Nebraska Public Health Laboratory Newsletter

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NPHL Updates

By Peter C. Iwen, PhD, D(ABMM), Director, NPHL

"Expect the unexpected" seems to be the motto for the laboratory with this year's surprise, an outbreak of cyclosporiasis that occurred in Nebraska and Iowa during the height of summer activities. The article by Dr. Sarah Buss, Clinical Microbiology Fellow provides some insight into the complexities of diagnostics for this unusual protozoan. It was reassuring to know that communication among the private laboratories in Nebraska, the NPHL, and the CDC allowed for our laboratories to work efficiently in the diagnosis and follow-up evaluations for this outbreak. Additional information concerning the outbreak can also be found is a recent Letter to the Editor written by Dr. Buss and published in the *Journal of Clinical Microbiology* (51:3909).

To add to the disease outbreak scenarios that appear in Nebraska, Andi Brockman, NPHL Laboratory Specialist, provides an oversight on *Salmonella* serotyping, with the introduction of new molecular testing. The NPHL continues to provide the data necessary to allow for epidemiologists to identify both local and national outbreaks in which many Nebraska residents unfortunately continue to be participants. Karen Stiles, the State Training Coordinator also provides a summary of the changes that have occurred with protocols for the Laboratory Response Network. She continues to work with the laboratorians throughout the state to provide training and to enhance communication.

Finally, as the year comes to a close, we look back on the first 100 years of the public health laboratory in Nebraska with excitement in preparation for the next century of providing diagnostic services for the citizens of Nebraska.

INSIDE THIS ISSUE:

NPHL Updates ..……..……..……....1
Cyclosporiasis Outbreak 2013..…....……..……....1
Changes in LRN Sentinel Laboratory Protocols…….....3
Salmonella Serotyping at NPHL……...……...…...…..4
The State PHL in Nebraska: The First Hundred Years…..5

Cyclosporiasis Outbreak 2013

By Sarah Buss PhD, Clinical Microbiology Fellow

In mid-June of 2013 an outbreak of cyclosporiasis occurred in Iowa and Nebraska[1]. By October 23rd, 25 state health departments had reported roughly 643 *Cyclospora* infections to the CDC. No fatalities occurred, but 45 patients were hospitalized. Most cases were reported from TX (278), IA (153) and NE (86) with illness onset dates ranging from mid-June through early July. In Nebraska, affected counties primarily included Douglas (45), Lancaster (16), and Sarpy/Cass (11)[2]. Epidemiological investigations in Iowa and Nebraska linked infections to salad consumption, and an FDA trace-back investigation identified Taylor Farms de Mexico as the source of the outbreak in those states[1]. It remains unclear whether cases in all 25 states were related to the same source, however further epidemiological investigations are pending.

Cyclosporiasis is a nationally notifiable enteric disease caused by the coccidian parasite, *Cyclospora cayetanensis.* The organism is endemic to tropical and subtropical regions of the world including Mexico, Central and South America, Indonesia, Asia, Africa, Southern Europe, the Middle East, Nepal, India, and the Caribbean. Humans become infected when sporulated *Cyclospora* oocysts are ingested. In endemic areas, contaminated soil, water, and food are sources of infection. However in the US and other non-endemic regions, most infections have been linked to the consumption of imported and contaminated fruits and vegetables[3]. Cooking food may prevent infection, but most of the food items previously linked to *Cyclospora* outbreaks in the US are eaten raw. Consequently, consumers should always thoroughly wash fresh produce, even when labeled as pre-washed and ready to eat.

Although humans are the only known host for *Cyclospora cayetanensis*, the infection is not directly spread from person-to-person. Only non-infectious and unsporulated oocysts are passed in human feces. Oocysts become infectious over a period of 2-4 weeks, after they mature and sporulate in the environment. Symptoms of cyclosporiasis typically begin about 1 week after ingestion of sporulated oocysts, and may include watery diarrhea, nausea, gas, and fatigue. Less frequently patients experience vomiting or low grade fever, and some people may even remain asymptomatic. The infection is not usually life threatening, however without treatment the duration of symptoms may be prolonged, lasting up to 60 days. *Cyclospora* can be successfully treated with trimethoprim-sulfamethoxazole and patients should also focus on rehydration[3].

Diagnosis of cylclosporiasis can be made by microscopic identification of unsporulated oocysts in stool specimens. Wet mounts are not confirmatory. To confirm the identification of *Cyclospora,* a modified acid-fast stain (mAF), using a 1% H_2SO_4 decolorizer, should be performed. Using the mAF technique oocysts will stain variably and appear pink to dark purple against a blue background. Unstained oocysts, referred to as ghost cells (Ghc), may also be present in the mAF stained slide (Figure 1).

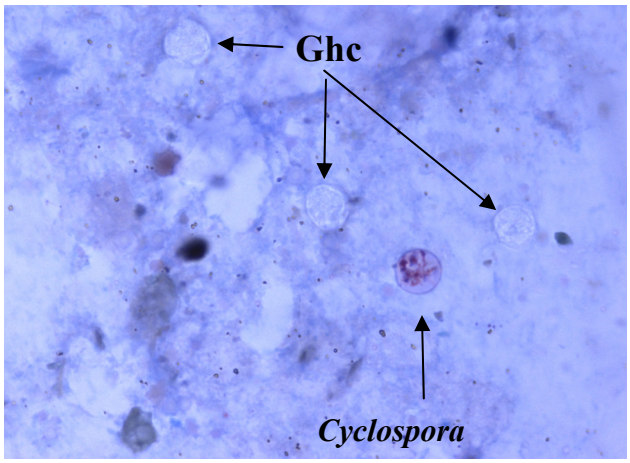

Figure 1 Modified acid-fast stain on stool showing *Cyclospora* oocysts, 400X

Techniques such as examination of autofluorescence (Figure 2 - non-specific fluorescence of the oocysts under UV light), hot safranin or Auramine O staining may also assist with the identification. Moreover, the CDC has a PCR-based molecular test that can be used to confirm the diagnosis of *Cyclospora*.

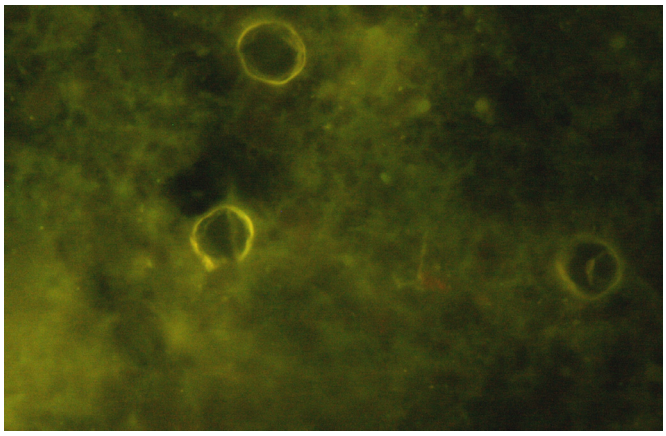

Figure 2 Autofluorescence of *Cyclospora* oocysts in stool, 400X

Clinicians need to be aware that a standard Ova & Parasite exam of stool does not specifically detect for *Cryptosporidium, Cyclospora* or *Cystiospora*. For *Cryptosporidium*, the optimal test is the *Cryptosporidium* Antigen by EIA. For *Cyclospora* and *Cystiospora*, the optimal test is the mAF. The submission of stool specimens for *Cyclospora* testing can be a confusing issue because microscopic and molecular tests have different collection and storage requirements. Fresh specimens are acceptable for both microscopic (if testing will be completed within 2 hours) and molecular (if stored refrigerated or frozen) testing. Alternatively, stool specimens fixed in buffers that contain-Formalin (ex: Protofix) are acceptable for microscopic evaluation. However, Formalin can be inhibitory to PCR so Formalin containing fixatives should not be used when molecular testing is required. Conversely, specimens preserved in PVA are acceptable for molecular testing, but not mAF staining.

Questions regarding the submission of specimens for *Cyclospora* testing can be directed Dr. Iwen at piwen@unmc.edu.

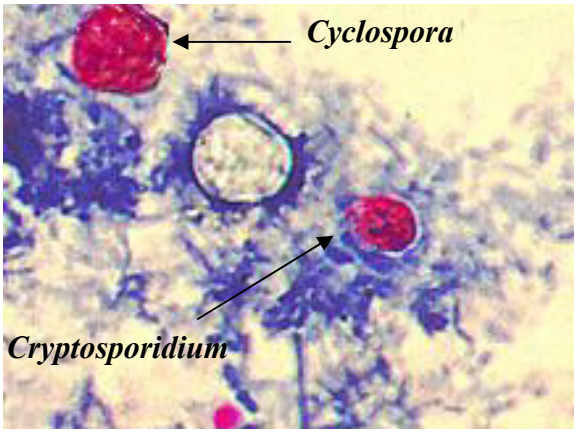

Figure 2 Modified acid-fast stain on stool showing protozoal oocysts, 1000X (Lynn Garcia/Hardy Diagnostics)

NPHL Test Directory for Ova and Parasite, Stool for *Cyclospora* & *Isospora* (*Cystoisospora*) Stain

Synonym	Parasitology Stain by Modified Acid-Fast
Method	Concentration procedure with modified acid-fast stain
Availability	Monday, Wednesday, Friday; results within 24h
Specimen	Stool
Collection device	Fresh stool or if transport > 2 hours use Proto-fix vial for preservation and add stool to fill line marked on Proto -fix vial. Seal and shake vigorousy. Add bloody or watery portions of stool if present.
Volume	Walnut size specimen or 10 ml of stool in preservative.
Storage/Transport	Ambient, Category B
Unacceptable	Specimen in PVA with Formalin, dry specimens, leaking containers, specimens contaminated with oil, barium, or urine, multiple specimens in 24 hour period, rectal swabs, delayed transport to the laboratory, specimens other than stool.
Specimen stability	Preserved specimen stable for up to 9 months at ambient conditions.
Reference Interval	No ova or parasites seen
Reportable Disease	Detection of *Cryptosporidium* spp. or *Cyclospora cayetanensis* is reportable.
Comments	Proto-fix vials available from Regional Pathology Service

This test is recommended for the examination of stool for the presence of *Cyclospora* or *Isospora* (*Cystoisospora*) by modified acid-fast stain. Recommended testing for *Cryptosporidium* is by use of the *Cryptosporidium* Antigen EIA test. Revised 07/02/2013

References:

1. CDC – Parasites - Cyclosporiasis - Outbreaks - Investigation of an Outbreak of Cyclosporiasis in the United States. http://www.cdc.gov/parasites/cyclosporiasis/outbreaks/investigation-2013.html. Last accessed 08/16/2013.
2. Nebraska Department of Health and Human Services. Emerging Health Issues – Nebraska *Cyclospora* Outbreak. http://dhhs.ne.gov/publichealth/EPI/Pages/EHICyclospora.aspx. Last accessed 08/16/2013.
3. CDC –Parasites – Cyclosporiasis. http://www.cdc.gov/parasites/cyclosporiasis/. Last accessed 08/16/2013.

Changes in LRN Sentinel Lab Protocols

by Karen Stiles SM(ASCP)[CM], State Training Coordinator NPHL

A document which articulated the role of the sentinel laboratory in the Laboratory Response Network (LRN) was recently approved by multiple agencies. It included representatives from state and local public health laboratories, the Centers for Disease Control and Prevention (CDC), Association of Public Health Laboratories (APHL), American Society for Microbiology (ASM), and the American Society for Clinical Pathology (ASCP)[1]. This document also outlined the responsibilities of the LRN Reference Public Health Laboratories (state health laboratory) in support of the sentinel clinical lab. Environmental, food, veterinary, agriculture, military, public health, as well as clinical laboratories have the potential to encounter samples that may contain agents which threaten the public's health and are often the first to interface with physicians and the public health system.

The role of the sentinel laboratory was defined as "a laboratory certified to perform high complexity testing under the Clinical Laboratory Improvement Amendments (CLIA)[1]" which provides in-house testing including Gram stains and at least one of the following: lower respiratory tract, wound or blood cultures. If your laboratory fits this definition, you play a vital role in the LRN network. To be prepared for this role, the following are requirements of the laboratory:

1. Be familiar with reportable disease guidelines within your state and county health departments,
2. Maintain capability to perform testing outlined in the *ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases* (available on the ASM or NPHL websites). This would include phenotypic tests for oxidase, catalase, beta-lactamase, indole, urea, and motility. Supplies to perform these tests are provided by NPHL at no cost.
3. Demonstrate annual competency by participation in proficiency testing or exercises, such as the CAP Laboratory Preparedness Exercise (LPX) or state-developed challenge sets.
4. Have policies and procedures in place to refer diagnostic specimens or isolates suspected to contain hazardous agents to the state public health laboratory,
5. Ensure personnel have met the applicable federal regulations for packaging and shipping of infectious substances,
6. Have a Class II or higher certified biosafety cabinet, complies with Biosafety Level II (BSL-2) practices and
7. Follow Occupational Safety and Health Administration (OSHA) regulations for respiratory protection.

To assist the sentinel clinical laboratory in meeting the above definition, the NPHL will:

1. Maintain a database of the sentinel laboratories, including emergency contact numbers and testing capabilities.
2. Provide training to encourage sentinel labs to maintain a knowledgeable staff in the LRN protocol (to include recognition, rule-out or referral of hazardous agents, packaging & shipping, chain of custody and biosafety and risk assessment).
3. Utilize real events or develop exercises to assess functionality of the sentinel lab to refer samples to the state,
4. Provide 24/7 availability of information and technical consultations, as well as necessary confirmatory testing to the sentinel laboratories and
5. Ensure a robust electronic system for communication or routine and emergency alerts and critical information to all sentinel labs.

The LRN continues to evolve, but faces challenges of diminishing resources and expanding expectations to mitigate the consequences of emerging infectious diseases. Meeting this demand requires enhancing partnerships between private and public health communities, with a greater emphasis on reportable diseases at the state and local levels. Clinical laboratories continue to be an integral part of the LRN, and their engagement as active partners is a top priority of each states public health LRN Reference Laboratory in partnership with the CDC. Building and maintaining strong relationships with the sentinel laboratories is crucial to accomplishing the primary function of the LRN: rapid detection and reporting of biothreat and hazardous organisms. Without the prompt rule-out or referral by the sentinel laboratory, the rapid identification and response to potential biothreat agents would be jeopardized.

LRN protocols are found on the ASM website under the policy tab: http://www.asm.org/index.php/guidelines/sentinel-guidelines . They are also linked on our NPHL website under the biological preparedness tab: http://nphl.org/bioTerror.cfm .

In coordination with the CDC and APHL, the ASM has recently updated their protocols designed to offer standardized, practical methods and techniques to rule out suspected hazardous microorganisms, or refer to the public health laboratory. More importantly, they empathize maintaining the highest level of safety practices when working with suspected agents. The following revisions and/or additions are contained within these updated protocols:

1. DO NOT USE AUTOMATED OR KIT-Based Systems for identification and susceptibility if slow growing, tiny Gram negative rods/coccobacilli or diplococci fail to grow on MacConkey agar.
2. Guidelines for handling specimens are updated for each agent with greater emphasis on specimen type.
3. Guidance regarding Chain-of Custody compliance in the event that a biothreat or emerging infectious agent occurs.
4. Guidance is included for safe and effective destruction of specimen materials and organisms.
5. Testing of *Francisella, Brucella, Burkholderia, Bacillus anthracis* and *Yersinia pestis* must be performed using a BSL-2 lab with BSL-3 precautions. Because these may be detected in blood cultures, the same precautions should be used when working up positive cultures.
6. The Safety/Biosafety section of each protocol empathizes the use of the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

NPHL has concurrently updated the *Bench Guide for Hazardous Pathogens*. The new version and updated Bioterrorism and Chemical Terrorism posters will be mailed to all Nebraska sentinel laboratories this fall. LRN training onsite is available upon request. Contact Karen Stiles at kstiles@unmc.edu for further information.

Reference:

1. CDC and APHL, Sentinel Clinical Laboratories, October 2012

Salmonella serotyping at NPHL

by Andrea Brochman-Williams MS, Laboratory Specialist, Microbiology/Molecular

NPHL began using a new molecular assay to serotype *Salmonella* in January 2013. Previously, *Salmonella* serotyping was performed using agglutination assays to detect for specific O antigens (lipopolysaccharide) and H antigens (flagella) using approximately 200 different antisera produced by the CDC. The cost and time limitations for making and maintaining all the antisera necessary for serotyping, no longer makes this technique the most useful option.

The molecular test is based on Luminex technology, a multiplex, bead-based suspension array which allows for the detection of up to 100 different molecular targets in a single reaction. Briefly, PCR is performed on each isolate using biotinylated primers. The denatured amplicons are then hybridized to bead sets that are covalently labeled with antigen-specific probes. Each bead is labeled with a different proportion of red and infrared fluorophores resulting in a unique spectral address that allows the instrument to determine which antigen-specific probe is attached to the bead. After streptavidin is added, each bead is read by two different lasers in the Luminex instrument, which is similar to a flow cytometer. The classification laser will excite the beads, determining which antigen-specific bead is being read and then the reporter laser will read the fluorescent reaction between the biotin-labeled PCR fragments that have hybridized to the bead.

Benefits for using the molecular Luminex Assay include increased turn-around-time (the molecular assay can be performed in one day) and increased sensitivity (ability to identify isolates that can't be fully identified by conventional agglutination methods).

Serotyping continues to be used in conjunction with DNA fingerprinting (using pulsed field gel electrophoresis) to monitor *Salmonella* outbreaks across Nebraska. Since January, the following outbreaks have occurred in our state:

***Salmonella* Outbreaks in Nebraska 2013**

Serotype	# of NE Cases	# of National Cases	Source
Typhimurium	14	358	Baby chicks and poultry
Enteritidis	4	549	Eggs (as possible source)
Heidelberg	6	240	Source Unknown
Newport	11	94	Sushi (as possible source)
Infantis	3	41	Source Unknown

NEED TO CONTACT NPHL?

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24/7 Pager: 402-888-5588
http://www.NPHL.org

Training/Education
402-559-3590

Client Services
866-290-1406 (Toll Free)
402-559-2440

NPHL
Upcoming 2014 Events

Packaging/Shipping
Division 6.2
March 18 - Kearney NE
March 20 - NPHL Omaha NE

Chemical Laboratory Response Network (LRN-C):
Hospital Training Guidance
Tabletop Exercise
TBA

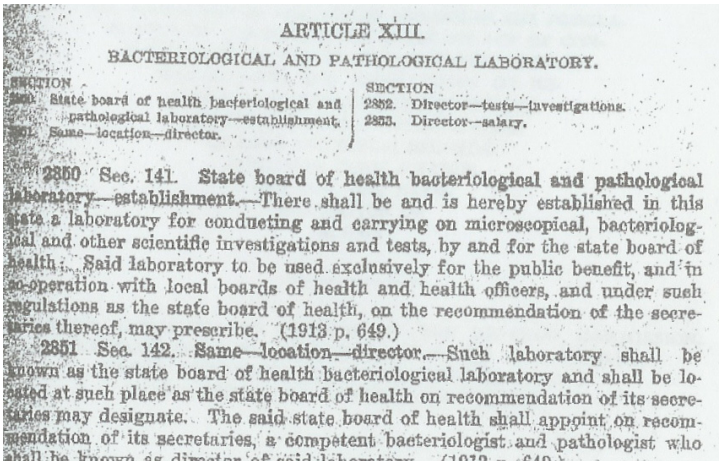
The State Public Health Laboratory in Nebraska: The First Hundred Years

by Karen Stiles SM(ASCP)CM
State Training Coordinator NPHL

The year 2013 has been highlighted with the 100th Anniversary celebration of the Nebraska Public Health Laboratory. A reception was held in the Durham Research Center in April, as part of the celebration for National Laboratory Week. This achievement was also recognized at the Annual Center for Preparedness Symposiums held in Gering, Norfolk, Lincoln and Kearney. Through the years, the NPHL mission statement has been "Dedicated to protecting the health and safety of Nebraskans through diagnostic laboratory science, technology and education."

Dr. Peter Iwen, Dr. Steven Hinrichs and Anthony Sambol

Kristin Watkins, administrator in Regenerative Medicine, compiled historical documents to write the history of our public health laboratory. Dr. Iwen presented a timeline of events that brought the laboratory to what it is today. In 1909, there was mention of a "state laboratory" at UNL in the memoirs of Dr. Francis Long. Shortly after, Omaha and Lincoln experienced a typhoid fever outbreak. In Feb 1913, a "Health Train" promotion arrived in Lincoln, while local and state public health officials held a joint meeting. By April 1913, the "Bacteriological Laboratory" was approved.



ARTICLE XIII.

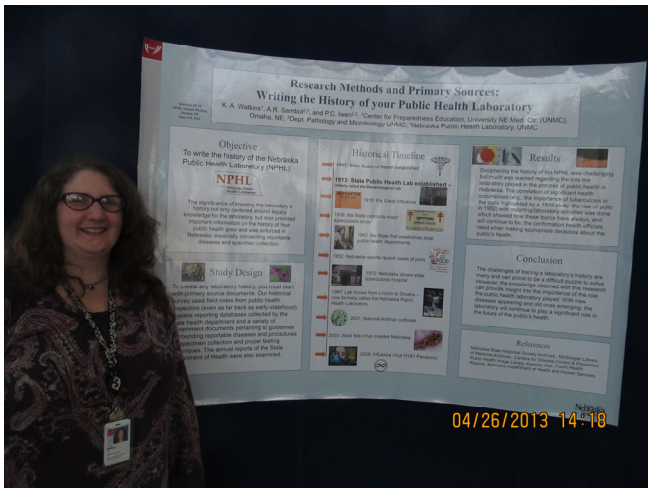
BACTERIOLOGICAL AND PATHOLOGICAL LABORATORY.

SECTION
2850. State board of health bacteriological and pathological laboratory—establishment.
2851. Same—location—director.

SECTION
2852. Director—tests—investigations.
2853. Director—salary.

2850 Sec. 141. State board of health bacteriological and pathological laboratory—establishment.—There shall be and is hereby established in this state a laboratory for conducting and carrying on microscopical, bacteriological and other scientific investigations and tests, by and for the state board of health. Said laboratory to be used exclusively for the public benefit, and in co-operation with local boards of health and health officers, and under such regulations as the state board of health, on the recommendation of the secretaries thereof, may prescribe. (1913 p. 649.)

2851 Sec. 142. Same—location—director.—Such laboratory shall be known as the state board of health bacteriological laboratory and shall be located at such place as the state board of health on recommendation of its secretaries may designate. The said state board of health shall appoint on recommendation of its secretaries, a competent bacteriologist and pathologist who



Kristin Watkins Historical Timeline

1891: State Board of Health established
1913: State public Health Lab established - initially called the Bacteriological Lab
1918: the Great Influenza
1939: the State conducts major tuberculosis study
1943: the State first establishes local health departments
1952: Nebraska reports record cases of polio
1972: Nebraska closes state tuberculosis hospital
1997: Lab moves from Lincoln to Omaha - established the Nebraska Public Health Laboratory
2001: National anthrax outbreak
2003: West Nile virus invades Nebraska
2009: Influenza A virus H1N1 Pandemic
2013: Cyclosporiasis outbreak

Kristin's objective in writing the history of the NPHL was "not only centered around legacy knowledge for the laboratory, but also to provide important information of the history of how the public health laboratory grew and was enforced in Nebraska, especially concerning reportable diseases and specimen collection." She felt that tracing the laboratory's history was challenging. However, the knowledge obtained with her research provides insight into the importance of the role the laboratory played in the health of Nebraskans. With new diseases appearing and old ones emerging, the laboratory will continue to play a significant role in the future.

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IN THIS ISSUE

NPHL Updates

Cyclosporiasis Outbreak 2013

Changes in LRN Sentinel Laboratory Protocols

Salmonella Serotyping at NPHL

The State PHL in Nebraska: The First Hundred Years

The Nebraska Public Health Laboratory Newsletter is a publication of the Department of Pathology and Microbiology, Steven H. Hinrichs, MD, Professor and Chairman, at the University of Nebraska Medical Center. The views expressed here do not necessarily reflect the opinions of the Nebraska Department of Health and Human Services or the University of Nebraska Medical Center.

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