Kansas K-STATE Kansas State University
VETERINARY Quarterly
FOR THE PRACTICING VETERINARIAN
Winter 2011
Volume 14, Number 1

Imported bulls subject to new Kansas trichomoniasis regs

The following information was taken from the Kansas Animal Health News, December 2010, edited by Dr. Bill Bryant and Karen Domer. It is a newsletter published by the Kansas Animal Health Department and USDA/APHIS Veterinary Services and is available on line at: http://www.kansas.gov/kahd/newsletter/

To help protect the Kansas cattle industry from trichomoniasis, new cattle import regulations concerning *Tritrichomonas foetus* went into effect in Kansas after September 24, 2010. Currently, the regulations pertain only to bulls imported into Kansas and not to intrastate sales. In addition, bovine trichomoniasis was added to the list of Kansas reportable diseases.

Bulls imported into Kansas must go to a licensed slaughter plant or be accompanied by a completed certificate of veterinary inspection issued within the previous 30 days and signed by an accredited veterinarian. The certificate must individually list each imported animal and state whether, to the best of the veterinarian's knowledge, trichomoniasis has occurred in the herd of origin within the past two years.

Virgin bulls 18 months of age or younger must have a statement attached to the certificate that is signed by the owner or the owner's representative stating that the bulls have not been sexually exposed to breeding age females.

Non-virgin bulls, bulls 19 months and older, and bulls of unknown status must be certified negative for *Tritrichomonas foetus* and the negative test results should be attached to the certificate of veterinary inspection. "Certified negative" means that the samples were submitted to and tested by a laboratory accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD). The samples must be collected into and transported to the laboratory using the "InPouchTF" test kit system and one of two requirements must be met:

1. Three successive negative samples collected at least one week apart, if the positive/ negative status of the "InPouch TF" samples is determined by microscopic examination; or
2. One negative sample, if the positive/ negative status of the "InPouch" sample is determined by the real-time polymerase chain reaction (PCR) test. The owner must certify that the bull had at least two weeks of sexual rest before the sample was collected.

Whichever test is used, it must be conducted within 30 days before entry of the animals into Kansas and the producer has to verify that the bull had no female contact after the qualifying test.

Bulls that go to a sanctioned rodeo or a livestock show where they will be shown and returned to the state of origin without being sexually exposed to a breeding-age female are exempt.

Trichomoniasis "InPouch TF" System

The "In Pouch TF" test kit system is a self-contained system for the detection by culture of *Tritrichomonas foetus* from bovine preputial or vaginal samples. The proprietary medium is selective for the transport and growth of the trichomonad, while inhibiting the growth of yeast, mold and bacteria that might interfere with a reliable diagnosis. The "InPouch TF" test kit system is a product of BIOMED Diagnostics and is available from that company, veterinary distributors or in limited numbers from the K-State Veterinary Diagnostic Laboratory. For more information on the test system you may access the company's website at www.biomeddiagnostics.com or email them at: info@biomeddiagnostics.com.

You can also obtain information on the InPouch system, sample collection, proper shipment (i.e., they should be kept at room temperature rather than refrigerated or frozen), and any other question concerning trichomoniasis testing from Patricia A. Payne, D.V.M., Ph.D., assistant professor, Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine. Phone: 785-532-4604 or e-mail: payne@vet.k-state.edu

Also in this issue

Hepatozoonosis in Kansas dog linked to Gulf Coast tick

Jerome C. Nietfeld
Veterinary Diagnostic Laboratory

In October 2010, the K-State Veterinary Diagnostic Laboratory (KSVDL) received a blood smear and a muscle biopsy from a seven year-old, spayed Australian cattle dog with a request that both be examined for *Hepatozoon*. No evidence of blood parasites was found. However, there was multifocal, pyogranulomatous myositis with protozoal merozoites in areas of inflammation. There also were multilayered, onion skin-like cysts which are unique to and considered diagnostic of *Hepatozoon americanum*. The submitting veterinarian, who is located in the southeastern corner of Kansas said in a phone call that he is getting good at recognizing hepatozoonosis in dogs. This was his third client with affected dogs.

Hepatozoon americanum is a tick-borne infection of dogs caused by a protozoon of the apicomplexan phylum that includes *Eimeria, Isospora, Toxoplasma, Babesia, Plasmodium, Cryptosporidium* and other genera. The disease is considered to be emerging in the southeastern and south-central United States.[1,2] Transmission is by ingestion of *Amblyomma maculatum*, the Gulf Coast tick, which until recently was limited to the southern U. S. In recent years the tick has apparently been moving northward, and a recent review states that the range has expanded as far north as Kansas and Kentucky.[2] Dogs can also be infected by *H. canis*, which occurs worldwide, but in the U. S., *H. americanum* is the more common and important species. While *H. canis* infections are usually mild, *H. americanum* typically causes severe debilitating disease that if untreated is usually fatal.

After ingestion of infected ticks, sporozoites are released from macrophages and taken up by macrophages and transported throughout the body. The target tissue appears to be striated muscle, both skeletal and cardiac, where the organisms replicate and form cysts in macrophages between muscle fibers. The host cells deposit concentric rings of mucopolysacchride material around the cysts, thus forming the onion skin-like structures that are unique to *H. americanum*. Mature merozoites are released from the cysts and incite intense pyogranulomatous inflammation.

Clinical signs include fever, generalized muscle and bone pain, lameness that can progress to recumbency, weight loss, and cachexia that do not respond to antibiotic therapy. Because of the generalized muscle inflammation there is marked, persistent neutrophilia. Periosteal new bone proliferation is a common radiographic finding, with the proximal ends of long bones most commonly affected. The bony proliferation is thought to be secondary to inflammation at the insertion of skeletal muscles.

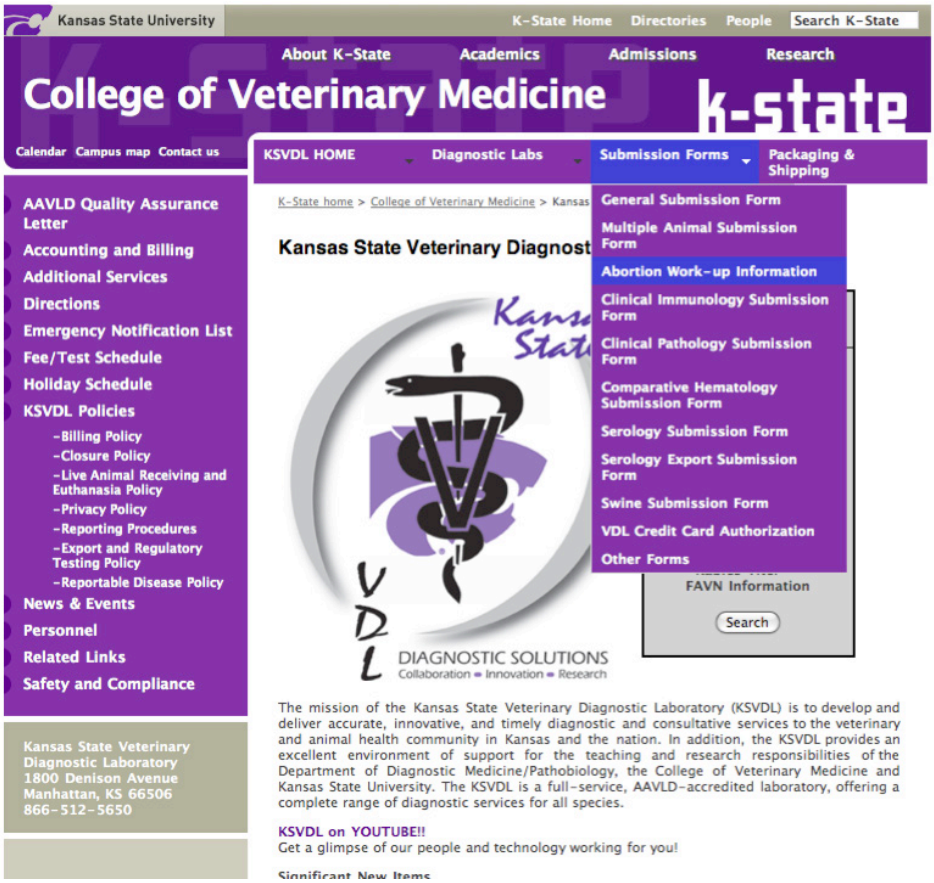
Diagnosis is by identification of *H. americanum* in circulating monocytes in smears of peripheral blood or by muscle biopsy. Because very few circulating monocytes contain organisms, the success rate for examination of blood smears is very low, and false negative results are common. Examination of multiple muscle biopsies is the more recommended method of diagnosis. Because infection is widespread and the numbers of organisms very high, false negative results by muscle biopsy is rare. Researchers at Auburn

See Hepatozoonosis, page 4

Abortion work-up guidelines posted

You may now access our recommended abortion work-ups via the diagnostic lab website at www.ksvdl.org. They are located in the drop down menu under "Submission Forms" (see below).

Each species is listed separately with general instructions, a list of supplies needed to collect samples, and what tests are recommended. To access complete information on each of the listed tests simply click on the test name to see estimated turnaround times and current prices along with various other details. Be sure to submit samples in proper packaging with ice packs (if needed) along with a completed general submission form. If you have questions contact us at 785-532-5650 or toll free at 1-866-512-5650.



Faulty premix formula causes selenium toxicosis in pigs

Kellie Almes, D.V.M., Diplomate ACVP
Veterinary Diagnostic Laboratory

Three pigs were submitted to the KSVDL for necropsy and diagnostic testing in September 2010. There was a history of feed refusal on the farm along with pigs being down in the rear limbs. A mycotoxin screen had been perfomed elsewhere on feed samples and was reported to be negative.

One dead and two live pigs were submitted to the diagnostic laboratory. The live pigs were unable to rise on the hind limbs, but they retained proprioceptive reflexes and a normal response to deep pain. Both animals were salivating with obvious bruxism. The pigs were humanely euthanized and all three animals were necropsied.

A full set of tissues including brain and spinal cord were placed in 10% buffered formalin and processed for histological examination. Fresh tissues including liver, kidney, brain, spinal cord, whole blood, and serum were saved for potential further testing.

Pig number 1 (the dead pig) had gross lesions consisting of chronic adhesions within the pleural and abdominal cavities along with a chronic umbilical abscess, which were all consistent with bacterial septicemia. There were also bilateral yellow malacic areas within the grey matter of the ventral horns of the lumbar region of the spinal cord.

The two live pigs were grossly normal except for the spinal cord. Within the cerviothoracic region in both pigs, as well as the lumbosacral region of pig number three there were bilaterally symmetric areas of reddening within the grey matter of the ventral horns.

Histopathologic evaluation of all three spinal cords revealed bilaterally symmetric poliomyelomalacia (Fig. 1) with lesions consisting of vacuolation of the neuropil, necrosis of neurons, gliosis, large numbers of gitter cells, and variable lymphoplasmacytic perivascular cuffing and plump endothelium lining prominent vasculature (Fig. 2). The latter two findings are indicative of chronic change and all of the observed lesions were consistent with selenium toxicosis. Whole blood, liver, and kidney samples were processed by the KSVDL toxicology laboratory for evaluation of selenium levels.

Samples of feed components were also submitted for quantification of selenium levels, which included corn, salt, soybean meal, complete grower diet, vitamin premix, and trace mineral premix. All samples were within the acceptable range except the trace mineral premix which contained 2,282 ppm which was estimated to be approximately 11.5 times the expected selenium levels.

Focal symmetrical polioencephalomalacia due to selenium toxicosis is unique to swine and is uncommonly seen in today's swine industry. Cases such as this with incorrect ration formulations are the typical cause and lead to clinical signs which are dependent upon the selenium concentration and duration of intoxication.

Acute intoxication often leads to signs that include decreased feed intake or feed refusal leading to decreased weight gain, hindlimb ataxia, dog-sitting posture, sternal recumbency, and occasionally generalized flaccid paralysis. Subchronic intoxication is characterized by signs such as hindlimb ataxia that progresses to posterior paralysis, tetraparesis, and generalized paralysis.

Affected pigs remain alert and attempt to ambulate while dragging their hindlimbs. Separation of the hoof wall at the coronary band also can be seen. Histologically these animals will have the characteristic symmetric poliomyelomalacia. Chronic intoxication leads to lameness, hoof overgrowth and deformation.

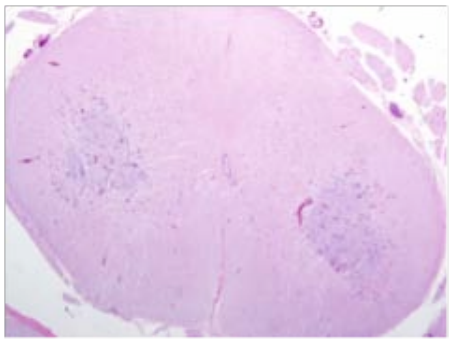


Figure 1: Spinal poliomyelomalacia

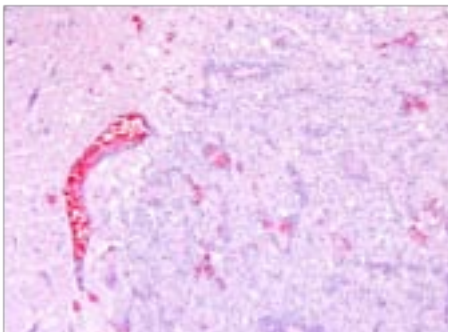


Figure 2: Perivascular cuffing

Typical necropsy findings in these cases are similar to the ones seen in these three pigs and the histologic lesions are unique to this condition. Confirmation of selenium toxicosis can be achieved as it was in this case with analysis of whole blood or fresh liver and kidney.

(*Editor's Note:* Because the mineral premix purchased by the pig producer contained excessive selenium, producers who had purchased the premix or feed formulated with the mineral premix were contacted. Multiple producers ended up having feed with toxic levels of selenium and pigs with clinical signs of selenium toxicity. The case is a good example of what can happen when an error is made in formulating feed components.)

Selenium Levels

Pig Number	Whole Blood	Liver	Kidney
1	N/A	5.66ppm	5.57ppm
2	12.77ppm	9.04ppm	6.59ppm
Toxic Range	.08-.35ppm**	>3ppm*	>3.8ppm*

* KSVDL reference values; ** Clinical Veterinary Toxicology

Tremorgenic mycotoxicosis in Kansas dogs caused by consumption of garbage containing penitrem A

Brad M. DeBey, Deon van der Merwe, Veterinary Diagnostic Laboratory

Several dogs in a Kansas household developed a sudden onset of severe clinical signs that included repeated vomiting, seizure-like activity described as a stiffening of the limbs, and behavioral changes that were initially believed to be caused by consumption of commercial dog food.

Based on the clinical signs, the differential diagnoses included pesticides (strychnine; organophosphates; carbamates; metaldehyde; bromethalin, zinc phosphide, chlorinated hydrocarbons), tremorgenic mycotoxins (penitrem A; roquefortine; various mushrooms), and stimulant foods or drugs (methylxanthines from chocolate/coco; caffeine; ADHD medications etc.).

The clinical signs most closely resembled classic strychnine poisoning because of the apparent inability to control reflex extension of the limbs. This placed strychnine, penitrem A and metaldehyde at the top of the differentials list because these poisons share a similar mechanism of action by suppressing the neuro-inhibitory and reflex-inhibiting functions of glycine and/or GABA in the brain and spinal cord.

There was evidence of garbage consumption in the stomach content of the affected dogs. The stomach contents tested negative for strychnine. A gas chromatography screen revealed viridicatin, which is a metabolite associated with *Penicillium* molds that often also produce penitrem A. Unfortunately, penitrem A breaks down in the high temperatures needed to perform gas chromatography, so a separate test for penitrem A was performed using high pressure liquid chromatography. Penitrem A was identified in the stomach contents, but not the dog food.

Penitrem A is produced by fungi in the genus *Penicillium*, and is regarded as the most toxic tremorgenic Penicillium mycotoxin. The most common substrates for toxin production are dairy products including cheese and sour cream, nuts, and grains. Poisoning is often associated with consumption of discarded moldy food or garbage. In most cases, grossly visible mold is present on the foodstuff that results in poisoning. Roquefortine is a tremorgenic mycotoxin produced by *Penicillium* spp. that can be present along with penitrem A. It was first discovered in association with *Penicillium roqueforti*, which is used to make Roquefort and blue cheese.

Penitrem A is one of several tremorgenic mycotoxins that have the potential to cause severe clinical disease and death. In dogs, clinical signs of tremorgenic mycotoxin consumption include vomiting, salivation, tremors, ataxia, agitation and seizures. The clinical signs closely resemble strychnine poisoning; therefore, tremorgenic mycotoxin poisoning should be considered as a differential diagnosis in suspected strychnine cases, as well as other seizure-inducing poisonings.Less is known about the clinical signs of roquefortine toxicosis.

Although death is a potential outcome, many dogs that have consumed penitrem A totally recover within 24 to 48 hours of the onset on clinical signs. There are occasional reports of a more protracted recovery. Lack of controlled reflexes in the throat while vomiting increases the risk for aspiration pneumonia.

Treatment of patients with clinical signs includes diazepam to control seizures. Barbiturates may be needed to control seizures if diazepam is not effective. Methocarbamol can be used if diazepam and barbiturates are insufficient to control the muscle fasciculations and hyperesthesia. Supportive care includes intravenous fluids, control of body temperature, and oxygen therapy if respiration is compromised. Activated charcoal can be helpful to prevent absorption before the onset of clinical signs if an animal was observed to consume potentially poisonous material.

Hepatozoonosis, from page 2

University have developed a PCR test for use with whole blood that is offered commercially.

Treatment is initially symptomatic aimed at correcting life-threatening problems and supporting cachectic dogs. There are no medications available that completely eliminate the organism. However, a protocol utilizing a combination of trimethoprim-sulfadiazine, clindamycin, and pyrimethamine for two weeks followed by chronic administration of decoquinate has been used effectively to treat overt clinical disease.[2] Treatment effectively eliminates the merozoite stage, which removes the stimulus for myositis and results in remission. Because all organisms are not eliminated, relapses often occur.

The following references are excellent reviews of the disease and offer much additional information.

1. Ewing SA, Panciera RJ. 2003. American canine hepatozoonosis. *Clinical Microbiology Reviews*, 16(4):688-697.
2. Potter TM, Macintire DK. 2010. Hepatozoon americanum: an emerging disease in the south-central/ southeastern United States. *Journal of Veterinary Emergency and Critical Care*, 20(1): 70-76.

Diagnosing leptospirosis in Holstein feedlot cattle

Brad M DeBey, D.V.M., Ph.D.
Veterinary Diagnostic Laboratory

Holstein feedlot cattle are more commonly diagnosed with clinical leptospiral infections than beef cattle breeds maintained in similar conditions. Whether this is because Holstein cattle are more susceptible, or because they are typically backgrounded in conditions more likely to result in infection by leptospira, or because of other conditions is speculative.

Typical clinical signs of leptospirosis include those expected with hemolytic anemia; hemoglobinuria, icterus, and pale mucous membranes. It is not unusual to find calves dead before any clinical signs are observed. Expected gross postmortem findings of cattle dying of acute leptospirosis include pale, edematous lungs, a friable, anemic and bile-stained liver, dark urine due to hemoglobinuria, and dark, swollen kidneys.

When leptospirosis is suspected, collection of blood (serum) for testing for serum antibodies, and urine and kidney for identification of *Leptospira* spp. is recommended. Depending on how acute the leptospiral infection is at the time of blood collection, there may be significant elevation of serum antibodies (to a titer greater than 1:800). It is not unusual to have a serologic response to multiple serovars in an infection with just a single serovar. The serologic response will usually become more specific with a single serovar developing a higher titer as the immune response matures.

It is not unusual for calves dying acutely to be seronegative to all serovars. PCR assay is replacing immunofluorescence (FA) testing as the method of choice for laboratory confirmation of leptospira. Silver-staining of tissue is sometimes used, but has poor sensitivity and specificity. PCR procedures for leptospira are generally more reliable for urine than for tissues, so it is recommended to collect urine when doing necropsies for suspect leptospirosis cases.

Leptospiral infection usually causes more severe disease in the incidental host rather than the maintenance host for that serovar. For example, the pig is recognized as the maintenance host for serovar *pomona* and even though *pomona* is often very virulent in cattle it is less so in pigs. For that reason, serovar *hardjo*, which is adapted to cattle, would not likely cause severe disease in cattle. Furthermore, some leptospiral serovars, including *hardjo*, do not cause intravascular hemolysis because they lack hemolysin, and therefore will not be associated with hemolytic anemia.

There are several potential differential diagnoses for cattle with icterus and hemolytic anemia, including bacillary hemoglobinuria (*Clostridium haemolyticum* infection), copper toxicosis, and toxins that can cause hemolytic anemia (rape and kale).

Bacillary hemoglobinuria diagnosis relies on identification of an infarct in the liver and isolation of the causative organism from the infarct. Copper toxicosis is diagnosed by chemical analysis of liver and/or kidney tissue for copper content.

Editor's note: In November 2010 (after Dr. DeBey wrote this article), the Diagnostic Laboratory identified Leptospirosis in a group of Holstein steers in a feedlot in central Kansas.

New KABSU facility offers range of services

Kansas Artificial Breeding Services Unit (KABSU) has moved into the new facility and begun bull semen collections. The facility is located north of the University main campus within the Animal Sciences and Industry livestock units complex. The address is 3171 Tuttle Creek Blvd., which allows easy access directly off Highway 24 (Tuttle Creek Blvd).

KABSU offers the following options for bull semen collection:

Bull housed at KABSU for CSS semen collection.

- Pre-entry health testing required (TB, Brucellosis, Leptospirosis, BVD-virus isolation)
- CSS health testing required
- Minimum 60-day residency required to complete CSS testing protocol

Bull housed at KABSU for Non-CSS semen collection

- Pre-entry health testing required (TB, Brucellosis, Leptospirosis, BVD-elisa test)
- No minimum residency requirements
- Bull is taken home whenever desired amount of semen is collected
- Weekly trichomoniasis tests are required during first 3 weeks of residency

Haul-in semen collection

- No health testing required
- Bull collected and taken home same day

Field service on-farm semen collection

- No health testing required

KABSU also offers semen storage and shipping options. Contact KABSU by calling 785-539-3554 or e-mail at kabsu@ksu.edu

Stop by for a visit of the new facility.

Collecting diagnostic samples for PCR and viral tests

Jerome C. Nietfeld
Veterinary Diagnostic Laboratory

We frequently receive questions concerning collection and submission of specimens, especially those to be tested for viruses or by polymerase chain reaction (PCR). Considerable time and money goes into collection, shipping, and testing of specimens, and veterinarians naturally want to do the best job possible in order to maximize the potential of obtaining the correct results for clients.

PCR procedures detect DNA or RNA, and samples suitable for bacterial or viral isolation are, with one exception, suitable for molecular tests. The exception is swabs in gel bacterial transport media, such as gel Amies medium. Culturettes with gel transport media are useful because they are suitable for isolation of aerobic and anaerobic bacteria. However, the gel renders samples unsuitable for PCR and virus isolation.

Postmortem decomposition, heat, desiccation, and pH extremes inactivate many viruses and degrade nucleic acids, especially RNA. Cool temperature, moisture, and protein have a stabilizing effect. Small tissue samples (≤1 cm^3) chilled as soon as possible and kept cool during transport are suitable, if received by the laboratory in two or three days. Submit tissues in sterile containers and do not place contaminated tissues, such as pieces of the gastrointestinal tract, with clean samples.

If the lag time will be more than two or three days, many people feel that swabs give better results. This actually depends on the viruses in question as they vary markedly in their stability while being transported.

In general, swabs in liquid are suitable for virus isolation, ELISA, PCR, and other tests. Physiologic saline is suitable, and phosphate buffered saline is better for isolation of relatively hardy viruses, and they give good PCR results. Transport systems designed for isolation of viruses, *Mycoplasma* spp, and *chlamydiae* are available commercially (M4®, Remel, Lenexa, Kansas, USA; BD™ Universal Viral Transport System, BD Diagnostics, Sparks, Maryland, USA) and give excellent results. Commercial systems consist of a tube of medium and two swabs. The media contain antibiotics to prevent bacterial overgrowth, protein to stabilize viruses, and buffers to prevent drastic pH changes. Brain-heart infusion broth with 50 µg gentamicin/ml also works well for virus isolation and PCR.

If contacted ahead of time, most diagnostic laboratories, including the K-State lab, will supply tubes with transport media. If you prepare your own transport tubes, use synthetic swabs with plastic or metal shafts and 2-3 ml of liquid to prevent over dilution. Swabs tipped with cotton alginate or with wooden shafts are not recommended.

If swabs are to be used only for PCR, they can be allowed to dry before shipment, but they will not be suitable for virus isolation. Often we want to know only if a particular agent is present or not. In those cases, dry swabs for PCR are perfectly suitable.

In many cases there is a desire to type the virus. For instance, many veterinarians and owners want to type BVD and swine influenza viruses. Typing is most commonly done by PCR or by sequencing segments of viral nucleic acid after PCR amplification. Sometimes there is enough viral nucleic acid in the sample to do the sequencing without isolation, but in many cases the virus must be isolated and the isolate used for typing.

To Pool or Not to Pool Samples

A common request is to pool samples from multiple animals to keep costs down. Pooling can be helpful because it allows testing of more animals without increasing costs and gives a better overall picture of the status of the group.

However, pooling samples is not always a good idea. If you want to do virus isolation, it is usually best to keep samples from individual animals separate. The reason is neutralizing antibodies, which prevent viruses from infecting cells. If one animal in the group has neutralizing antibodies to the virus in question, antibodies in tissues can prevent isolation of the virus, even if tissues from multiple animals contain the virus.

PCR and ELISA results are normally not affected by pooling. The only effect of pooling is possible over dilution of the agent if most of the samples are negative (isolation is also subject to over dilution). Sometimes diagnostic laboratory personnel might be reluctant to pool samples or to make large pools if they do not have data to indicate that results will be accurate.

If a pool is positive, is it important to know which sample was infected? If so, the lab must individually test all samples in the pool. In cases where the proportion of positive animals is relatively high, you will end up spending more money by pooling.

If there is no chance that individual animals will need to be tested, it is fine for the submitter to pool samples before shipment. If there is a chance individual animals will be tested, send the samples separately and ask the diagnostic lab to do the pooling. They will save a portion of each sample and if a pool is positive can test the individual samples.

New molecular tests available

Available Now

Real-Time PCR Panel for Bovine Respiratory Diseases. A real-time PCR panel to identify common viruses and bacteria commonly associated with Bovine Respiratory Disease complex has been developed and available at the KSVDL.

The current bovine respiratory panel identifies the presence of nucleic acid from the following bacterial and viral agents: *Mycoplasma bovis, Chlamydial* species, Bovine Viral Diarrhea Virus (BVDV), Bovine Respiratory Syncytial Virus (BRSV), Bovine Respiratory Coronavirus (BRCV), Infectious Bovine Rhinotracheitis Virus (IBR), and Bovine Parainfluenza Virus-type 3 (PI3).

The panel targets DNA or RNA isolated from individual animal samples, preferably nasal swabs but also pharyngeal swabs/washes, or fresh lung tissues (unfixed). Cost for individual animal submissions is $75 per animal. Contact Mike Hays (785-532-4425, or hays@vet.ksu.edu) or Dr. Dick Oberst (785-532-4411, or oberst@vet.ksu.edu) for more information.

In Development

Real-Time PCR Test for Canine Brucellosis Caused by Brucella canis. A real-time PCR test to identify and possibly quantify the bacterial load of *Brucella canis*, which causes canine brucellosis is in the final stage of development. The test is a duplex real-time PCR procedure targeting the 16S rRNA gene that is common to all Brucella species, and a DNA fragment that is specific to *Brucella canis*. Blood in a sodium citrate tube, and a vaginal swab in bacterial transport medium are preferred sample types. Vaginal swabs can be directly used for DNA extraction and PCR reactions, which is more sensitive, and the bacterial load can be quantified. Blood samples need to be cultured prior to PCR, thus is not quantitative.

For a limited time, we are accepting potential positive samples for free testing to further validate the test. Contact Dr. Jianfa Bai (785-532-4332, or jbai@vet.ksu.edu) for more information.

Multiplex PCR Panel for Canine Respiratory Diseases. A multiplex PCR panel to identify bacteria and viruses frequently associated with canine respiratory cases is being developed at the KSVDL. The current targets for the canine respiratory panel include: *Streptococcus canis* , *S. zooepidemicus*, *Bordetella bronchiseptica*; canine adenovirus type 2, canine herpes virus, canine respiratory coronavirus, canine influenza virus, canine parainfluenza virus, canine distemper virus and an internal test control Canine GAPDH.

The test is being designed to detect these common canine respiratory pathogens following collection of nasal swabs of clinically effected dogs and shipping the swabs in a standardized viral transport media to the KSVDL for evaluation. *The test is still in validation to evaluate the sensitivity and specificity of the panel in comparison to bacterial and viral isolation, and therefore not currently available.* We will have the assay available in the near future. Contact Dr. Lalitha Peddireddi (785-532-4425, or lpeddire@vet.ksu.edu) or Dr. Dick Oberst (785-532-4411, or oberst@vet.ksue.edu) for more information.

Johne's Disease updates

On the following pages you will find the winter Johne's Disease updates designed for beef and dairy producers. Please feel free to copy either or both articles and send them to clients whose operations may be at risk or who would like education on the topic.

Upcoming Events

Cattlemen's Day – March 4

Cattlemen's Day is set for March 4 in Weber Arena, Kansas State University, Manhattan.

On-site registration, trade show and educational exhibits begin at 8 a.m. Educational program begins at 10 a.m. Forms for veterinary continuing education credits will be available at registration.

For additional information and registration information see: http://www.asi.ksu.edu/cattlemensday

Kansas State Veterinary Quarterly
FOR THE PRACTICING VETERINARIAN

Newsletter Coordinators

Larry C. Hollis, Extension Beef Veterinarian
785-532-1246 • lhollis@oznet.ksu.edu

Jerome C. Nietfeld
785-532-4460 • nietfeld@vet.ksu.edu

Contributors — K-State Research and Extension

Dale Blasi, Scott Beyer, Mike Brouk, Joel DeRouchey, Brian Faris, Bob Goodband, Karl Harborth, Sandy Johnson, Twig Marston, Chris Reinhardt, Jim Nelssen, John Smith, Mike Tokach, Justin Waggoner

Contributors — Veterinary Diagnostic Laboratory

G.A. Andrews, S.S. Dritz, R. Ganta, T. G. Nagaraja, J.A. Pickrell, M. Wight-Carter, M.M. Chengappa, M.W. Dryden, R. Hesse, F.W. Oehme, S. Stockham, B. DeBey, W. Fortney, D.A. Mosier, P. Payne, D. vander Merwe, M.J. Wilkerson

K-State Research and Extension
137 Call Hall
Manhattan, KS 66506

NATIONAL JOHNE'S EDUCATION INITIATIVE

Johne's Disease – Beef

Volume 1, Number 3 Winter 2010

A cooperative effort of the National Institute for Animal Agriculture, USDA, APHIS, Veterinary Services, in association with the National Johne's Working Group & United States Animal Heath Association

Better to be Proactive Than Sorry

While some seedstock producers argue that testing for Johne's disease is an outward sign that you are concerned that your herd might be infected with *Mycobacterium avium* subspecies *paratuberculosis*. Dave Judd of Judd Ranch, Pomona, Kan., sees things differently. This Kansas purebred breeder argues that testing for Johne's disease is a proactive step that every seedstock producer should undertake.

Dave says testing for Johne's disease and knowing no positive have been found lets him sleep better at night.

"Although we had not seen any cases of Johne's disease in the herd, I want to be confident that Johne's disease is not unknowingly present in our herd and might result in infecting the herds of our customers when they purchase a Judd Ranch bull or female," Dave explains.

Dave, who owns Judd Ranch in partnership with his wife Cindy and sons Nick and Brent, adds that Judd Ranch sells 200-plus Gelbvieh, Red Angus and Balancer bulls every March and 100-plus females every October. With those sales comes responsibilities.

"Your reputation is on the line with every bull or female sold to fellow seedstock producers and commercial cow-calf operators," Dave elaborates. "It just makes sense to participate in a Johne's disease prevention, control and testing program."

Judd Ranch initiated testing for Johne's disease five years ago. The initial testing was recommended by their herd health consulting team out of Kansas State University, with the testing cost partially funded by USDA/APHIS/VS.

Since then, government assistance for testing has ceased. Now the cost of testing is underwritten in full by Judd Ranch and deemed a smart investment.

"Ignorance is not bliss when it comes to Johne's disease," Dave states. "It's a responsibility of seedstock producers such as ourselves to know the prevalence or non-prevalence of Johne's disease in our herds. I would compare this knowledge and confidence level equal to being a certified brucellois-free herd or a PI-free herd."

Judd Ranch turned to a veterinarian from Kansas State University to handle its initial Johne's disease testing. All cattle three years of age and older were blood tested, with samples submitted to the Kansas State University Diagnostic Lab (an approved Johne's disease testing facility). The result: No positive animals were found.

Seedstock producers have lots of responsibilities, and Dave Judd of Judd Ranch says knowing the prevalence or non-prevalence of Johne's disease in your herd is one of those responsibilities. "You don't want to unknowingly pass Johne's disease on to your customers," he states. Upper righthand corner: Dave Judd, Judd Ranch

Annual Johne's testing has continued at Judd Ranch since that first testing five years ago.

"Judd Ranch is definitely proactive when it comes to herd health," states Dr. Larry Mages who handles the ranch's day-to-day herd health. "I wish every beef seedstock herd in the country would be this proactive. If you're selling seedstock or bulls to commercial cattlemen, then you owe it to your customers to test for Johne's disease and be comfortable that you're not unknowingly introducing Johne's disease into their herds."

Attention to Recips

Johne's disease testing proponents stress that it's important that seedstock producers using embryo transfer programs test recipient females. After all, Mycobacterium avium subspecies paratuberculosis can be passed in utero.

"We use Judd Ranch-raised females as recips so that isn't a problem here," Dave adds. "But, if we ever ran out of recips and had to buy recips, they would be tested straight way before they are used in the ET program. That's just a smart biosecurity measure."

This e-newsletter is brought to you by the National Johne's Educational Initiative funded by USDA/APHIS/VS and managed by the National Institute for Animal Agriculture. Editor: Teres Lambert. To learn more about Johne's, visit ***www.johnesdisease.org***.

Vaccine Project Underway

With only one USDA-approved vaccine available to help protect against Johne's disease, many veterinarians and producers would like more available vaccines—particularly since the current approved vaccine has limitations and is not approved for use in all states. With funding from USDA-APHIS-VS, the Johne's Disease Integrated Program has undertaken an effort to identify viable vaccine candidates and evaluate those with the greatest potential for commercial development.

"The project is in the initial stages of a three-step process," states Tiffany Cunningham with JDIP. "Currently, JDIP is in Phase I of the vaccine-testing program and has added an additional participating institution, AgResearch Limited, to the program."

As part of Phase I of the program, scientists have submitted strains of live vaccine candidates and recombinant proteins, and a laboratory at The Pennsylvania State University is coordinating the collection and growing the strains that have been received. The strains will then be distributed to candidate vaccine-testing centers at the University of Wisconsin and the University of Minnesota for blinded evaluation.

"The JDIP Epidemiology and Biostatistics Core at Cornell University will analyze the results of the testing in a blinded manner and identify the 'best candidates'," Cunningham states. "Once the analysis is complete and the blind key is opened, all of the program participants will receive the data at the same time."

During Phase II of the vaccine-testing program, "best candidates" will be evaluated using a mouse model. If all goes as planned, two laboratories will conduct the infection and protection studies in the mouse.

The "best candidates" identified though the mouse studies will be evaluated using a goat model in Phase III.

"This will provide data similar to that from cattle, but the data will be available in a much shorter time frame and at a lower cost," states Robab Katani , a JDIP scientist with The Pennsylvania State University.

"The coordinated three-stage evaluation will take approximately three years to complete. It is expected that this rigorous screening process will identify one or more viable candidates to move forward for commercial development."

The Johne's Disease Integrated Program, often referred to as JDIP, is a comprehensive consortium of scientists whose mission is to promote animal biosecurity through the development and support of projects designed to enhance knowledge, promote education, develop real-world solutions and mitigate losses associated with Johne's Disease. By joining together, efficiencies through collaborative research can be achieved and intellectual and physical resources critical to overall success can be shared. The consortium includes leading scientists in the field, drawn from the world's top academic and government institutions representing the diverse disciplines of microbiology, immunology, pathology, molecular and cellular biology, genomics, proteomics, epidemiology, clinical veterinary medicine, public health, extension and public policy.

Funding from USDA-APHIS-VS allows JDIP to provide competitively awards for meritorious research, education and extension projects addressing Johne's disease. Approximately $1 million will be distributed in the current funding cycle, with $400,000 designated for Johne's disease diagnostics projects, and the remainder available across the remaining JDIP Core and Project areas.

Commercial cow-calf producers and seedstock producers can lessen the chance of introducing Johne's disease into their herds by purchasing bulls and/or females only from Johne's tested herds. The bulls used on these females come from a Johne's tested herd.

For information about Johne's disease, contact your Designated Johne's Coordinator Donald Evans, *Donald.E.Evans@aphis.usda.gov*, **Ph (785) 270-1305 or your Beef Quality Assurance Coordinator Clayton Huseman,** *clayton@kla.org*, **Ph (785) 273-5115.**

NATIONAL JOHNE'S EDUCATION INITIATIVE

JOHNE'S DISEASE – DAIRY

Volume 1, Number 3 Winter 2009

A cooperative effort of the National Institute for Animal Agriculture, USDA, APHIS, Veterinary Services, in association with the National Johne's Working Group & United States Animal Heath Association

Johne's Disease a Focus of RDQMA Study

Mycobacterium avium subspecies *paratuberculosis*, the organism known to cause Johne's disease, was among organisms studied by the Regional Dairy Quality Management Alliance which tracked endemic disease dynamics over time in Northeast dairy herds with well-characterized animals and herd management practices. The RDQMA project is in cooperation with USDA.

Since starting its research, the RDQMA has identified MAP super-shedders on study farms and have recognized the role that super-shedders play in the creation of passive shedders.

"Over time, many passive shedders become infected with MAP in their intestinal tissues (adult infection)," states the RDQMA report. "We recognized that virtually all active shedders have the potential to become super-shedders and super-spreaders of MAP."

In the most recent study, researchers sought to estimate the effect of Johne's disease status on individual cow milk production using longitudinal data collected over a four-year period from three U.S. dairy herds enrolled in the RDQMA project. Quarterly ELISA serum testing, biannual fecal culture and culture of tissues at slaughter helped determine Johne's disease status: uninfected, low shedding or high shedding. Milk production data were collected from the Dairy Herd Improvement Association, with the effect of Johne's disease status on milk production analyzed.

Highlights of the RDQMA report:

- "Johne's disease status was found to have a significant effect on milk production, and this effect was not uniform across Johne's disease status categories. Our data indicate that cows that eventually will show low and high shedding of MAP are out-producing MAP-negative animals in the herd. Although latent animals produce more milk than uninfected animals, that difference decreases over time in the latent infection state."
- "When an animal starts shedding low levels of MAP, the model predicts an initial milk production that is slightly higher than that of uninfected herdmates, but there is a greater rate of decrease in milk production compared with the latently infected animals."
- "Animals in the high-shedding category have a meaningfully lower milk production than uninfected herdmates, with large decreases in production over time when remaining in the herd."
- "Greater milk yield is evident during latency compared with uninfected herdmates, but the discrepancy in yield decreases as the disease progresses over time. This MAP-induced decrease in milk production is supported by the clinical progression of Johne's disease."
- "As the organism invades the intestinal epithelium and begins to affect nutrient absorption, feed efficiency decreases and milk production in negatively affected."
- "This analysis provides strong support that Johne's disease status affects milk production in all infected animals, with increasing losses in milk production as disease progresses."
- Culling decisions should be made "on an individual animal, economic level, particularly since animals shedding MAP also spread the infection through environmental contamination."

RDQMA finding: Johne's disease status affects milk production in all infected animals, with increasing losses in milk production as disease progresses.

Looking Closer at Super-Shedders

To better understand the epidemiology of Johne's disease and MAP dynamics in dairy cattle, researchers used a DNA-based molecular subtyping technique. Using this technique with the observed MAP shedding level, they evaluated whether low shedders of MAP were passive shedding (pass-through) animals or whether they were truly infected and whether these animals were possible infected by the super-shedders within the herd.

From among the 142 isolates from fecal and tissue samples from the

(continued on page 4)

This e-newsletter is brought to you by the National Johne's Educational Initiative funded by USDA/APHIS/VS and managed by the National Institute for Animal Agriculture. Editor: Teres Lambert. To learn more about Johne's, visit ***www.johnesdisease.org***.

Researchers Team with Producers in Battle Against Johne's

When the National Animal Health Monitoring System released its comprehensive report on Johne's disease in 1997, the majority of dairy producers had only a general idea of what the disease was, and fewer still thought it affected their herds. But with an estimated 50% of the dairy animals in Michigan infected with Johne's disease, there was no doubt it was a serious industry priority.

Several Michigan State University researchers sought out funding to learn more about this economically damaging animal health issue, which has an estimated $200 million annual impact on the U.S. dairy industry.

Central to the initial research efforts was Dan Grooms, MSU associate professor of large animal clinical sciences and a large animal veterinarian. Along with learning more about the disease, Grooms and his colleagues from MSU and other universities would work for several years to determine the best management practices to employ on a dairy farm to prevent the spread of the disease and lower the percentage of animals infected (prevalence rate).

In 2003, researchers and veterinarians from the MSU Department of Animal Science, College of Veterinary Medicine and the Diagnostic Center for Population and Animal Health, and the Michigan Department of Agriculture joined researchers from 16 other states to monitor dairy herd management practices. The Michigan team was chosen to be a part of the USDA's National Johne's Disease Demonstration Project. The purpose of the national project was to evaluate the long-term feasibility and effectiveness of management-related practices designed to control infection by Mycobacterium avium paratuberculosis (MAP), the causative organism for Johne's disease.

Grooms selected seven herds to serve as his Johne's disease demonstration herds. The herds, located in various regions of the state, underwent whole-herd testing to measure baseline levels of infection. From there, a disease risk assessment was conducted, and management practices were put in place to help control on-farm spread of the disease.

Calf management was the top priority of a seven-herd Michigan research project focused on best management practices to prevent the spread of Johne's disease and lower the percentage of animals infected.

"We know that animals are most susceptible to Johne's infection at a very young age, so calf management was our first priority," he says. "There is no cure for Johne's, so the best way to manage the disease is to prevent it."

At the same time that Grooms was assembling his herds for the demonstration project, Galen Schalk, a dairy farmer in Hillman, Mich., encountered his herd's first diagnosed case of Johne's disease.

"I had heard about Johne's disease but thought, 'That's not me,'" Schalk says. "We have had a closed herd since 1974, so because I was not bringing new animals into the herd, I didn't feel we were at risk for the disease."

The first Johne's test from the Schalk's herd, run at the request of his veterinarian, came back positive for Johne's. The diagnosis concerned Schalk, who contemplated how many other cases he might have in the herd, so when Grooms approached him to be part of the Johne's disease demonstration project, Schalk did not hesitate to sign up.

"I had a very minimal understanding of Johne's and minimal prevention practices when we started with this project," Schalk says.

The first round of fecal cultures from the Schalk herd came back with a 21% prevalence rate among the 168 animals tested; the second year, 2004, the rate jumped to 42%. The more Schalk learned about the disease and the test results on his herd, the more he realized that he had seen cows develop clinical signs of the disease in the past but hadn't realized it was Johne's.

(continued on page 3)

For information about Johne's disease, contact your Designated Johne's Coordinator Donald Evans,

Donald.E.Evans@aphis.usda.gov,

Ph (785) 270-1305 or visit

www.johnesdisease.org

Johne's Battle *(continued)*

"We would have cows get really thin and drop in productivity, so we would cull them from the herd," Schalk says. "Now I know they were Johne's animals."

Seeing the high prevalence rate, Schalk was eager to eliminate the problem as quickly as possible. Shalk, Grooms and other MSU scientists put together new management strategies to help control the disease.

The area of highest concern on the Schalk farm was the calving area. Cows calved on a manure pack, which created the perfect environment for disease organisms to survive and spread to newborn calves. Though the Schalks had already drawn up building plans for a new transition barn, they opted instead to construct a new maternity and housing area for close-up cows.

"It was good that we were already looking to put up a new building because we really needed a better place for the animals to calve in," Schalk says.

Along with building the new maternity area, Schalk started withholding the colostrum from Johne's-positive cows and feeding newborn calves colostrum from only non-infected cows.

"Johne's can be transmitted to the calf through the colostrum or from the contaminated environment," Grooms says. "Knowing which cows are positive for Johne's is critical in stopping the disease from spreading."

The new maternity area also provided an opportunity for each cow to calve in its own pen and allowed Schalk time to clean and disinfect each pen between calvings. Because animals contract Johne's disease early in life, properly caring for calves is one of the most critical steps in preventing disease transmission, even though measuring immediate results from changing management practices is difficult.

"Even though we culled a number of animals during the first two years of the project, we still need to manage for the disease because we know some of the older animals are carriers," Schalk says.

Visually identifying the Johne's carriers helps Schalk manage the disease. Schalk now tags all animals that test positive for Johne's disease with a special red neck chain. Any heifers born to positive dams are also tagged with the red neck chain until they receive a negative test reading.

"It is not perfect," Schalk says. "Occasionally an animal is born early in the close-up area and not in the assigned calving pen, but we are really making progress."

Researcher Dr. Dan Grooms notes that "this project shows us that, though there is no cure for Johne's disease, with proper management, farmers can prevent the spread of the disease on their farms and reduce its prevalence over time."

Animals can shed the organism that causes Johne's even if they are not showing clinical signs of the disease. Research indicated that the disease-causing organism is shed through the manure. So Schalk implemented another critical management practice -- taking preventive measures to ensure that no manure comes in contact with animal feed.

To prevent cross-contamination, the Schalks bought a second skid steer and use one only to clean and scrape manure and the other only to handle and move feed. They also make sure not to cross over feed alleys with equipment to minimize the risk of any manure on the tires coming into contact with the feed.

Since the Schalk herd became part of the Johne's demonstration project, the prevalence of Johne's in the herd has dropped to less than 5%. The results on this herd are similar to the outcomes realized by the other test herds.

"We saw a reduction in the number of Johne's-positive animals in all the herds we worked with," Grooms says. "This project shows us that, though there is no cure for Johne's disease, with proper management farmers can prevent the spread of the disease on their farms and reduce its prevalence over time."

As the demonstration project winds down, Schalk is looking ahead to how he will continue implementing the recommended management practices on his farm. Now that he has the prevalence rate down to less than 5 percent, he will continue to test the herd to monitor for any new infections.

"We were surprised to learn that we had the disease at all. If we don't continue to test the herd, we won't know if we're continuing to make progress," Schalk says.

Funding for Grooms' position with an emphasis on cattle disease management was made possible by the Animal Agriculture Initiative at MSU. The AAI was established in 1996 as part of the grass-roots-driven Revitalization of Animal Agriculture in Michigan Initiative.

Vaccine Project Underway

With only one USDA-approved vaccine available to help protect against Johne's disease, many veterinarians and producers would like more available vaccines—particularly since the current approved vaccine has limitations and is not approved for use in all states. With funding from USDA-APHIS-VS, the Johne's Disease Integrated Program has undertaken an effort to identify viable vaccine candidates and evaluate those with the greatest potential for commercial development.

"The project is in the initial stages of a three-step process," states Tiffany Cunningham with JDIP. "Currently, JDIP is in Phase I of the vaccine-testing program and has added an additional participating institution, AgResearch Limited, to the program."

As part of Phase I of the program, scientists have submitted strains of live vaccine candidates and recombinant proteins, and a laboratory at The Pennsylvania State University is coordinating the cultures and growing the strains that have been received. The strains will then be distributed to candidate vaccine-testing centers at the University of Wisconsin and the University of Minnesota for blinded evaluation.

"The JDIP Epidemiology and Biostatistics Core at Cornell University will analyze the results of the testing in a blinded manner and identify the 'best candidates'," Cunningham states. "Once the analysis is complete and the blind is opened, all of the program participants will receive the data at the same time."

During Phase II of the vaccine-testing program, "best candidates" will be evaluated using a mouse model. If all goes as planned, two laboratories will conduct the infection and protection studies in the mouse.

Phase II will involve the "best candidates" identified through the mouse studies being evaluated using a goat model.

"This will provide data similar to that from cattle, but the data will be available in a much shorter time frame and at a lower cost," states Robab Katani, a JDIP scientist with The Pennsylvania State University.

"The coordinated three-stage evaluation will take approximately three years to complete. It is expected that this rigorous screening process will identify one or more viable candidates to move forward for commercial development."

The Johne's Disease Integrated Program, often referred to as JDIP, is a comprehensive consortium of scientists whose mission is to promote animal biosecurity through the development and support of projects designed to enhance knowledge, promote education, develop real-world solutions and mitigate losses associated with Johne's Disease. By joining together, efficiencies through collaborative research can be achieved and intellectual and physical resources critical to overall success can be shared. The consortium includes leading scientists in the field, drawn from the world's top academic and government institutions representing the diverse disciplines of microbiology, immunology, pathology, molecular and cellular biology, genomics, proteomics, epidemiology, clinical veterinary medicine, public health, extension and public policy.

Funding from USDA-CSREES-NRI allows JDIP to provide competitive awards for meritorious research, education and extension projects addressing Johne's disease. Approximately $1 million will be distributed in the current funding cycle, with $400,000 designated for Johne's disease diagnostics projects, and the remainder available across the remaining JDIP Core and Project areas.

RDQMA *(continued)*

three farms were 15 different strains: 9 types on Farm A, 7 types on Farm B and 6 types on Farm C. The results indicated herd-specific infections: a clonal infection in Herd C with 89% of animals sharing the same strain (Type 2) and different strains in Herds A and B.

Type 4 was the most predominate one on Farm A (59%), and most super-shedder isolates belonged to this type.

Farm B was found to have a variety of strains from a limited number of isolates, and animals from which these samples were collected were purchased from different sources.

On Farm C, 100% of the infected cows shed the same strain as that of contemporary super-shedders. On Farm A, 17% to 70% of cows shed the same strain as that of contemporary super-shedders. Tissues from about 82% of cows other than super-shedders were culture-positive for MAP, indicating a true infection.

Based on results of MAP strain-typing and shedding levels, at least 50% of low shedders have the same strain as that of a contemporary super-shedder.

"The results of this study indicate that very few cows had characteristics of a possible pass-through animal. Many more cows were truly infected," the researchers stated in their report. "Sharing of the same strain of low shedders with the contemporary super-shedders suggest that low shedders may be infected by the super-shedders."

The next RDQMA research project: model the efficacy of vaccination against Johne's disease.

This e-newsletter is brought to you by the National Johne's Educational Initiative funded by USDA/APHIS/VS and managed by the National Institute for Animal Agriculture. Editor: Teres Lambert. To learn more about Johne's, visit ***www.johnesdisease.org***.