Boston in 1721 epidemic comes from the work of Benjamin Franklin, who at that time was an apprentice at a newspaper run by his brother James. The paper took an anti-inoculation position at that time in order to improve circulation. Later, as inoculation took hold and made its way to New York and Philadelphia, Franklin became an outspoken supporter of inoculation, especially as his 4 year old son died of smallpox in 1736. He published a paper in 1759 entitled *Some account on the success of inoculation for the small-pox in England and America* (London.W. Strahan), including instructions on how to perform variolation and care of the patient afterward. His friend, English physician William Heberden wrote the pamphlet; Franklin added an introduction documenting the success of the process in Boston and distributed the pamphlets in America for free.

Smallpox tended to occur anytime there was a war, where large groups of recruits were gathered together in small areas. Pontiac's Rebellion occurred in the Great Lakes Region in 1763. Native American tribes, after the French-Indian war, were offended by British policies in the area and threatened the border of the English frontier. They were led by the Ottawa chieftain, Pontiac, and attacked Fort Pitt in western Pennsylvania. Colonel Henry Bouquet wrote to his commanding officer, Sir Jeffrey Amherst, the new North American governor-general, suggesting that smallpox-infected blankets be used (as fomites) to infect the Indians. Amherst agreed, and this was the first attempt to use biological materials in a war setting. The Massachusetts town of Amherst and Amherst College are named after the British commander.

Dr. Sanchez next spoke of the colonial history of smallpox. There was an epidemic in

North America from 1775-1782, coinciding with the Revolutionary War. William Howe, Commander-in-Chief of British Forces during the American Revolution, fought in Boston in 1775 at the Battle of Bunker Hill in which British losses were extremely heavy. There was a standoff for nine months when George Washington arrived. The Americans fortified Dorchester Heights with heavy cannons in March 1776 - and the British fled (Evacuation Day) sailing to their naval base in Nova Scotia. Howe later went to New York and captured both New York City and Philadelphia. Apparently smallpox broke out among the English in Boston even though most of the British had been variolated or exposed to the disease by that time. Heavy losses from the disease were seen in the American army. Washington himself, had pock marks on his face after having contracted the disease in Barbados during a visit.

Continental troops, fearing an assault from the north by the British, allied with the French and Indians, were sent to attack Montreal, which they conquered. Their attempts to conquer Quebec City ended in disaster however, as they encountered more smallpox. The troops retreated, leaving Canada to the British Empire. There were many deserters from the American army and Governor John Trumbull of Connecticut said they had trouble recruiting troops because soldiers feared catching the dread disease more than they feared the enemy. In 1777 Washington ordered mandatory inoculation for all existing Continental troops, even though some states, Virginia, for example, had anti-inoculation laws. Andrew Jackson had joined the War as a courier when a teenager; he was captured and caught smallpox while interred in an English prison camp.

Dr. Sanchez noted the effect of smallpox on higher education in the United States. People of means were always traveling to England and Europe to obtain a better education but Americans feared going to Europe as they, like the American troops, were more susceptible to smallpox. This led to the expansion of higher education in the US as colleges were established or enlarged. Eleazer Wheelock founded Dartmouth College in Hanover, NH, for example, which expanded its curriculum as did the college of William and Mary in Virginia.

Smallpox (continued)

Enrollment rose as Americans desired to remain at home.

Meanwhile, Edward Jenner in England in 1796 noticed that dairymaids who were naturally infected with cowpox did not get smallpox. Using a method he called “vaccination” [vaccinus means relating to the cow], Jenner scratched material from a cowpox lesion on the hand of milkmaid Sarah Nelmes into the skin of 8-year old James Phipps, who he subsequently demonstrated was immune to smallpox infection. He vaccinated other children and his own son, and in 1798 published a series of studies as to how he used cowpox material to provide immunity to the related smallpox virus, arguing that variolation was dangerous. He sent this treatise and some cowpox material to his classmate Dr. John Clinch in Newfoundland, who gave the first smallpox vaccinations in North America. Jenner’s work was initially criticized but later was rapidly accepted widely, and within a decade, the procedure replaced variolation and was being performed worldwide.

Benjamin Waterhouse, MD, professor at Harvard Medical School, received vaccination materials from Europe in 1800. He was trained overseas and was then the chief physician in Boston. Waterhouse first vaccinated his family and then conducted a controlled experiment endorsed by the Board of Health; his data was presented before the American Academy of Arts and Sciences. Waterhouse first approached President John Adams about supporting vaccination, but it was Thomas Jefferson in 1801, upon succeeding Adams, who supported Waterhouse’s idea of using Jenner’s method of cowpox vaccination in the US to eliminate smallpox. Thus vaccination was established as a public health procedure. Jefferson went so far as to try to inoculate some of the Native Americans that were still left in the South. Ulysses Grant at that time was stationed in the west and had encountered smallpox in the Native Indian population. Lincoln delivered the Gettysburg address in 1863 and shortly after returning to Washington acquired smallpox, was severely impaired, but recovered. Throughout the Civil War, Boston, Pennsylvania, and states

to the south had pockets of populations that would not accept vaccination.

The French Canadians were one of the populations most resistant to the idea of vaccination and a large unprotected population existed in Montreal in 1885 when a large smallpox outbreak occurred. Poor isolation policies existed in the civic smallpox hospital and ultimately resulted in over 3,000 fatalities in nine months. There were riots when public health officials tried to vaccinate people. A Canadian-born physician, who had studied in Europe and at Johns Hopkins in the US initially took care of smallpox patients and developed a mild case himself. He wrote to a friend describing the smallpox epidemic and noted that the friend should not worry about becoming ill because he had taken the liberty of disinfecting the letter. Even at that time people were concerned about disseminating the disease by contaminated mail. He used a device that punctured the letter with small holes, and then sprayed the letter with suitable disinfectant, allowing disinfection of the outside and inside!

In 1894 there was a massive epidemic in Milwaukee. Authorities tried to implement vaccination, but again the people rebelled and there were riots.

The last smallpox outbreak in Boston occurred at the South Hampton Hospital in 1901. The poor were taken to institutions; those of better means stayed at home. The Board of Health had numerous physicians vaccinating people, threatening to bring those that refused to court. There was a well recognized and very outspoken anti-vaccination lobby. Autopsies were done by noted pathologist William Thomas Councilman, professor of pathological anatomy at Harvard, who published a number of papers on the pathology of smallpox. From 1901-1903, the number of smallpox cases decreased due to such efforts as forced vaccination of the homeless, the start of house-to-house vaccination, and the defeat of anti-vaccination bills. The last epidemic cases were seen in MA in March 1903.

Massachusetts enacted the first mandatory vaccination law in 1809, giving Boards of Health authority to require that persons over 21 be vaccinated against smallpox. Opposition to vaccination continued and in Jacobson

Smallpox (continued)

(individual freedom) versus Massachusetts (compulsory vaccination), Jacobson was fined and briefly imprisoned. The petitioner resided in Cambridge and opposed a 1902 Cambridge Board of Health mandate. The case was taken to the Supreme Court, with John Marshall Harlan presiding. Harlan in 1905 found for Massachusetts (compulsory vaccination), ruling that the State had established reasonable regulations that would protect the public health and public safety. The Court ruled that Jacobson's constitutional rights and liberty were not invaded. This was a powerful precedent and the verdict was quoted repeatedly.

The last epidemic in New York occurred in New York City in 1947. A male born in Maine returned to New York from Mexico City in early February; he became ill and febrile, but toured New York. He had a rash and questionable pneumonia, and had been vaccinated against smallpox as a child. He was hospitalized and died in early March. Several staff and patients with whom he had contact developed smallpox and authorities realized it was imported from Mexico. Mayor William O'Dwyer instituted a massive campaign to inoculate every person in New York City, which was done at hospitals, fire departments, police stations, etc. About 6 million people were vaccinated in a month. This epidemic approach worked: twelve were ill and only two died. This demonstrated the effects of a good public health system, which required effective communication in all areas.

Many states in the US required smallpox vaccination beginning in 1843, and the disease continued to diminish. By 1897 it had been largely eliminated from the country but the practice continued as protection against reintroduction. Routine childhood smallpox vaccination was stopped in the US in 1972. The World Health Organization (WHO) undertook a global smallpox eradication program in 1967, with worldwide mass vaccination, detection and containment campaigns; at that time 10-15 million cases were occurring annually worldwide, with about 2 million deaths. The last natural case was in Somalia in 1977, and a serious laboratory accident occurred in an English research laboratory in 1978 when

airborne spread led to the death of a laboratorian and the subsequent suicide of the laboratory director. WHO declared the disease eradicated in December 1979. Currently only a Russian research center and the Centers for Disease Control in Atlanta, GA, have stores of smallpox for research purposes. Plans to destroy all samples have been repeatedly postponed as there are concerns that some of the stockpiles generated for bio-weapons have not been eliminated. Also, other nations may have undeclared smallpox stockpiles. Many people are calling for the eradication of all remaining stocks and the controversy continues.

In conclusion, Dr. Sanchez showed an anti-vaccination poster, one of many done in the early 1800's. Irish playwright George Bernard Shaw called smallpox vaccination "a particularly filthy piece of witchcraft" and regarded traditional medical treatment as dangerous quackery. Shaw himself had contracted the disease in 1881 and nearly died.

Editors Note: A statue commemorating the 30th anniversary of smallpox eradication was unveiled on May 17, 2010 in front of WHO headquarters in Geneva.

The City of Boston and the Commonwealth of Massachusetts played an integral role in the development of Public Health. As early as the 1700's, Boston, serving as the first line of defense against smallpox, held ships quarantined in the harbor to protect the general population. In 1796, Boston established the first Board of Health in the nation; its first sitting President was Paul Revere.

Science Fairs

The NEB annually contributes an award of \$100 to each of five MA regional fairs and the VT Science Fair, and \$200 to the MA Science Fair. Congratulations 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.

The Following Programs Were Jointly Sponsored with Other Professional Organizations

***The 62nd American Society for Clinical Laboratory Science Central New England (ASCLS:CNE) Annual Convention**

The ASCLS:CNE Annual Convention was held at the Rhode Island Convention Center in Providence, RI on May 4-5, 2010. It was jointly sponsored with the Bay State Chapter CLMA (CLMA); Rhode Island Cytology Association (RICA); Northeast Branch, American Society for Microbiology (NEB-ASM); Rhode Island Blood Bankers Society (RIBBS); and the Rhode Island Society for Histology (RISH).

***Fighting Bad Bugs (MDR-GNR) – A Team Approach**

Fighting Bad Bugs (MDR-GNR) – A Team Approach was held at the Lahey Clinic Auditorium in Burlington, MA on May 6, 2010, and had an audience of about two hundred registrants. The intermediate level program focused on a multidisciplinary approach to diagnosing, preventing, treating and managing infections due to multidrug-resistant gram-negative rods (MDR-GNR) and controlling the spread of these problematic bacteria.

Faculty included: Larry Madoff, MD, Director, Division of Epidemiology and Immunization, Massachusetts Department of Public Health, Professor of Medicine, University of Massachusetts Medical School, Jamaica Plain, MA; Janet Hindler, MCLS, MT (ASCP), Senior Specialist, Clinical Microbiology, University of California, Los Angeles Medical Center, Los Angeles, CA; Kenneth Lawrence, BS, PharmD, Senior Clinical Pharmacy, Specialist, Tufts Medical Center, Assistant Professor of Medicine, Tufts University School of Medicine, Boston, MA; Robert Duncan, MD, MPH, Associate Professor of Medicine, Tufts Univer-

sity School of Medicine, Hospital Epidemiologist, Lahey Clinic, Burlington, MA; and Alfred DeMaria, Jr., MD, Medical Director, Bureau of Infectious Disease, Prevention, Response and Services, State Epidemiologist, William A. Hinton State Laboratory Institute, Jamaica Plain, MA.

Program sponsors included the Bureau of Infectious Disease Prevention, Response and Services, Bureau of Laboratory Sciences, Massachusetts Department of Public Health; Northeast Branch-American Society for Microbiology (NEB-ASM); Northeast Association for Clinical Microbiology and Infectious Disease (NACMID), Lahey Clinic, and National Laboratory Training Network (NLTN).

***Hospital Response to Chemical Emergencies**

This program was designed for emergency room specialists and laboratory staff who may provide patient care during a public health emergency, and was presented at South Shore Hospital on May 21, 2010 and June 23, 2010; a fall program is also planned. The program gave a comprehensive overview of response roles during a suspected chemical exposure event and included a comprehensive overview of chemical agents, 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 Gloria Cheng, MS, Assistant Coordinator, Chemical Threat Response Laboratory, William A. Hinton State Laboratory Institute, MDPH; Michael A. Feeney, RPh, JD, CHO, Director, Indoor Quality Program, MDPH; and Jennifer Jenner, PhD, Coordinator, Chemical Threat Response Laboratory, William A. Hinton State Laboratory Institute, MDPH.

The programs were sponsored by the Massachusetts Department of Public Health, (MDPH), the Northeast Branch-ASM, and South Shore Hospital.

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