Animal Health MATTERS

David H. Zeman
South Dakota State University

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Head/Director's Message
David H. Zeman, DVM, PhD
Director, ADRDL/OBL

Anthrax, High Risk Pathogen Biocontainment and the ADRDL

As most of you know, South Dakota and North Dakota experienced the worst anthrax outbreak in our region since the early 1900’s. The ADRDL received scores of suspect specimens and confirmed anthrax in over 50 different submissions from South Dakota premises. The outbreak in North Dakota was even more extensive. In this issue, more in-depth information about the anthrax outbreak is provided by Extension Veterinarian Dr. Russ Daly. The lab modified its routine receiving, testing and reporting procedures during the outbreak to provide field veterinarians and regulatory officials timely data. The outbreak appears to have ended, with our last case occurring on September 23.

This was a significant outbreak for many individual cattle producers, but was relatively small when compared to what could happen should South Dakota ever be faced with an accidental or maliciously introduced foreign animal disease (FAD). The same could be said for potential bioterrorism incidents, since ~75% of the agents on the bioterrorism possibility list are zoonotic pathogens and therefore such outbreaks could rapidly overwhelm both veterinary and human health laboratories.

The anthrax outbreak and events since 911 continue to remind us of critical infrastructure needs at the ADRDL. Modern animal health laboratories today must have higher level biocontainment and biosafety capabilities than ever before, and the ADRDL is no exception. High level laboratories (BSL 3+) are designed to not only provide the lab workers with a safe place to work with more dangerous specimens, but are designed also to protect the local environment from secondary outbreaks. The ADRDL will need the support of all stakeholders to move this need forward, so that we can truly be prepared to serve during future crises involving dangerous pathogens.

Extension News - SDSU ADRDL

Anthrax in South Dakota, Summer 2005
R Daly, D Zeman

During the summer of 2005, South Dakota experienced an unprecedented number of anthrax cases throughout certain areas of the state. Submissions of anthrax suspect specimens at the SDSU ADRDL reached an all-time high, with peaks in early and mid-August (see chart on next page). July 20, 2005 saw the first suspect submitted, and the most recent sample was received on October 11 (as of date of press).

In all, 54 South Dakota premises in 17 counties had cattle losses due to anthrax. Of this total, 47 herds lost cattle, 4 lost strictly bison, 1 herd lost cattle and bison, and 1 herd lost cattle, bison, and horses. Anthrax was also identified in one white-tailed deer population.

Positive anthrax cases seemed to arise initially from two distinct areas of the state: central South Dakota, notably Dewey, Potter, and Sully counties; and from northeast South Dakota: Brown, Marshall, Day, and Spink. These seven counties saw over 70% of the affected herds.

According to South Dakota Animal Industry Board records, 538 animals were reported lost from anthrax from a total of 11,831 animals at risk in those herds, for an overall fatality rate of 4.5%. Of those 538 fatalities, 221 came from one herd; animals lost within herds ranged from 1 to 221, with a median number lost of 4. Individual mortality rates within herds ranged from a high of 33.5% to 0.2%. Median mortality within affected herds was 2.3%.

The ADRDL received samples from 39 different South Dakota counties and two other states. In addition to samples from cattle, bison, and deer, submissions included samples from antelope, elk, goats, and a dog which were all negative.
## Anthrax Submissions by County, SDSU ADRDL, July-October, 2005

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**TOTALS** 57       122     179

**Notes:**
- Five positive submissions and one negative submission were from herds that had already confirmed infection in the herd.
- Two additional herds were classified by the Animal Industry Board as positive herds based on signs within the herd and/or confirmation of anthrax in share cattle on the same premises.
Positive anthrax cases were defined as cases in which appropriate clinical history in the herd.

Giemsa-stained blood smear when coupled with the plate. In those cases, diagnosis was made on the basis of the making it difficult to identify the pathogen growing on the plate. In those cases, diagnosis was made on the basis of the Giemsa-stained blood smear when coupled with the appropriate clinical history in the herd.

ADRDL Anthrax Outbreak Procedures – The large number of anthrax-suspect samples received at the ADRDL and the urgency in which their results were needed resulted in the ADRDL adapting to the situation by employing several changes in how those cases were treated upon arrival:

1. The Giemsa Anthrax Screen test was “separated” from the Anthrax Culture Test. This meant that preliminary screen test results could be reported much faster to herd veterinarians via the VADDS Report Generator on the internet, and to the Animal Industry Board via fax and phone.
2. Procedures were changed to ensure that anthrax suspect samples were passed immediately after log-in to the bacteriology section for processing, with the goal of reporting anthrax results by noon, thus allowing the field vets and AIB time to react the same day to test findings.
3. Improved and consistent results terminology was developed to improve communications regarding test results.
4. Saturday mail from August through early October was opened and screened for anthrax submissions, and testing was completed on Saturdays as required. Bacteriology staff also worked Sundays as needed to finalize anthrax tests.

The producers that sustained losses due to anthrax this summer were placed under a great deal of strain during the outbreak in terms of economic loss, increased labor and medication costs, and personal anxiety. To a smaller degree, veterinarians, state officials, and lab personnel were placed under unusual stress also, as they worked to assist the affected producers. At SDSU’s ADRDL, it is felt that dealing with the outbreak strengthened the lab’s ability to respond to such an event, and will surely be useful in the future as other animal health challenges present themselves.

What’s ahead:

The number of submissions and positive cases this summer presents a unique opportunity to study the epidemiology of anthrax in our state. Work will take place to further characterize the affected herds, with the hopes of making comparisons: 1) between affected and non-affected herds, and 2) between this summer and previous summers within affected herds. Factors under consideration include: environment, climate, soil type, and host factors, among others. B. anthracis isolates from affected herds are being subtyped by strain. In addition, meetings have been scheduled with officials from North Dakota, Minnesota, and Manitoba in order to compare findings and procedures as those areas study their own anthrax cases. Anthrax is an almost annual occurrence in South Dakota, and it is hoped that an even clearer understanding of this disease may result from further study of the cases of the summer of 2005.

Transmission of Equine Influenza Virus to Dogs
Science. 2005 Sep 26; [Epub ahead of print]

Abstract:

Molecular and antigenic analyses of three influenza viruses isolated from outbreaks of severe respiratory disease in racing greyhounds revealed that they are closely related to H3N8 equine influenza virus. Phylogenetic analysis indicated that the canine influenza virus genomes form a monophyletic group, consistent with a single interspecies virus transfer. Molecular changes in the hemagglutinin suggested adaptive evolution in the new host. The etiologic role of this virus in respiratory disease was supported by the temporal association of rising antibody titers with disease and by experimental inoculation studies. The geographic expansion of the infection and its persistence for several years indicates efficient transmission of canine influenza virus among greyhounds. Evidence of infection in pet dogs suggests that this infection may also become enzootic in this population.

Synopsis of Paper:

Initial Studies and Characterization of the Virus:

• In January 2004, an outbreak of respiratory disease occurred in 22 racing greyhounds at a Florida racetrack. Two clinical syndromes were evident:
  1. a milder illness characterized by initial fever and then cough for 10-14 days with subsequent recovery (14 dogs), or
  2. a peracute death associated with hemorrhage in the respiratory tract (8 dogs)

The SDSU Veterinary Extension Website is being updated regularly. Visit often for updates on animal health issues in South Dakota and the region.

Website address = http://vetsci.sdstate.edu/vetext/ (or access through http://vetsci.sdstate.edu and click on Veterinary Extension)
Experimental Studies:

- Postmortem examinations revealed extensive hemorrhage in the lungs, mediastinum, and pleural cavity. Histological examination of the respiratory tract revealed tracheitis, bronchitis, bronchiolitis, and supplicative bronchopneumonia. The epithelial lining and airway lumens in these tissues were infiltrated by neutrophils and macrophages.
- Virus was isolated from the lung homogenate from one dog and was characterized as an equine influenza A H3 subtype using ELISA, PCR, and serology. Sequencing indicated that all genes of the canine isolate were of equine influenza virus origin, and it was concluded that the entire genome of an equine influenza virus had been transmitted to the dog.

Outbreak Investigations:

- To determine involvement of the virus in the respiratory disease outbreak, paired acute and convalescent sera from 11 sick dogs and 16 asymptomatic contacts were analyzed for antibodies specific to the virus in question. Seroconversion occurred in 8 of 11 sick dogs and 8 of 16 asymptomatic contacts. This demonstrated that infection of the dogs with the virus coincided with the onset of respiratory disease in most animals.
- Three months after the outbreak, single serum samples were collected from an additional 46 asymptomatic dogs housed with the sick dogs. Of these, 93% were seropositive. The history of respiratory disease among racing greyhounds, suggested sustained circulation of a canine/FL/04-like virus in this population.

Editor’s Note: This paper shows that the canine influenza virus in question has spread to many different parts of the United States, apparently rather insidiously, as many dogs, with and without respiratory symptoms, show evidence of exposure. Canine influenza therefore may be considered a possible cause for respiratory symptoms in dogs in our area. Reports from veterinary clinics in other states indicate that symptoms are very similar to kennel cough, but patients do not show the normal response to antibiotics that a kennel cough case would. Dire reports of high mortality due to the virus would seem to be overblown, given that even experimentally infected animals did not show severe symptoms, and the evidence that a high proportion of the population shows evidence of exposure already.

- From June to August 2004, respiratory disease outbreaks occurred at 14 tracks in 6 states. Groups of dogs from West Virginia, and Kansas were seropositive.
- From January to May 2005, respiratory disease outbreaks occurred at 20 tracks in 11 states. Dogs from Florida, West Virginia, and Wisconsin were seropositive.
- The isolation of three closely related influenza viruses from fatal canine cases over a 16-month period and from different geographic locations, together with the substantial serological evidence of widespread infection among racing greyhounds, suggested sustained circulation of a canine/FL/04-like virus in this population.
- Serological tests were performed on 70 dogs with respiratory disease in a Florida shelter facility, four Florida veterinary clinics, and one New York veterinary clinic. Ninety-seven percent of the shelter and pet dogs were positive for antibody to canine/FL/04.
- This indicated the lack of genetic barriers to infection in the dog population and the spread of the virus to pet populations of regions of the country without greyhound racing.

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Experimental Studies:

- Four 6-month-old beagles were each inoculated with the virus by the intratracheal and intranasal routes.
  - All dogs developed a fever, but no respiratory signs were detected.
  - Postmortem examination on 2 of 4 dogs revealed histologic lesions, and viral H3 antigen detection.
- These results established the susceptibility of dogs to infection with the canine/FL/04 virus. The failure to reproduce severe disease and death in the experimentally inoculated beagles is not surprising since a large proportion of the naturally infected greyhounds were asymptomatic.

Epidemiologic Investigations:

- To investigate whether the virus (canine/FL/04-like influenza) had circulated among greyhound populations in Florida prior to the January 2004 outbreak, serologic examination was performed on archival sera from 65 racing greyhounds.
  - There were no detectable antibodies in 33 dogs sampled from 1996 to 1998.
  - Of 32 dogs sampled between 2000 and 2003, 9 were seropositive.
    - The seropositive dogs were located at Florida tracks involved in outbreaks of respiratory disease of unknown etiology from 1999 to 2003, and virus was isolated from Archival tissues from a greyhound that died March 2003, indicating that the virus had infected greyhounds prior to 2004.
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Diagnosis of canine influenza at SDSU’s ADRDL is possible through virus isolation attempts. Post-mortem samples (fresh and fixed lung) are most optimal for isolation. Practitioners with sick patients may submit pharyngeal swabs for ELISA antigen detection. Keep in mind, however, that virus detection on antemortem swabs may be difficult due to the small window in which viral shedding usually takes place in the dog. Please call the ADRDL for advice on collecting antemortem samples. Serology for canine influenza is not currently available at SDSU, but is being done at a few laboratories in the US.

Of interest to human and animal health practitioners is the demonstration of the interspecies transfer of a whole mammalian influenza virus to an unrelated mammal species, which is a relatively rare event. The concern is that with
Evidence of canine influenza infection in pet dogs, close companions to people, that possibilities may exist for transmission of novel influenza A viruses to humans.

Additional information on this and other topics can be found at: [http://vetsci.sdstate.edu/vetext/](http://vetsci.sdstate.edu/vetext/)

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**Diagnostic News - SDSU ADRDL**

**Reminder: Avoid Contaminated Milk Samples**

**R Daly, R Parmar**

Contaminated milk samples can be a frustrating problem for practitioners, milk producers, and laboratory personnel. Strict aseptic procedures must be used when collecting milk samples in order to prevent contamination with the many microorganisms present on the skin of cow’s flanks, udder and teats, on the hands of the sampler, and in the barn environment. Please share the following guidelines with your producers to help avoid milk sample contamination.

**Collecting From Individual Cows**

1. Organize your tubes and materials before obtaining samples.
   - Sterile screw-cap tubes are preferred. Plastic test tubes with snap-on lids will also work if lids are completely closed.
   - Whirl-pak plastic bags are not to be used because of leakage that occurs during shipment.
   - Collect and organize: sample tubes, rack for tubes, alcohol swabs, marker for sample identification, styrofoam cooler or insulated box for transport to refrigerator or freezer.
2. The hands of the person collecting the sample must be clean and dry. Wearing latex or vinyl gloves is preferable. Clothes should be clean. The person collecting samples should not be milking at the same time.
3. The most important factor is that teats need to be clean and dry. Prep the cow as usual, but teats need to be dried completely by the person doing the sampling.
4. Scrub the teat ends thoroughly with an alcohol swab or sterile cotton ball saturated with alcohol.
   - Clean the teats on the far side of the udder first.
   - Use a separate swab for each teat.
   - If prior prepping is not done, scrub until a new surface of the cotton or sponge remains clean. More than one pledget or sponge may be needed to clean a teat end properly.
5. Be careful not to touch cleaned teat ends before the sample is taken.
6. Sample tubes should be handled properly to ensure sterility at all times. Do not put caps into pockets, touch the tops or touch the inside of the collection tubes. Avoid getting particles of dust, dirt or manure into the sample tube.
7. Sample the teats closest to you first. Discard two squirts of milk before sample collection.
8. Tilt the sample container at a 45-degree angle to one side of the udder to prevent contaminating substances from falling into it while the sample is being taken.
10. Tighten the cap and properly label the collection tube using a waterproof marker.
11. Refrigerate samples as soon as possible. If samples are to be stored longer than 24 hours, they should be frozen.
12. Ship milk samples in a manner so they will arrive at the lab cold or frozen. Place vials in a Ziploc bag. Place bag between frozen freezer packs and fill empty space with newspaper. Preferably samples should arrive at ADRDL, SDSU, on or before Wednesday of a given week.

**If sampling outside the parlor:**

- Open barn doors or tunnel ventilation can cause massive air movement, resulting in major contamination problems from bedding and dust.
- Feeding during sampling should be avoided.
- It is best to sample at milking time (before milking the cow). If the sample is taken during midday, it should be taken at least 4 hours after the last milking.

**Collecting Bulk Tank Samples**

Collect samples 5 days in a row.
1. Agitate the tank well before sampling.
2. Use a sterile syringe and needle or clean dipper to draw the sample from the top of the tank.
   - Do not collect samples from the outlet valve; samples collected in this manner often will be contaminated
3. Fill the syringe or tube ½ full. (Remember, milk expands when frozen)
4. Replace the protective cap if using a syringe and needle (Remove needle prior to shipping).
5. Properly label the syringe or vial using a water proof marker.
6. Place immediately in the freezer. Any delays will allow bacteria to grow giving erroneous results.
7. Ship milk samples in a manner so they will arrive at the lab cold or frozen. Place vials in a Ziploc bag. Place bag between frozen freezer packs and fill empty space with newspaper. Preferably, samples should arrive at ADRDL, SDSU on or before Wednesday of a given week. If many (more than 5) farms’ samples are to be submitted at once, contact the laboratory (605-688-5171) prior to sending the samples.

Reasons for No Growth on Culture
Quite the opposite from contaminated milk cultures is the problem of no growth from a submitted sample. Two explanations are commonly associated with this phenomenon:
- The milk sample is taken too soon after the quarter was treated. Samples taken sooner than ten days following last treatment may not exhibit bacterial growth due to interference from antibiotic.
- The milk sample is taken from a quarter at the most acute stage of mastitis. In this case, bacteria may have been killed by the cow’s host defense mechanisms and will not grow on culture.

Selenium Toxicosis in Horses and Cattle: Sampling, Diagnosis and Clinical Signs
R Daly, N Thiex, R Neiger

Veterinarians in many parts of South Dakota and surrounding states are often presented with cases of possible selenium toxicosis ("alkali disease") in individuals or groups of animals. Diagnosis of this condition is dependent upon submitting optimal samples to the Olson Biochemistry Lab for selenium analysis:

1. Hair:
   - Submit 2-3 grams of hair (roughly speaking, a pile at least the size of a golf ball) in a plastic bag. The most frequent error in hair submissions is insufficient quantity of hair.
   - Shave hair from the flank area of the suspect animal. Do not send mane or tail hair.
   - Ensure that the hair sampled is clean. If caked with mud or manure, the analysis will include the soil or manure if it is present, resulting in inaccurate values.

2. Whole Blood: 5-7 ml in an EDTA tube
3. Serum: 3-5 ml spun off and poured off the clot.
   - Must not be hemolyzed, or falsely high values may result. Spin off serum promptly and pour into empty tube for submission.
4. Feeds and Forage:
   - Take a good, representative sample. Guidelines for proper sampling can be found in SDSU Extension Extra, “Take an Accurate Forage Sample,” which can be accessed at http://agbiopubs.sdstate.edu/pub_description.cfm?Item=ExEx4001

5. Liver or Kidney (liver may be preferred, since it is more versatile for other analyses also):
   - At least 2-3 grams, submit fresh on ice packs.

Interpretation of Results: Cattle and Horses
Simple, exact guidelines that apply to all animals and all situations cannot be devised. In diagnostic cases, pathologists will consider specimen type, geographic location of affected animals, and clinical signs in interpreting selenium levels as excessive.

It is prudent to remember that the level of selenium in the tissues is directly related to the duration of illness. This differs according to the sample submitted. For example, whole blood gives us a 2 to 3 month window into selenium exposure, while serum and plasma levels correspond to roughly the past 10 days. Hair samples reflect longer term exposures (over 2 to 3 months) depending on hair shedding.

Chronic Selenium Poisoning: Susceptibility
Chronic selenium poisoning in animals depends on the amount and rate of absorption of selenium from the intestinal tract. Horses appear to be more susceptible to chronic selenosis than cattle and sheep.

Individual animal susceptibility, the chemical form of selenium present, and the bioavailability of selenium as a result of interaction with other elements (e.g. sulfur, arsenic) in the diet are also important in the pathogenesis of selenium poisoning. It is also important to realize that animals raised in selenium-rich areas have different tolerances to high-selenium feeds than animals raised in selenium-deficient areas.

Chronic selenosis in animals results from consumption of forages or feeds grown in seleniferous soils that have accumulated toxic levels of selenium. Plants or diets with 5 to 50 ppm selenium are most likely to cause chronic selenosis. Problems may result when high-selenium forages are consumed along with feed containing supplemental selenium.

Acute selenium poisoning is usually the result of oversupplementation or accidental overdosing of animals with parenteral preparations.

Chronic Selenium Poisoning: Clinical Signs
In horses, the most distinctive clinical signs result from abnormalities in the keratin of the hoof and hair. The long hairs of the tail and mane tend to break off at the same level, resulting in a “bob” tail and “roached” mane. Lameness is due to the abnormal rapid uneven growth of hoof wall in all feet, resulting in circular ridges and subsequent cracking of the hoof wall. Some horses may slough the hoof wall entirely. Cattle will show similar defective hoof wall growth but will rarely lose the hoof wall. Other symptoms related to chronic selenium toxicity are: reduced reproductive performance, anemia, liver cirrhosis, heart atrophy, and degeneration of bones and joints in horses and cattle.
Symptoms of acute selenium poisoning are initially lethargy, anorexia, and generalized weakness. These symptoms progress to abdominal pain, sweating (in horses), diarrhea, dyspnea, and eventual circulatory and respiratory failure.

### ADDITIONAL REMINDER: VITAMIN E SUBMISSIONS

Vitamin E analysis (in suspected deficiency cases) is a commonly requested analysis, often in conjunction with selenium. Please note that:

- Vitamin E in serum rapidly deteriorates following collection.
- After collection, serum samples should be spun down as soon as possible, poured off from the clot, and frozen.

### Holiday hours:

November 11 – Veteran’s Day
November 24 – Thanksgiving
December 26 – Christmas
January 2 – New Year’s Day
January 16 – Martin Luther King Day
February 20 – President’s Day

- Optimally, the sample should remain frozen until the time of analysis at the lab. Overnight shipment on dry ice will result in the most accurate values possible.
- Hemolysis, failure to freeze samples promptly, or thawing of samples during shipment will likely result in falsely decreased levels of Vitamin E upon analysis.

### ANOTHER ADDITIONAL REMINDER: WATER SAMPLE SUBMISSIONS

One liter of water is necessary for the proper analysis. Water preferably should be submitted in a sealed plastic container. Whirl-paks are not appropriate for water submissions.

As with any submission, please call before sampling the animal if you have any questions: SDSU ADRDL 605-688-5171, or Olson Biochemistry Lab at 605-688-6171.

Sources:
Olson Biochemistry Laboratory, SDSU
Osweiler G, Carson T, Buck W, Van Gelder G. Clinical and Diagnostic Veterinary Toxicology

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**Research News - SDSU Veterinary Science Department**

**Center for Infectious Disease Research & Vaccinology Holds Conference on Enteric Diseases**

D. Francis

The Third International Rushmore Conference on Enteric Diseases: Strategies in the Prevention of Enteric Disease and Dissemination of Food-Borne Pathogens was held at the Rushmore Plaza Hotel in Rapid City, September 29-October 1, 2005. The conference was sponsored by the Center for Infectious Disease Research and Vaccinology anchored at SDSU, and the USDA Experiment Station Regional Technical Committee NC-1007 “Enteric Diseases of Swine and Cattle: Prevention, Control and Food Safety”, with financial support from the resources of those two organizations, plus the USDA, Novartis Animal Health, Larchwood, IA and Hematech, Inc, Sioux Falls.

The Conference included 18 invited presentations, 9 additional oral and 22 poster presentations. The conference addressed new developments from animal model studies regarding the pathogenesis and/or carriage of enteric and food-borne pathogens, vaccine technologies and alternative strategies to prevent or lessen the effects of enteric disease on domestic animals and people. New strategies in vaccine delivery discussed include skin patch technology (transcutaneous vaccine delivery); transgenic plant-based vaccine production and use of harmless (food grade) bacteria and transgenic delivery systems of antigens from enteric organisms. Strategies for animal protection alternative to vaccines included passive immunotherapy, probiotics, and production and utilization of transgenically produced antibacterial proteins such as lysozyme. Abstracts of conference presentations will be placed on the conference website: [http://rushmoreconference.sdstate.edu](http://rushmoreconference.sdstate.edu).

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Calendar of Events

November 10-11 – Swine Disease Conference for Swine Practitioners, Scheman Building, Iowa State University, Ames, IA
http://www.ucs.iastate.edu/mnet/swinedisease/home.html

December 1-3 – Academy of Veterinary Consultants Winter Meeting, Renaissance Denver Hotel, Denver, CO
http://www.avc-beef.org/

December 3–7 – American Association of Equine Practitioners, Washington State Convention & Trade Center, Seattle, WA
www.aaep.org

December 6–8 – Range Beef Cow Symposium XIX, Rushmore Plaza Civic Center, Rapid City, SD (605) 394-2236

January 9, 2006 – Diagnostic Laboratory Update, Animal Disease Research & Diagnostic Laboratory, Brookings, SD. Call 605-688-6649 for more information.

February 2-4, 2006 – Minnesota Veterinary Medical Association 109th Annual Meeting, Duluth Entertainment Convention Center, Duluth, MN.
http://www.mvma.org/convention_info.asp

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