

# VETERINARY

FOR THE PRACTICING VETERINARIAN

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## Veterinarians' role in COOL still uncertain

Larry C. Hollis, D.V.M., M.Ag.  
Extension Beef Veterinarian

Country of origin labeling (COOL) was passed into law by Congress as part of the 2002 Farm Bill. This law states that each package of fresh red meat (beef, pork, lamb) offered for retail sale must indicate the product's country of birth, country where raised, and country where harvested. Only those animals born, raised and harvested in the United States can bear the label "USA Beef," "USA Pork," or "USA Lamb." The burden of proof that meat offered for sale has met these requirements has been placed on the retailer by this law. As the law currently reads, retailers face a penalty of \$10,000 per infraction if meat products offered for sale are labeled as a product of the United States when the retailers cannot prove that all three requirements listed above have been met. These requirements become voluntary Sept. 30, 2003, and mandatory on Sept. 30, 2004.

Major retailers have notified meat packers that they will be required to provide proof of origin for meat supplies, and several major beef packers have already sent letters to feedlots indicating that they will require proof of origin of cattle that they harvest. This requirement for proof of origin will ultimately trickle down to cow/calf, pork and lamb producers, and is anticipated to become a condition of sale as these animals leave the farm or ranch. Stated bluntly, not having the records in place to prove the origin of individual animals could disrupt the ability to sell livestock in a timely fashion or sharply reduce the price offered for non-verifiable livestock.

As veterinarians serving red-meat producing clients, we can help clients maintain full marketability of their animals by making them aware of the need for all animals, regardless of their age, to keep documentation of where they were born and raised. This includes older animals that may be sold as culls as we near September 30, 2004, as well as younger stock. The

details have not been worked out yet, but it appears that records that can be used to produce a verified, auditable, traceable system will be required (much like an IRS audit). Livestock purchase records, individual animal records, herd books, health certificates, pregnancy checking records, financial records, and inventory records probably will be acceptable. Vaccine and feed purchase records may be cross-referenced against inventory records. Individual animal identification will simplify the process and will rapidly become a requirement of this system.

Unless the legislation is changed from its current wording, it appears that independent third-party verification of origin of livestock will also become a requirement when COOL is finally implemented. Veterinarians are in a logical position to provide this verification as a service to their clients. As final details are worked out, it will be more apparent what the veterinarian's role will be in the implementation of COOL.

## K-State lab now offers *Clostridium perfringens* test

Richard Oberst, D.V.M., Ph.D.  
Diagnostic Medicine/Pathobiology

A multiplex PCR for toxin genotyping of *Clostridium perfringens* is now available from the Molecular Diagnostic Section of the Veterinary Diagnostic Laboratory at KSU College of Veterinary Medicine. The multiplex PCR detects the genes for alpha, beta, epsilon, iota, beta2 and enterotoxin in a single tube reaction to provide a useful typing tool in the diagnosis of *C. perfringens* related diseases.

Cost for the *Clostridium perfringens* multiplex PCR toxin genotyping is \$30 per sample.

Genotyping results are finalized and reported within one week of sample receipt.

A confirmed *Clostridium perfringens* isolate should be submitted on an agar plate or non-thioglycollate broth culture for the genotyping assay. Cultures should be sealed with parafilm and shipped overnight on ice packs to KSU-VDL with a completed submission form. Tissues may be submitted, but will be charged the anaerobic culture fee of \$15 for isolation of *C. perfringens*.

For more information, contact Dr. Richard Oberst at 785-532-4411 or Mike Hays at 785-532-4425.

### Also in this issue

- Chronic wasting disease in deer ..... 2
- Foreign heartwater disease ..... 3
- Porcine circovirus type 2 ..... 4
- Drought increases nitrate toxicity ..... 5

Thank you to the Pfizer Animal Health Group, Livestock Division, Cattle Products Group, for financial assistance in publishing this newsletter.

# Diagnostic testing of chronic wasting disease of deer

George A. Kennedy, D.V.M., Ph.D.  
Diagnostic Laboratory

The Kansas State Veterinary Diagnostic Laboratory is doing the immunohistochemistry (IHC) test for chronic wasting disease (CWD) of deer and elk. Although there are other officially recognized, ELISA-based tests that have recently come on the market for CWD testing, the IHC test is the one preferred by the USDA. A section of formalin-fixed brain stem at the level of the obex is the area of brain required for a valid test. Medial retropharyngeal lymph node is also recommended, but not required. Evidence is accumulating that lymph node may be as good, or even better than brain for testing deer, but not for elk. However, brain stem at the level of the obex is still recommended by the USDA for a valid negative test. Ideally, the brain stem and medial retropharyngeal lymph node should be used. These tissues are required for the laboratory to officially declare an animal negative for chronic wasting disease.

The USDA, Kansas Animal Health, and Kansas Wildlife and Parks have been collecting specimens for surveillance of CWD. Last hunting season, approximately 1,200 samples were tested, and no positive specimens were found in Kansas deer. Kansas is adjacent to two states with known positive animals, so that status could change.

Individual hunters can also submit deer tissue for testing at their own expense. Hunters who would like to have deer tested should contact their local veterinarian to take the appropriate tissues. It is critical that the obex area of the brain be included. This is the area just behind the cerebellum where the fourth ventricle goes into the spinal canal. This is the location of the dorsal motor nucleus of the vagus nerve and it is the earliest and the most severely affected area of the brain in animals infected with chronic wasting disease. There is evidence that in deer, but not in elk, that some

lymphoid tissues may be affected even earlier and is the reason for recommending both brain and lymph node testing.

If you are asked by a hunter to collect and submit samples for CWD testing, follow this procedure.

**Step 1** – Remove the animal's head at the atlanto-occipital joint. This would be at the level of the first cervical vertebra. Try to sever the brain stem as far caudally as possible during removal to be sure the obex area is not damaged.

**Step 2** – Once the head has been removed, the foramen magnum and severed brain stem should be visible. Grasp the fibrous dura with forceps and pull caudally for leverage.

**Step 3** – This step involves cutting the cranial nerves and attachments of the brain stem to the inner aspect of the dura. A number of instruments can be used to reach in and cut these attachments. A grapefruit knife with serrated cutting edges and a slightly bent tip works well. Other instruments that have been used include a laboratory spatula with a bent tip or a common teaspoon with sharpened edges that has been ground down to fit in the foramen magnum. Small curved Metzenbaum scissors also can be used but are harder to get all the way back to the obex area.

Once the attachments are cut as far cranial as possible (resistance is usually encountered when one gets as far as the cerebellum), rotate the cutting instrument and tip the curved portion upward pulling the brain stem out. Check to be sure the V-shaped depression of the fourth ventricle where it goes into the spinal canal is present. Place brain stem in 10 percent neutral buffered formalin.

If also submitting the medial retropharyngeal lymph node, these are the pair of large elliptical node located just dorsal to the nasopharynx. These nodes can be difficult to retrieve without splitting the head, which will be a problem if the owner wants to keep the rack or head for mounting. If the head is

split before removing the brain stem, it can damage the area of the obex, which is the more important of the two tissues. The medial retropharyngeal node can be reached from the posterior ventral aspect of the head, but takes some practice to locate.

Please submit the name, address, telephone number and hunting license number of the hunter and where the deer or elk was shot.

Negative results will be reported to either the submitting veterinarian or client, whichever is requested. However, positive results have to be reported first to the USDA, which will contact the hunter.

It is also important for the hunter to realize that this is not necessarily a food safety test. It just means the laboratory did not find evidence of chronic wasting disease. Chronic wasting disease has not been shown to cause disease in people or animals other than deer and elk. However, this is a relatively new disease, and there are still a lot of unanswered questions. Prudence suggests people not consume positive animals.

For hunters looking for more information on chronic wasting disease and some good links to other CWD sites, the Kansas Wildlife and Parks Web site offers up-to-date information at <http://www.kdwp.state.ks.us>

**It is crucial to have either the obex of the brain stem or medial retropharyngeal lymph node for the laboratory.**

## To submit samples for CWD testing include:

- The obex of the brain stem
- The medial retropharyngeal lymph node (*recommended*)
- Name, address, telephone number and hunting license number of the hunter
- County where the deer was shot

Negative results will be reported to either the submitting veterinarian or client. Positive results will be reported to the USDA, which will report them to the hunter.

# Foreign heartwater disease poses threat to U.S. livestock

George A. Kennedy, D.V.M., Ph.D.  
Diagnostic Laboratory

*Editor's Note: The following article is part of the Quarterly's continuing series on foreign animal diseases. Heartwater is a serious and costly tick-borne disease present in many countries in sub-Saharan Africa. It is also present on many islands in the Caribbean. Because there are species of ticks present in the United States capable of carrying the causative agent, this disease is of concern to the USDA and our livestock and wildlife industries.*

## Definition and Etiology

Heartwater is a noncontagious infectious disease of ruminants affecting cattle, sheep, goats, and a variety of species of African antelope. It is caused by a rickettsial organism *Cowdria ruminantium*. In the United States, the white-tailed deer has been found experimentally to be highly susceptible to this disease.

The organism is transmitted by various ticks in the genus *Amblyomma*. Two American species of *Amblyomma* have been shown to be capable of transmitting the organism. One of these, *Amblyomma maculatum*, or gulf-coast tick, has been found as far north as Kansas. The gulf-coast tick is a common parasite on white-tailed deer, which creates the possibility for this disease to become established in this country.

## Clinical Signs

Heartwater occurs in four different clinical forms. The peracute form is usually seen when naive animals are introduced into an enzootic area, or infected ticks carry the disease to a new area. The peracute form presents as sudden death or sudden onset of high fever and severe respiratory distress preceding convulsions and death.

The more common acute form is characterized by fever, anorexia, depression, and dyspnea, followed by neurologic signs. The neurologic signs include chewing movements, salivation, twitching of the eyelids, protrusion of the tongue, circling, gait, and postural abnormalities progressing to terminal convulsions. Diarrhea may be seen, particularly in cattle. The course of the disease varies from several days to a week.

A milder sub-acute form occurs with fever, cough, lack of coordination, and recovery or death in one to two weeks. A

sub-clinical form also occurs, mostly in partially immune animals. Calves, lambs and kids less than three to four weeks old have a high resistance to clinical disease. Some breeds also tend to have more innate resistance than others.

Mortality is variable depending on the strain of the organism, species and breed, and also previous exposure, but can approach 100 percent. Estimates of mortality in U.S. cattle on initial exposure are that it could be 50 percent or more. Recovered animals develop immunity lasting several months to a couple of years.

## Pathogenesis and Lesions

*Cowdria ruminantium* infects cattle, sheep, goats, and wildlife through the bite of an infected tick. The organism initially invades leucocytes, and then invades and multiplies in vascular endothelium. At this point, the animal becomes febrile and the blood is infective. Clinical signs and gross lesions are the result of the subsequent damage to vascular endothelium. The resulting increase in vascular permeability leads to widespread hemorrhages and edema, particularly pulmonary edema, hydropericardium, hydrothorax and ascites. Brain edema and encephalitis are the cause of the neurologic signs. The spleen tends to be enlarged.

## Diagnosis

Diagnosis of heartwater depends on demonstration of the organism. Traditionally, cytologic examination of a "squash prep" from meningeal vessels or endothelial lining of the jugular, or other vascular tissue, stained with Giemsa stain has been the most common diagnostic technique. The organisms appear as variably sized clusters of intracellular blue to reddish-purple cocci within endothelial cells. Autolysis destroys the organism. An immunohistochemical technique is available on formalin fixed tissues. A PCR technique has been recently developed.

An indirect fluorescent antibody (IFA) and competitive-ELISA have been used to detect antibody response, but cross-reactivity with *Ehrlichia sp* can cause problems with interpretation. The organism can be demonstrated via PCR in infected ticks.

As with other suspected foreign animal disease cases, if heartwater is suspected, contact the USDA or a Kansas Animal Health Veterinarian.

## Differential Diagnosis

The peracute form of heartwater can be confused with any sudden death, including anthrax. The acute form may need to be differentiated from rabies, meningitis, encephalitis, babesiosis and various toxicities such as strychnine, lead, organophosphates and chlorinated hydrocarbons. Heavy parasite infestation also may resemble heartwater.

## Treatment and Control

Tetracyclines, particularly oxytetracycline, are effective early in the course of the disease. There is no vaccine available, but dipping can help prevent introduction into new areas. Prevention through tick control has been largely unsuccessful because *Amblyomma* are multi-host ticks that may take up to four years to complete their life cycle and can harbor the organism for long periods.

## How is heartwater a risk to the United States?

Heartwater could be introduced into the United States via importation of infected wildlife from Africa or the Caribbean, or infected ticks on other species including reptiles. The cattle egret is a migratory bird that travels great distances and is seen in Kansas. These birds can be infested by and potentially carry infected ticks from infected countries. Once in the indigenous tick and wildlife population, this disease could prove impossible to eradicate.

Previous articles on foreign animal diseases that have appeared in the Kansas Veterinary Quarterly include Foot and Mouth Disease and Vesicular Stomatitis, Volume 4, Number 2 (Spring 2001); Rinderpest, Volume 5, Number 1 (Winter 2002); Classical Swine Fever (Hog Cholera) and African Swine Fever, Volume 5, Number 2 (Spring 2002); Exotic New Castle Disease and Reporting a Suspected Foreign Animal Disease, Volume 6, Number 2 (Spring 2003). Old issues of *Kansas Veterinary Quarterly* can be accessed on the Internet at <http://www.vet.kstate.edu>, by clicking on News and Events, and then on *Kansas Veterinary Quarterly*.

*Material for this article has been taken from "Foreign Animal Diseases" 1998, compiled by the United States Animal Health Association committee on foreign animal diseases, and used with the permission of the secretary of the USAHA.*

# Abortions associated with porcine circovirus type 2 in swine

John M. Ragsdale, D.V.M.  
and Norbert Takacs, D.V.M.  
Diagnostic Medicine/Pathobiology

Three litters of swine fetuses were examined shortly after they were aborted. The affected gilts were from a confinement swine herd that was experiencing an outbreak of late-term abortions with mummified fetuses, stillborn fetuses, and fetuses that were born alive, but weak. The gilts were vaccinated twice against porcine parvovirus, and there was no history or serological evidence of porcine reproductive and respiratory syndrome (PRRS) virus infection in the herd.

At postmortem examination, the litters contained mummified fetuses, partially mummified fetuses, and fresh fetuses that appeared to be stillborn. All of the fetuses appeared to have died during different stages of gestation ("stairstep" mummies). One of the fetuses had ventral subcutaneous edema along the abdominal body wall and severe ascites; however, no macroscopic lesions were present in any of the other fetuses. Microscopically, the myocardium of all the fetuses contained multiple areas of nonsuppurative myocarditis, cardiomyocyte loss, cardiomyocyte mineralization, fibrosis, and intranuclear basophilic inclusion bodies. A few of the fetuses had centrilobular hepatic congestion and necrosis consistent with chronic passive congestion of the liver.

**Include PCV 2  
in list of  
differential  
diagnoses when  
presented with  
"stairstep"  
mummified  
fetuses.**

The serum from the fetuses in each litter were pooled (three serum samples total), and all were positive for IgG. All three serum samples were serologically negative for porcine parvovirus and PRRS virus. Testing for porcine parvovirus, porcine reproductive and respiratory syndrome (PRRS) virus, *Leptospira* sp.

and bacterial causes of abortion was negative. Porcine circovirus type 2 (PCV 2) was isolated on viral culture from tissues of two of the three litters. The other litter was not cultured for viruses. Cardiomyocytes in the hearts from fetuses in all three litters (including the intranuclear inclusion bodies) stained positive using an immunohistochemical stain for PCV. The

nonsuppurative necrotizing myocarditis with basophilic intranuclear inclusion bodies along with the isolation of and positive immunohistochemical staining for PCV are diagnostic for abortion associated with PCV 2 fetal infection.

Circoviruses are 15-24 nm nonenveloped viruses containing a single strand of circular DNA. Circoviruses belong to the family Circoviridae, which includes porcine circovirus type 1 (PCV 1), porcine circovirus type 2 (PCV 2), chicken anemia virus and psittacine beak and feather disease virus.

Porcine circovirus was first identified in 1974 as a contaminant of pig kidney cell culture. This isolate has since been designated as PCV 1. PCV 1 has not been associated with a disease condition. Porcine circovirus type 2 was

first identified in 1996 in tissue culture of pigs with a postweaning wasting syndrome. PCV 2 has also been associated with respiratory disease in young pigs and, more recently, myocarditis in aborted (including mummified fetuses), and stillborn pigs. As the literature and this case illustrates, when presented with "stairstep" mummified fetuses, abortion associated with PCV 2 needs to be included in your list of differential diagnoses for swine abortions.

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# Recent drought increases nitrate toxicity, puts animals at risk

J.A. Pickrell, R. Pannbacker, K. Altwegg,  
N. Wienandt, F.W. Oehme,  
Comparative Toxicology Laboratories,  
Veterinary Diagnostic Laboratory

Ruminants (cattle, sheep, goats, and exotic zoo ruminants) are most sensitive to nitrate toxicity because their rumen microflora convert relatively non-toxic forage nitrate into the more toxic nitrite. With their large cecums, horses and rabbits are 3 to 4 times less sensitive. Monogastric animals are just as sensitive to nitrite as ruminants, but have less efficient systems of generating it within themselves. Os-triches are also quite sensitive.

## Mechanism

Nitrite oxidizes the iron of hemoglobin from  $Fe^{2+}$  to  $Fe^{3+}$  to form methemoglobin. Methemoglobin (oxidized hemoglobin) will not function to carry oxygen.

## Source

*Amaranthus* (common pigweed), and *Che-nopodium* (Lambs Quarter) are the most efficient accumulators of nitrate. However, of equal or greater concern are some monocoty-ledonous plants (corn, sudan, shatter cane, milo, sudex, johnsongrass).

Nitrate is soluble in water. It is taken up into plants through the roots and is thought to concentrate in the roots and lower stems. *The lower stems are predicted to have the highest concentration of nitrate.* Excess nitrate from overfertilization is taken up after fertilization, irrigation or a recent rain. Plants overfertilized and stunted by drought can take up nitrate dissolved in water rapidly when they experience a gentle rain. Nitrate in excess of what is necessary for synthesis of amino acids and protein is deposited in plant cell walls. It is sel-

dom mobilized for reuse in protein synthesis, although it is possible. Excess nitrate accumulates progressively throughout the nitrate-accumulating plant's lifetime. *Leftover nitrate is greatest when growth has recently been stunted,* such as from drought or early fall weather. Plant consumption at this time of the year, especially in light of the drought, may be risky for the animal's health.

## Clinical Situation

Animals with acute methemoglobinemia following consumption of forage containing more than 12,000 to 15,000 ppm nitrate on a dry weight basis will often die within a few hours to two days. Methemoglobin is chocolate-brown colored and carries no oxygen. The discoloration of blood and mucous membranes is directly proportional to the content of methemoglobin. Animals with significant amounts of methemoglobin (~35 percent) will hyperventilate with no rales heard on auscultation. As methemoglobin increases to 50 percent, animals become hypoxic after walking short distances and may drop; they lack the oxygenation and energy to rise. Animals still standing with methemoglobin of 60 to 75 percent will have necks extended and be hyperventilating. At necropsy, the chocolate-brown color is often lost with advanced post mortem change. Levels at or above 5,000 ppm nitrate in pregnant animals often will cause abortion. Intoxicated ruminants with acute methemoglobinemia have a fair to good prognosis with treatment if they remain standing. The prognosis worsens if they are down.

## Therapy

Methylene blue converts methemoglobin to 90 percent hemoglobin: 10 percent methemoglobin. One to 4 percent methylene blue

is given intravenously at 4 to 22 mg/kg to reach this equilibrium quickly. Retreatment the following day is required in approximately one third of the animals.

## Diagnostics

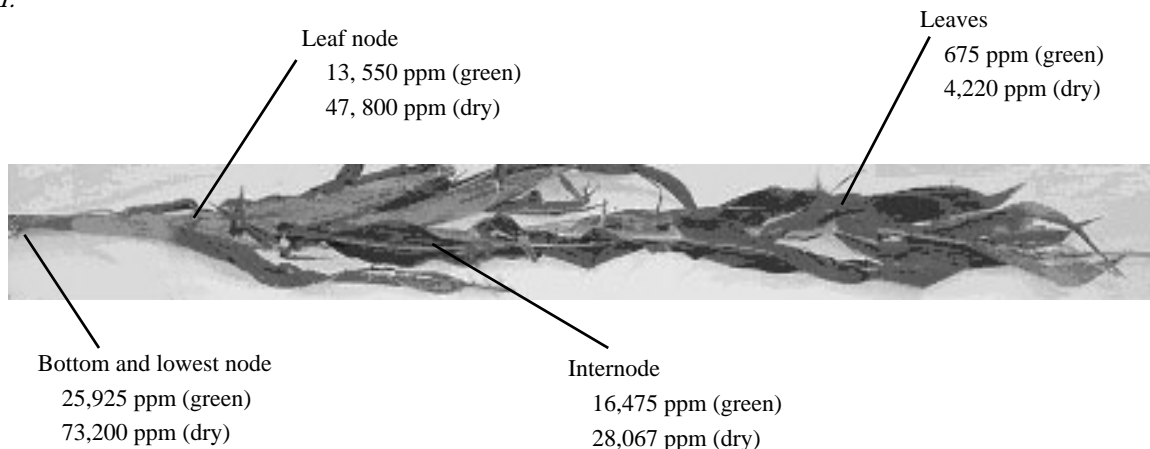
The ocular fluid of dead animals may be collected for nitrate analysis. Concentrations of nitrate greater than 20 ppm are confirmatory for a diagnosis of nitrate toxicosis.

Forage nitrate levels can also be assayed in our laboratory. We advise sampling forage from multiple locations in the pasture, multiple hay (forage) bales or multiple locations in silage; ¼ to ½ pound will be a large enough sample to analyze precisely and accurately.

We are in the process of studying the distribution of nitrate in forages. Our results from limited samples for "green" (irrigated) and "dry" (drought-damaged) corn plants are shown below (*Figure 1*). Similar distributions have been found in other laboratories. Obviously, the lower regions of the mature plant are highest in nitrate, and drought-damaged plants are higher throughout in nitrate (expressed as parts per million based on dry weight). For the calendar year 2003, we will perform these profile analyses (four analyses per whole-plant stalk) on whole-plant specimens of corn and sorghums at our usual single test rate of \$15 plus a \$4 accession fee per case. We hope the data will be of value to stockmen considering grazing animals on drought-damaged forages and stalks.

**Address samples to:** Comparative Toxicology Laboratories; Veterinary Diagnostic Laboratories, College of Veterinary Medicine, Kansas State University, 1800 Denison Ave, Manhattan, KS. Specific questions may be addressed by calling 785-532-5678.

Figure 1.



# Canine lepto causes may be changing

Diagnosticians at the Texas Veterinary Diagnostic Lab conducted an epidemiological survey of 405 clinical cases to determine the prevailing species associated with canine leptospirosis when testing for Lepto was requested as part of the differential diagnosis. Testing was performed predominantly on large or hunting breed dogs. The two serovars with the highest incidence of titers of 1:200 or greater and 1:3200 or greater were grippityphosa and pomona. Until the mid-80s, canine leptospirosis was thought to be caused primarily by the serovars canicola or icterohaemorrhagiae. However, more recent surveys, supported further by this survey in Texas, have demonstrated that the serovars grippityphosa and pomona are becoming more common as causes of clinical leptospirosis than canicola or icterohaemorrhagiae. Diagnosticians also found serovar bratislava titers in some animals tested.

When canine leptospirosis is suspected, testing for these additional serovars may be indicated. Additionally, it may be necessary to utilize a 5-way Lepto product for vaccination to provide protection against these additional serovars.

Don't forget that Lepto is a zoonotic disease, transmitted to humans primarily by contact of leptospire on abraded skin or mucous membranes. In one of the cases above, the owner was also diagnosed as having leptospirosis after the dog was diagnosed with the disease.

When canine leptospirosis is suspected, testing for these additional serovars may be indicated. Additionally, it may be necessary to utilize a 5-way Lepto product for vaccination to provide protection against these additional serovars.

*Texas Veterinarian, April 2003*

## Continuing Education

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### Newsletter Coordinators

*Larry C. Hollis*

Larry C. Hollis, Extension Beef Veterinarian  
785-532-4246 • [lhollis@oznet.ksu.edu](mailto:lhollis@oznet.ksu.edu)

*George A. Kennedy*

G.A. Kennedy  
785-532-4454 • [kennedy@vet.ksu.edu](mailto:kennedy@vet.ksu.edu)

### Contributors—K-State Research and Extension

|               |               |              |
|---------------|---------------|--------------|
| Dale Blasi    | Ron Hale      | Twig Marston |
| Mike Brouk    | Sandy Johnson | John Smith   |
| Joel DeRouche | Gerry Kuhl    |              |

### Contributors—Veterinary Diagnostic Laboratory

|                |               |                |
|----------------|---------------|----------------|
| G.A. Andrews   | R. Ganta      | R. Pannbacker  |
| M.M. Chengappa | S. Kapil      | J.A. Pickrell  |
| B. DeBey       | K.S. Keeton   | J. Sargent     |
| S.S. Dritz     | D.A. Mosier   | M.F. Spire     |
| M.W. Dryden    | T.G. Nagaraja | S. Stockham    |
| B.W. Fenwick   | J.C. Nietfeld | M.J. Wilkerson |
| F.W. Oehme     |               |                |

### K-State Research and Extension

137 Call Hall  
Manhattan, KS 66506

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