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FDA announces label change for approved antimicrobial: Extra-label drug use prohibition still in effect

Virginia Fajt, Chair
American Association of Bovine Practitioners
Committee on Pharmaceutical and
Biologic Issues

Baytril® 100 (enrofloxacin) injectable solution has received FDA approval for the treatment of bovine respiratory disease (BRD) in dairy replacement heifers less than 20 months of age. A label restriction that previously stated "Not for use in cattle intended for dairy production" prohibited the use of Baytril 100 in dairy heifers.

Due to a recent comprehensive review FDA-CVM granted a modification of that restriction. The label now reads "Not for use in dairy cattle greater than 20 months of age. Use of enrofloxacin in this class of cattle may cause milk residues."

American Association of Bovine Practitioners (AABP) Committee on Pharmaceutical and Biologic Issues reminds bovine practitioners that the prohibition of extra-label drug use for fluoroquinolone antibiotics is still in effect. The only condition or disease for which enrofloxacin is labeled is bovine respiratory disease.

The use of this product, like the use of all fluoroquinolone antimicrobials, for any condition or disease not on the label is a violation of federal law. Baytril 100 is still prohibited in lactating dairy cows, and should be segregated on the dairy farm from those drugs used for lactating animals. It will continue to be a prescription drug, to be used by, or on the order of a licensed veterinarian.

Safeguarding public health by appropriately and legally using therapeutics in cattle is the bovine practitioner's responsibility. Our continued prudent use of antimicrobials will help maintain their availability for treatment of disease in food-producing animals.

KSU diagnostic laboratory welcomes three faculty

Dr. Jianfa Bai is a new member of the molecular diagnostics team in the K-State Veterinary Diagnostic Laboratory. He received a bachelor of science degree from the Northwest Agricultural University in People's Republic of China, a master of science degree in genetics from the University of the Philippines at Los Banos and a doctor of philosophy, plant pathology/molecular genetics, from Kansas State. Dr. Bai has authored or co-authored nearly 50 publications, presentations, and book chapters. In addition, he has submitted 475 new cDNA and genomic DNA sequences to GenBank and also has made 36 microarray data submissions. Dr. Bai belongs to numerous professional organizations and has partnered with collaborators to bring nearly two million dollars for funding important molecular research during his young career.


Dr. Jianfa Bai

Dr. Bai began his career doing extension work but soon began working in the Institute of Crop Breeding and Cultivation, Beijing, China, where he held many positions, including deputy director of a division. He came to K-State for Ph.D. graduate work and post-doctoral training in functional and comparative genomics. Dr. Bai was director of the K-State gene expression facility prior to joining the Department of Diagnostic Medicine/Pathobiology and the KSVDL. Most of his career has involved research on plants/plant pathogens and utilization of cutting-edge technology for discovery and new test development. Dr. Bai's experience fits well with the needs and goals for molecular diagnostics in the KSVDL. His experience with genomics and microarray technology will be pivotal in developing new tests and striving to lead molecular diagnostics for animal health and food safety.

Dr. Lisa Pohlman joined the faculty of the Department of Diagnostic Medicine/Pathobiology at Kansas State University in November of 2007 as assistant professor of clinical pathology. She earned a DVM in 2001 from the Ontario Veterinary College, University of Guelph,


Dr. Lisa Pohlman

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Copper toxicity in sheep up in Kansas, Nebraska

Meredyth Jones DVM, MS, DACVIM
Kansas State University Veterinary Medical Teaching Hospital
Deon van der Merwe BVSc, MSc, PhD
Comparative Toxicology, Kansas State Veterinary Diagnostic Laboratory

Over the past two months, we have received several phone calls from veterinarians and sheep producers across Kansas and Nebraska requesting information on ill and dead sheep describing signs consistent with copper toxicity. In many of these cases, toxic levels of copper have been documented in the tissues and we have become aware of a sheep-labeled feed that is at the center of this outbreak of cases.

There are two forms of copper toxicity: Acute copper toxicity results from ingestion of high copper feeds, copper salts, pesticides, poultry litter, and other high copper substances. Acute copper poisoning can occur at copper intakes of 20-100 mg/kg in sheep and young calves, and 200-800 mg/kg in adult cattle. Chronic copper toxicity occurs when high levels of copper are ingested over a period of time, but at doses below the acutely toxic level. Sheep are the species most susceptible to chronic copper toxicity, because their liver cells have a high affinity for copper, and they excrete copper into the bile at a very low rate, leading to a build-up of liver copper concentration over time. One of the most common causes of toxicity in sheep is the accidental feeding of feedstuffs intended for other livestock. Molybdenum reduces the accumulation of copper in the liver. The ratio of copper to molybdenum in the feed is, therefore, an important factor determining the risk of copper poisoning. Chronic copper toxicity typically involves the ingestion of feeds that have a high copper: molybdenum ratio. Any feed that tests to have copper levels greater than 25 ppm or has a copper : molybdenum ratio of greater than 10:1 is considered potentially toxic for sheep.

Copper is a strong oxidizing agent. It binds to proteins in the liver cells and is stored in lysosomes within hepatocytes. As long as the copper remains stored in lysosomes it does not cause tissue damage. Copper can, however, be spontaneously released or released at times of stress, including shearing, weather extremes or transport. Chronic copper poisoning is, therefore, often described as a stress-related disease. When copper enters the blood it partitions into red cells, elevating red cell copper levels 15-20 times, while plasma copper levels only increase 2-3 times. It causes oxidative injury to hemoglobin, inducing Heinz-body formation and converting it to methemoglobin, which cannot bind O2 or CO2. The sulfhydryl groups of the red blood cell membrane also undergo oxidative change, resulting in significant hemolysis and anemia. Finally, this massive release of hemoglobin can result in hemoglobinuric nephrosis and renal failure.

Many animals affected by copper toxicity are simply found dead. Necropsy findings will include icterus and gun metal blue kidneys (Figures 1 and 2). In the live animal, icterus, red or brown urine (Figure 3), anorexia, pallor, weakness and recumbency are common signs. Brown blood (Figure 4) or pink serum may be noted on blood collection and processing; anemia and, in some cases, evidence of red blood cell regeneration, will be present on blood work. Elevations in creatinine are expected in animals with renal involvement. Hepatocellular injury and bile duct occlusion occur as the copper release and the enzymes AST and GGT have been shown to be elevated as far as 9 weeks before development of clinical signs.

Once clinical signs are recognized, the current feed for the flock should be withdrawn pending testing for both copper and molybdenum. Because copper may be stored in the liver for up to 18 months, it is common to find that the current feed is not the source. On necropsy, fresh samples of liver and kidney should be submitted to a diagnostic laboratory for copper levels. Serum copper levels are unreliable in live animals due to the primary storage in liver. If serum copper levels are elevated (greater than 2.0 ppm), this is diagnostic. If the levels are below this level, copper toxicity cannot be excluded because the

Figure 1: Severe icterus in a sheep with copper toxicity.

Figure 2. Characteristic gunmetal blue kidneys of a sheep with chronic copper toxicity.

Figure 3. Dark hemoglobin-containing urine from a sheep with copper toxicity.

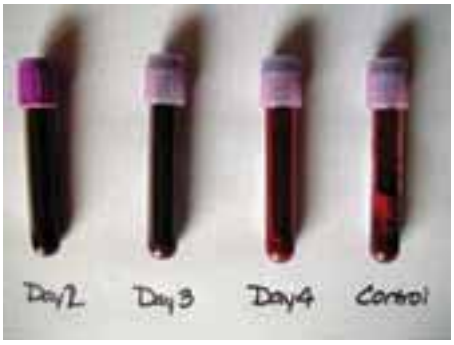

Figure 4. Blood from a sheep with copper toxicity and from a control sheep. Note that the blood from the affected sheep is much darker than the control and from the sample taken on day 4.

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elevation in serum copper concentration is often transient. Liver copper levels should also be interpreted with caution, because the release of copper from the liver during the disease process can significantly reduce liver copper concentrations.

Treatment is complicated by economic restrictions and antidote availability. For each drug, the current slaughter withholding for food animals1 is listed. Methylene blue (4-10 mg/kg slow IV; given to effect) is important in controlling the acute methemoglobinemia. Response is typically rapid with a noticeable effect expected within 15 minutes. The low end of the dose range may be repeated if additional doses are required. Methylene blue is a potential carcinogen, and because of the lack of residue studies that accounts for bound methylene blue in tissues, a slaughter withholding of 180 days has been recommended by the FDA in any species. Free methylene blue is not readily retained in the body and is virtually completely eliminated by 14 days, this being the current recommendation for withholding in cattle suggested by FARAD. Sodium thiosulfate (1000 mg per animal) is administered orally once daily for 3 weeks. This usually comes in an injectable form, which is administered orally. This drug is considered by FARAD to not be a concern for slaughter, but it is recommended to impose a slaughter withdrawal of 24 hours. D-penicillamine (26 mg/kg orally twice daily for six days) is a heavy metal chelator and increases copper excretion via urine. The recommended slaughter withdrawal is 21 days. Ammonium tetrathiomolybdate (1.7 mg/kg IV every other day for three treatments) decreases absorption of copper and increases removal from liver, decreasing liver copper within six days. A 10-day slaughter withdrawal is recommended. Vitamin C (500 mg subcutaneously) may also be useful in treating copper toxicity as ascorbic acid counters red blood cell oxidative damage. Supportive treatments, including blood transfusions and aggressive intravenous fluid therapy should be considered as indicated by clinical and economic parameters.

When addressing individual ill animals, it is also important to consider flock management. It is recommended that sodium thiosulfate, at the above listed dosage, be administered to all at-risk animals daily for three weeks to facilitate copper removal from the liver. This disease is of particular concern in extreme summer heat and exhibition of lambs. These stressors contribute significantly to the development of clinical disease and complicate therapeutic intervention in the individual animal and flock.

If you have questions regarding this or any other matter regarding livestock health, please call the Kansas State University Veterinary Teaching Hospital at 785-532-5700 or the Veterinary Diagnostic Laboratory at 866-512-5650.

WELCOME, from page 1

Ontario, Canada. She spent 3 years in small animal practice in Ottawa, Canada, and began attending graduate courses at the University of Ottawa's medical school in her spare time. In 2004 Dr. Pohlman moved to Alabama to pursue a residency and master's degree in clinical pathology at Auburn University. She taught students clinical problem-solving skills and clinical pathology in all four years of the veterinary curriculum. For 2 years she was the faculty advisor for the Animal Welfare Action Committee/ Shelter Medicine Club. The latter involved bimonthly visits with students to local shelters to examine the animals, along with vaccination of the healthy and treatment of the sick.

The American College of Veterinary Pathologists and American Society of Veterinary Clinical Pathology awarded Dr. Pohlman the prestigious ASVCP Young Investigator award in 2006 for her presentation, titled "Classification of 50 Cases of Feline Gastrointestinal Lymphoma." She was the first scientist to report a predominance of B-cell immunoblastic lymphoma in the stomach of domestic cats, a finding that may have significance in the causes of this relatively common form of cancer in cats. In the fall of 2007 she completed her residency and became a Diplomate of the American College of Veterinary Pathologists.

Dr. Pohlman shares instructional responsibilities with Dr. Steve Stockham in clinical pathology in the veterinary curriculum, and she works with the other clinical pathologists, the clinical pathology residents, medical technologists, and other laboratory personnel to provide clinical laboratory services for clinicians within the College of Veterinary Medicine, for veterinarians in private practice and for experimental investigations.

She is also continuing her research in lymphoma in dogs and cats and is currently working on a project looking at populations of T regulatory lymphocytes in normal dogs and dogs with lymphoma before and after treatment. Other research interests include the immunological response to cancer and anti-tumor immunotherapy, hypercalcemia of malignancy and mechanisms of oxidative damage to red blood cells.

Dr. Kyathanahalli Janardhan joins the diagnostic laboratory as a veterinary pathologist. Dr. Janardhan earned his BVSc and MVSc (veterinary pathology) degrees from the University of Agricultural Sciences, Bangalore, India in 1994 and 1997, respectively. He worked as a veterinary pathologist for four years in a contract research organization dealing with safety evaluation of various pharmaceuticals and agrochemicals. In 2001 he moved to Saskatoon, Canada where he obtained his Ph.D. from the University of Saskatchewan in 2006. From July 2006 to June 2008 he worked as an anatomic pathology resident at Kansas State University.

Dr. Kyathanahalli Janardhan

Dr. Janardhan's Ph.D. research was focused on understanding neutrophil and monocyte recruitment in lung inflammation in response to various stimuli such as *Streptococcus* pneumonia, bacterial lipopolysaccharide and *Escherichia coli*. He was also associated with studies exploring the pathogenesis of *Mannheimia hemolytica*-induced pneumonia in calves, lung inflammation induced by swine barn air and endotoxin-induced lung inflammation in horses, with an emphasis on the role of pulmonary intravascular macrophages.

You can contact Dr. Janardhan by phone at 785-532-4129 or by email: kjanardh@vet.k-state.edu

U.S. scientists investigate high fever disease in China

Dr. Richard A. Hesse
Kansas State University
College of Veterinary Medicine

Dr. Rodney (Butch) Baker
Iowa State University
College of Veterinary Medicine

The following has been condensed from a report submitted to the National Pork Board on return to the United States following the December 2007 investigation

A team of scientists from academia and industry recently returned from China after conducting an investigation of high fever disease in that country. The National Pork Board, American Association of Swine Practitioners, and Tetracore, Inc. sponsored Ying Fang (South Dakota State University), Johnny Callahan (Tetracore Inc.), Butch Baker (Iowa State University), Eric Neumann (Massey University, NZ) and Dick Hesse (Kansas State University) as participants in an investigative trip that included visits to the Veterinary Research Institute in Shanghai, the China Agricultural University in Beijing, the Swine Diseases Division of the Veterinary Medicine Institute in the Guangdong Academy of Agricultural Sciences, and the Institute of Animal Husbandry and Veterinary Medicine at the Fujian Academy of Agricultural Sciences in Fuzhou.

Sample collection and testing

The investigative team interacted closely with prominent Chinese investigators to gain better insight into the etiology, epidemiology, and severity of the High-Fever Disease outbreak that has affected most provinces in China since it was first reported in ProMED on April 12, 2006. The researchers had the opportunity to visit farms affected by high fever disease and collect samples to test for various viral agents at the different regional laboratories. Samples previously collected by the regional laboratories and/or freshly collected samples were tested using real-time PCR assays developed by Tetracore for the presence of African swine fever (ASFV), classical swine fever (CSFV), porcine reproductive and respiratory syndrome (PRRSV) and for porcine circovirus (PCV) 2a or 2b using a PCR assay developed at KSU.

Real time PCR results indicated that CSFV and PRRSV alone or in combination with PCV2b were closely associated with clinical cases of high fever disease in the limited number of samples that were tested. PRRSV/PCV2b was the most common co-infection identified in diseased pigs followed by CSFV/PCV2b. No ASFV or PCV2a was found in any of the samples tested.

Lingering questions

Although the team of experts did not find evidence of African swine fever virus during their two weeks in China, its presence cannot be entirely ruled out. The team was informed from several sources that some of the earliest and most severe outbreaks of high fever disease were on small pig holdings located in the west-central provinces. Due to time and logistical constraints, these areas were not visited. Investigators were able to collect biologic samples from one actively infected farm in Guangdong and perform a necropsy at one additional commercial farm near Beijing. Further samples were made available for testing from archived stocks held by the each of the laboratories that were visited. The means by which animal disease data (scanning surveillance, investigative surveillance, geospatial occurrence of disease, and epidemiologic descriptors of outbreaks) is collected and reported in China remains unclear. This leaves ample room for speculation on the true state of pig health in the country.

Discrepancies in deaths reported

The view of people inside universities and research institutes was quite different from individuals actively involved in the Chinese pig industry. Although the team did not have an opportunity to visit many pig producers, those met with provided views that contrasted both with official government data and information provided by some in universities and research institutes. The most glaring example of the discrepancy was in reference to the estimated excess annual pig mortality caused by the high fever disease outbreak. Official reports made by the Chinese government suggested somewhere around 500,000 excess pig deaths in the first year of the outbreak; the unofficial estimates from people within the industry suggested an additional 50 to 100 million pigs had died during the same period. Additional mortality in sows was thought to have reduced the national breeding herd by almost 10 million sows (35 million sows currently as compared to a normal of around 45 million sows in the inventory).

In China, a lack of producer organization (except perhaps in the southern provinces), an absence of private veterinary service, and the unregulated movement and transaction of pigs and production inputs (feed, vaccines, medication, transportation, etc) remain significant hurdles to the improvement of pig health.

Diagnostic services

Although the universities and research institutes believed they were connected reasonably well to the pig industry, there appeared to be little routine diagnostic activity that was stimulated by the needs of producers or local veterinarians. There were two diagnostic laboratory pathways in China. One included the laboratories associated with veterinary research institutes (such as the ones we visited) or universities.

The role of laboratories was largely undertaking basic research into pathogens, pathogenesis, immune response, and vaccine development. They were involved with farms to the extent the interaction facilitated new or ongoing research. Postmortem examination and diagnostic testing services were not routinely offered to farmers.

The second laboratory pathway included the provincial and national laboratories administered by the national animal disease control officials. These laboratories appeared to be geographically scattered throughout the country and along with duties related to occurrence of OIE-listed diseases they were able to interact with the government veterinarian(s) that were stationed in most local towns and villages. Local government veterinarians are not involved in clinical work but in monitoring farmers' compliance with

mandated disease control programs (eg CSF and FMD vaccination).

Within the realm of university and institute research laboratories that were visited, talented and motivated scientists were easy to find. Additionally, there appeared to be a good quantity of students interested in advanced study of animal diseases and pathogens. The quality of the physical space and equipment in specific laboratories varied widely. Scientists did appear to be free to publish results of their research.

While at the Fujian Academy of Agricultural Sciences, data generated from some field cases of high fever disease was presented by the Chinese scientists. In one study, they reported 61 positive virus isolations from 57 pigs that were suspected of having died from high fever disease. Type 2 PRRSV (having a unique 30 nucleotide deletion in nsp2) and CSFV was isolated from 35% and 24% (respectively) of the affected pigs. PCR results from tests the team ran while in China detected PRRS and CSFV in about half of the small number of samples that were tested. Pigs examined had many of the same gross lesions found in PCVAD pigs observed during the early days of high mortality in the United States during 2005 and 2006.

Key pathogens identified

Based on the team's clinical and laboratory testing and interpretation of discussions that occurred during the visit, high fever disease appeared to be a multi-agent syndrome that likely involved co-infection with a specific or varied set of infectious agents. The key pathogens involved in the syndrome appeared to be a newly emerged and highly virulent Type 2 PRRSV, PCV2b, and CSF virus. Indeed, this was an opinion shared by many of the Chinese scientists met with. Petechial and ecchymotic hemorrhages (heart, gastric mucosa, lymph nodes, liver, spleen, kidney, others) were consistently mentioned as common gross lesions in affected pigs. Among known pig pathogens, ASFV and CSFV are two pathogens that are likely to cause gross hemorrhage. Hemorrhage (limited to the umbilical cord) has been reported in the scientific and anecdotal PRRSV literature. The emergence of a novel infectious agent responsible for high fever disease has not been ruled out. After a virulent PRRSV variant was identified in China, exposure of experimental pigs to the virus apparently reproduced the clinical signs and efforts to identify any novel agents ceased. Unfortunately, because of the unavailability of disease-free pigs, the inoculation studies that were done could not definitively rule-out the presence of an unidentified agent.

Farm biosecurity

Farm biosecurity in China was limited mainly to progressive pig producers. Not surprisingly, our examination of pig transport vehicles used by Chinese farmers (everything from wheelbarrows to motorbikes to large trucks) were as difficult to clean and disinfect as those used in the U.S. People involved in the pig industry confirmed there was little emphasis on biosecurity, even in the larger operations. Based on experience with the rapid spread of PCV2b across the United States and the likely role of transport vehicles in that spread, there is little reason to doubt that the same phenomena may have occurred with the organism(s) responsible for high fever disease in China.

The U.S. pork industry remains at risk for new disease agent introductions. Organisms that are environmentally stable can easily pass across international borders as hitchhiker organisms on footwear, clothing, and materials. Given the mobility of people between countries, relying on degradation of stable viruses or bacteria simply as a result of transit time is unlikely to offer much protection. Travelers need to continue to be educated about biosecurity risks associated with travel. Vigilance must be maintained by requiring an active disinfection process of all clothing and items that may have come into contact with foreign animal production units and requiring a no-contact period with domestic livestock once back in the United States. An additional, but difficult to quantify risk, is that posed by people and products that move into the U.S. from countries known to have livestock diseases exotic to the U.S. via a third country. As an example, high fever disease appears to have arrived in Vietnam through some unknown mechanism, and it is reasonable to assume that further spread has occurred to other countries of southeastern Asia but has yet to be reported. For this reason, it is difficult to design foolproof country-specific biosecurity precautions and instead suggests awareness and education programs for travelers combined with routine compliance checks at the border will be important measures to protect U.S. livestock.

Preparation for an exotic animal disease emergency has been significant with most of the effort going toward design of control programs. There remains significant opportunity at the border to improve our own biosecurity safeguards for prevention. Given the sheer number of long-distance movements that happen daily in the U.S. pig industry, geographic containment of an exotic disease after it arrives remains questionable and will be incumbent on widespread adoption of industry-driven processes that limit disease transmission both for endemic and exotic agents.

A significant opportunity for the industry lies in re-inventing our transportation philosophy (everything from information systems to trailer design). This is an aspect of national biosecurity that exists exclusively in the domain of people involved in the pig industry and requires no assistance or support from government.

A better understanding of the swine diseases currently circulating in China and the associated biosecurity issues which could put the U.S. pork industry at risk was achieved as a result of this investigative visit. Additionally, strong collaborative relationships between Chinese and U.S. scientists were established. Future research, training and exchange opportunities between the U.S. and China will be valuable in development of strategies to limit international spread of diseases.

Researchers identify virus behind mysterious parrot disease

Researchers at the University of California, San Francisco, have identified a virus behind the mysterious infectious disease that has been killing parrots and exotic birds for more than 30 years.

The team, led by UCSF professors Joseph DeRisi, Ph.D., and Don Ganem, M.D., also has developed a diagnostic test for the virus linked to Proventricular Dilation Disease, or PDD, which will enable veterinarians worldwide to control the spread of the virus.

The new virus, which the team named avian bornavirus (ABV), is a member of the bornavirus family, whose other members cause encephalitis in horses and livestock. Working with veterinarians on two continents, the group isolated this virus in 71 percent of the samples from infected birds, but none of the healthy individuals.

"This discovery has potentially solved a mystery that has been plaguing the avian veterinary community since the 1970s," said DeRisi, a molecular biologist whose laboratory aided in the 2003 discovery of the virus causing Severe Acute Respiratory Syndrome, or SARS, in humans. "These results clearly reveal the existence of an avian reservoir of remarkably diverse bornaviruses that are dramatically different from anything seen in other animals."

The discovery could have profound consequences on both domesticated parrots and in the conservation of endangered species, according to DeRisi and Ganem, both Howard Hughes Medical Investigators at UCSF. Those species include the Spix's Macaw, currently one of the most endangered birds in the world, whose number has dwindled to roughly 100 worldwide and whose continued existence is threatened by PDD.

The research analyzed affected birds using a high-throughput screening technology that uses a DNA microarray to test viral samples. The team was able to recover virus sequence from a total of 16 diseased birds from two different continents. The complete genome sequence of one isolate was captured using ultra deep sequencing.

The virus they identified is highly divergent from all previously identified members of the Bornaviridae family and represents the first full-length bornavirus genome ever cloned directly from avian tissue. Analysis of the avian bornavirus genome revealed at least five distinct varieties.

PDD is a fatal disease that causes nervous system disorders in both domesticated and wild birds in the psittacine, or parrot, family worldwide. The disease has been found in 50 different species of parrots, as well as five other orders of birds, and is widely considered to be the greatest threat to captive breeding of birds in this family, the researchers said.

The disorder often leads to the birds' inability to swallow and digest food, with resulting wasting; many birds also suffer from neurologic symptoms such as imbalance and lack of coordination. Regardless of the clinical course the disease takes, it is often fatal.

Scientists have theorized for decades that a viral pathogen was the source of the disease, but until now, no one had been able to identify the likely culprit.

Continuing Education

October 31-November 1
Veterinary Career Opportunities Workshop

November 1 and 2, 2008
15th Mid-Western Exotic Animal Medicine Conference

For the most complete, up-to-date conference information visit our Web site at: *www.vet.ksu.edu* and click on Continuing Education, or contact: Linda M. Johnson, Ph.D., at 785-532-5696 or *johnson@vet.ksu.edu*

Upcoming Events

September 26
KLA/K-State Ranch Management Field Day, Medicine Lodge

October 2
KSU Stocker Field Day, Manhattan

October 8-10
HACCP Plan Workshop
Kansas City, KS

October 11
State 4-H Meat Judging Contest (tentative date)

November 20
KSU Swine Day, Manhattan

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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