Gupta: New Instructor in Clinical Pathology

Dr. Aradhana (“Ara”) Gupta has joined the Kansas State Veterinary Diagnostic Lab (KSVDL) as an instructor in clinical pathology. She is originally from Punjab state of India, which is famous for agriculture and Punjabi music. She graduated from Punjab Agricultural University, India in 2000 and then completed a master’s in epidemiology and preventive veterinary medicine in 2002.

Her research focused on infectious bovine rhinotracheitis and bovine brucellosis. Upon completion of her master’s program, she worked as a veterinary microbiologist for one year, where she was an essential part of the veterinary disease investigation team.

Dr. Gupta accompanied her husband for three years in Sydney, Australia. While her husband was pursuing a Ph.D. at the University of Sydney, she worked as a research associate at the veterinary clinics there. She was as an integral part of a research project on the investigation of bacteriological isolates and their sensitivity patterns in different animal species. Another project at the university involved mucosal immunity following the oral delivery of vaccine in poultry.

Dr. Gupta was employed by New South Wales Department of Primary Industries in Sydney, Australia, before moving to Oklahoma State University in 2006. Dr. Gupta successfully completed one year of the American Veterinary Medical Association Educational Commission for Foreign Veterinary Graduates program at OSU in 2007. After working for a year in a small animal practice in Florida, she began a three-year clinical pathology residency at Louisiana State University. She completed her residency and became a Diplomate of the American College of Veterinary Pathologists in 2011.

Gumber: New Anatomic Pathologist

The Kansas State Veterinary Diagnostic Laboratory is pleased to announce the addition of Dr. Sanjeev Gumber to its staff as an assistant professor of anatomic pathology. Dr. Gumber earned his BVSc degree from Punjab Agricultural University, India in 2000. After graduation, he completed a master’s in veterinary science in epidemiology and preventive veterinary medicine from PAU in 2002. His master’s research was primarily focused on epidemiology of bovine brucellosis. Sanjeev worked as a veterinary microbiologist in the same department for one year and was a key member of the disease investigation team.

In 2003 he moved to the University of Sydney, Australia, to pursue a Ph.d in veterinary microbiology. His Ph.D. research was based on various aspects of the pathogenesis and diagnosis of Johne’s disease. The major research contribution was identification of stress/dormancy proteome signatures of Mycobacterium avium subsp. paratuberculosis (MAP) using artificially engineered microenvironments. These proteins were further characterized for their use as potential candidates for the earlier diagnosis of MAP.

After completion of his Ph.D., Dr. Gumber moved to Oklahoma State University in January 2007 and successfully completed the AVMA Educational Commission for Foreign Veterinary Graduate program. From May 2008 to October 2011 he worked as an anatomic pathology resident at Louisiana State University. Sanjeev became a Diplomate of the American College of Veterinary Pathologists in 2011. He has authored and coauthored nearly 30 publications and two patent applications.
Recently a newly identified virus in the family Bunyaviridae, genus Orthobunyavirus that has been provisionally named Schmallenberg virus has been the focus of attention in Western Europe. Cases have been reported in Germany, the Netherlands, Belgium, France, United Kingdom, and possibly other European countries. Clinical signs in acutely infected adult cattle, sheep, and goats are often absent, but can consist of fever, loss of appetite, reduced milk yields, and, rarely, diarrhea. The clinical signs were first observed in the late summer and early autumn of 2011. Beginning in mid-December outbreaks of malformed newborn ruminants began to appear in affected areas, and in utero infection with Schmallenberg virus has been confirmed. So far sheep are primarily affected, but cases have also been reported in cattle and goats. The syndrome is described as an arthrogryposis-hydranencephaly syndrome (AHS). The United States Department of Agriculture is rightly concerned and has asked people to be on the lookout for cases of possible Schmallenberg virus infection in the United States. This seems like a good time to review Cache Valley virus, which is also an Orthobunyavirus in the family Bunyaviridae.

Virus History
Cache Valley virus was first isolated from mosquitoes collected in Utah. Except for the southeast it is widespread throughout the United States. Schmallenberg virus is a member of the Simbu serogroup, which includes Akabane virus, and Cache Valley virus is a member of the Bunyamwera serogroup. Schmallenberg virus is believed to be transmitted by Culicoides spp. and possibly by mosquitoes. Cache Valley virus is transmitted by several genera of mosquitoes and possibly other insects.

In the late 1980s there was a large outbreak of reproductive failure in sheep in Texas that was associated with congenital malformations. Researchers at Texas A & M University did some detective work and were able to associate the outbreak with in utero infection by Cache Valley virus and used the virus to reproduce the syndrome. Features of the outbreak included increased open ewes at lambing, increased stillborn lambs, and the birth of stillborn and live lambs with congenital malformations. Experimentally, infection of fetuses before 48 days of gestation resulted in fetal death or central nervous system and/or musculoskeletal defects, depending on the age of the fetus. After 48 days of gestation the virus seemed to have little adverse effect on the fetuses. Experimentally, infected lambs, including those that become malformed, are able to mount an immune response and eliminate the virus from their bodies before birth. Therefore, they are born virus negative but with Cache Valley virus antibodies. Diagnosis is by collection of serum from lambs that have not nursed and testing it for antibodies to Cache Valley virus.

Cases and Symptoms
The first cases of Cache Valley virus that I saw were in the early 1990s when I was at South Dakota State University. During one lambing season we had malformed lambs from five states that were confirmed by the Texas A & M University Veterinary Diagnostic Laboratory to have been infected in utero by Cache Valley virus. Since coming to K-State in 1993 we have routinely diagnosed Cache Valley virus in lambs. We do not see cases every year (I do not know of any cases in 2012), but in a five-year period we probably see cases in two or three of the five years. Typically, affected lambs have arthrogryposis (Figure 1) and decreased muscling. Microscopically, much of their skeletal muscles are replaced by fat. There may or may not be brain lesions. When present, the most common brain lesions that I have found have been porencephaly or hydranencephaly. When I have removed the spinal cord, it has been much smaller than normal (Figure 2). Histologically, there are decreased numbers of neurons in the grey matter of the spinal cord. The arthrogryposis and muscle hypoplasia are likely secondary to a lack of normal innervation.

Cache Valley virus has been isolated from the blood of cattle, and researchers...
Ecstasy Poisoning in a Dog

Kenneth Harkin, D.V.M., DACVIM, KSU Veterinary Medicine Teaching Hospital and Deon van der Merwe, Ph.D., Veterinary Diagnostic Lab

The abuse of psychoactive substances is a problem that shows no signs of diminishing. It is, in fact, growing in complexity due to the proliferation of poorly-regulated, new psychoactive drugs including synthetic amphetamine-like substances, synthetic cannabinoids (e.g., “K2”; “Spice”), synthetic opioids, synthetic cathinones (“bath salts”), and novel herbal drugs such as Salvia divinorum and Mitragyna speciosa (“Kratom”). More substances are added to the list every year, staying ahead of regulatory controls. These products are often marketed as legal alternatives to banned substances, using deliberate strategies to avoid regulation, such as inclusion into products sold as potpourri, fragrances, bath salts etc., and labeling them as “Not for human consumption.” Unfortunately, ingestion of these products may lead to serious, sometimes life-threatening, adverse effects. Ingestion by pets, either deliberately or by accident, often leads to large overdoses because of the relatively small body size of most pets.

We describe a case of poisoning in a dog by a synthetic amphetamine-like substance, methylenedioxymethamphetamine (MDMA; “Ecstasy”). MDMA causes the release of endogenous stores of serotonin, epinephrine, and dopamine, leading to a wide range of effects related to over-stimulation of the sympathetic and central nervous systems.

A 1-year-old, 2-kg, male Pomeranian-mix dog was presented to the Kansas State Veterinary Medical Teaching Hospital (KSUVMTH) in a state of continuous seizures. The dog had no prior medical issues, was current on vaccinations, lived as the only pet in a one-person household, and had outdoor access restricted to leash walks. The dog was reportedly normal in the morning, and was walked in the afternoon showing no abnormalities. In the early evening, however, the dog began barking prompting the owner to take the dog out for a second walk. Barking continued on the walk, which was unusual, and the dog sat down numerous times with whole-body tremors. At home the tremors intensified and became continuous. At this point the dog began to have a

Further Reading:


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Increased Listeriosis Cases

Jerome C. Nietfeld, D.V.M., P.h.D.
Veterinary Diagnostic Laboratory

Listeria monocytogenes has been long recognized as a cause of abortion and central nervous system disease in ruminants. Genital infection by Listeria results in placentaitis, abortion, and premature delivery of week neonates that die soon after birth. Affected cattle have placentitis and, in many cases, retained placenta. Affected fetuses often die in utero and are retained for 2 to 3 days before being expelled and are very autolytic at birth. Typically, L. monocytogenes is readily isolated from the fetal tissues and uterine fluids. Central nervous system infection is in the brainstem and often not symmetric. The most characteristic clinical signs are due to multiple unilateral cranial nerve deficits that result in facial paralysis, droopy ear, and circling, especially when animals are confined. Infection is from ingestion of the organism and the most important predisposing factor is feeding silage. Properly cured silage is rarely a problem because Listeria growth is inhibited at pH less than 5.5. Most years we see very few if any cases of listeriosis in cattle. This past winter and spring has been unusual for the number of cases. I did not poll the other pathologists or the bacteriology lab but I know of at least two cases of listerial abortion and four cases of encephalitis that involved multiple animals. I am not certain of the reason for the increased cases. My first thought was that it might be due to the dry summer resulting in corn that was unusually dry when cut for silage and that it did not ferment properly. However, several cases were from areas that had more normal rainfall. Listeria was isolated from the two abortion cases and one meningoencephalitis case.

The remaining three meningoencephalitis cases were diagnosed on the basis of microscopic pathology in the brainstem and positive immunohistochemical (IHC) staining for Listeria spp. Bacterial culture of the brainstem for Listeria is still pending, but so far nothing has grown in spite of the fact that small gram positive bacilli that are IHC positive for Listeria spp. are visible. Over half of the central nervous system cases are culture negative, and the most common method of diagnosis has long been the presence of characteristic microscopic pathology in the brainstem. Immunohistochemical staining is an improvement because it allows immunologic identification of bacteria as Listeria species.
“Non-Lactating Dairy Cattle”

According to the FDA’s Center for Veterinary Medicine (CVM) has become aware that the term, “non-lactating dairy cattle,” may be confusing and producers and veterinarians could mistakenly interpret it to mean that drugs approved for use in non-lactating dairy cattle are safe when used in dry dairy cows, i.e., in cows between two lactations. The term “non-lactating dairy cattle” includes replacement dairy heifers, replacement dairy bulls, and dairy calves, according to current animal industry standards and a long standing FDA practice. These classes of dairy cattle have not yet, or would never produce, milk for human consumption. The term non-lactating dairy cattle does not include dry dairy cows. Dry dairy cows have previously produced milk for human consumption and will again in the future after completion of the “dry period” between lactations. These standards are reflected in CVM’s Guidance for Industry (GFI) #191 (Appendix III, Species and Classes of Major Food Animals).

This is an important human food safety issue because of the potential for residues of drugs labeled for use in non-lactating dairy cattle to be present in milk of the treated cows, as well as in the tissue of the calves born to the treated cows. In order for these drugs to be approved for use in dry dairy cows, residue depletion studies would be necessary to determine whether there are residues in calves born to the treated dry dairy cows and in the milk produced by the treated cows in their subsequent lactation.

FDA is working with sponsors of products approved for use in non-lactating dairy cattle to revise labeling to clarify that dry dairy cows are not non-lactating dairy cattle and therefore should not be treated with drugs labeled for use in non-lactating dairy cattle.

Source: http://www.fda.gov/AnimalVeterinary/NewsEvents/ucm292761.htm

Sheep and Goat Abortions: Handle with Care

Jerome C. Nietfeld, D.V.M., P.h.D.
Veterinary Diagnostic Laboratory

Winter and spring are the time when we see the majority of ruminant abortion cases. Everyone handling samples from aborted sheep and goats should remember that the most common causes of abortion in these species are zoonotic. A study of 1784 sheep abortions examined at South Dakota State University from 1980-1989 identified Toxoplasma gondii, Campylobacter spp., and Chlamydophila abortus (formerly Chlamydia psittaci serotype 1) as the most common causes. There were two cases of Coxiella burnettii. Between 2003 and 2007 Iowa State identified Campylobacter spp as the most common cause of sheep abortion. Toxoplasma gondii was second and there were a few cases of C. burnetti and C. abortus. A study of 211 goat abortions examined by the California Veterinary Diagnostic Laboratory System from 1991-1998 identified C. abortus, C. burnettii, and T. gondii as the most common infectious causes. There were two cases of C. jejuni abortion in the goats. All of these agents are zoonotic and care needs to be taken in handling the fetuses, placentas, and the aborting females.

Toxoplasma gondii.

Toxoplasma gondii was the most common and second most common cause of sheep abortions in the South Dakota State and Iowa State studies, respectively. It was the third most common cause of goat abortions in California. Sheep are considered to be one of the more common sources of human T. gondii infection. Uncooked or undercooked meat is probably the main source, but the fetal tissues are another potential source. Human infection is by the oral route, so it is imperative that anyone handling materials from aborting small ruminants wash their hands carefully after handling the material. Preferably they will wear gloves and then wash their hands.

Campylobacter spp.

In the South Dakota Study, C. fetus subspecies fetus was the most common Campylobacter isolated during the first eight years of the study, but in the final two years C. jejuni cases outnumbered C. fetus cases. Campylobacter jejuni was isolated from 89 percent and C. fetus subspecies fetus from 11 percent of the Iowa Campylobacter cases. Depending on the study, C. jejuni is the most common or second most common cause of foodborne enteritis in humans. Human infection with C. fetus subspecies fetus is uncommon, but it is the Campylobacter species most likely to cause bacteremia in humans. Campylobacter fetus subspecies venerealis is host adapted to the bovine reproductive tract and is not considered zoonotic.

Chlamyphila abortus:

Chlamyphila abortus, also referred to as enzootic abortion of ewes, was the third most common agent identified in the South Dakota study of sheep and the most common in the California study of goats. Human disease is rare, but all known natural cases have involved pregnant women. Affected women developed disseminated intravascular coagulation and disease was life threatening. The fetuses have become infected, often fatally. There have been cases where the fetus has survived when mother was treated early with an effective antibiotic and the fetus was old enough to survive following a C-section. Although infection is rare, pregnant women or women who might be pregnant should never work with aborting sheep or goats or handle materials from aborting sheep or goats. It is probably best that pregnant women not work at all with pregnant sheep and goats.

Coxiella burnetti.

Coxiella burnetti was the second most common cause of goat abortions in California and a sporadic cause in South Dakota and Iowa. We also see it most commonly as a cause of goat abortion. We see a few cases of sheep abortion and rare cases of bovine abortion due to C. burnetti. C. burnetti also cause of Q fever in

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humans, which usually results in influenza-like symptoms of variable severity, but it can also result in chronic endocarditis. I know of two cases where the veterinarian became involved because a member of the family was diagnosed with Q-fever and the family owned aborting goats. Like Campylobacter and Chlamydia the

Teflon Toxicity in Chickens

Potter, Magunda and Crespo, Washington Animal Disease Diagnostic Lab, Pullman, Washington

A backyard chicken flock in North Idaho suffered a sudden, catastrophic death loss in February of 2012. The flock was free range and consisted of 25 birds. Although free range, most of the flock was locked into a raised, well-insulated coop at night. One morning, all fowl in the coop were dead, while those who remained outside were alive. All dead fowl were found in normal postures with no signs of disturbance. Relevant history included addition of a new heat lamp bulb into the coop the previous day. Six dead birds were submitted to the Washington Animal Disease Diagnostic Laboratory for analysis.

Based upon the history of the new heat lamp bulb, Teflon toxicity was suspected. Polytetrafluoroethylene (PTFE) is the main ingredient in Teflon, which is known to be toxic to pet birds when the product is overheated. The PTFE is also used in shatter resistant coatings on light bulbs, including some infrared bulbs used at heat lamps for poultry. When heated to temperatures above 280°C, PTFE coated objects, whether non-stick cook-ware or light bulbs, will emit a variety of toxic fumes. Birds, due to their small size and efficient respiratory system, are highly susceptible and die, often without warning. Humans can also be affected.

Based upon these findings, it has been recommended that PTFE coated infrared bulbs not be used in poultry facilities. Unfortunately, it is not easy to determine if PTFE is a component of any particular bulb. In general, bulbs labeled as “shatter resistant likely have PTFE in the coating and should not be used in poultry houses. Find vendors who indicate that their bulbs are approved for avian safety.

General Guide to Interpreting Cycle Thresholds

The state-of-the-art PCR facilities in the Molecular Diagnostic Unit use a new advanced form of PCR technology called quantitative real-time PCR (QPCR) to detect target DNA, according to Dr. Richard Hesse, director of diagnostic virology, Kansas State Veterinary Diagnostic Lab. QPCR differs from conventional PCR in that it is quantitative, enabling the veterinarian to know how much target DNA is present in a sample.

Hesse provided the following explanation of the technology, courtesy of Langford Veterinary Services, the company that developed the test.

During QPCR, the amount of PCR product formed is measured each cycle and reported in fluorescence units. The more target DNA present in a sample, the more quickly the PCR product (and therefore fluorescence) is generated. A sample is positive if the amount of fluorescence produced rises above the threshold level. The threshold cycle (Ct) value denotes how many PCR cycles are required for the sample fluorescence to reach the threshold level. The more target DNA present in a sample, the lower the Ct value will be, because the threshold is reached sooner. QPCR results are reported by stating the Ct value for the sample. This helps the veterinarian interpret results by providing information on the amount of target DNA present. For example, retrovirus proviral loads can be measured and repeat samples used to monitor a cat’s progress; or response to haemoplasma treatment can be assessed by measuring haemoplasma loads after treatment.

An example

The figure to the left illustrates how Ct values are generated. The QPCR traces for two samples are shown. Sample A contains 250 times more target DNA than Sample B, so it reaches the threshold level at a lower cycle number (20) than Sample B (28). Samples containing no target DNA never reach the threshold level.

Figure 3. This graph illustrates how Ct values are generated.
Real Time Ct Values

What does Ct mean?

In a real time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The Ct (cycle threshold) is defined by the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e. the lower the Ct level the greater the level of target nucleic acid in the sample). WVDL real time assays undergo 40 cycles of amplification.

Cts ≤ 29 are strong positive reactions indicative of abundant target nucleic acid in the sample.

Cts of 30-37 are positive reactions indicative of moderate amounts of target nucleic acid.

Cts of 38-40 are weak reactions indicative of minimal amounts of target nucleic acid which could represent an infection state or an environmental contamination.

Source: http://www.langfordvets.co.uk/lab_pcr_ct_values.htm

Sheep Abortions Caused by Resistant C. jejuni

Jerome C. Nietfeld, D.V.M., Ph.D.
K-State Veterinary Diagnostic Laboratory

Campylobacter is one of the most common causes of abortion in sheep in the United States. Traditionally, Campylobacter fetus subspecies fetus has been the species most commonly associated with abortion. In recent years Campylobacter jejuni has been the most common species. Traditionally tetracycline is the treatment of choice for abortions in sheep. In fact, tetracycline is the only class of antibiotics approved for treatment of Campylobacter abortion in sheep. In 2008, the Iowa State University veterinary diagnostic laboratory reported that from 2003 to 2007 Campylobacter jejuni was the most common cause of sheep abortion at their laboratory. They acquired isolates from veterinary diagnostic laboratories in South Dakota, Idaho, and California and performed antibacterial sensitivity profiles on the isolates. All 74 isolates were resistant to oxytetracycline and to ceftiofur. The resistance to ceftiofur was not unexpected, because cephalosporins are often added to media for isolation of C. jejuni from heavily contaminated samples. All 74 isolates were sensitive to lincosamides, florfenicol, and tulathromycin. Seventy-two (97%) were sensitive to tylosin. The ISU group also did molecular testing and found that 66 of 71 isolate belonged to a single genetic clone. This indicates that a single tetracycline-resistant clone of C. jejuni has spread across much of the United States and become an important cause of sheep abortions. The ISU group has also reported experimental results that indicate that one of the commercially available C. jejuni vaccines may not protect against the tetracycline-resistant sheep abortion clone. This year we had several Campylobacter abortions in sheep and the ones that we were able to identify as C. jejuni were resistant to tetracycline. In at least one of the cases, the sheep were vaccinated against C. jejuni and C. fetus subspecies fetus.

The researchers at Iowa State followed up their study by looking at sequences of C. jejuni isolates reported to the Center for Disease Control and found that 9 percent of the human isolates were indistinguishable from the sheep abortion clone. The most common source in cases of human outbreaks was raw milk. In sporadic human cases the source was usually unknown. The sheep abortion clone was also isolated from raw milk, sheep and cattle feces, bile from healthy sheep, and goat and cattle abortion cases. Although sheep abortions do not appear to be a common source of Campylobacter infection in people, the clone is pathogenic for humans, and people should handle the materials from aborting sheep with care to avoid sheep-to-human transfer.
Schmallenberg virus (SBV) hits Northern Europe

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Extension Beef Veterinarian

Those in livestock practice should be aware of a new ruminant disease outbreak occurring in northern Europe. The disease-causing organism, called Schmallenberg virus (SBV) because of the area where it was first detected, has been reported in cattle, sheep and goats from Germany, the Netherlands, Belgium, the United Kingdom, and France (see map right). There is currently limited knowledge specifically related to SBV. Available information suggests that this virus is part of the Simbu serogroup of the Bunyaviridae family. Main clinical signs observed in cattle are fever, loss of appetite, up to 50 percent reduction in milk yield and, in rare cases, severe diarrhea for approximately one week. SBV has also been detected in association with a variety of congenital abnormalities observed in stillborn or newborn lambs and calves (see picture below). Many reports are coming in at this time as lambing and calving proceed in the affected areas. The disease is thought to be vector borne via biting midges, similar to Bluetongue or Akabane virus, so transmission may increase as warmer weather approaches.

While at this time the disease is confined to the regions mentioned, it may hop aboard a plane and show up in the United States. Let your veterinarian know if you see something suspicious. The most striking first signs have been the birth defects in stillborn or newborn lambs or calves.

Current knowledge suggests that it is unlikely that SBV can cause disease in humans.

Source: http://www.promedmail.org

Upcoming Events

July 13–14, 2012
Dr. Bob Hines’ Kansas Swine Classic, Manhattan, Kan.

July 22–24, 2012
Beef for Profit Alliance Conference, Manhattan, Kan.

August 9, 2012
The 2012 K-State Beef Conference will be held in the Frick Auditorium of the College of Veterinary Medicine in Manhattan. For the convenience of those who are not able to travel to Manhattan, the conference will be broadcast remotely to several sites around Kansas, including El Dorado, Parsons, Pratt, and Wakeeny. More information about the conference will be available in coming weeks at www.asi.ksu.edu/beefconference.

August 17–19, 2012
The Flint Hills Beef Fest is an annual celebration of the grass cattle industry for which the Flint Hills region is Kansas is known. For details and a schedule of events, visit http://www.beefest.com.

August 25–26, 2012
Kansas Livestock Sweepstakes, Manhattan, Kan.

September 27, 2012
The 2012 KSU Beef Stocker Field Day is set for Thursday, Sept. 27 at the KSU Beef Stocker Unit in Manhattan. Registration will begin at 9:30 a.m. and the day will conclude with a good old-fashioned prairie oyster fry. Watch for complete details on www.KSUbeef.org.

Randall Peterson
Section Editor