# Nebraska Public Health Laboratory Newsletter

A publication of the Nebraska Public Health Laboratory (NPHL) at the University of Nebraska Medical Center
Summer/Fall 1998

## Introduction:

This issue of our newsletter continues the theme of discussing seasonal diseases and includes the topic of tick-bome diseases. The role of the local or state laboratory irrtick-bome disease may well be limited, as a majority of specimens for Rocky Mountain Spotted Fever or Lyme disease are sent to reference laboratories for processing. However, as a source of education and information for the community, the laboratory plays a critical role in providing information to the public, as well as, local physicians. As discussed in the article by Thomas Safranek, M.D, State Epidemiologist, all cases of classic Lyme disease have been imported into Nebraska with the tick contact occurring in an adjacent state. The most common tick vector of *Borrelia burgdorferi*, the agent which causes Lyme disease, is *Ixodes scapularis*. This tick has not been detected in Nebraska. However, continued surveillance is important and the state entomologist, Dr. Wayne Kramer is willing to identfy any ticks associated with cases of suspected humann disease. You are also no doubt aware that Nebraska has now had a case of Hantavirus infection. To facilitate rapid testing for Hantavirus we have included phone numbers of key individuals who can assist hospitals or laboratories who need to submit specimens.

Steven Hinrichs, M.D.

## Tick-Borne Diseases

by Thomas Safranek, M.D.

Tick-borne illnesses of interest to Nebraska residents, healthcare providers and laboratorians include Rocky Mountain Spotted Fever, Lyme disease, ehrlichiosis, and tularemia. Only two tick species are believed to be associated with these diseases in Nebraska: *Dermacentor variabalis* and *Amblyomma americanum*. *Ixodes scapularis* (formerly *Ixodes dammini*) is the only known vector of *Borrelia burgdorferi* and *Ehrlichia phagocytophila*, the causative agents of Lyme disease and human granulocytic ehrlichiosis, respectively. This tick has never been detected in Nebraska.

### Rocky Mountain Spotted Fever (RMSF)

Persons with RMSF have been reported in Nebraska for many years. The Office of Epidemiology receives an avenge

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of 2 to 5 cases of such reports annually. Because it is an uncommon disease, health

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## Vancomycin-Resistant Enterococcus

by Peter C. Iwen, MS

The enterococci have emerged as major causes of nosocomial infections, recognized as the 3rd most common cause of bacteremia. This increase in infection is due in part to resistance to standard therapies, such as high level aminoglycosides and the beta-lactam antimicrobial agents--and more recently, to the glycopeptides, including vancomycin and the non-FDA approved agent, teicoplanin . Currently, there are no known effective antimicrobial Vancomycin-Resistant Enterococcus agents to treat infections caused by the vancomycin-resistant entorococci (VRE), with prevention and early detection the best approaches to control.

VRE can remain viable in the environment for an extended time period, and therefore poise a problem for infection control in hospitals and

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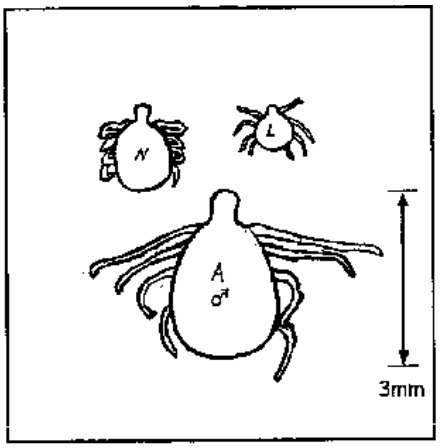


*Size comparison of **Amblyomma americanum**: Adult (A), Nymph (N) and Larval (L) forms.*

*One of the most important issues relative to ticks in Nebraska is the small size of the immature stages of **Amblyomma americanum** (lone-star ticks). This tick is found in southeastern Nebraska.*

*In the SE quarter of Nebraska, the lone-star tick can be locally abundant and it is common for people to come into contact with the immature stage (most commonly the nymphal stage) and not realize it is a tick because of the small size. Some individuals may call them deer ticks which is a term more accurately used in other parts of the country to refer to **Ixodes scapularis** (the most important vector of Lyme disease in the U.S) but that tick is not found in Nebraska. Lone-star tick nymphs and adults are active from April through September. Lone-star tick adults are similar in morphology but slightly smaller than the most common tick in Nebraska **(Dermacentor variabalis)***

***A. americanum** ticks do like to feed on deer, especially as nymphs and adults, and there may be some correlation between deer populations and tick populations in certain areas since deer are such abundant hosts.*

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care providers have a tendency to overlook this diagnosis. RMSF should be a diagnostic consideration in any person with a fever and a history of exposure to an environment where ticks might be encountered. The skin rash which gives this illness its name, is not universally present at the time of presentation to the physician. There are numerous reports in the literature where health care providers have missed diagnoses of RMSF with unfortunate patient outcomes. The laboratory diagnosis is traditionally made by detecting a rise in antibody titer to *Rickrettsia ricketsii* on acute and convalescent sera specimens. The organism can also be detected using fluorescent antibody methods applied to tissue (e.g., skin biopsy) specimens. The disease responds to tetracycline-like antibiotics and chloramphenicol. Treatrnent should be started empirically while awaiting diagnostic test results.

## Lyme Disease

Our knowledge of Lyme disease continues to develop following its first description as "pseudojuvenile rheumatoid arthritis" in young boys in Lyme, Connecticut. Lyme disease is now the most prevalent tick-borne disease in the United States and is caused by a spirochete called *Borrelia burgdorferi*. *B. burgdorferi* is transmitted by the tick *Ixodes scapularis* which has not been identified in Nebraska. This makes it doubtful that any one has ever acquired classic Lyme disease caused by *B. burgdorferi* from a Nebraska exposure.

Confusion about whether classic Lyme disease could be acquired in Nebraska occurred because of technical issues related to the diagnostic tests. There are two laboratory diagnostic approaches to confirm the diagnosis of Lyme disease: serologic tests looking for antibody to *B. burgdorferi*, and tissue culture or other antigen detection methods. There has never been a tissue culture or other antigenic confirmation of *B. burgdorferi* in a person who acquired Lyme disease in Nebraska. There have been Nebraskans whose serologic tests for Lyme disease have reported as positive. While some of these people reported a tick-borne exposure in regions of the country where classic Lyme disease is clearly established, many of these people had never left Nebraska. The positive Lyme disease serologies in this latter group of patients is attributed to the lack of specificity in the early versions of the test: the false positive rate was unacceptably high. These false-positive tests could have resulted from underlying medical conditions such as rheumatoid arthritis, or they may have reflected prior exposure to other spirochetal organisms sufficiently similar to *B. burgdorferi* to result in a cross reaction with the serologic test (e.g., leptospirosis, treponemal species, or to the presence of Borrelia species other than *B. burgodorferi*).

One notable example of such cross-reactivity is the recently recognized existence of a poorly charactarized *Borrelia*-like organism felt to be transmitted by *Amblyomma americanum*, often referred to as the Texas lone-star tick. This tick, which is quite common in southeast Nebraska has been reported to harbor a *Borrelia* species different from *B. burgdorferi.* Researchers are currently attempting to characterize this organism and its life cycle, and to develop tissue culture and serologic tests. Infection with this organism, tentatively assigned the name "*Borrelia lonestarri*", appears capable of causing a clinical syndrome similar to Lyme disease, including erythema migrans skin lesions. Though the full spectrum of its clinical manifestations is currenty poorly defined, persons infected with this organism appear to have less severe long-term sequelae compared to those infected with *B. burgdorferi*. Additionally, infection appears to respond to antibiotics used to treat classic Lyme disease.

The currently licensed serologic tests for Lyme disease include an initial ELISA procedure followed by a Westernblot for confirmation. The assays have greatly improved sensitivity and specificity in untreated patients tested two to three weeks following exposure. The extent to which current serologic tests may cross-react with *Borrelia* species other than *B. burgdorferi* (such as *B. lonestarri*) is currently under investigation.

## Ehrlichiosis

Ehrlichiosis is caused by an intracellular bacteria that grows within cytoplasmic phagosomes of white blood cells. Laboratorians should be familiar with the classic inclusion or monrlae in neutrophils and lymphocytes which suggests the diagnosis of ehrlichiosis. In many cases, review of the peripheral blood smear provides the clue sequelae to the diagnosis, which is confirmed by serologic testing or by molecular detection of *Ehrlichia* DNA.

The symptoms of this disease include a maculo-papular rash as well as fever, chills, and leukopenia. The illness may progress with hypotension, coagulopathy, hemorrhage of intemal organs and renal failure. Prior to 1986,

| TICK | DISTRIBUTION | ASSOCIATED ILLNESS | INFECTIOUS AGENT |
|---|---|---|---|
| *Dermacentor variabalis* (American dog tick or wood tick) | Statewide | Rocky Mountain Spotted Fever<br>Human monocytic ehrlichiosis<br>Tularemia | *Rickettsia ricketsii*<br>*Ehrlichia chaffeensis*<br>*Francisella tularensis* |
| *Amblyomma americanum* (Texas lone-star tick) | Southeast NE | Variant Lyme disease*<br>Human monocytic ehrlichiosis | "*Borrelia lonestarii*"<br>*Ehrlichia chafeensis* |
| Ixodes *scapularis* (Deer tick) | Has not been in Nebraska | Lyme disease<br>Human granulocytic ehrlichiosis | *Borrelia burgdorferi*<br>*Ehrlichia phagocytophila* |

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ehrlichiosis was recognized primarily as a disease affecting horses or dogs which is caused by organisms related to but distinct from *E. chaffeensis*.

Tracking of tick-borne disease is an important function of the state health department and questions may arise regarding the speciation of a tick found on humans or animals. Dr. Wayne Kramer, the State Medical Entomologist emphasizes the importance of continued surveillance. Dr. Kramer encourages the submission of tick specimens to his offrce for identification. This office provides a valuable resource for the identification of these ticks.

## Summary

Because native *B. burgdorferi* infection is not felt to occur in Nebraska, and because so little is known about variant Lyme disease believed to be transmitted by the *A. americanum* tick and caused by "*B. lonestarii* ", we currently recommend that a skin biopsy be obtained from any person with erythema migrans. Physicians and Tick-Borne Diseases laboratorians should contact either the State Entomologist (Dr. Wayne Kramer), the State Epidemiologist (Dr. Thomas Safranek, 402-471- 0550), or the NPHL Director (Dr. Steven H. Hinrichs, 402-559-8301) at the time of diagnosis, and make arrangements for the collection and processing of the skin biopsy. Similar arrangements should be made if other tissue thought to harbor *Borrelia* organisms, are collected/ biopsied (e.g., joint tissue, cerebralspinal fluid, etc). The CDC will test the specimens at no cost to the physician or patient in an attempt to identify a specific causative agent.

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**Vancomycin-Resistant Enterococcus**

nursing homes. In addition, these enterococci have been detected as part of the enteric flora in non-symptomatic patients. These colonized patients serve as potential sources for transfer of this organism to other patients and medical personnel.

## Classification

Currently, 14 species of enterooocci have been recovered from humans. *Enterococcus faecalis* accounts for 80 to 90% of enterococcal infections from all sources, with *E. faecium* responsible for a majority of the rest. The number of other species is generally less than 5%, although this may be higher, since methods to identify enterococci other than *E. faecalis* and *E. faecium*, are not widely used by clinical laboratories. In a study conducted at University Hospital evaluating enterococcal isolates recovered from blood cultures over eight years, *E. faecalis* was responsible for 68.5%, *E. faecium* for 26.2%, and the other enterococci for 5.3% **(Table 1)**. In this study, resistance was most evident with *E.faecium*, which was also responsible for all cases of vancomycin resistance. Nationally, resistance to vancomycin also occurs most frequently with *E.faecium*, even though other species of enterococci have become resistant.

Intrinsic low-level vancomycin resistamce occurs with *E. casseliflavus* and *E. gallinarum*, generally the most common *nonfaecalis/faecium* enterococcal species detected. These VRE species are found as normal stool flora and are not usually considered clinically significant even though sporadic blood stream infections have been detected in severely immunocompromised patients.

Recently, a phenotypic classification system was devised to categorize the VRE into three groups: *van*A strains, which show high-level vancomycin resistance (minimum inhibitory concentrations [MIC] of >32 mcg per ml) and resistance to teicoplanin; *van*B strains, which have variable resistance to vancomycin (MICs of 4 to >128 mcg per ml) and susceptibility to teicoplanin; and *van*C strains, which show intrinsic resistance to low-levels of vancomycin (MICs of 2 to l6 mcg per ml) and susceptibility to teicoplanin. These *van*C enterococci which include *E. casseliflavus* and *E. gallinarum* can be differentiated from other enterococci since they are usually positive for motility. It is important for the laboratorian to distinguish these motile species from the other enterococci which show high-level vancomycin resistance, since the former are not considered an epidemiological threat for nosocomial transfer and are usually susceptible susceptible to standard therapies.

## Laboratory identification

VRE at the Nebraska Public Health Laboratory (NPHL) are generally detected by routine "Aerobic Culture" of a normally sterile body site, by a "VRE Culture Screen", or as an incidental finding during the culture of stool for "Enteric Pathogens". Gram positive cocci with atypical macroscopic appearance, which are catalase-negative and spot pyrrolidonyl arylamidase- (PYR) positive are suspected *Enterococcus* species. Also, growth of an isolate with these characteristics on CVA medium, which is a selective medium used in an enteric pathogen screen culture to detect *Campylobacter* in stool, should be considered suspicious for the presence of VRE. This medium supports the growth of enterococci and contains vancomycin in a concentration adequate to screen for resistance.

Suspected enterococcal isolates considered clinically significant or isolates which grow on CVA medium are subsequently tested by biochemicals for identification and by tests for susceptibility to, high-levels of gentamicin, high-levels of streptomycin, and ampicillin. Additionally, an agar dilution test - containing vancomycin is also inoculated as an initial screen for vancomycin resistance. Isolates identified as *Enterococcus* species which grow in the presence of vancomycin on the agar dilution plate are subsequently confirmation tested. This includes vancomycin and teicoplanin disk

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diffusion and motility tests to screen for the low-level vancomycin resistant motile enterococci (*van*C strains). Isolates confirmed as nonmotile and by the disk diffusion as resistant to vancomycin, are identified as VRE. New patients with VRE detected, whether colonized or infected, are subsequently reported to Infection Control to initiate isolation procedures.

Teicoplanin results are used only for epidemiological purposes to classify isolates as a *van*A or *van*B phenotype.

## Conclusion

The first vancomycin resistant Enterococci was detected in August 1993 at NHS-University (formally University Hospital). Since that time, numerous additiorral isolates have been reported. At present, *E. faecium* has been the only Enterococcus species associated with high-level resistance to vancomycin, with an approximate 70 to 30 ratio between *van*A and *van*B phenotypes, respectively. A majority of patients have been identified as colonized by a "VRE Culture Screen", or as an incidental finding from slool. Blood and peritoneal fluid have been the most common source of VRE-caused infection.

Personnel at the NPHL are interested in conducting a a statewide surveillance for VRE to evaluate clonality among isolates and they would welcome submission of these isolates from through out the State of Nebraska for banking and additional testing. Additionally, the NPHL offers verification testing for the identification of VRE. To submit isolates for verification or for banking , complete a "Special Microbiology Requisition Form", and submit this along with the isolate to the NPHL. For additional information or to receive a copyof the requisition form by FAX, call Peter Iwen at (402) 559-7774.

# Hantavirus in Nebraska

by Steven H. Hinrichs, M.D.

Most laboratories are aware that the first case of Hantavirus infection in Nebraska was reported this summer. The infection occurred in a 40-year-old male who had possible exposure to mice while cleaning out grain bins and trucks. Previous to this summer, Nebraska was the only state west of the Missouri river that Hantavirus had not occurred. Although it is known that Hantavirus is carried by rodents, particularly the deer mouse and passed on to humans through urine, saliva or droppings, the lack of a case in Nebraska suggests that all the parameters causing the infection are not fully understood. A screening for Hantavirus in rodent populations has been conducted by the Epidemiology, Toxicology and Vector Surveillance Section of the Nebraska Health and Human Section for a number of years. Antibodies to Hantavirus are generally found in a statewide proximately 6% of animals. In Dundy County were the human Hantavirus infection occurred this summer, the rate is no higher and possibly lower than other counties. The most important issue for the laboratory is accurate testing in humans. Althrough the original specimen was sent to a reputable reference laboratory, a definitive diagnosis was obtained only after an additional specimen was sent to the University of New Mexico and the CDC. Therefore, the possiblity exists that previous cases have occurred in Nebraska but were not accuately diagnosed. This emphasizes the importance of involving the Epidemiology section of the Nebraska Health and Human Services Departnent or the Nebraska Public Health Laboratory through which the specimen can be expedited.

Several warnings were issued early in the year following for possible increased numbers of cases of Hantavirus infection due to the possibility that El Nino would increase vegetation in certain areas of the U.S. leading to an increase in the rodent population. These warnings may have made people aware of the risk associ-

**Table 1.**

**Antimicrobial susceptibility of *Enterococcus* species isolated from blood cultures (1988 through 1995).** [a,b,c]

| Species and time period | Total no. identified | % Resistant AM | GM | ST | VA |
|---|---|---|---|---|---|
| *E.faecalis* | | | | | |
| 1988-91 | 142 | 0 | 4.9 | 7.7 | 0 |
| 1992-95 | 148 | 0.7 | 32.4 | 17.7 | 0 |
| *E.faecium* | | | | | |
| 1988-91 | 35 | 17.1 | 0 | 20.0 | 0 |
| 1992-95 | 75 | 60.0 | 32.0 | 42.7 | 22.7 |
| *E.gallinarum*[d] | 6 | 0 | 0 | 0 | 0 |
| *E.casseliflavus*[d] | 5 | 0 | 0 | 0 | 0 |
| *E.raffinosus* | 5 | 60.0 | 0 | 0 | 0 |
| *E.durans* | 3 | 0 | 0 | 0 | 0 |
| *E.hirae* | 2 | 0 | 0 | 0 | 0 |
| *E.avium* | 1 | 0 | 0 | 0 | 0 |

Abbreviations: AM = ampicillin, GM = high-level gentamicin, ST = high-level streptomycin, VA = vancomycin

[a]Condensed from; Iwen, et al. 1997. Change in prevalence and antibiotic resistance of *Enterococcus* species isolated from blood cultures over an 8-year period. **Antimicrob. Agents Chemother.**, 41:494.

[b]One isolate per patient only.

[c]Species were identified using conventional macrotube biochemical tests.

[d]These isolates have intrinsic low-level resistance to vancomycin.

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The client
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ated with cleaning barns or removing rodents nests because the anticipated number of cases did not occur.

Person contemplating trapping or performing necroposieson rodents in Nebraska should contact Dr. Wayne Kramer for recommendations of transmissions of the virus. It is known that Hantavirus Pulmonary Syndrome (HPS) is caused by a previously unknown group of Hantaviruses of which the Sin Nombre virus is the most common. Previous to 1993, Hantaviruses were known only as the etiologic agents of hemorrhagic fever transmitted by rats.

## EHEC Surveillance Study: Update

by Paul D. Fey, Ph.D.

The NPHL has been conducting an enterohemorrhagic *Escherichia coli* (EHEC) surveillance study this past summer. The study has been accomplished by collecting diarrheal stools from 10 participating microbiology laboratories throughout the state. Following are some preliminary results of the study: to date, we have received 320 diarrheal samples and *E. coli* O157:H7 was isolated from nine (2.8%) of these samples. Interestingly, 7 additional samples (2.2%) were shiga-toxin positive using an ELISA test. However, we have been unable to isolate *E. coli* Ol57:H7 from these 7 samples. It is a distinct possibility that these samples contain non-O157:H7 EHCH, and this possibility will be investigated this fall and winter. The NPHL is willing to provide screening for *E. coli* O157:H7, and for other serotypes of EHCH, if screening is not already being performed. For more information, please call Dr. Paul D. Fey at (402)559-8104 or read the Spring 1998 edition of the NPHL Newsletter.