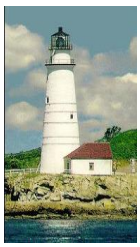

Northeast Branch Newsletter

Number 145 2023

Can Poliovirus be Eradicated?

Can Poliovirus be Eradicated? was presented on January 31, 2023 by Vincent Racaniello, PhD, Columbia University Medical Center, New York, NY. Dr. Racaniello has been studying the poliovirus since the 1980s and is the host of This Week in Virology, a podcast created by himself and Columbia microbiologist Dickson Despommier, PhD, in 2008. The lecture was co-sponsored with the Department of Medical Laboratory Science, University of MA Dartmouth.

Dr. Racaniello first described the disease and then spoke of the history of the poliovirus vaccines--made by passage of the 3 serotypes of poliovirus in different animals, cell cultures and so forth, and eventually resulting in Sabin 1, 2, and 3 oral vaccine strains (OPV), which are still used today. Ingested, they provide protection in the mouth, in the intestines, and then in the blood. They reproduce in the intestine and provide, perhaps for a few months, some mucosal immunity; severe disease will be prevented but not infection. OPV was introduced in the United States (US) in 1962 and led to the eradication of wild polio in the US. Protection in the mouth and intestines is important as polioviruses infect the mouth and multiply in the intestines.

Inactivated polio vaccine (IPV) is injectable, and is the only polio vaccine given in the United States since 2000. It is given by a shot in the arm or leg, depending on the person's age. IPV

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Black Spot, Black Death, Black Pearl: Tales of Bacterial Effectors

American Society for Microbiology Distinguished Lecturer Kim Orth, PhD, from Howard Hughes Medical Institute and University of Texas Southwestern Medical Center, spoke on *"Black Spot, Black Death, Black Pearl: Tales of Bacterial Effectors",* on February 1, 2023. She used three interwoven examples to illustrate how bacterial pathogens rewire host cells using their effector proteins. Black Spot refers to work with *Xanthamonas campestris*, which causes black spots on tobacco and tomato leaves. Black Death highlights work with *Yersinia pestis*, which causes "the black plaque," and how *Yersinia* Yop proteins hijack host cells. Black pearl covers work with effector proteins from *Vibrio parahaemolyticus*, which contaminates oysters.

Dr. Orth's laboratory studies how virulence factors expressed by bacterial pathogens cause disease. Studies using microbial genetics, biochemistry, cell biology and bioinformatics on effectors from *Yersinia* and *Vibrio* bacteria have

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Inside This Issue - Programs in Review

NORTHEAST BRANCH-ASM OFFICERS and STANDING COMMITTEE CHAIRS
(Offices effective until June 30, 2024)

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NEB Council Meetings

Council Meetings this year will continue to be held virtually until further notice. Members and all interested microbiologists and scientists are welcome to attend. Please notify Irene George, Secretary at (508) 785-0126 in advance.

Membership Notes

Dues reminders for 2024 will be sent to our membership via e-mail. Members who did not provide an e-mail address will be contacted 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 membership information.

Although membership in a national organization 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.* Many Northeast Branch are also national ASM members.

Council Election Results

Congratulations to the following NEB members whose terms as Branch Officers began July 2023. President, Ramy Arnaout, Secretary, Irene George, and Local Councilor, Mark W. Silby. Thank you for another great year of programs and we are looking forward to planning a busy 2023-24!

Student Chapters

The NEB is associated with an active student chapter, the Maine Society of Microbiology, Orono, ME.

FUTURE PROGRAMS

***Local Programs**:*

Announcements of Local Meetings and registration materials are posted on our website:
http://northeastbranchasm.org

March 21, 2024.
In Person Dinner- Meeting.
Location: Children's Hospital. Waltham
Annual co-sponsored meeting with NEADLM (Formerly NEAACC).
Details to be announced.

New England Microbiology Laboratory Directors Spring Meeting
Location: Publick House, Sturbridge, MA. Date/time to be announced.

National Meetings:

June 13-17, 2024. ASM Microbe, Atlanta, GA
https://asm.org> Events

October 7-11, 2024
Clinical Virology Symposium, Long Beach, CA
https://asm.org> Events

November 15-17, 2024 ASMCUE, Pittsburgh, PA
https://asm.org> Events

2023 Travel Awards Available for Early Career Northeast Branch Members

The 2024 ASM Peggy Cotter Travel Award Program for Early Career Branch Members provides funds for outstanding early career Branch members to attend ASM Microbe 2024 to be held on June 13-17, 2024 in Houston, TX. Three $1,650 cash awards are available to pay for registration, accommodation and travel costs associated with Microbe 2024.

All submissions must be dated no later than February 16, 2024. The Selection Committee will announce the winners by February 27, 2023.

Send submissions to:
Frank J. Scarano, PhD, at
fscarano@umassd.edu .

Can Poliovirus be Eradicated?

(continued)

and OPV both block infection in the blood because both induce antibodies. Vaccination should last a lifetime.

Despite the use of these two vaccines, polio remains a global problem because vaccines were only given to a small percentage of children in most countries. Sabin went to Brazil where he showed that mass immunization campaigns, in which children were immunized in June and August of every year, dramatically reduced disease in the following years. Based on such results the World Health Organization (WHO) established an organization named EPI to increase the distribution OPV. Dr. Racaniello also spoke of his interactions with Albert Sabin, who always spoke his mind.

In 1988 the WHO said "We are going to eradicate polio" and by 2010 are going to stop immunizing so we can move on to other things.

Can Poliovirus be Eradicated?
(continued)

Smallpox was eradicated, the only major human disease to be done so, but where are we now? Can you actually eradicate a disease Dr. Racaniello asked? Three features are needed for a virus to be eradicated. First, replication in only one host; second, vaccination must induce lifelong immunity; and third, asymptomatic infections must not contribute to viral spread. For polio, the first and second features are good, the third, is not. Asymptomatic infections do not usually contribute to spread but they do for polio, and this is why Dr. Racaniello thinks we probably will not eradicate polio.

The Sabin vaccines have mutations located throughout the genome that Sabin selected for, that prevent the virus from causing disease. The three serotypes have in common changes in the highly structured noncoding region that are essential for its attenuation phenotype. Experiments in mice showed if these are changed, paralysis will occur; a single base change in a wild type virus is enough to prevent paralysis. This is important because this base changes in people who get the vaccine.

Shortly after the introduction of OPV in the US in 1962, cases dropped from few thousand a year to almost nothing from 1961-2003, however, there continued to be cases of vaccine-associated paralytic polio (VAP). The last case of wild polio in the US was in 1979. In 2000 we switched to IPV, which is used to this day, and does not cause polio. Much of the world still uses OPV and that is part of the problem. In most vaccine recipients, the weakened virus in the vaccine reproduces in the intestines and can mutate in a few days to become dangerous, and is shed in the feces. Only very rarely—one in 1.4 million doses given to children—does it cause polio in the recipient. It is not due to the vaccine but to the genetics of the child.

Dr. Racaniello cited two cases of polio that raised concern and showed that poliovirus can spread anywhere via global travel. The first case was in June 2022, when poliovirus was found in London sewage; the United Kingdom (UK) uses only IPV. The intestines of everyone who gets IPV are susceptible to OPV which is used in many other countries. People entering the UK from these countries are shedding OPV, transmitting it to the resident population, and OPV winds up in the sewer. It will not cause polio in immunized people. The second, was a polio case in in Rockland County, a New York City suburb, in July 2022, caused by vaccine-derived Sabin OPV 2 strain. Local wastewater analysis showed it was related to virus found in the UK and Israel. Again, people entering the US from other countries shed OPV; if someone is not vaccinated (and the person In NY was not vaccinated), they can catch polio. People inoculated with IPV are susceptible to OPV, because IPV does not protect the gut from infection. Also, >90% coverage in an area is needed to be effective; polio vaccination in several areas in this county and another county nearby was $\leq$ 70%.

Vaccinated people shed virus for many years and it is most likely widely spread in US sewage. The US has recently expanded passive wastewater surveillance for poliovirus and it was detected in wastewater in Rockland County and in New York City, which means it's circulating among the general public. Poliovirus probably circulates in many other places in the US as well, but we have become complacent about polio, thinking it has been eradicated, and just haven't been testing for it.

Worldwide, we have done well with polio eradication; wild types 2 and 3 are declared eradicated by WHO. However, wild poliovirus type 1 (WPV1) is still endemic in Afghanistan and Pakistan, and vaccine derived type 1 poliovirus cVDPV causes outbreaks in Africa. The US uses IPV, not all countries can, but neither IPV or OPV confers long-term gut immunity or protects from infection - everyone sheds. As long as we continue to use the oral vaccine, the virus will continue to circulate and some unvaccinated people will get so-called vaccine-derived polio.

The current WHO eradication plan suggests using two new bivalent OPV strains, switching to IPV, and eliminating OPV vaccination globally. The new vaccines are expected to provide comparable protection against poliovirus while being more genetically stable and not cause polio. Most countries that use IPV will probably immunize indefinitely. Dr. Racaniello's solution is to continue to use IPV and stop focusing on

Can Poliovirus be Eradicated?

(continued)

eradicating poliovirus, because circulating virus is not a problem as long as you are vaccinated. We can't eradicate a virus where 99% of infections are asymptomatic.

Dr. Racaniello then spoke briefly of other enteroviruses that are global pathogens, such as Enterovirus, Coxsackievirus and Rhinovirus. There were few infections with Enterovirus D68 until 2014, when large outbreaks occurred, and "cold-like" symptoms could result in respiratory induced acute flaccid paralysis/myelitis. The virus is not detected in blood, feces or cerebrospinal fluid, yet travels from the respiratory tract to the central nervous system (CNS). He noted that viruses that invade the CNS, such as poliovirus, EV-D68, West Nile virus, and Zika are efficiently transmitted to new hosts by shedding (gut, respiratory tract) or by mosquitos. CNS invasion is a viral dead end in humans. Prevention of oral-fecal contamination includes good hygiene; washing your hands with soap and water after using the restroom and before touching your mouth or eating, using caution in what you eat, and sterilizing common surfaces. He noted that hand sanitizer doesn't inactivate poliovirus.

Tales of Bacterial Effectors

(continued)

effectors from *Yersinia* and *Vibrio* bacteria have uncovered many mechanisms that bacteria use to subvert host signaling pathways, including the discovery of two novel post translational modifications: YopJ Ser/Thr Acetylation and VopS AMPylation. Her team's work has uncovered unexpected strategies that bacteria use to survive and spread. Additionally, her work has also helped reveal normal signaling pathways.

Bacterial pathogens disrupt and deregulate molecular switches during infection, and by uncovering the activity of their virulence factors, we discover new molecular switches and tools. Dr. Orth studies Gram negative bacteria, that encode a type 3 secretion (T3SS) that is a syringe-like apparatus, and the bacteria make proteins called effectors. When bacteria come into contact with the host they poke a hole in the membrane of the target host cell and actually unfold and translocate these proteins thru this needle, delivering them into the host, where they refold and manipulate a cellular response to infection.

These Type 3 effectors are tools to elucidate bacterial signaling because they mimic or capture an endogenous eukaryotic activity, and then they use that activity to target and manipulate eukaryotic signaling machinery. Importantly, these effectors are quiescent in the bacteria in which they are made in several ways. They can be associated with a chaperone so they are loosely folded, they might need a cofactor which is only found in the host cell, or they may be very active enzymes; but without the proper substrate in the bacteria that produce them, they can't do harm.

Dr. Orth first spoke of the Black Death, and a very active effector protein Yersinia YopJ, that years ago, was found to inhibit all MAP kinase and the NFkB signaling pathways. They realized that the secondary structure of the protein, when aligned with known secondary structure from crystalized protein, contained a catalytic triad, similar to one found in cysteine proteases such as Ulp1 (*Saccharomyces cerevisiae* protease) and AVP (*Adenovirus* protease). They hypothesized that YopJ and homologues depend on their catalytic residues for activity and that it is probably a protease, as it shares homology and similar secondary structure with Ulp1, AVP, and Vibrio VopA. Genetic experiments for several more years failed to show how YopJ worked, and they turned to studying homologues.

Another student meanwhile, had been studying XopD protein from *Xanthomonas campestris*, a bacterium that causes black spot. Enzymology studies showed that XopD is a type 3 effector protein like YopJ, is a cysteine protease, has catalytic activity, and exhibits substrate specificity. Looking at these, and comparing VopA, YopJ, AVP, Ulp1 and XopD, they now questioned their hypothesis about YopJ working like Ulp1, because YopJ was

Tales of Bacterial Effectors
(continued)

missing a substrate binding domain. They now asked if it was a hydrolase or protease.

A colleague then suggested that Dr. Orth use biochemistry, and in vitro assays were used to look at the signaling pathways. Numerous experiments using lysates, kinases and mass spectrometric analyses, showed acetylation on serine and threonine residues never seen before. The importance of this was that the serine and threonine were totally conserved in all the kinases that YopJ inhibits, and these are the same residues that are on the activation loop and are phosphorylated by upstream kinases. YopJ was adding an acetyl group onto a hydroxyl in these amino acids, it was an acetyltransferase They proved the new hypothesis, and published that saying: YopJ acetylates critical residues on the activation loop and inhibits activation of the kinases by phosphorylation.

This was confusing as their original hydrolysis hypothesis was: was YopJ a protease or transferase? She then explained two ways in which acetyltransferases can add an acetyl group to a protein. One way is when cysteine comes in and cleaves the bond, leaving an acyl intermediate, but this enzyme is different from a transferase, because it allows water in its catalytic site, therefore it is a hydrolytic event; the water attacks and cleaves the high energy bond.

From this they discovered there is a new co-translational modification by *Yersinia* effector protein, and that proteases and transferases have the same chemistry but use different substrates. The most important thing learned from this study was how to study virulence factors that have any kind of new chemistry. You need to identify the target, determine how the target is changed by these effectors, and then elucidate the biochemical mechanism.

Dr. Orth then described studies on the Black Pearl, *Vibrio parahaemolyticus.* The organism has two T3SSs and many effector proteins that have not yet been characterized. Vibrios are controlled by temperature and leave their dormant state in warm months; global warming is contributing to the spread of these pathogens. The organisms can leave their dormant state when heat waves occur out of season. *V. parahaemolyticus* has caused many outbreaks of gastroenteritis associated with consumption of contaminated raw seafood.

The clinical isolate they studied contains a number of virulence factors. It has thermostable

ASMDL Lecturer Kim Orth, PhD

hemolysins, a non-toxic T3SS (T3SS1), and a-toxic T3SS (T3SS2). The non-toxic system is found in every *Vibrio parahaemolyticus* on chromosome 1, is ancient, has about four or five effectors, and does not make humans sick. It's turned on by temperature and changes in calcium, and is more like the *Yersinia* system. The second system is toxic, can cause gastroenteritis and can be lethal to people who are immuno-compromised. It is on the 2nd chromosome, is encoded in a pathogenicity island, and is turned on by bile salts, such as those in the human digestive tract.

Dr. Orth spoke only of one effector from this system, VopS, a new post translational modification they discovered, and it follows the same profile they used for YopJ. Another student discovered that VopS inhibits Rho GTPAses, small G proteins that are in an inactive state and in one conformation when they bind GDP. When they exchange GTP, they change their conformation, become an active molecule, and fit into a downstream host effector protein. This causes changes in the active cytoskeleton. They were post-translationally modifying the G protein with an AMP on a hydroxyl residue (causing steric hindrance). This new modification was called AMPylation, because AMP is being added, as in phosphorylation, an

Tales of Bacterial Effectors
(continued)

observation not seen since its discovery using another enzyme in the 1960's.

The T3SS2 *Vibrio* system, toxic to humans, has its own set of effector proteins, induced by bile. They discovered that *Vibrio* invades a cell, replicates to 300-400 organisms/cell and then escapes into the cytoplasm, turns off host alarms and escapes from the cell. It has an effector protein VopC, a deaminase, the most conservative post-translational modification. It changes a glutamine to a glutamase, a 1 dalton change, and that activates Rac (a small GTPase) by deamination, thus allowing cell invasion by the organisms. Other effectors are also involved in this process.

They also looked at the effector VopL, responsible for making stress fibers in cells. The gentamicin protection assay (GPA), and consulul microscopy, were used to support their hypothesis that VopL suppresses the ability of cells to make intracellular reactive oxygen species (ROS) production by targeting actin. They studied these effectors further and still have many unanswered questions.

Dr. Orth lastly spoke of how *Vibrio* escapes from the host cell membrane compartments using the molecule VPA0226; it does this by compromising the plasma membrane. The pathogenicity island contains no lipase, but there is a lipase, VPA0226, that is conserved in the genome of all *Vibrios.* They found that after deleting VPA0226 and performing the GPA, the mutant bacteria could not escape while the wild type could. They also found that both VPA0226 and its catalytic activity are required to mediate bacterial egress from the host. Additional studies showed it co-localized with the mitochrondrial COXIV and ACAT1 markers, and fragments mitochondria. VPA0226 is a Type 2 secreted lipase that they also showed esterifies cholesterol with host fatty acids.

Dr. Orth's final comments were that effectors use clever mechanisms to manipulate a host, and that microbes are resourceful and can use an interplay between virulence systems and host cell resources to involve ingenious schemes for pathogenicity.

Wastewater Intelligence as Public Health Infrastructure

Mariana Matus, PhD, CEO and Co-Founder at Biobot Analytics, Cambridge, MA, spoke on *Wastewater Intelligence as Public Health Infrastructure* at a March 16th dinner-meeting co-sponsored by the Northeast Branch-ASM and the Northeast Section of the American Society for Clinical Chemistry.

Dr. Matus is a computational biologist and started working on wastewater epidemiology during her PhD at MIT. She and Newsha Ghaeli, an urban studies researcher from MIT, are cofounders of Biobot Analytics, the first company to offer wastewater monitoring commercially. Their mission is simple Dr. Matus said. Wastewater is a data asset, not waste. Whenever a toilet is flushed, a high-quality medical sample is being sent into the public wastewater infrastructure where it gets mixed, combined and anonymized to produce a wonderful data source. Sewers are the conscience of the city and everything we do and everything we are leaves a chemical and biological footprint in the wastewater. They thought of this as a global, not only a domestic platform.

Their vision is to build a wastewater intelligence platform to deliver untapped insights on human health and behavior. Much work was done around the Covid pandemic, that was a global crisis, but much more can be done with wastewater. Continuous ongoing surveillance can be set up, anomalies can be detected, and outbreaks and pandemics can be predicted before they happen. This leads into understanding mental health in communities, for example, drug use, stress levels, dietary information, etc., over time, and across geographies.

Clinical surveillance data is important as it makes public health more equitable; wastewater gives a voice to everybody, and not everyone can access medical care for financial or cultural reasons. Public health data is also heterogeneous; the data includes clinical cases, 911 calls, hospitalizations, syndromic data, etc.

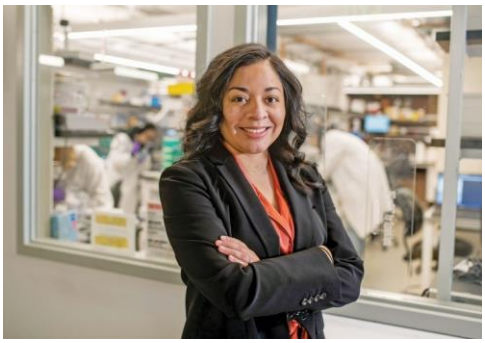
Wastewater Intelligence as Public Health Infrastructure (continued)

Wastewater can be used to look at multiple things in parallel and helps understand population health more inclusively. It is predictive, and a leading indicator for infectious diseases and epidemics. It is inclusive, in that everyone is involved, and it is versatile, as a source of health data for COVID, flu, diet etc. That was seen in 2022 when Boston area wastewater Covid data spiked to extremely high levels, ahead of the clinical data. Wastewater intelligence can also be thought of as national security because it can help understand threats, whether natural or man-made.

Biobot was founded in 2017, and in the early years, it was difficult to convince public experts that their data was credible and accurate. Covid gave them an opportunity to say wastewater is valuable, and currently, Biobot currently has surveillance in all US states and territories with partners, and Canada. Their data currently covers about 50% of the Massachusetts population.

Dr. Matus, as a first-year PhD student, first looked for influenza virus in Boston sewage, and found few positive samples. They discovered a system level design was needed, which manhole should be used, how to collect and concentrate samples, which detection technology to use, etc. She wrote a proposal that became her PhD thesis, and she received a research grant from the Kuwait Foundation for Science that involved working in Boston and Kuwait.

The grant addressed whether wastewater data is only noise; what fraction of the microbial and chemical markers in the wastewater are of human origin and what is part of the sewer ecosystem. Their first experiment was in Cambridge, MA, and looked for bacteria and chemical markers that correlated with diurnal activity and those which were independent from it. Data was incredible. On the chemical side, 80% of chemicals in the wastewater correlate with human activity. On the bacterial side, 40 to 60% correlate with human activity, and this is difficult to quantify. The rest are part of the sewer ecosystem. They needed assistance with



Mariana Matus, PhD

collection, concentration and analysis; thus, they left MIT and Biobot was started. Now robots, not buckets, are used to collect wastewater samples.

They worked on substance abuse, the opioid epidemic, until March 2020, and then, in partnership with the MWRA, the first COVID detection in the world in wastewater was at MWRA, and currently MWRA has the longest time series of wastewater data in the world. There are now over 40 sentinel sites in MA, and they cover about 40% of the US population. She lauded the MWRA staff for their assistance and how much these unsung heroes contribute to the community They also have partnerships with various state agencies and nationwide

Dr. Matus explained graphs demonstrating how wastewater data is used and data is reported. She explained how specimens are normalized, depending on how much stool is present in the water and the methods used. Protocols are optimized, changed accordingly, and variants can be identified in real time.

Wastewater data is collected on the neighborhood level and she spoke of how it was used in Chelsea, Cambridge and Boston; in Chelsea, this resulted in mobile testing and vaccination clinics being strategically deployed. The main objective here was group intervention and public health. Analyzing wastewater before it mixes downstream at treatment facilities permits Biobot to measure drugs at specific locations, and identify compounds of direct human excretion, which are typically degraded too quickly to be detected at treatment plants.

She also showed national-scale SARS-CoV-2 variant data from Dec. 2021-Dec. 2022 and explained the benefit of making data available publicly in real time. Data is shared with CDC d

Wastewater Intelligence as Public Health Infrastructure (continued)

and other national agencies. Many Boston hospitals are now looking at this type of data also in regard to their own institutions.

She described the 2018 Cary, North Carolina pilot study in which wastewater data identified the distribution of prescription opioids used across town, resulting in an educational campaign designed around prescription opioids, and decreasing overdoses by 40%.

Biobot works with Public Works departments of municipalities, and look at sewer maps to determine where a manhole must be opened; these become sentinel sites, and are representative of a bigger group. (prisons, airports). Smaller communities have less noise, and less dilution by large entities.

Samples collected by partners throughout the US are sent by FedEx to Biobot in Cambridge, MA for testing. Many pathogens of public importance can be detected in wastewater stools or urine by looking for pathogen biomarkers. They test for Covid variants, monkeypox, Poliovirus, influenza, RSV, hormones, adrenaline, antidepressants, cocaine, methamphetamines, nicotine naloxone, narcan and methadone, and xylazine.

Dr. Matus hopes this type of data will lead to more transparency and data sharing.
There is a need for discussion as to whether the data is personally identifiable and how to protect identities. There were many questions for her throughout the presentation.

Potential Influence of Climate Change on the Northward Expansion of Primary Amebic Meningoencephalitis Cases

This annual joint meeting co-sponsored by the Northeast Branch-ASM, The American Society for Clinical Laboratory Science of Central New England, and the University of Massachusetts Dartmouth Department of Medical Laboratory Science was held at Rachel's Lakeside in Dartmouth, MA, on April 24, 2023. Caterina M. Miraglia, D.C. MLS(ASCP)^{CM} , Associate Professor, Department of Medical Laboratory Science, University of Massachusetts Dartmouth, spoke on *Potential Influence of Climate Change on the Northward Expansion of Primary Amebic Meningoencephalitis Cases.* She discussed how climate change may affect the environmental factors influencing *Naegleria fowleri* and development of primary amebic meningoencephalitis.

Naegleria fowleri, causes primary amebic meningoencephalitis (PAM), an infection that destroys the brain. It was first described in 1965 in four Australian patients by Fowler and Carter. The disease was named PAM by Cecil Butt, after his 1966 NEJM article identified PAM in the brains of three children with a history of prolonged swimming in freshwater lakes in Florida. The new species of amoeba was later named *Naegleria fowleri* after Malcolm Fowler.

Naegleria fowleri is a free-living opportunistic organism in the environment with no major reservoir host. There are three stages in its life cycle; cyst, trophozoite and flagellated forms. The parasitic trophozoite is the motile, reproductive, feeding, and infective form. It is found in fresh water and soil and eats bacteria, fungi and human cells. Nutritional deficiency in the environment will cause the trophozoite to differentiate into a flagellate, the non-feeding stage, that has two apical flagella. The flagellate swims to locate food, and when found, transforms back into a feeding trophozoite. *N. fowleri* encysts (dormant stage) when harsh adverse conditions occur, such as temperature extremes, pH extremes, and intolerable salinity levels.

Trophozoites penetrate the nasal mucosa most commonly during fresh-water recreational activities, but infection can also occur with sinus irrigation, rising with contaminated tap water, or exposure to cyst-laden dust. Dry infections account for about 6% of PAM worldwide and occur in warmer parts of the globe, such as Africa. The organisms penetrate the cribriform plate in the nasal cavity and enter the brain

Primary Amebic Meningoencephalitis
(continued)

cortex. Trophozoites are found in the cerebrospinal fluid (CSF) and tissue, and flagellated forms are occasionally found in the CSF; cysts are not formed in human tissue.

PAM is characterized by severe central nervous system (CNS) dysfunction with rapid degeneration. *N. fowleri* uses sucker-like structures (feeding cups) to ingest brain tissue, and releases various cytolytic molecules like proteases that cause additional cell destruction in the CNS. An intense immune response is also elicited.

Symptoms develop 1-9 days after infection, and the disease progresses rapidly with a 95 to 99% mortality rate. Lethargy, disability, unconsciousness and coma occur and can easily be mistaken for other types of meningitis, therefore early diagnosis is essential. PAM is treated with intravenous and intrathecal amphotericin B, fluconazole, rifampin, erythromycin, miltefosine and dexamethasone. Hypothermia is also used to help limit damage from edema, and intracranial pressure needs to be monitored.

PAM diagnosis can be made by microscopic visualization of motile trophozoites in a wet mount of fresh, unfrozen, unrefrigerated CSF. CSF smears can be stained with hematoxylin and eosin (H and E), periodic acid-Schiff (PAS), Giemsa-Wright staining, or modified trichrome stains. Gram stain is not useful. Diagnosis needs to be confirmed with PCR.

Diagnosis can also be made by visualization on stained smears of brain biopsy or autopsy material or by using an immunohistochemical stain on formalin-fixed tissue or CSF. Serologic testing is not recommended as patients usually succumb to PAM before detectable antibodies are formed. Dr. Miriglia showed trichrome and H and E stained slides of the organisms.

Since it's identification in the 1960's, there have been about 400 cases worldwide with only seven survivors, and these are most likely underreported. The organism has been detected on all continents except Antarctica, but its presence appears to be sporadic across the globe and there appears to be a complex

Caterina M. Miraglia, D.C. MLS(ASCP)^{CM} , (R), Frank J. Scarano, PhD, MLT(ASCP)M^{CM} (2nd from R), and UMA Dartmouth Students

interaction between the organism and its environment.

Environmental factors like temperature, pH, salinity, and food sources impact the life cycle stage of *N. fowleri.* It lives in warm moist environments and is very sensitive to desiccation. It has been found in fresh water, soil, swimming pools, tap and well water, brackish water, and flourishes in ground water bodies. Biofilms in water distribution systems provide it with protection from chlorine and with a bacterial food source. The organism is thermophilic, and grows at a temperature of 32-46°C and in a broad pH range. Cysts will remain dormant in winter months in water sediments and emerge as trophozoites as the temperature increases. The organism is not detected in marine environments, and encysts with increasing salinity.

An emerging infections disease is defined by CDC as one whose incidence in humans has increased in the past 2 decades and threatens to increase in the near future. It is argued that PAM qualifies as such, as the incidence is likely to increase due to climate change.

Climate change models predict global warming and *N. fowleri* is thermophilic. Both drought and more rainfall can potentially cause an increase in PAM cases. Drought can increase the use of rooftop rainwater systems or artesian wells that rely on groundwater. Many pathogens have been isolated from rainwater, which is usually not treated prior to use for household activities, and *N. fowleri* is often isolated from

Primary Amebic Meningoencephalitis
(continued)

rainwater tanks in Africa and Australia. Drought would also increase airborne dust and potential cyst exposure. Increased rainfall would cause nutrification, an increase in plant and bacterial growth in estuaries and coastal waters, that would also favor growth of the organism.

PAM is more common in developing countries, where there are more favorable conditions for growth, and it can be masked by other diseases or be misdiagnosed and underreported. Currently 0-8 cases of PAM are diagnosed in the US annually. Statistics from 1999 through 2010 estimated 16 deaths annually from PAM. However, more than half of the deaths from CNS infections are unspecified, therefore the true incidence is unknown and probably underestimated and underreported.

Generally, PAM cases in the US occur in the summer months after recreational exposure to warm untreated fresh water. The number of cases reported varied from year to year from 1978-2018, but there was no increase. Significant however, is that most exposures occurred in the south, but there was a rise in the Midwest, where all cases except one occurred in 2010 or later.

Texas and Florida have the highest number of cases, but cases have been confirmed in more northern states after 2010, suggesting a northward expansion. As air temperatures rise, water temperatures will also, providing a more favorable environment for the organism to grow and PAM diagnoses will likely become more common. Dr. Miraglia commented that we still lack a good understanding of its microbial ecology, and need better knowledge regarding diagnosis, treatment and eradication.

Science Fairs

The NEB annually donates an award of $100 to each of five MA regional fairs and $300 to the MA Science Fair. Congratulations again to the students for their outstanding work.

ADDITIONAL ACTIVITIES

Northeast Branch Records Available

The complete collection of Northeast Branch-ASM records at the Public Health Museum in Tewksbury, MA has been cataloged, and the catalog will be on line soon for scholars researchers, and others interested in the history of the Northeast Branch.

For information call: 978-851-7321 x 2606, or by email: info@publichealthmuseum.org

New England Microbiology Laboratory Directors Meetings

The New England Microbiology Laboratory Directors group has been meeting at the Publick House in Sturbridge twice a year for over thirty years in order to share information and their experiences in the laboratory. The informal half-day agenda consists of presentations by attendees and the spring meeting was held on April 25, 2023. The agenda included *Three Cool Cases; Mechanism of Action of Streptothricins, the First Gram-Negative Antibiotic, Lessons in Confronting the Challenge of Multidrug-Resistant Pathogens*; *Multi-Drug-Resistant N. gonorrheae*; and a description of the new CDC funded *US Pathogen Genomics Center of Excellence* in Massachusetts.

The fall meeting was held on October 24, 2023. The agenda included *Proposed VHF Toolkit for Laboratories; A Prolonged and Troublesome Outbreak Due to Burkholderia cenocepacia; Discussion on Current and Future Application of Next Generation Sequencing;* and *Rabies Post-Exposure Prophlaxis.*

The meetings are attended by physicians, laboratory directors, epidemiologists and laboratorians from New England., and are supported in part by the NEB.

Please contact Alfred.DeMaria@state.ma.us if you would like to receive meeting information.

MEMBERSHIP RENEWAL FORM

January 1, 2024 – December 31, 2024

Please check personal information.

Name: _____ ASM Member? _____ ASM Membership No _____

Preferred Mailing Address _____

Home/ Business Address _____

Phone (Day) _____ Preferred Email: _____

Phone (Other) _____ Other Email: _____

Professional Position: _____ Specialty: _____

Primary Area of interest: __Biotechnology __Education __Marketing/Sales
__Clinical/Public Health __Industrial Other: _____

Are you interested in any of the following Branch activities? __Working on Committees __Running for Office

MEMBERSHIP OPTIONS:

__Individual ($ 15.00 annually) __Individual ($ 40.00 / 3 years) __Student ($ 10.00 annually)

__Emeritus* (No Charge) *Emeritus membership is defined as a member who is in good standing for 20 consecutive years, and who is retired from their profession.

__ UPDATE ONLY ENCLOSED (changes can be emailed to NEBranch-ASM@comcast.net)

Renewals postmarked after September 1, 2023 will be effective 9/1/23-12/31/24.

Please renew either with your annual ASM membership or mail this form and dues check (payable to NORTHEAST BRANCH-ASM) to:

Patricia E. Kludt
6 Abigail Drive
Hudson, MA 01749

Date Dues Received: _____

Check No.:_____

Sept 2023