Outbreak of porcine circovirus, associated diseases

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

In 1996 Harding and Clark described a new disease syndrome that they had been seeing since 1991 in Canadian pigs. Affected pigs lost weight rapidly and were pale, listless, dyspneic, and had swollen inguinal lymph nodes. Morbidity was low in affected herds, but case mortality was high. Affected pigs were weaned and in the nursery or early finishing phases of production. Harding and Clark also identified a new circovirus (porcine circovirus type 2 (PCV2) that was present at high levels in affected tissues. They proposed the name porcine multisystemic wasting syndrome (PMWS) to describe the condition and proposed that it was caused by PCV2. A consistent microscopic lesion is a marked loss of lymphocytes from germinal centers of follicles in lymphoid organs, such as lymph nodes, tonsils, spleen, thymus, and Peyer’s patches. In more severe cases there is pronounced loss of lymphocytes from paracortical areas as well. Replacing the lymphocytes are large macrophage-like cells, including multinucleated giant cells, and eosinophilic stromal cells. Less consistent lesions are granulomatous inflammation in parenchymal organs such as the lungs, liver, and kidneys, and acute coagulative necrosis in lymphoid follicles. Because of the lymphoid depletion and disruption of normal lymphoid architecture by PCV2, there appears to be marked immunosuppression in affected pigs. Coexistent with PCV2 infection there is a large increase in the incidence of other bacterial and viral diseases. Porcine reproductive and respiratory syndrome virus (PRRSV) especially increases the pathogenicity of PCV2.

In recent years, PMWS attracted a lot of attention in North America, but it was not common and was not considered economically important. In the late 1990’s PMWS spread across much of Europe and caused severe economic losses. In many herds, mortality was 10 to 20 percent or even higher and the disease did not simply sweep through a facility and disappear. Instead the condition stayed and affected subsequent groups of weaned pigs. At the same time there were increasing reports of a syndrome characterized by skin lesions that ranged from small red papules on the...

New faces in the College of Veterinary Medicine

Welcome Drs. Richard (Dick) Hesse, Matt Miesner and Deon van der Merwe to the College of Veterinary Medicine.

Dr. Hesse is the new Director of Diagnostic Virology at K-State’s veterinary diagnostic laboratory. He holds a master’s degree in biology from South Dakota State University and a Ph.D. in virology from the University of Nebraska. Hesse has authored or co-authored more than 40 publications, numerous presentations and a few patents. He has led the development of at least 12 USDA-licensed vaccines. He is an adjunct faculty member at the University of Kansas, Iowa State University, and the University of Nebraska. Honors include the Army Distinguished Service Medal for Lassa Fever research, membership in the Gamma Sigma Delta honor society, and the Schering Plough Excellence Award for development of a porcine reproductive and respiratory syndrome (PRRS) virus vaccine.

Hesse began a scientific career as an undergraduate assisting in college laboratories and in the local medical center. He served at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick, Md., working with highly hazardous pathogens in total containment facilities (Biosafety level-4).

After the Army, Hesse began graduate studies at South Dakota State University with a focus on bovine respiratory viruses. Continued on page 2

Also in this issue

K-State addresses rural food supply veterinarian shortage .................. 2
Cellulitis secondary to injections in finishing cattle ......................... 3
Case study: Poor quality hay leads to nitrate intoxication ....................... 6
Canine leptospirosis ........................................ 7
Roland leads multistate PRRS virus research project ............................ 7
Start early to control stable flies in pastures ................................... 7

Thank you to the Pfizer Animal Health Group, Livestock Division, Cattle Products Group, for financial assistance in publishing this newsletter.
K-State addresses rural food supply veterinarian shortage

K-State University Colleges of Veterinary Medicine and Business Administration brought food animal practitioners and veterinary students together for a Veterinary Career Opportunities Workshop November 3-4, 2006. The goal of the event was to help practitioners and prospective new associates find the correct career placement for future success.

Food supply veterinary practitioners from seven states and current veterinary students participated in the conference. Participants heard from the college of veterinary medicine and business administration faculty and practiced new techniques through facilitated interactions.

Food animal veterinarians that attended the conference will invest more than $1 million into hiring new associates for their practices.

Practitioners enhanced skills related to finding the right associate. Attendees learned about meeting job seeker expectations, the legal aspects of interviewing, fair benefits packages and appropriate associate recruitment techniques. Practitioners created a successful job announcement with input from conference veterinary and business faculty.

“Incorporating a solid business foundation into the practice of food supply medicine can help this essential piece of veterinary medicine continue to thrive,” said Kevin Gwinner, Ph.D., College of Business Administration marketing department head.

K-State veterinary students learned and practiced appropriate interview techniques. The event was a valuable networking opportunity for students, and attendees were linked to numerous employment and internship prospects.

Participants took part in mock interviews. Practitioners and students visited one-on-one for 5 minutes in a round-robin style event. Prospective employers and new associates discussed career expectations and made viable contacts.

“Veterinary practices and students have varied goals and aptitudes. This conference was successful in helping both groups identify methods to find the right new job or associate for their situation,” said Brad White, D.V.M., Assistant Professor, K-State College of Veterinary Medicine.

Bayer Animal Health, Schering-Plough Animal Health, and Intervet helped support this event. Conference organizers were pleased and plan to make this workshop an annual event.
Cellulitis secondary to injections in finishing cattle

Jerome Nietfeld, D.V.M., Ph.D.
Veterinary Diagnostic Laboratory

Over the past few years there has been a big push in the cattle industry to cut down on muscle damage, infections, and abscesses that develop secondary to injection of vaccines or medications, and there has been a lot of progress in this regard. Most veterinarians and cattle producers no longer give injections in the muscles of the rear legs, because of possible damage to some of the better cuts of beef.

Today most injections are given in the neck to reduce potential economic losses from injection damage. But not all are given in the neck, and we still necropsy cattle with large abscesses in the gluteal muscles, but this is not common. Injection damage in the subcutaneous tissue of the neck is not uncommon in fattening cattle in the early stages of finishing. By far most of this damage appears to be a direct result of injected substances and is localized, but occasionally we find evidence of bacterial infections.

During my last necropsy rotation we posted two calves weighing 600 to 750 lbs. One had been anorexic for a couple of days, but the owner did not think that the calf looked that bad before the calf was found dead. The second calf was treated for lameness and according to the owner was otherwise normal. The next day it was found dead. This calf had been purchased a week before dying.

In both cases there was severe cellulitis with grey and brown pus extending the length of the left side of the neck, around (both dorsal and ventral) the left forelimb, and in the subcutis of the ventral chest and abdomen to the inguinal region (see figure 4). In one calf the inflammation surrounded the trachea and esophagus and there were exudate-filled pockets in the muscles of the neck just dorsal to the trachea. In both cases there was little feed or water in the rumen indicating that neither calf had been eating.

These cases are reminders that it is important to keep syringes clean and that, while it is often impractical, reusing needles can and does cause sometimes cause problems. My guess is that storage and reuse of partially used vaccines is the biggest culprit in these cases. Over the years, both in practice and at diagnostic laboratories, I have been involved in several incidents where there were multiple abscesses and even deaths that occurred because contaminated vaccines were injected into cattle or pigs. Probably the most serious case was one where a number of sows died following vaccination in the neck. All of the dead sows had gangrene of the neck, and Clostridium perfringens was isolated from the neck of several sows and from the 50 dose vial of vaccine. The vaccine had been partially used and stored prior to this incident. Of course nothing was isolated from an unopened vial of vaccine from the same lot. In the other cases, affected cattle and swine were not killed, but in each there were multiple animals with localized infections. In each case, bacteria were isolated from a partially used bottle of vaccine, but not from unopened bottles from the same lot. Producers should be reminded that storage and reuse of vaccines is not without danger, especially if care is not taken to prevent contamination. The cost of even one abscess will pay for a lot of vaccine.

By far most damage appears to be a direct result of the injected substances and is localized, but occasionally there is evidence of bacterial infections found.

Figure 4. Abscesses that do not occur frequently but occur for reasons such as injecting in places other than the neck or from reusing needles, etc.
rear half of the body to large, irregular areas of hemorrhage and necrosis scattered over the entire body. These pigs usually died soon after skin lesions developed, and at necropsy they had perirenal edema, swollen, wet kidneys, and pinpoint hemorrhages on the surface of the kidneys. Microscopically, these pigs had systemic, necrotizing vasculitis with the most severe manifestations in the glomerular capillaries, renal arteries, and dermal arterioles. This condition was named porcine dermatitis and nephropathy syndrome (PDNS).

PDNS has been recognized under different names as a sporadic, idiopathic condition for several decades, but as PMWS spread across Europe the incidence of PDNS increased dramatically, and in a few herds it reached near epidemic proportions. PDNS is usually believed to be a type III hypersensitivity reaction with antibody-antigen complexes deposited in the walls of arteries and capillaries, which results in activation of complement and vascular necrosis.

Although the cause of PDNS is unproven, it is classified as a PCV2 associated disease. Other conditions associated with PCV2 infection are porcine respiratory disease complex, proliferative and necrotizing pneumonia, necrotic enteritis, and reproductive failure. The complex of diseases associated with PCV2 is referred to as porcine circovirus associated diseases (PCVAD) in North America and porcine circovirus diseases (PCVD) in Europe.

In late 2004 and in 2005 the Canadians began to report a large increase in cases of PMWS. In December 2005 we began to receive pigs from several Kansas herds experiencing large losses compatible with PMWS (Figure 1) and PDNS (Figures 2, 3).

Between December 2005 and May 2006, PCVAD was identified in more than 15 Kansas herds. Outbreaks have been in finisher pigs with death losses beginning to increase within 2 to 3 weeks and peaking 6 to 7 weeks after placement. In many houses mortality has been 10 to 20 percent, and in some cases mortality has been higher. This can be an incredibly costly disease. Most cases are clinically compatible with PMWS, but in most herds there has also been an unusually high incidence of PDNS. In fact, in several herds the incidence of PDNS is greater than that of PMWS.

In addition, we have seen cases of proliferative and necrotizing pneumonia, severe pulmonary edema with pleural effusions, and necrotic enteritis. The key to diagnosis in these cases is the prominent replacement of lymphocytes by macrophages and the very large amounts of PCV2 visualized by immunohistochemical staining of affected tissues.

At about the same time that we began to see a dramatic increase in PCV2 asso-
associated diseases, reports began to come in about similar increases of PCVAD in North Carolina and a little later in Nebraska, Iowa, and other Midwestern states.

In August, Smithfield Farms announced that PCVAD losses raised their cost of production by 3 percent and almost all of these losses were in their North Carolina operations. The one big difference between the Kansas outbreak compared to those in North Carolina and other regions of the midwest is the large number of cases of PDNS.

In other areas of the United States, PDNS remains sporadic with an incidence of less than 1 percent. Generally PDNS is not considered to be important. However, we are seeing an unusually high incidence of PDNS; of 87 pigs submitted from 18 premises, 26 (29.9%) were PDNS. This is similar to some outbreaks in Europe where cases of PDNS outnumber PMWS.

Although PCV2 was first identified in the 1990s, there is serologic evidence that the virus has been present since at least the late 1960s and that it is present in virtually all pig herds in Europe and North America and probably the rest of the world. Some authors claim that all pigs are infected by the time they reach slaughter weight.

However, epidemiological studies suggested that PCVAD spread across Europe like a new infectious agent in a naïve population, which does not make sense if the virus was previously ubiquitous in swine. Then workers in Sweden announced that they had identified two major strains of PCV2 and that only one of the strains was associated with clinical outbreaks of PCVAD. Studies of outbreaks in Canada also revealed two strains of PCV2 and that outbreaks were only associated with one strain. Based on the patterns obtained from restriction fragment length polymorphism (RFLP), the Canadians refer to their strains as “422” and “321”, with “321” being associated with clinical disease.

Since the beginning of the Kansas outbreak, members of the K-State Veterinary Diagnostic Laboratory have been working with the Abilene Animal Hospital and with Dr. Robert Rowland, a researcher in the Department of Diagnostic Medicine/Pathology. In a short time Dr. Rowland and members of his lab sequenced the genome of several PCV2 isolates obtained from multiple herds. He noted that the isolates were virtually identical and that they were different than previously reported sequences of PCV2 from the United States. These isolates were closely related to an isolate from an outbreak of PCVAD in France.

The K-State lab then isolated and sequenced PCV2 from a pig that did not have clinical signs or lesions of PCVAD. This isolate was related to previously reported isolates from the United States. We consulted with the Canadians and found that the strain of PCV2 that we were isolating from clinically ill pigs was the same as the Canadian “321” strain. The lab has developed a PCR test that will differentiate between “321” and “422” strains of PCV2.

Results to date indicate that both viruses are relatively widespread in pigs, with the “422” strain seeming to have the greater distribution. But one thing that has also been consistent is that we find the “321” strain associated with clinically affected pigs. Pigs with PCVAD are infected with “321” PCV2 or with both strains, but not the “422” strain only.

Members of the K-State group and the Abilene Animal Hospital are collaborating in PCV2 vaccine trials. The trials have not been completed, but so far the results look promising.

If you have questions concerning porcine circovirus type 2, PCVAD, their diagnosis, and control, contact Drs. Robert Rowland, Richard Hesse, and Jerome Nietfeld at the K-State Veterinary Diagnostic Laboratory (785) 562-5650 and Drs. Steve Henry, e-mail: shenry@ahspa.com and Lisa Tokach ltokach@ahspa.com at the Abilene Animal Hospital, Abilene, Kan. (785) 263-2301.

For information on control of PCV2D contact Dr. Tokach and Dr. Henry. Because they are out of the office so much, e-mail is usually the best way to contact them.

A good source of information is the booklet “A Producer’s Guide to Managing PCVAD Porcine Circovirus Associated Disease,” which is available from the National Pork Board at (515) 223-2600, fax: (515) 223-2646, or www.pork.org and the American Association of Swine Veterinarians at (515) 465-5255, fax: (515) 465-3832 or www.aasv.org.
Case study: Poor quality hay leads to nitrate intoxication

Pickrell JA, Nietfeld, J, Kuroki K, Blevins L, van der Merwe D, Oehme FW

Nitrogenous wastes from animals form organic amines in soils. Unconsumed plants that die also can form organic amines in soils. Amines in soils are reduced to ammonium, oxidized to nitrites and nitrates that are absorbed by plants and provide nitrogen to plants for plant growth. Animals consuming plants as part of their diets also ingest nitrates contained in plant tissues.

Both nitrites and nitrates are soluble in water and can be leached into underground aquifers, increasing their concentrations in the water. Excess nitrates in water come from leachates, natural nitrate sources, livestock manure or external nitrogen fertilizers that have been applied to that land. Nitrates in the environment can be denitrified by microbial processing and other natural processes and released to the atmosphere as nitrogen or oxides of nitrogen.

Nitrates in plants are incorporated into proteins or converted to ammonia and released to the atmosphere. The importance of these processes in plant tissues depends on nitrate reductase activity in plant tissues. Drought or lack of sunlight can lead to the suppression of metabolic processes in plants and excessive accumulation of nitrates in plant tissues. Other conditions that promote excessive nitrate accumulation in plants include insufficient plant growth to use high levels of nitrate fertilizer or manure applied to a field. After a rain, nitrate is rapidly taken up and stored. After a frost, plant growth is also slowed and excess nitrate is diverted and stored in plants.

Excess dietary nitrate is a frequent cause of toxicity in ruminants because their rumens efficiently convert nitrate to nitrite. This reduction is much more rapid than the subsequent conversion of nitrite to ammonia, so an excess of nitrite is present for some time in animals consuming forage high in nitrates.

Nitrite oxidizes the iron in hemoglobin from a +2 to the +3 state present in methemoglobin that does not carry oxygen. When 30% methemoglobinemia is present, ruminants will compensate by hyperventilation. At 50% methemoglobinemia, ruminants become vulnerable to hypoxemia, because there is not sufficient functioning hemoglobin to carry oxygen to tissues which need it. Ruminants so affected may be unable to remain standing. If ruminants are standing, they carry a fair to good prognosis of recovery (70-75%). If ruminants cannot remain standing, they have a poor prognosis for full recovery (25-30%). Adequate dietary vitamin A will lessen the effects of consuming excess dietary nitrate, but will not eliminate them.

To aid in diagnosis, dietary nitrates were measured as ppm nitrate in a mixed, representative sample of forage. Diets with 100% of the plants > 3,000 ppm nitrate may cause ruminants to form sufficient methemoglobin to reduce productivity. In diets with 100% of the plants at 5,000 ppm nitrate, the methemoglobin formed is sufficiently high to affect the fetuses of pregnant animals. Diets with 100% of the plants containing 10,000 to >12,000 ppm may generate sufficient methemoglobin to make the ruminant vulnerable to hypoxemia. Thus, the level of dietary nitrate constitutes a relatively accurate predictor of impending nitrate toxicity. If nitrate in dietary forage is elevated, we must also consider the extent to which water also may be elevated in nitrate.

A major limitation of dietary nitrates is that they provide no indication of which animal consumed them. Secondly, dangers to pregnant animals go unnoticed if the animals are asymptomatic. Finally, minor impairments in productivity are often unnoticed.

In a recent field case, 12 cows surrounded and consumed the contents of one round bale of poor quality grass or alfalfa hay and died acutely. The owner drove the cattle away from the remaining high nitrate forage. We burned the offending hay; we could not sample the high nitrate bale. However, the concentration of nitrate in the remaining poor quality alfalfa hay was near that needed to make the cows vulnerable to acute nitrate toxicity.

Had treatment been an option, we would have recommended removing cattle from any high nitrate forage. The poor quality alfalfa hay was withdrawn from the feeding schedule. Nitrate accumulates in ocular fluid; post-mortem samples of ocular fluid are used to test for nitrate toxicity. Nitrate concentrations in ocular fluid greater than 25 ppm nitrate suggest nitrate intoxications. Samples of ocular fluid were analyzed by color change on preformed nitrate test strips.

All measurements of ocular fluid exceeded 50 ppm nitrate. Posterior chamber ocular fluid from the two submitted cows had 100-250 ppm nitrate and anterior chamber ocular fluid from the submitted eyeball was 50-100 ppm nitrate. Results suggested increased likelihood of nitrate intoxication in these cows.

The nitrate level of the alfalfa sample correlated with increased nitrate in ocular fluid.

The poor quality grass hay had low levels – 40 ppm – of nitrate, while the poor quality alfalfa hay had 9,600 ppm nitrate on a dry weight basis. The nitrate level of the alfalfa sample correlated with increased nitrate in ocular fluid. Since the owner burned the offending hay, we could not sample that bale. However, the concentration of nitrate in the remaining poor quality alfalfa hay was near that needed to make the cows vulnerable to acute nitrate toxicity.

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continued on page 7
Rowland Leads Multistate PRRS Virus Research Project

Dr. Raymond (Bob) Rowland of the Department of Diagnostic Medicine/Pathobiology of the Kansas State College of Veterinary Medicine is the new leader of the USDA Cooperative Agricultural Project on porcine reproductive and respiratory syndrome (PRRS CAP). PRRS is a worldwide disease caused by an arterivirus that is closely related to the virus that causes equine viral arteritis and is the most important disease of swine in North America.

The disease is responsible for losses of approximately $600 million and $12 million annually in the United States and Kansas, respectively. The PRRS CAP project is a multi-institutional and multidisciplinary program devoted to PRRS research, education, and outreach. The $4.8 million four-year program is currently undergoing renewal. Rowland is heading renewal efforts and will manage the program afterwards. The primary source of funding is the USDA, with additional support from the National Pork Board and private companies. The focus of the project is development of improved PRRS virus vaccines, immunotherapeutics, and new diagnostic tests.

Canine leptospirosis cases confirmed in Kansas

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

Several cases of canine leptospirosis have been confirmed recently at the K-State Veterinary Diagnostic Laboratory (KSVDL). Abundant late summer and early fall rainfall may have contributed to the appearance of these cases, as leptospirosis frequently follows flooded or wet environmental conditions.

When leptospirosis is suspected, urine and serum are the best specimens to be submitted to the laboratory for testing. Serum is used for the microscopic agglutination test (MAT), which has been the standard serologic testing for antibodies to leptospiroa for many years. In early stages of infection before production of serum antibodies, urine testing is necessary to confirm infection. Generally it is most efficient to submit serum for antibody titers and urine for leptospira detection at the same time. The KSVDL is testing urine with a PCR test that is specific for pathogenic leptospira. The cost for the PCR test is $25 and turnaround time is 2 to 3 days. The cost of the MAT for serum antibody titers is $10 for the 6 serovars used for screening canine serum, with a turnaround time of 2 to 3 days.

Start early to control stable flies in pastures

Alberto Broce, Ph.D.
Livestock Entomologist

Spring-early summer populations of stable flies in pastures develop at winter feeding sites of hay in round bales. When hay wasted during feeding is mixed with cattle or horse manure it develops into ideal larval habitats for stable flies. The production of stable flies from these habitats is a function of the amount of wasted hay and the accumulation of the hay/manure medium.

Hay:manure ratios of 1:1 to 5:1 provide ideal media for developing stable fly larvae. Thus, any measure to reduce the amount of wasted hay and/or to control the accumulation of the hay:manure medium will help decrease the production of stable flies at these feeding sites.

This can be achieved by various practices, including the following: frequently moving the placement of the feeding tub to prevent the accumulation over one spot of the hay-manure medium; use of feeders, such as cone feeders, demonstrated to reduce (although not prevent) the amount of wasted hay; unrolling the round bales on pastures, but not over the same site; spreading accumulated hay:manure medium to allow it to dry.

Economic levels of stable fly populations in pastures appear to be of significance only during a 4 to 6 week period during the spring-early summer, yet during this period they can reduce weight gain of stockers by 0.5 lb/head/day.
Continuing Education

**March 3**  
Veterinary Technicians Conference  

**March 5-16**  
VetBytes – Outpatient Pain Management in Dogs and Cats: Beyond NSAIDs and Fentanyl Patches  

**March 11**  
Frank W. Jordan Seminar on Zoonoses and Pets of Immunocompromised Clients  

**April 9-20**  
Vet-Bytes – Bovine Spongiform Encephalopathy (BSE): An update on the current U.S. situation  

**April 20-21**  
Bovine Conference  

**June 3-6**  
69th Annual Conference for Veterinarians and KVMA Veterinary Trade Show

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

**March 2**  
KSU Cattlemen’s Day  
Manhattan, Kan.  

**March 7-9**  
Western Dairy Management Conference  
Reno, Nev.  

**March 10**  
KSU Sheep Day and Youth Sheep Day  
Manhattan, Kan.  

**March 17-18**  
2-Day Equine Reproductive Management Shortcourse  
Manhattan, Kan.  

**March 23 and 24**  
2-Day Equine Reproductive Management Shortcourse  
Manhattan, Kan.  

**March 31**  
Wildlife Habitat Evaluation Contest  
Manhattan, Kan.  

**April 9-20**  
Vet-Bytes – Bovine Spongiform Encephalopathy (BSE): An update on the current U.S. situation  

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