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Animal Health MATTERS

David H. Zeman
South Dakota State University

SDSU Veterinary Science Department • Animal Disease Research & Diagnostic Laboratory

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Head/Director's Message

David H. Zeman, DVM, PhD

Equipment Grant to Support Bioterrorism Preparedness and TSE Testing at the ADRDL

The ADRDL recently received a $264,000 grant from the South Dakota Department of Public Safety & Emergency Management to purchase lab testing equipment that would enhance the state's efforts to prepare for a Bioterrorism incident. The grant was facilitated by State Veterinarian Sam Holland and Assistant State Veterinarian Tom Cline, with funds originating from the Department of Justice. The equipment purchased included:

- Real-time PCR equipment that can be used to test for maliciously introduced foreign animal diseases or assist with testing during a public health zoonotic disease crisis
- Food safety molecular testing equipment (the ADRDL conducts tests for the SD State Meat Inspection program
- Bio-Rad rapid ELISA prion screening equipment
- Prion immunohistochemistry test equipment

It appears that the preferred test technology in the face of any disastrous animal or zoonotic disease situation will be molecular based. The molecular tests are highly specific and relatively fast (compared to standard culture procedures). They are easier to adapt to high volume situations. This equipment will truly enhance our capability to respond to an infectious disease disaster. In addition, rapid test technology for prion testing (ELISA based technology) will likely be the preferred test for high volume prion screening, with positive samples tested further by traditional immunohistochemistry (IHC). The lab is now poised much better than previously to respond to an Agroterrorism or zoonotic Bioterrorism event. The support of the SD Animal Industry Board and the SD Department of Public Safety are greatly appreciated.

Call for Cases for the Summer Meeting

We want to put you on the Pierre program – and you can win. We are looking for 12 cases for presentation on Tuesday. All you have to do is take pictures of an interesting case (or cases) between now and then. Small animal, large animal, exotic, equine, it doesn't matter. Bring the pictures with you to the June meeting and describe the case – the signs, treatment, and outcome, etc.

If the audience votes your case among the top 3 most interesting, you will win cash. Prizes are $300 for first, $200 for second, and $100 for third. All you have to provide are the photos and a description of the case. We will scan the photos (or radiographs, if you have them) into the computer for projection. So throw a disposable camera in each truck and stay on the lookout for anything interesting – the unusual, bizarre, extreme, or maybe just a really good look at the common.

NOTE:

We have not had much interest from SDVMA members on our above proposal to have case presentations at the summer meeting.

If we do not have adequate interest by April 23, 2004, we will not have this addition to our program.

Please contact Dr. Daryl Thorpe at 605-229-5472 or Janice at 605-688-6649 by the above date if you want to participate.

Diagnostic News - SDSU ADRDL

BVD Ear Notch Test— Submission Guidelines

Submission: Collect ear notches using a pig ear-notcher or similar tool. Be SURE that each notch consists of skin and not just strands of hair. If you can feel the tool cut through the cartilage of the ear, you have correctly notched the ear. Avoid collecting tissue from the tip of the ear or any traumatized areas that are covered with a scab and may consist of ulcerated skin only.

See the ADRDL User Guide for instructions on making 10% neutral buffered formalin

http://vetsci.sdstate.edu/

Place each ear notch in a separate 10 ml red top serum tube or similar-sized centrifuge tube. Fill each tube with 7 to 8 ml of 10% neutral buffered formalin. Please do not use whirl-pak bags for the ear notch test.

Number the red-top tubes 1, 2, 3 ….etc. Write the animal identification number that corresponds to each tube number on the submission form—please print carefully. Do not write the animal identification number directly on the tube.

Tattoo ink will not interfere with the test and the sample does not have to be kept under sterile conditions.

Shipping the samples: If the ear notch is stored in 10% formalin for more than 5 or 6 days, false negative results may occur. Therefore, please ship the samples as soon as they are collected to the address above. The tissue will undergo fixation while the samples are en route to ADRDL. If you are testing a large herd and will need more than 1 to 2 days to collect the entire set of ear notches, please call Dr. Tanya Graham (Tanya.Graham@sdstate.edu) in advance for details.

Results: Results will be available in approximately 10 to 14 days during the busy spring season; sooner at other times of the year.

Interpretation: The BVD ear notch test is a screening test designed to detect persistently infected cattle. By tradition, persistently infected cattle have been defined as those cattle that have remained infected with the virus for longer than 4 weeks, usually indefinitely. Normal cattle—that are not persistently infected—would typically clear the infection within 4 weeks time. It is believed that the vast majority (upper 90 percentile) of IHC ear notch positive animals are persistently infected, and many veterinarians will recommend culling after one positive test. If the animal is highly valued or the test result is unexpected, testing again 4 to 12 weeks later by an alternative procedure such as virus isolation or PCR is recommended (purple top blood submitted for buffy coat culture or fresh serum for VI). Producers may wish to separate ear notch positive animals from their herd mates if they choose to re-test positive animals.

Out of multiple test submission forms?

Download a copy from the ADRDL User Guide

http://vetsci.sdstate.edu/

Tritrichomonas Pouch Culture Procedures at the ADRDL

The ADRDL has been utilizing the InPouch™ TF test kit provided by Biomed Diagnostics for several years. These pouches have been valuable for preserving and propagating *Tritrichomonas foetus* from preputial smegma. The ADRDL will send pouches with instructions for culturing preputial cavities upon request. Once these pouches are inoculated, they are incubated at 98.6 degrees F and examined daily for trichomonads via light microscopy. The test is considered negative if there are no trichomonads observed after 6 days on test.

Some points to be aware of concerning this test:

- The test was considered highly specific for *T. foetus* when inoculated with preputial cavity material. However, in recent years it has been discovered that on rare occasions, organisms other than *T. foetus* may be cultured. These are presumably contaminating GI tract trichomonads, most often found in young bulls. Because of this, whenever we culture trichomonads in the pouch, we follow up with a PCR test to be sure the organism is truly *T. foetus* versus some closely related trichomonad.
- Once pouches are inoculated, veterinary clinics may choose to read their own pouches. However, trichomoniasis is a reportable disease in South Dakota and therefore tests must be "official" i.e. collected by a licensed veterinarian, and the Animal Industry Board only considers it an "official" test result if it is tested at the ADRDL or another recognized accredited lab.
- Remember the trichomonads are cold sensitive. Do not refrigerate or freeze the specimens after inoculation.

If you have any questions about this test you may contact Dr. David Zeman

Equine Infectious Anemia Testing

The AGID and ELISA tests are available for EIA testing. The ELISA test is more sensitive than the AGID test and provides more rapid results. ADRDL is encouraging the use of the ELISA test and will perform the ELISA unless the AGID is specifically requested. Results for samples submitted in the morning for the ELISA test will usually be available at the end of the same day. Results for samples submitted in the morning for the AGID test will usually be available at the end of the following day. The ELISA is $6 per sample and the AGID is $7.50. In addition, there is a $7 case accession fee for each case.

Research News - SDSU Veterinary Science Department

VSD and the Governor's 2010 Research Initiative

Governor Rounds has launched a proactive program to enhance the level of research at SD universities. One of the program's goals is to promote economic development by increasing the level of university research activity by encouraging research collaboration with local industry. The department under the leadership of Dr. David Francis has submitted a proposal to develop a *Center for Infectious Disease Research and Vaccinology*. Multiple departmental faculty researchers, as well as collaborators with other departments and industry are involved. We will be notified this summer on the status of this competitive proposal.

Extension News - SDSU ADRDL

Toxoplasma gondii and schizophrenia?

"Toxoplasma gondii is an intracellular parasite in the phylum Apicomplexia. Its life cycle can be completed only in cats and other felids, which are the definitive hosts. T. gondii also affects a wide variety of intermediate hosts, including humans. The effect of Toxoplasma infection on any given person may differ, depending on such factors as individual genetic predisposition, the state of the immune system, the dose, the virulence of the infecting strain, the timing (e.g. infections in the first trimester of pregnancy) and the part of the brain affected.

Humans may become infected by contact with cat feces or by eating undercooked meat. In humans, Toxoplasma is an important cause of abortions and stillbirths after primary infection in pregnant women. The organism can also cross the placenta and infect the fetus. The symptoms of congenital toxoplasmosis include abnormal changes in head size (hydrocephaly or microcephaly), intracranial calcifications, deafness, seizures, cerebral palsy, damage to the retina, and mental retardation. Some sequelae of congenital toxoplasmosis are not apparent at birth and may not become apparent until the second or third decade of life.

A study was…conducted by analyzing serum samples from pregnant women, obtained shortly before delivery, who gave birth to children in whom schizophrenia or other psychoses developed. Preliminary analysis indicates an increased rate of immunoglobulin (Ig) M (but not IgG) class antibodies to Toxoplasma gondii in mother with infants in whom schizophrenia developed later, suggesting that the mothers were experiencing an active infection or that they had persistent IgM antibodies, as described in other studies.

Numerous studies indicate that the [schizophrenia] disease process has its origins in early stages of brain development, [even though] the symptoms of schizophrenia generally do not manifest until late adolescence or early adulthood. The ability of Toxoplasma organisms to infect the perinatal brain is thus consistent with this aspect of schizophrenia pathogenesis. Prospective studies also support a possible role of postnatal infections in some cases of schizophrenia.

Multiple studies have demonstrated that the brains of persons with schizophrenia show structural and functional changes and that these exist even in patients who have never been treated with antipsychotic medications…thus schizophrenia is a chronic disease of the central nervous system. Neuropathologically, studies of T. gondii in cell culture have shown that glial cells, especially astrocytes, are selectively affected. Postmortem studies of schizophrenic brains have also reported many glial abnormalities, including decreased numbers of astrocytes. Similarly, animal studies of Toxoplasma infections have demonstrated that this organism affects levels of dopamine, norepinephrine, and other neurotransmitters, which are well know to be affected in persons with schizophrenia. Seropositivity to Toxoplasma has also been associated with "lack of energy or tiredness" in school children.

Some cases of acute toxoplasmosis in adults are associated with psychiatric symptoms such as delusions and hallucinations. Additional studies have documented that persons with serologic evidence of Toxoplasma infection have evidence of psychiatric changes in the absence of a history of clinically apparent Toxoplasma infection…[and that] serum antibodies to T. gondii are associated with alterations in behavior and psychomotor skills.

Epidemiologically, two studies have reported that adults who have schizophrenia or bipolar disorder had a greater exposure to cats in childhood. A recent study found that persons with schizophrenia who have serologic evidence of Toxoplasma infection have increased levels of cognitive impairment compared to age-matched Toxoplasma-seronegative patients with similar degrees of psychotic symptoms.

In all of the [6 recent] studies, the patients had more antibodies to Toxoplasma than the control groups and in the three studies…of patients who were having their first-episode

of schizophrenia, the differences were statistically significant. One of the [studies] divided the patients into those who had never received antipsychotic treatment and those who had received some treatment. The antibody levels for the treated group were intermediate between the levels of the never-treated group and those of the control group, suggesting that antipsychotic medication may have decreased by the antibody levels. This conclusion is supported by a study that indicated that some antipsychotic medications inhibit the growth of T. gondii in cell culture.

Studies are ongoing in attempts to better define the relationship of Toxoplasma infection to schizophrenia. Clinical trials are under way of antimicrobial drugs with anti-Toxoplasma activity, such as trimethoprimsulfamethoxazole and azithromycin, as adjunct treatment for persons with schizophrenia. These studies may lead to new methods for the treatment of schizophrenia and other psychiatric disorders that may be associated with Toxoplasma and related organisms."

Excerpts from Toxoplasma gondii and Schizophrenia by EF Torrey and RH Yolken. Emerging Infectious Diseases, Vol. 9, No. 11, November 2003
Compiled by Tanya D. Graham, DVM, Diplomate ACVP

A survey of agents associated with neonatal diarrhea in Iowa swine including *C. difficile* and PRRSV

This survey was undertaken to determine the relative frequency of agents that are currently associated with neonatal diarrhea in swine, including *Clostridium difficile* and porcine reproductive and respiratory syndrome virus (PRRSV). The subjects for this study were the first 100 live 1–7-day-old piglets submitted to the Iowa State University Veterinary Diagnostic Laboratory with a clinical signalment of diarrhea, beginning on January 1, 2000. The evaluation of each pig included bacterial culture of a section of ileum, 2 sections of jejunum, and a single section of colon; a fluorescent antibody test (FAT) or immunohistochemistry (IHC) for transmissible gastroenteritis virus (TGEV); ELISA's for rotavirus and *C. difficile* toxins; IHC for PRRSV; and microscopic examination of ileum, midjejunum, spiral colon, liver, spleen, and lung. Survey results demonstrate a decline in the relative number of diagnoses of TGEV, *Escherichia coli*, and *Clostridium perfringens* type C compared with retrospective data. The combined case frequency rate for these 3 pathogens dropped from 70% in 1988 to 21% in 2000. This survey also demonstrated the emergence of *C. difficile* as an important pathogen of neonatal swine. *Clostridium difficle* toxin was detected in the colon contents of 29% of the piglets, and at least 1 toxin-positive animal was identified in 55% of the cases. All 29 *C. difficile* toxin-positive piglets had mesocolonic edema, and colitis was observed in 21 of 29 toxin-positive animals. PRRSV-positive macrophages were detected in the lamina propria of intestinal villi by IHC in 10 piglets with diarrhea. In 6 of these cases, PRRSV was the only pathogen detected. Gross and microscopic lung lesions were not a reliable indicator of PRRSV infection in these neonatal pigs with diarrhea. The addition of tests for *C. difficile* and PRRSV to a routine neonatal diarrhea diagnostic protocol resulted in a significant increase in the diagnostic success rate on both individual animal and case bases.

Abstract from A survey of agents associated with neonatal diarrhea in Iowa swine including *Clostridium difficile* and porcine reproductive and respiratory syndrome virus, Michael Yaeger, et al, J Vet Diagn Invest 14:281-287 (2002)

Avian Influenza: Should We Be Concerned About Poultry In Asia?

Highly pathogenic avian influenza virus type A, strain H5N1, referred to in the popular press as bird flu, has been detected in several Asian countries recently. The exact origin of this particular strain of avian influenza is unknown. Various strains of bird flu have been around for many decades and H5N1 was first detected in Hong Kong in 1997. Two deaths due to H5N1 occurred in Hong Kong in February of 2003 (shortly after the victims returned from a trip to China)—the significance of these infections was not widely reported due to the emergence of SARS.

Avian influenza is an airborne virus that is shed in the feces and nasal and ocular secretions of infected birds. Most strains of avian influenza are carried subclinically by migratory birds such as ducks and geese.

Avian influenza is rarely pathogenic to humans. In the current outbreak a limited number of human cases of bird flu have been reported. Unfortunately, the majority of these human cases infected with bird flu have been fatal. (No human vaccine for this bird flu exists at this time.)

Public health officials are concerned that if a person infected with human influenza simultaneously became infected with avian influenza H5N1 that the human and avian viruses could easily swap genes. This could lead to a world wide pandemic if a new virus emerged that had the pathogenicity of the H5N1 avian virus and was easily transmitted from human to human. (The pandemic of 1918 that killed 20 to 40 million people world-wide was caused by an avian influenza virus that mutated and became readily transmittable to humans. SARS, which killed nearly 800 people world-wide last year, was a "wake up call" about what could happen with the next influenza virus pandemic.)

The World Health Organization sees 3 possibilities associated with the current outbreak of H5N1 bird flu in Asia:

- H5N1 (avian influenza A) gradually mutates and develops the ability to readily infect humans
- The H5N1 virus in poultry combines with human influenza viruses to cause a flu pandemic

- The bird flu is contained—thru mass culling, vaccination of remaining flocks, and good biosecurity—and does not pose a threat to humans.

The strain of avian influenza (H7) that has recently been detected in Delaware is different from the bird flu strain (H5) currently circulating in Asia. This strain of avian influenza is similar to one that has been detected before in New York live bird markets and is not a threat to humans. The Delaware strain of the virus is, however, a substantial threat to the poultry industry and will result in restrictions in trade between the US and other countries.

Currently, poultry workers, cullers, and veterinarians in Asia are wearing protective clothing and masks or respirators. In some areas, the workers are also being vaccinated for human influenza with this season's human influenza virus vaccine. This human flu vaccine does not protect against bird flu but should minimize the likelihood that a person could be infected simultaneously with a human strain of the flu and the H5N1 bird flu. Thus the chance for the two viruses to swap genes is minimized. Experts believe that existing avian influenza vaccines will offer some protection for domestic poultry in Asia and additional vaccines are being developed. Vaccination of birds "outside" the infected areas must be used in conjunction with culling of all diseased and exposed poultry, strict biosecurity protocols, and measures designed to limit the movement of birds between countries. Culling and vaccination of poultry flocks will also reduce the amount of avian influenza virus in the environment which will in turn help prevent the spread of bird flu to large numbers of humans.

No specific restrictions have been placed on international travelers at this time but anyone who plans to travel abroad should see their doctor 4 to 6 weeks prior to their travel date for any necessary vaccinations, medications, and advice. With regard to the current epidemic of bird flu, travelers should avoid contact with live poultry at farms or live bird markets and wash hands frequently with soap and water or an alcohol-based waterless hand cleanser.

H5N1 can survive in chilled / frozen meat and in uncooked eggs—meat and eggs must be cooked before consumption. The risk to humans is greatest in the countries in which cooks purchase live birds at their local markets and take the bird home to dress it and prepare meals. The World Health Organization does not believe that processed poultry products such as refrigerated / frozen carcasses or eggs (products we routinely purchase at our grocery stores) pose a risk to public health. It is still important, however, to practice good hygiene during preparation of food such as frequent hand washing, preventing cross-contamination of food products, and thorough cooking of poultry products. The internal temperature of meat products should reach at least 158 F degrees during cooking.

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At this time, it is unlikely that South Dakota poultry flocks will be affected. Flock owners & managers should be aware, however, that many strains of avian influenza are carried by wild birds such as ducks and geese. Therefore, free-range (open-range) rearing of domestic poultry is more risky than rearing birds in a confined environment.

Pigs have immune systems similar to humans – if bird flu infects pigs they can serve as a "mixing vessel" for the bird flu and strains of human flu – farms should separate pigs and poultry.

At all times poultry operations should practice good biosecurity techniques such as:

1. Limited access by visitors and restricted parking
2. Routine disinfection of all equipment and buildings
3. Up-to-date vaccination program
4. Rodent and insect control programs
5. Routine monitoring of production, consumption, and mortality records
6. All-in, all-out production.

Tanya D. Graham, DVM, Dipl. ACVP

SOUTH DAKOTA STATE UNIVERSITY

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SDSU Veterinary Science Department
Animal Disease Research & Diagnostic Laboratory
Box 2175- North Campus Drive
Brookings, SD 57007-1396

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Calendar of Events

June 6-8, 2004 – SDVMA Summer Meeting, Pierre, SD. For more information call 605-688-6649.

June 24-26, 2004 – Allergy & Otology Symposium and Video Otoscopy Laboratory, Gateway Center in Ames, IA. For information please contact Sandy Popelka at 515-294-2531; spopelka@iastate.edu

August 18-21, 2004 – SDVMA Annual Meeting, Rapid City, SD. For more information call 605-688-6649.

September 10-12, 2004 – 12th Complete Course in External Skeletal Fixation, Gateway Center, Ames, IA. For more information call Dr. Jim Toombs at 515-294-2199. For registration information, contact Sandy Popelka at 515-294-2531.

Editor: Bill Epperson, DVM

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