# Nebraska Public Health Laboratory Newsletter

A publication of the Nebraska Public Health Laboratory (NPHL) at the University of Nebraska Medical Center.

FALL **www.nphl.org** **1-866-290-1406** 2004

## Raising the Bar for Diagnostic Capabilities

*By Steven Hinrichs M.D., Director, NPHL*

In this issue, readers will again find strong arguments for the need to continually improve the diagnostic capability of laboratories throughout the state as well as improve the communication between the public and the private sector. At the same time that public health and the private sector are facing yet another challenging infectious disease (see the article regarding *Bordetella pertussis* by Catherine Gebhart, Ph.D., the state has also encountered its first case of Vancomycin-Intermediate *Staphylococcus epidermidis* (VISE). Both of these examples illustrate the significant challenges facing front-line diagnostic laboratories. In the case of *B. pertussis*, although new technologies have increased the capability for accurate diagnosis, the technology has not been streamlined for application to most laboratories. In the case of VISE, laboratorians must be constantly on the lookout for new patterns of antibiotic resistance.

We have received an analysis of laboratory preparedness for emerging infectious diseases that was conducted for the Centers for Disease Control and Prevention by the Battelle Corporation. In a written survey with follow up by phone call, the investigators from Battelle asked laboratory managers and technologists around the state to several questions regarding their level of training and interactions with the Nebraska Public Health Laboratory (NPHL). The report indicated that not only do a majority of private labs know about NPHL, but they have received training or educational materials within the past year that improved the knowledge base and ability to meet the challenges of their daily work.

## *Bordetella pertussis*

*By Catherine Gebhart, Ph.D., Technical Director, Molecular Diagnostics Laboratory, UNMC*

*Bordetella pertussis* is the cause of one of the most contagious human diseases known as whooping cough. *B. pertussis* causes severe coughing spells, with a characteristic "whoop" made as the affected person struggles to breathe through narrowed airway passages between coughing spasms. *B. pertussis* is a small gram-negative aerobic coccobacillus that colonizes the cilia of the nose and throat of infected humans. Toxins produced by *B. pertussis* paralyze the cilia and cause inflammation of the respiratory tract, interfering with the clearance of pulmonary secretions. This disease was first described in the 16$^{th}$ century and was one of the most frequent and severe diseases in infants in the United States, commonly resulting in morbidity and mortality among children prior to introduction of an effective vaccine. The incidence decreased dramatically following the introduction of the vaccine; however, incidence has been gradually increasing since the early 1980's.

Who is most at risk?

Pertussis can occur at any age; however infants and young children, particularly those who are not immunized, are at the highest risk for severe disease and potentially life-threatening complications. The infection is most perilous in babies less than 6 months of age. At this young age, the coughing episodes may leave them not only breathless but without oxygen. Infants are also at high risk for secondary bacterial pneumonia. Additionally, infants are at risk for neurologic complications such as seizures and encephalopathy as a result of hypoxia from coughing or possibly from the bacterial toxins.

What are the symptoms?

The clinical symptoms vary significantly depending on the age and the immune status of the affected person. Babies and unimmunized children will generally exhibit classical symptoms, while in otherwise healthy adolescents, adults and vaccinated children, symptoms may be indistinguishable from other common respiratory infections. The incubation period of pertussis for all age groups is generally 7 to 10 days, with a range of 4 to 21 days. During the first and most contagious stage patients exhibit a runny nose, sneezing, low-grade fever, and a mild occasional cough similar to the common cold. In infants and children, the cough gradually becomes more severe over a 1-2 week period, until the classical "whooping cough" becomes apparent. This occurs when thick, sticky mucus develops in the windpipe. Characteristically, the patient has bursts of numerous, rapid coughs, usually followed by a long inspiratory effort accompanied by a characteristic high-pitched whoop. The whoop may not occur in infants younger than 6 months of age due to lack of strength. During such an attack, the patient may become cyanotic (turn blue). Infants and children often appear very ill and distressed at this stage, commonly displaying vomiting and exhaustion following coughing episodes; however, they may appear normal between attacks. These attacks, which occur more frequently at night, usually produce thick, glue-like mucus, which makes it difficult for infants and children to eat, drink and breathe. Symptoms in this stage generally persist for 1 to 6 weeks and then gradually decrease.

How is the disease spread?

Transmission of pertussis is most commonly person-to-person via airborne droplets of respiratory secretions and in rare instances by contact with freshly contaminated articles. Transmission rates may increase in summer and fall, but does not follow a distinct seasonal pattern. Schools and day care centers are common sources of infection for older children and adolescents. Parents, caregivers, and older siblings are important reservoirs for *B. pertussis* because their symptoms are generally mild and members of these groups may not seek medical treatment; however, their disease is still highly contagious allowing for continued

*(B. pertussis, Continued on page 2)*

*(B. pertussis Continued from page 1)*
transmission of the disease to infants and young children.

*B. pertussis* vaccine

The vaccine for whooping cough is given initially at 2, 4, 6, and 12-18 months of age. Children are routinely vaccinated again between 4-6 years of age. Individuals who have not received the complete vaccine series of five doses are most susceptible to the disease. Immunity resulting from vaccination seems to weaken after 5-10 years. Therefore, recommendations for vaccination of teenagers and adults will likely come in the near future in order to decrease the non-symptomatic carrier pool.

How is *B. pertussis* detected?

There are three approaches to test for *B. pertussis* in the laboratory, including direct fluorescent antibody (DFA), culture, and polymerase chain reaction (PCR). DFA was useful in the past as a rapid screening tool, however, it is no longer recommended as a screening test due to its low sensitivity, however, DFA is still valuable in the laboratory to confirm the identify of cultured isolates. Isolation of the organism by culture has been the standard test for confirmation of diagnosis, and is essential if evaluation of antimicrobial resistance or molecular typing is required. However, successful isolation of the organism declines significantly beyond the first 2 weeks of illness and with prior antibiotic therapy. Furthermore, the organism is very fastidious, requiring 3-6 days to form detectable colonies by culture and often will not grow if cultures are not initiated within 48 hours of sample collection. In addition, a swab containing charcoal is also recommended in addition to plating to appropriate media immediately after collection. To address these limitations, molecular tests have been developed. Primer specific amplification, also known as PCR testing of nasopharyngeal swabs or aspirates is a rapid, sensitive, and specific method for diagnosing pertussis that does not require live organism. This assay is currently available in most reference laboratories and may also be obtained through the Nebraska Public Health Laboratory. While PCR is the recommended test of choice, it does not provide material for additional epidemiologic studies and therefore it is important that culture is attempted on suspected cases of *B. pertussis* when possible.

Treatment

When *B. pertussis* is suspected, recommended antibiotic therapy includes azithromycin for 5 days or either erythromycin or trimethoprim-sulfamethoxazole for 14 days. Depending on the situations, household members and other close personal contacts of known positive patients may require appropriate therapy including a course of antibiotics, even if they are not symptomatic. More information about *B. pertussis* can be found by going to the Centers for Disease Control and Prevention's website (http://www.cdc.gov/health/pertussis.htm).

## Veterinarian Joins NHHSS

*By Josh Rowland, MBA, MT(ASCP), State Training Coordinator, NPHL*

*This is a continuation of the NPHL newsletter article series that introduces new members of the Nebraska Health and Human Services System (NHHSS) to the state laboratory community. These people will often be key contact people for organizations and testing programs they represent.*

In recognition of the importance of zoonotic infections and their risk to both humans and the Nebraska agricultural economy, the NHHSS established a new position of State Public Health Veterinarian and recruited Annette Bredthauer, Doctor of

*(Vet, Continued on page 5)*

## *Training Opportunities from Your Computer* - Introducing NE.TRAIN

*By Sue Raymond, Librarian, Nebraska Center for Bioterrorism Education*

A Learning Management System (LMS) has recently been made available through the Nebraska Center for Bioterrorism Education (NCBE) known as Nebraska's TrainingFinder Real-time Affiliate Integrated Network (NE.TRAIN). NE.TRAIN is part of a national effort to provide and track training opportunities for health care professionals. Subject areas include public health, disaster, emergency management, bioterrorism, and emergency response information for audiences including laboratorians, public health professionals, first responders, and emergency personnel. The LMS is set up so that each participant has a unique profile so they can keep track their progress and certificates of completion.

Although the majority of the courses are offered free of charge, some courses offering Continuing Educational Units (CEU's) or university credit may require payment. NE.TRAIN is a searchable repository of educational material, many with an emphasis on public health. An example of a search of "laboratory professionals" would generate articles such as: The Role of the Public Health Laboratory in Preparedness from the Iowa Center for Public Health Preparedness and Arboviral Infections: Focus on West Nile Virus.

Many organizations in Nebraska, as well as other states participating in TRAIN, are developing unique course which will be accessible using NE.TRAIN.

For questions, please contact Sue Raymond, NE.TRAIN Administrator, at 402-552-3091. You can access NE.TRAIN by going to http://necenterforbted.org and selecting NE.TRAIN from the menu. Feel free to peruse the Nebraska Center for Bioterrorism Education's web page for additional training opportunities.

## Update from NPHL's Chemical Terrorism Preparedness Laboratory

*By Dana El-Hajjar, MBA, Chemical Terrorism Preparedness Technologist, NPHL*

The Centers for Disease Control and Prevention (CDC) has continued to emphasize the importance of developing capability for meeting the many challenges of chemical terrorism. Through the chemical terrorism preparedness program, with the Nebraska Health and Human Services System at the State of Nebraska, the NPHL initiated testing protocols for chemicals of primary concern.

The Chemical Terrorism Preparedness Laboratory (CTPL) at the NPHL recently completed validations for heavy metals in urine and for cyanide metabolites in whole blood.

Validations for these tests were certified by the CDC following a testing challenge process. The CTPL was certified as a Level-2 facility for testing two sets of analytes. For this program, blood cyanide will be measured by gas chromatography-mass spectrometry (GC-MS), whereas urine metals will be measured by inductively-coupled plasma mass spectrometry (ICP-MS). The next focus of effort will be on meeting the requirements for testing of nerve agent metabolites in urine.

Questions about the CTPL can be directed to Dr. Doug Stickle at 402-559-8785 or dstickle@unmc.edu. Dana El-Hajjar may also be contacted if you have questions (402-559-9421 or delhajja@unmc.edu).

## The Waxing and Waning of West Nile Virus Activity in Nebraska … A Tale of Two Seasons

*By Tony Sambol, MA, SM, Assistant Director, NPHL*

In 2003, Nebraska experienced a true epidemic with the occurrence of significant disease caused by the West Nile Virus (WNV). The 2003 season started with the first human case being reported in July. Records kept for 2003 by the Nebraska Health and Human Services System (NHHSS) indicate that an unprecedented number of humans, sentinel chickens, mosquito pools, and wild birds tested positive for WNV (see **Table 1** for 2003 and 2004 data). Antibody testing for WNV IgM was performed at the Nebraska Public Health Laboratory (NPHL), on 10,887 specimens obtained between August 1 and October 31, 2003. Of the specimens tested, 2,177 (20%) were determined to be positive. During this time frame, 29 human deaths were reported resulting in the 3rd highest number of deaths, following only Colorado (61) and Texas (37), both states with much higher populations.

In 2004 the first case was again identified in July, however, WNV activity was markedly decreased in all areas of the state in comparison with 2003 as indicated by surveillance testing and the reporting of human cases. As of November 2004, the NPHL had tested over 2,200 patients for WNV IgM antibody, with approximately 120 patients being IgM positive. Additionally, several hospital laboratories in Nebraska offered WNV IgM testing. Epidemiologists at NHHSS have confirmed 49 human cases occurring statewide (see **Figure 1** from the NHHSS) as being attributed to recent WNV infection.

The waxing and waning of WNV activity that Nebraska has experienced over the last two years has been reported throughout the country as affected states pass through their second and third seasons. One explanation given for the decreased activity was the average temperature coupled with a cooler, wetter summer occurring throughout the state. It is also possible that immunity of the bird, and human populations has also increased. In comparison, the states of Arizona and California encountered significant WNV activity in 2004.

It is expected that WNV activity will not be as high in 2005 as it was in 2003, however, if hot, dry conditions were to occur, the state might experience an increase in WNV activity. Time will reveal the answer. Questions about WNV testing at the NPHL can be directed to Tony Sambol, 402-559-3032.

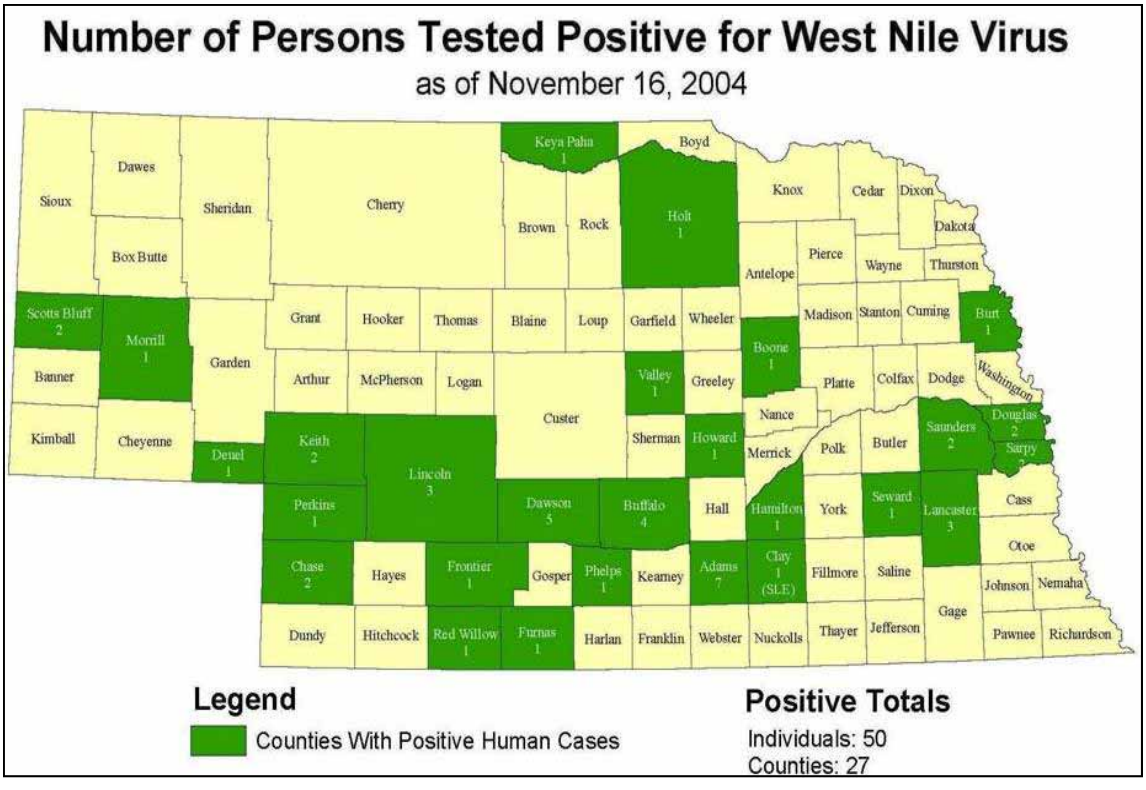
**Table 1**

| Comparison of WNV Activity in Nebraska | | | | | | |
|---|---|---|---|---|---|---|
| **Category** | **2003** | | | **2004*** | | |
| **Human Deaths** | 29 | | | 0 | | |
| **Positive Blood Donors** | 189 | | | 3 | | |
| | **Tested** | **Positive** | **Positivity Rate** | **Tested** | **Positive** | **Positivity Rate** |
| **Human Cases** | 10,887 | 2,177 | 20% | ** | 49, 1 SLE | N/A |
| **Mosquito Pools** | 2,699 | 936 | 35% | 3,299 | 128 | 3.8% |
| **Sentinel Chickens** | 100 | 81 | 81% | 116 | 48, 1 SLE | 42% |
| **Dead Birds** | 1,030 | 576 | 56% | 372 | 92 | 25% |

* Data from NHHSS website, through 11/16/04

** Refer to article for the number tested

**Figure 1**

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## Meet the Laboratorian - Major Lucia More

*Compiled by Josh Rowland, MBA, MT(ASCP), State Training Coordinator, NPHL*

Major Lucia More is the Laboratory Director of Erhling-Bergquist Hospital at the Offutt Air Force Base. Her career provides an exciting example of the many opportunities for laboratory specialists. She has been involved in a wide variety of activities in the United State Air Force (USAF) including a stint as the laboratory consultant for all USAF laboratories in Europe (one in Turkey, two in Italy, three in England, and four in Germany). She was in that position on "9/11" and was subsequently heavily involved in providing support for bioterrorism threats at all those bases.

**What got you interested in pursuing a career in laboratory science**?

I was interested in biochemistry but wanted a job that would pay well enough for me to support my family if necessary. The information about Southwest Minnesota State's Medical Technology program sounded like it would be interesting as well as provide a good salary. Little did I know where that instinct would lead me.

**Where did you attend medical technology school?**

The college courses were at Southwest Minnesota State College in Marshall, MN (now Southwest State University) and I did a 12 month internship at Sioux Valley Hospital in Sioux Falls, SD. I graduated in July 1976; I can't believe it's been 28 years already!

**What do you like most about your job?**

I learn new things every day. As a clinical laboratory scientist I get to learn about the new advances in laboratory medicine such as in coagulation testing. As the laboratory manager I evaluate new methods and improvements and determine whether I can implement them in my lab. I feel like I can make my little corner of the world a better place every day.

**How does the laboratory at Offutt AFB support the U.S. military and the citizens of Nebraska?**

We provide routine clinical laboratory services to active duty and retired military personnel and their families who live in this area. We are a Level-A (Sentinel) Laboratory in the CDC's Laboratory Response Network (LRN). We work closely with NPHL to prepare for a local bioterrorism attack to handle specimen processing. We are also a Homeland Defense Laboratory for the USAF and are equipped with instrumentation to identify potential agents of bioterrorism.

**What is unique about a career in the military?**

Laboratorians with bachelor's degrees in clinical laboratory science enter the USAF and are placed into management positions. Their first assignment is usually at a larger facility where there are multiple laboratory officers (laboratory managers in civilian terms); giving them time to learn how the military works and how to be a manager from a more senior laboratory officer.

Enlisted technicians are trained by the USAF after basic training. Trainees selected for the laboratory course are the highest scorers on the entry exams. After six weeks of basic training they attend a 17 week Phase I didactic course. Phase II takes place in a medical treatment facility that has inpatient beds and is a nine month course of classes and on-the-job training. After graduation the students receive an assignment to another laboratory. After one year of experience they qualify to take the MLT exam to receive certification as a two year degreed technician.

**What is the biggest challenge you face in your job today?**

We experience all the same things civilian labs do: completing the workload in the most efficient manner possible, determining how to get the test done most economically in-house or at a referral lab, maintaining regulatory requirements, complying with CAP/FDA/JCAHO standards, maintaining performance improvement programs, quality assurance programs…all the things a civilian lab does. In addition to our "peacetime" mission, we have a "wartime" mission: maintaining our training for possible deployment to an expeditionary medical facility on the other side of the world. We have an annual two day medical readiness training in the field in which we set up medical tents and practice what we do when deployed. We carry patients on litters, decontaminate the patients, and treat their "injuries" all while wearing our chemical warfare gear (including gas masks). We learn security procedures so we can protect ourselves in a hostile environment. We are all qualified to fire either a rifle or a handgun. We also are required to maintain a healthy weight and fitness level and are tested annually. Part of our job is the awareness that we may be told to go somewhere hot and sandy for an extended period of time without our family. Although this sounds crazy, it's an adventure. There are many benefits to being in the military including medical benefits, educational benefits, and if you make it a career, a retirement pension.

**What are the biggest changes you have seen in the lab since you started?**

The explosion in development of testing for biological agents. The military was already working on sturdy or hardened portable PCR machines for use in the field when the September 11$^{th}$ terrorist attack took place. Since then, advances have escalated in developing hand-held "point-of-care" type instruments to identify chemical and biological agents for first responders.

**What would be your advice to a first-year med tech?**

Consider a career in the military. You can be a laboratory officer in the Air Force, Army, or Navy. If you have good people skills, are organized, can work well with others, like structure, don't mind wearing a uniform, and enjoy staying fit, this is the job for you. If you've been frustrated by the limitations to advancement within a civilian hospital, the military will give you more responsibility and opportunity to grow than you can imagine. The most difficult decision is whether you can live with moving every three or four years. You must learn to adapt to any environment, to find things to do in the area you live and enjoy it. A positive attitude is a must!

I entered the USAF 15 years ago because I wanted job security and no one else out there was offering the pay and benefit package that the USAF was. I was 35 years old and had three young children. I only had to commit to three years; I figured if I didn't like it, I'd leave after that. Staying was the best decision I've ever made. Fifteen years later I've had the pleasure of living in New York, Minnesota, Texas, Germany, and now Nebraska. The Air Force sent me back to college to get a master's degree, and paid my tuition, books, and usual salary. It's been a great adventure!

## Vancomycin-Intermediate *Staphylococcus epidermidis* (VISE) Isolated in Omaha

*By Nancy Cornish, M.D., Director of Microbiology, Nebraska Methodist Hospital, Kim Hemrick, MT(ASCP), Microbiology Team Leader, Nebraska Methodist Hospital, and Paul D. Fey, Ph.D., Associate Director, NPHL*

Although there have been no reports of vancomycin-intermediate *Staphylococcus aureus* (VISA) or vancomycin-resistant *S. aureus* (VRSA) in Nebraska, microbiologists at Methodist Hospital in Omaha have recently isolated a vancomycin-intermediate *Staphylococcus epidermidis* (VISE). A summary of the case and the microbiologic evaluation is presented.

A fifty year old male on renal dialysis for chronic renal failure had two positive blood cultures for oxacillin-resistant coagulase-negative *Staphylococcus* spp. Identification was based on gram stain, positive catalase test, and a negative *S. aureus* agglutination test (Pastorex Staph). The patient had been successfully treated with vancomycin twice in the past year for infections due to gram positive organisms. The original susceptibility test, performed on the Vitek 2® system, revealed an minimum inhibitory concentration (MIC) value of 4 µg/ml to vancomycin. This MIC value was confirmed by plating the isolate on BHI agar containing 6 µg/ml vancomycin, on which the isolate grew after incubation for 24 hours. An E-test MIC of 6 µg/ml further confirmed the original susceptibility result. The isolate was then submitted to the NPHL for further testing and confirmation of the MIC value. At the NPHL, the isolate was identified as *S. epidermidis* through conventional means as well as primer specific amplification of seven known *S. epidermidis* housekeeping genes. Since repeated MIC testing (growth on BHI agar containing 6 µg/ml vancomycin and an E-test vancomycin MIC of 6 µg/ml) at the NPHL confirmed the MIC value obtained with the automated system, an efficiency of plating (EOP) test was performed on this isolate. This test, which was originally designed for heterotypic methicillin resistant *S. aureus* (MRSA), detects subpopulations of a broth culture that are highly resistant to a particular antibiotic (in this case vancomycin). EOP testing demonstrated a typical vancomycin-intermediate curve for the VISE isolate under study compared to two reference vancomycin-susceptible control *S. aureus* strains (VSSA) **(Figure 1)**.

Although the incidence and prevalence of VISE is not known in the United States, data from the Centers for Disease Control and Prevention suggests that their prevalence is much higher than VISA. Dr. Paul D. Fey at the NPHL has a particular interest in antibiotic resistance in *Staphylococcus* spp. and he can be reached at (402) 559-2122 for any questions regarding antimicrobial susceptibility.

*(Vet, Continued from page 2)*

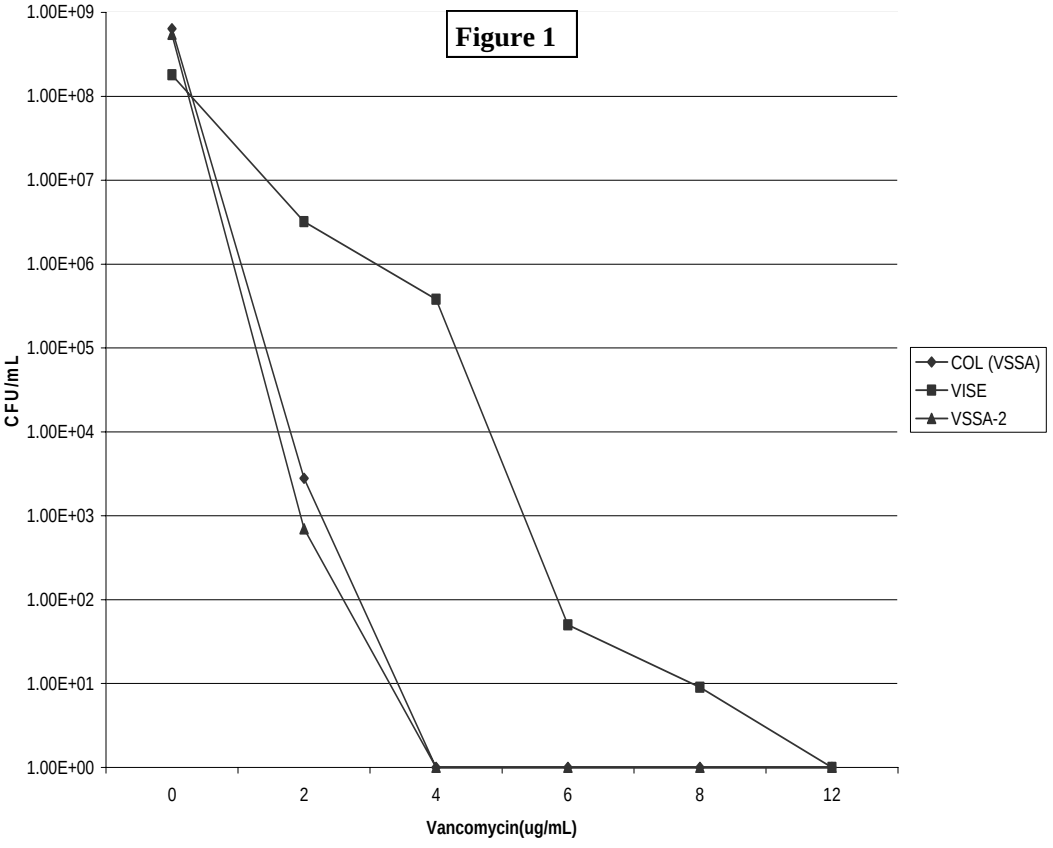
Veterinary Medicine (DVM) into this position as of December 2003.

Dr. Bredthauer grew up on her family's farm near Wood River, Nebraska. She attended Kansas State University and graduated with a DVM degree in 1982. Following graduation, Dr. Bredthauer worked in private practice treating both large and small animals for 18 years in north central Kansas and south central Nebraska. Dr. Bredthauer then accepted a position as a Veterinary Medical Officer for the United States Department of Agriculture Food Safety Inspection Service. While in this position, she worked in California for 2 months helping to eradicate Exotic Newcastle Disease, a foreign animal disease that affects birds.

In addition to being the Nebraska Public Health Veterinarian, Dr. Bredthauer will be the Rabies Control Coordinator for the state. The Rabies Control Coordinator will oversee rabies testing procedures and will work with the healthcare community and the public with continuing education programs. Other State Public Health Veterinarian duties include bioterrorism (especially agro-terrorism) issues, zoonotic diseases, and public health concerns involving animals. Dr. Bredthauer serves as the state's liaison with veterinarians, State and Federal governmental departments, livestock groups, and universities. She is also a member of two veterinary first responder groups: Nebraska Livestock Emergency Disease Response System (LEDRS) and the Kansas Veterinary Response Corps.

If your laboratory receives inquires regarding rabies testing or other animal/human related problems, these questions can be directed to Dr. Bredthauer's office at the NHHSS by phone (402-471-1374 or 402-429-6642).

**Figure 1**



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**-TOLL FREE NPHL CLIENT SERVICES NUMBER-**
**1-866-290-1406**

The Nebraska Public Health Laboratory Newsletter is a publication of the Department of Pathology and Microbiology, Samuel M. Cohen, M.D., Ph.D., Professor and Chairman, at the University of Nebraska Medical Center. The views expressed here do not necessarily reflect the opinions of the Nebraska Health and Human Services System or the University of Nebraska.

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