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Animal Health MATTERS

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**ADRDL Steps Up in Light of Highly Pathogenic H5N2 Avian Influenza Outbreaks**

Russ Daly DVM, SDSU

A well-used communication piece put out by the US Department of Agriculture (USDA) is that of a map of the counties affected by the highly pathogenic H5N2 avian influenza (HPAI) outbreak this spring (see page 2). Taking note of the location of veterinary diagnostic laboratories on that same map, one would easily see that the South Dakota Animal Disease Research and Diagnostic Laboratory (ADRDL) lies right in the epicenter of this US outbreak.

Partly because of that proximity, and mostly due to the surge capacity and expertise built into the ADRDL, the South Dakota lab emerged as a critical partner for the region’s poultry producers and regulatory officials, providing timely and accurate test results when HPAI was suspected in a flock.

The HPAI H5N2 strain is detected in samples from birds using real-time Polymerase Chain Reaction (rtPCR) techniques. Flock samples generally consisted of swabs taken from the throat or cloaca of affected birds. Initially, samples are run for the rtPCR detection of the virus’ matrix gene, which indicates the presence of influenza A. Samples that are positive for the matrix gene are then tested for the presence of the H5 gene specific to this strain of HPAI. A positive test here determines the presence of this HPAI strain in the flock. Samples positive at the ADRDL are then sent to the National Veterinary Services Laboratory (NVSL) in Ames, Iowa, for confirmation.

To the ADRDL, quick turnaround and timely results are important for all diagnostic samples, but in the case of HPAI, the timeliness of test results had a direct – and profound – effect on the economics of each affected flock. Because USDA indemnity payments compensate flock owners only for live birds euthanized for control purposes – and not birds dying from the virus itself – quick identification of the virus was critical. State regulatory officials made the decision early on to consider the positive test result from ADRDL (rather than confirmation from NVSL, which could come days later) as the trigger that started the regulatory process in motion. For affected flocks, delays in test results meant more birds dying from the rapidly-ensuing HPAI before regulatory officials could count the number of birds for which the producer could be reimbursed.

Staff at the ADRDL quickly responded to this urgency on the part of flock owners. It was no longer good enough to let samples from affected flocks wait until the next day, or over the weekend, for testing. Staff worked into the nighttime hours or early mornings, running samples that had been collected and driven over in the late afternoons, early evenings or early morning. Staff came in to process and run samples that came in on Saturdays and Sundays as well. Microbiologist Kelly Schmit states, “It can be a

*Avian Influenza*

(Continued on page 7)
The SD Animal Disease Research and Diagnostic Laboratory (SD ADRDL) has been testing for Highly Pathogenic Avian Influenza (HPAI) since March 2015. Currently, we have staff on “on call” and performing emergency testing during the day, evenings and weekends. In addition, testing is also being performed for surveillance purposes to make sure the virus hasn’t spread to nearby premises and to help prove that farm sites previously infected are now “clean” from the virus.

The laboratory could help more with the HPAI situation if there were a biosafety level 3 (BSL3) laboratory at the ADRDL whereby samples would not need to be sent to another laboratory for virus isolation (needed in the “clean up” procedures to make sure barns are not harboring live virus after disinfection) and for further work on vaccine design, other diagnostics, research and reagent development. This type of laboratory would also be important for biocontainment and worker safety purposes. Most veterinary diagnostic laboratories have BSL3 laboratory space for “high risk” pathogens such as HPAI. Both the USDA review of the SD ADRDL and the American Association of Laboratory Diagnosticians (AALVD) Accreditation Committee recommended that the SD ADRDL have a BSL3 space for high risk disease testing. Some of the diagnostic samples that do arrive at the laboratory may contain pathogenic agents that are important to work with in BSL3 laboratory space. In addition, these agents may be found in animals and the environment in South Dakota and the region. Some of these have been diagnosed in the SD ADRDL and it is important to have a biosecure and safe place to do the testing and find solutions to some of these diseases. Some of these “high risk” agents that are important to diagnose and work with in BSL3 facilities include bacteria such as Yersinia pestis (causative agent of plague), Francisella tularensis, Chlamydia psittaci, Bacillus anthracis (anthrax) Coxiella burnetii, bovine tuberculosis and Brucella, along with viruses such as Venezuelan equine encephalitis virus, Eastern equine encephalitis virus, influenza, rabies virus, and West Nile virus.

Due to the lack of a BSL3 laboratory at the ADRDL, funding from the USDA “Food Emergency Response Network” (FERN) will be ½ of what was previously received since “tier 1” laboratories are only those that have BSL3 facilities. Having a BSL3 laboratory is also in the criteria to be a “tier 1” laboratory for the National Animal Health Laboratory Network” (NAHLN). The SD ADRDL is a member of the USDA NAHLN which is a federal/state consortium of laboratories that are prepared to test for various endemic and foreign animal diseases when the need arises. It is expected that less funding from USDA will be available to the SD ADRDL for subsequent years in testing for any emerging and foreign animal diseases. It has been obvious that the SD ADRDL is in one of the best locations for receiving samples such as those submitted for HPAI since it was within driving distance of many flocks (see map) and

for other “high impact” livestock disease testing and surveillance.

The Molecular Diagnostic Section at the ADRDL which performs the HPAI PCR based test, has tested 100,500 samples (including other PCR and DNA sequencing assays) in fiscal year 2014 and to date, has performed almost double this amount (with 1 month left in the fiscal year). Some of the increase in testing has also been due to the emergence of porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV) in the United States in May 2013. When an infectious disease occurs, the best method of stopping the spread is to diagnose it early and prevent transmission. In this regard, we hope to continue to serve the animal health industry in the state and region. ■

indicates “SD Animal Disease Research and Diagnostic Laboratory (ADRDL)” located within the SDSU Veterinary & Biomedical Sciences Department ” http://www.sdstate.edu/vs/adrdl

Blue = Counties of HPAI outbreaks

Figure 1. All HPAI Detections As Of May 22, 2015 PM (as reported on www.agphs.usda.gov) *one or more detections may have occurred in county
The fourth annual SDVMA/SDSU Stethoscope Ceremony honoring SDSU pre-veterinary students was held on May 1, 2015, at the SDSU Student Union. A great crowd of family members, friends, and SDSU faculty and staff were on hand.

Seven students who had completed their pre-veterinary coursework at SDSU were presented Littmann Cardiology III stethoscopes courtesy of the SDSU Veterinary and Biomedical Sciences Department and the South Dakota Veterinary Medical Association (SDVMA). Each stethoscope was personalized with the student’s name and an SDSU insignia, and was presented to each student by an individual of their choosing who had played a special role in their education while at SDSU.

In addition, Veterinary and Biomedical Sciences Department scholarships were awarded to returning pre-veterinary students for the upcoming school year at this event.

Addressing the students, faculty, and family members present were Todd Carr, DVM, SDVMA President from Tea; Dr. Barry Dunn, Dean, SDSU College of Agriculture and Biological Sciences, and Dr. Jane Hennings, Veterinary and Biomedical Sciences.

The stethoscope ceremony is a partnership between the SDVMA and the SDSU Veterinary and Biomedical Sciences Department. Dr. Carr stated, “The SDVMA is grateful to play a role in this ceremony and we look forward to welcoming back these young people in a few years as new members to the SDVMA. The ceremony went very well, and I was impressed by how many of the students were already connected to the profession by their contact with their local veterinarian. I also enjoyed the obvious pride many of the presenters showed in students they had influenced now being accepted into veterinary school.”

SDSU Pre-veterinary students accepted to veterinary schools for Fall 2015. (Back row, L-R): Evan Koep, Britney Rauk, Breanna Stoltenburg, Kevin Gustafson. (Front row, L-R): Katelyn Stark, Hailey Ehlers, Rachel Wilking.


2015-2016 SDSU Veterinary and Biomedical Sciences Department Scholarship Award Winners:

- **Dr. J.B. Taylor Memorial Scholarship**: Madison Bieber, Bowdle, SD.
- **Dr. Harry Halverson Memorial Scholarship**: Ashley Swanson, Pukwana, SD.
- **Richard and Carol Dierks Scholarship**: Morgan Zaske, Renville, MN.
- **J. Michael Robbie Scholarship**: Paige Hinton, Winnipeg, MB; Megan Blanchard, Worthington, MN.
- **Freeman Lewis Scholarship**: Jace Philipson, New Underwood, SD; Lacey Quail, White, SD; Shelby Steiner, Fulda, MN; Olivia Kendall, Jordan, MN.
- **Rural Technologies Veterinary Science Scholarship in Support of the Jackrabbit Guarantee**: Haley Peterson, Spokane, WA.

**SDSU Pre-Veterinary Students Accepted to Veterinary Schools for Fall 2015:**

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>School</th>
<th>Presenter</th>
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<tbody>
<tr>
<td>Hailey Ehlers</td>
<td>Lakefield, MN</td>
<td>attending Iowa State University</td>
<td>(Dr. Todd Carr, SDVMA, presenter)</td>
</tr>
<tr>
<td>Kevin Gustafson</td>
<td>Madelia, MN</td>
<td>University of Minnesota</td>
<td>(Dr. Randy Ellis, St. James, MN)</td>
</tr>
<tr>
<td>Evan Koep</td>
<td>Lakefield, MN</td>
<td>Iowa State University</td>
<td>(Dr. Bob Thaler, SDSU)</td>
</tr>
<tr>
<td>Britney Rauk</td>
<td>Forest City, IA</td>
<td>Iowa State University</td>
<td>(Dr. Russ Daly, SDSU)</td>
</tr>
<tr>
<td>Katelyn Stark</td>
<td>Sanborn, MN</td>
<td>University of Minnesota</td>
<td>(Dr. Michael Gonda, SDSU)</td>
</tr>
<tr>
<td>Breanna Stoltenburg</td>
<td>Clear Lake, SD</td>
<td>Iowa State University</td>
<td>(Dr. Roger Genetzky, Brookings)</td>
</tr>
<tr>
<td>Rachel Wilking</td>
<td>Tracy MN</td>
<td>University of Minnesota</td>
<td>(Dr. Stacy Scramlin, SDSU)</td>
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The past year has seen a great number of shifts in workspaces within the ADRDL, all of which have resulted in improvements in case efficiency and customer service. One section in particular that has made great strides in improving their efficiency this past year has been the ADRDL clerical team.

With the completion of the remodeling of the former Olson Biochemistry Lab space in the SDSU Animal Science Complex for research laboratories, the serology section was able to take over the former ADRDL research laboratory space. Molecular Diagnostics then moved into the former Serology lab, which created greater lab space for these cramped sections. Subsequently, the clerical team was then able to expand into the space formerly occupied by the Molecular Diagnostics session.

This space was ideally located for the clerical team’s needs, as it is adjacent to the current receiving office. This new space is now called “specimen processing,” and it allows for a better division of labor and efforts. This area allows for much more efficient – and safer – workflow, as it also includes a biosafety cabinet under which packages that may contain riskier samples can be opened.

Boxes from UPS, FedEx, and the USPS containing diagnostic samples are delivered into this new specimen processing area, where they are opened, unpacked, labeled, and the documentation scanned and entered into folders. Then they go on to “specimen receiving” next door where further data is entered into the lab’s VADDS computer system and corresponding tests are ordered.

The division of these two areas has allowed for processing and labeling specimens in one area, with data entry and assistance for walk-in clients in the other area. In addition, to improve workflow for incoming cases, two additional work stations were added in a nearby office for clerical use.

To improve client service, the specimen receiving area was also remodeled, moving one work station to directly face the client walk-in window (see picture). A new copier/fax machine was added and a laser printer was moved for easier access by all sections. Bar-code readers have been added to all clerical work stations.

The outbreak of avian influenza this spring – along with the heavy demands for testing – has provided a unique challenge for the ADRDL, and in particular for the clerical section. Processing these cases means that each case must be associated with a Premise Identifi-
The recent detection of canine influenza in a South Dakota dog has raised the awareness of dog owners and veterinarians interested in detecting this novel virus in their pets and patients.

Since its first detection in Chicago this spring, the novel H3N2 virus has been detected elsewhere throughout the country. The strain is distinct from the previously-known H3N8 strain of canine influenza, and is closely related to strains known to infect dogs in certain parts of Asia. To date, this strain has not been detected in species besides dogs.

Signs of infection in affected dogs include coughing, nasal discharge, lethargy, and fever. Signs of infection resolve gradually over 2-4 weeks with good supportive care. In a few severe cases, the initial influenza infection can be complicated by a bacterial infection, resulting in pneumonia. Offentimes, however, the infection is mild and may not even be noticed by the owner.

Canine Influenza Testing at the SDSU ADRDL

Russ Daly DVM, SDSU

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## ADRDL Pathologists Team with Veterinarian to Address Cattle Lameness for Livestock Group

Drs. Dale Miskimins and Regg Neiger, professors and veterinary pathologists from the South Dakota ADRDL, along with Dr. C.A. Owen, Animal Health Clinic in Winner, recently addressed the annual meeting of the South Dakota Livestock Auction Markets Association. This meeting was held on Saturday, May 30 in Chamberlain.

Cattle lameness is gaining attention as a well-being issue in feedlot cattle, as well as an economic issue for cattle producers. Those in attendance at the meeting heard the SDSU veterinarians speak on the root causes and pathologic progression of the various forms of cattle lameness, while Dr. Owen spoke to the treatment and management of these conditions. As an important conduit for a large percentage of feeder cattle in South Dakota, auction markets were interested to hear about the condition and how they might assist in its prevention.

Future plans are for more discussions with other cattle groups in the state, as well as formation of a learning group of interested veterinarians and producers around this and other feedlot health topics.

### Canine Influenza Testing at the SDSU ADRDL

Russ Daly DVM, SDSU

The ADRDL can test samples from dogs for influenza. It’s especially important to understand that timing of sample collection is critical. The canine influenza virus is only shed 2-4 days after initial clinical signs, so if the duration of illness has been longer than that, virus is not likely to be present in samples taken later in the course of disease, even in dogs showing clinical signs.

Testing considerations:

1. Use a polyester, Dacron, or rayon swab with a plastic shaft (BD BBL sterile culture swabs are OK).
2. Collect both nasal and deep pharyngeal swabs.
   b. Pharyngeal swabs: swab the back of the throat, near the end of the soft palate.
3. Place swab back into tube and add several drops of saline to keep the tube moist.
4. If you have parafilm, cover the tube with it to seal it.
5. Keep the samples refrigerated until they can be mailed (preferably the day of sampling).
6. Mail with ice packs and mail overnight.

Testing is performed daily at a cost of $40/sample. The test is a real-time PCR procedure that detects Influenza A. If subtyping to characterize the virus as the canine H3N2 strain is desired, we can forward the sample on for further testing.

To submit, go to [http://www.sdstate.edu/vs/adrdl/index.cfm; click on “submission and other forms” on the left side of the page to find the “all species health form.” Write in “canine influenza testing” and include the form with the sample.](http://www.sdstate.edu/vs/adrdl/index.cfm)
ADRDL Hosts Tours for Legislators and State Agency Staff

The South Dakota ADRDL was one of the stops on a tour itinerary for a select group of legislators and state agency directors on May 28. Sponsored in part by state agricultural groups, the tour allowed approximately 30 individuals the chance to get a close-up look at the ADRDL and observe faculty and staff members in action.

Tour participants were able to see how samples and specimens are handled and how a variety of diagnostic testing methods are utilized to provide livestock and pet owners – through their veterinarian – answers to important animal health questions. Of particular interest was the lab’s recent work with avian influenza testing, in light of the current outbreak in the Midwest. Participants were able to view firsthand the ADRDL’s space issues and lack of BSL-3 space needed to further work with the Highly Pathogenic Avian Influenza (HPAI) virus.

Those touring the lab included State Representatives Tom Brunner, Dennis Feickert, Joshua Klumb, J. Sam Marty, Lee Qualm, Jim Schaefer, Kyle Schoenfish, Dean Schrempp and Dick Werner; and State Senators Gary Cammack, Jason Freichs, Betty Olson, Jim Peterson, Arthur Rusch, and Mike Vehle. In addition, representatives of the South Dakota Department of Agriculture, Game Fish and Parks, Department of Environment and Natural Resources, Natural Resources Conservation Service, Legislative Research Council, Crop Life Environment and Natural Resources, Natural Resources Conservation Center in Sioux Falls. Topics include:

- Dr. Jane Christopher-Hennings – Planning for the future of veterinary diagnostics in South Dakota
- Travis Clement – The SDSU ADRDL response to avian influenza
- Dr. David Knudsen – A 6-day old foal with diarrhea: An unusual diagnosis
- Dr. Dale Miskiminis (with Dr. Jim Beaty) – Losing their tails: Ergot toxicity in young bulls
- Dr. Chris Chase – Using SNPs to identify IBR viruses in field investigations- What are they and how do we use them?

Don’t forget to join ADRDL staff for the SDSU Ice Cream Social in the exhibit hall immediately prior to the Tuesday case reports.

ADRDL Offers Next Generation Sequencing

With the addition of two new pieces of cutting-edge equipment, the ADRDL is now providing gene sequencing services for their diagnostic clients. The instrumentation – and the expertise of the ADRDL faculty using it – also opens up research opportunities that will help investigators improve animal and human health.

The MiSeq whole genome sequencing instrument provides the ability to determine the amino acid sequence of all genes in a germ, whether it be a virus or bacteria. This “next-generation sequencing” goes way beyond the typical methods of organism identification. By fully laying out the nucleic acid fingerprint of a pathogen, it identifies small differences in nucleic acids that could lead to big differences in how that germ infects a person or animal, or becomes resistant to antibiotics. The technology can be used to detect specific strains of germs and is a critical tool in traceback studies as well as identifying new variant strains and antimicrobial resistance patterns in bacteria.

Some of the first uses of this technology are occurring in the realm of foodborne pathogen detection. ADRDL researchers are currently using the MiSeq to characterize the different strains of Salmonella and enterotoxigenic E. coli. By doing so, investigators are better able to link instances of foodborne illness to each other, with the goal of determining causes and preventing future illnesses. In addition, the next generation sequencing instrumentation is also finding use in providing whole genome sequences for PEDV (which the ADRDL is performing for the National Veterinary Services Laboratory) and eventually for PRRS, which will greatly aid area swine veterinarians in helping to characterize and stop the spread of this disease within their herds.

Another new addition to the ADRDL’s instrumentation is an ABI 3500 Genetic analyzer. This machine uses a more “classic” Sanger sequencing method and is useful when investigators want to sequence just one or two genes from a pathogen. This makes the ABI 3500 instrument useful for detecting a pathogen in a clinical sample.

Both of these instruments are examples of how the SDSU ADRDL is staying current with the needs of their clientele, along with seeking answers to the important questions that – nowadays – seem to pop up on a regular basis.

ADRDL at the SDVMA Annual Meeting

Once again, the SDVMA Annual Meeting will feature case reports from ADRDL faculty and staff during the Tuesday session, August 11, at 2:30 PM at the Ramkota Convention Center in Sioux Falls. Topics include:

- Dr. Jane Christopher-Hennings – Planning for the future of veterinary diagnostics in South Dakota
- Travis Clement – The SDSU ADRDL response to avian influenza
- Dr. David Knudsen – A 6-day old foal with diarrhea: An unusual diagnosis
- Dr. Dale Miskiminis (with Dr. Jim Beaty) – Losing their tails: Ergot toxicity in young bulls
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Dr. Joy Scaria pictured next to the MiSeq next generation sequencing machine.
Avian Influenza

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little stressful too, because there are people where their whole livelihood can be on the line. It’s therefore very important that they get their results in a timely manner. We talk to some of these people, and it can be pretty heartbreaking."

The extra workload came not only from flocks in South Dakota, but from flocks in surrounding states as well. Other diagnostic laboratories in the region were stretched as well by testing demand due to this outbreak, and the ADRDL was able to meet many of those unfulfilled needs.

The faculty and staff of the ADRDL also played a crucial role in educating the public about aspects of the outbreak and how the virus is detected in a flock. Staff were sought after to provide information to the public through broadcast news reports from KELO and KSFY, as well as print media coverage through the Minneapolis Star Tribune, Des Moines Register, and numerous state and local media outlets.

At the time of publication, over 7,100 samples from commercial and backyard birds in the Dakotas, Minnesota, Iowa, and Nebraska had been tested. The HPAI testing contributed to a record surge in the number of samples tested by the molecular diagnostics section. The section had run over 100,000 tests in fiscal year 2014 (July 2013-June 2014). Already, with more than a month to go in the fiscal year, the test count for this section FY 2015 is over 200,000.

In South Dakota, 9 commercial turkey flocks had been identified as infected as well as one commercial egg layer flock. As flocks move from detection to depopulation to recovery, the next push on HPAI sampling will come from environmental samples. In order for a premises to be cleared to repopulate, samples taken from the barn surfaces, waterers, feeders, etc. must be negative following the disposal of the depopulated birds. Samples from compost piles need to be tested if composting was utilized as a means of disposal. This ongoing need for environmental testing means that there is no end in sight for HPAI testing at the ADRDL anytime soon.

Environmental testing utilizes rtPCR in the same manner as for bird samples. However, any positive environmental samples then need to be tested using virus isolation in order to determine the presence of viable virus. Virus isolation involves the propagation of live HPAI virus, considered a significant select agent. In order to safely and legally perform virus isolation for HPAI, Biosafety Level 3 laboratory space is required. At this time, the South Dakota ADRDL does not have Biosafety Level 3 lab space, so these confirmatory tests need to be sent to NVSL, adding time and expense to the process. In addition, Biosafety Level 3 space is necessary in order to perform any sort of pathologic or vaccine development research projects on this particular virus. By necessity, these projects are performed at other institutions.

The situation this spring serves as a prime example of the readiness of the ADRDL to respond to disease incursions such as HPAI – not only in its laboratories but among its faculty and staff. Previous exercises and proficiency testing for influenza, as well as years of experience dealing with diseases such as PRRS and PEDV, meant the ADRDL was poised to serve as a critical resource for area poultry producers, backyard farmers, and regulatory veterinarians throughout this incident.
The SDSU Veterinary and Biomedical Sciences Department conducts research, teaching, professional service, and extension service to South Dakota and the surrounding region. An entity within the SDSU Veterinary and Biomedical Sciences Department, the South Dakota Animal Disease Research and Diagnostic Laboratory is a full-service, all-species diagnostic laboratory accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD). The AAVLD accreditation program complies with international expectations for quality diagnostic services under the guidance of the World Organization for Animal Health (the OIE). The ADRDL collaborates with the USDA National Veterinary Services Laboratory on many federal disease monitoring and eradication programs and is a member of the National Animal Health Laboratory Network. For information regarding the laboratory’s Quality System, contact Rajesh Parmar – ADRDL Quality Manager, at 605 688 4309.

Editor: Russ Daly, DVM

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