Journal of the American Association for Laboratory Animal Science
Copyright 2007
by the American Association for Laboratory Animal Science

Vol 46, No 3
May 2007
Pages 68–72

Porcine Dermatitis and Nephropathy Syndrome Associated with Porcine Circovirus 2 Infection in a Yorkshire Pig

Lise R Phaneuf,[1] Alejandro Ceccarelli,[2] Jenny R Laing,[2,†] Badru Moloo,[2] and Patricia V Turner[1,*]

We describe a case of porcine dermatitis and nephropathy syndrome (PDNS) in a 14-wk-old Yorkshire pig purchased from a commercial farm for research use. Physical examination of the affected animal upon arrival revealed multifocal, red, papular skin lesions on the rump, vulva, perineum, thighs, and lower hindlegs. At necropsy, gross lesions consisted of dermatitis, bilaterally enlarged kidneys and patchy pulmonary congestion. Histologic findings included multiorgan necrotizing vasculitis with prominent lesions in the skin, kidneys, lung, spleen, and liver. Immunohistochemical staining for porcine circovirus type 2 (PCV2) was strongly positive in affected areas of kidney and spleen. In light of the clinical assessment and gross and histologic findings, a diagnosis of PDNS was made. We emphasize the importance of considering PDNS as a differential diagnosis in laboratory swine with skin lesions.

Abbreviations: PCV2, porcine circovirus type 2; PDNS, porcine dermatitis and nephropathy syndrome; PRRSV, porcine reproductive and respiratory syndrome virus

Case Report

History. A 14-wk-old, 60-kg female Yorkshire pig arrived at a research facility for use in a teaching project. The facility is in compliance with the Animals for Research Act of Ontario, and all procedures involving animals were approved by the institutional animal care committee and are within the Guidelines of the Canadian Council on Animal Care.[2] The animal was purchased from a local dealer, who acquired pigs from several closed high-health-status commercial herds in southwestern Ontario, and was shipped by truck directly from the farm to the animal facility. Vendor health surveillance reports indicated that the animal came from a herd serologically negative for *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, porcine reproductive and respiratory syndrome virus (PRRSV), atrophic rhinitis, swine dysentery, and transmissible gastroenteritis virus and free of lice (*Hematopinus suis*) and *Sarcoptes scabiei*. The vaccination status of the herd was unknown. Upon arrival at the facility, the pig was noted to have multifocal to coalescing, raised, red, nonpruritic, papular to crusted skin lesions as large as 5 cm in diameter on the rump, vulva, perineum, thighs, and lower hindlegs (Figure 1 A). The animal's temperature was 38.1 °C, and it appeared to be slightly depressed and reluctant to move. On palpation, both hock joints were mildly warm and painful. Immediately after this assessment, the pig was separated from other animals received in the same shipment, and 0.01 mg/kg buprenorphine (Buprenex, Reckitt Benckiser Pharmaceuticals, Richmond, VA) was administered intramuscularly for pain relief. The 3 other pigs received in the same shipment did not show clinical signs of disease.

The animal was euthanized 24 h later with 120 mg/kg pentobarbital sodium, given intravenously, after sedation with 30 mg/kg ketamine hydrochloride and 2 mg/kg xylazine intramuscularly. A complete necropsy was conducted, and tissues (liver, kidney, spleen, heart, lung, small intestine, gall bladder, vulva, and skin) were collected into 10% neutral buffered formalin for microscopic evaluation. Blood was collected at necropsy and submitted to a commercial laboratory (Vita-Tech Canada, Markham, Ontario, Canada) for a complete blood count with differential (white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, bands, neutrophils, lymphocytes, monocytes, eosinophils, and basophils) and serum chemistry evaluation (total protein, albumin, globulin, albumin:globulin ratio, total bilirubin, conjugated bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatinine kinase, amylase, lipase, cholesterol, glucose, urea, creatinine, sodium, potassium, sodium:potassium ratio, chloride, calcium, and phosphorus). Urine was collected by cystocentesis and submitted to a commercial laboratory (Vita-Tech Canada) for analysis. Differential diagnoses based on the initial clinical assessment included erysipelas, *Actinobacillus suis* pneumonia and septicemia, exudative dermatitis (*Staphylococcus hyicus* infection), swinepox, and salmonellosis. Classical swine fever (hog cholera) and African Swine Fever also were considered and the Canadian Food Inspection Agency was notified, as these are reportable diseases in Canada.

Histopathology evaluations. Tissues were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Immunohistochemical analyses of representative formalin-fixed, paraffin-embedded tissue sections of heart, lung, kidney, spleen, and small intestine were performed by a local commercial laboratory (Animal Health Laboratory, University of Guelph, Guelph, Canada) using an autostainer (DakoCytomation, Mississauga, Ontario, Canada). Tissue sections were incubated at room temperature with either antiporcine circovirus 2 (PCV2) rabbit polyclonal antiserum (Dr P Halbur, Iowa State University, Ames, IA) at a dilution of 1:2000 for 60 min or antiporcine reproductive and respira-

Received: 18 Sep 2006. Revision requested: 8 Dec 2006. Accepted: 8 Dec 2006.
[1]Department of Pathobiology, University of Guelph, Guelph, Canada; [2]Animal Resources Center, University Health Network, Toronto, Canada.
[†]Present address: Trent University, Peterborough, Canada.
[*]Corresponding author. Email: pvturner@uoguelph.ca

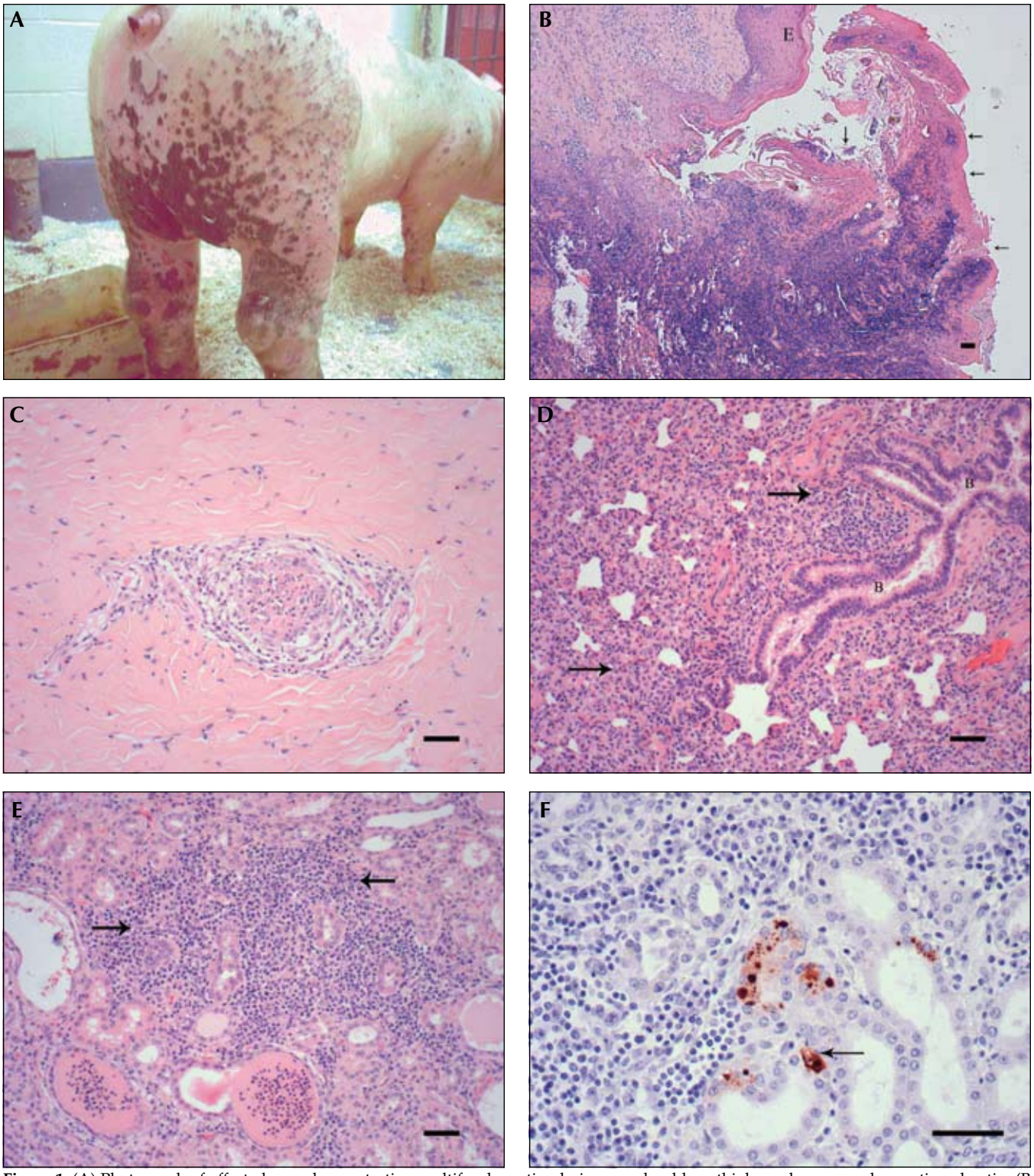

Figure 1. (A) Photograph of affected sow, demonstrating multifocal crusting lesions on shoulders, thighs and rump, and necrotic vulva tip. (B through F) Photomicrographs of affected tissues; marker, 50 µm. All sections stained with hematoxylin and eosin except for that in F, which demonstrates immunostaining for PCV2 antigen. (B) Section of skin lesion on thigh demonstrating locally extensive ulceration (denoted by arrows) with crusting and marked lymphocytic/plasmacytic infiltrate, subjacent to hyperplastic epithelium (labeled E). (C) Higher-power magnification of skin lesion demonstrating vasculitis within panniculus, affected vessel with fibrinoid necrosis of media, and mature and degenerate infiltrating neutrophils and lymphocytes. (D) Section of lung demonstrating marked interstitial thickening (arrows) and peribronchiolar (labeled B) lymphocytic-plasmacytic infiltrates. (E) Section of kidney from affected animal with marked nonsuppurative interstitial nephritis (arrows) and tubular dilatation. (F) Immunostaining of renal tissue with PCV2 antibody, demonstrating strong positive staining within the cytoplasm of tubular epithelial cells (arrow).

tory syndrome virus monoclonal antibody (SDOW 17A, Rural Technologies, Brookings, SD) at a 1:600 dilution for 30 min. For PRRSV negative controls, the primary antibody was substituted with an irrelevant monoclonal antibody at similar concentration in validation experiments or antibody diluent alone in test runs. In PCV2 assays, nonimmune rabbit serum diluted to a protein

Table 1. Serum biochemistry results from a female Yorkshire pig

Test	Result	Reference interval[a]	Unit
Total protein	69	61–81	g/l
Albumin	21	27–39	g/l
Globulin	48	34–43	g/l
Albumin:globulin ratio	0.4	0.1–1.2	Not applicable
Total bilirubin	1.5	0–4	µmol/l
Conjugated bilirubin	0.8	0–4	µmol/l
Alkaline phosphatase	46	None provided	U/l
Alanine aminotransferase	16	None provided	U/l
Aspartate aminotransferase	12	None provided	U/l
Creatine kinase	222	0–800	U/l
Amylase	925	None provided	U/l
Lipase	19	None provided	U/l
Cholesterol	3.49	2.0–5.0	mmol/l
Glucose	5.4	3.6–5.3	mmol/l
Urea	60	3.0–8.5	mmol/l
Creatinine	1679	90–240	µmol/l
Na^+	138	140–150	mmol/l
K^+	3.5	4.7–7.1	mmol/l
Na^+:K^+ ratio	39	None provided	mmol/l
Cl^-	95	99–105	mmol/l
Ca^{2+}	1.96	1.8–2.9	mmol/l
P	4.7	1.6–3.4	mmol/l
Hemolysis	Normal	None provided	Not applicable
Icterus	Normal	None provided	Not applicable
Lipemia	Normal	None provided	Not applicable

[a]Provided by the Animal Health Laboratory, University of Guelph, Ontario, Canada.

concentration similar to that of the PCV2 antiserum was substituted for primary antibody in negative control sections.

Results

Clinical chemistry findings. Serum chemistry analyses revealed markedly increased blood urea, creatinine, and phosphorus levels and decreased potassium levels (Table 1). Hematology findings indicated a mildly reduced red blood cell count, decreased hemoglobin and hematocrit values, mildly decreased mean corpuscular volume and mean corpuscular hemoglobin, and mild lymphopenia (Table 2). Urinalysis findings showed moderate hematuria and proteinuria, large amounts of urate crystals, and occasional leukocytes (Table 3). These results suggested moderate to severe renal disease with concomitant mild microcytic, hypochromic anemia.

Gross pathology findings. Noteworthy gross findings were limited to the skin, kidneys, and lungs. Extensive areas of dermatitis on the rump, thighs, perineum, and vulva were characterized by multifocal to coalescing, irregular and slightly raised, red to purple papules that were 3 to 5 cm in diameter, some of which were covered by a thin yellow crust (Figure 1 A). Similar but less severe skin lesions were present on the shoulders and ears. The kidneys were bilaterally enlarged (7 × 3 cm) and pale. The lungs were mildly consolidated with multifocal patchy red areas. There was mild, bilateral subcutaneous tarsal edema. No remarkable findings were observed in other tissues.

Histopathology findings. Histologic lesions occurred in the skin, lungs, liver, spleen, and kidneys. In the skin, lesions consisted of a severe, multifocal, nonsuppurative, necrotizing and ulcerative dermatitis and panniculitis (Figure 1 B). These lesions were characterized by marked, locally extensive ulceration with dense overlying crusts formed by sloughed, necrotic epidermis and protein admixed with frank blood, degenerate neutrophils, and cellular debris. Extending into the deep dermis and panniculus, blood vessels underlying ulcerated areas were thrombosed, with leukoclastic vasculitis and fibrinoid necrosis of the media (Figure 1 C). In less affected areas, there were marked mixed perivascular infiltrates and localized epidermal hyperkeratosis and acanthosis, characterized by prominent rete ridges.

Severe, multifocal, nonsuppurative, and necrotizing glomerulonephritis and interstitial nephritis were observed within the kidneys. Lesions consisted of marked, multifocal necrosis with scarring of glomeruli. Less affected glomerular tufts appeared swollen and hypercellular. There was multifocal tubular degeneration and necrosis consisting of hydropic swelling, flattening, and atrophy of renal tubular epithelial cells. Many tubules were dilated and contained bright eosinophilic material (protein). There were interstitial, perivascular, and periglomerular infiltrates of lymphocytes, plasma cells, and lesser numbers of neutrophils admixed with cellular debris (Figure 1 E).

Within the lungs, there was a severe, multifocal to coalescing, nonsuppurative interstitial pneumonia characterized by areas of consolidation with expansion of interlobular and interstitial spaces by edema and mixed infiltrates of lymphocytes, plasma cells, and macrophages (Figure 1 D). Within affected areas, there was alveolar edema and moderate hyperplasia of type II pneumocytes. In the perivascular and peribronchial regions, there were moderate lymphoplasmacytic infiltrates admixed with neutrophils and macrophages.

Within the liver, there was mild to moderate, multifocal interlobular hepatitis consisting of dissecting tracts of mixed leukocytic infiltrates (lymphocytes, plasma cells, and neutrophils) within the interlobular connective tissue. Moderate numbers of

Table 2. Complete blood count and differential results from a female Yorkshire pig

Test	Result		Reference interval[a]	Unit
White blood cell count	11.7		11–22	$\times 10^9$/l
Red blood cell count	4.4		5.0–8.2	$\times 10^{12}$/l
Hemoglobin	67		99–158	g/l
Hematocrit	0.2		0.32–0.5	l/l
Mean corpuscular volume	46		51–68	fl
Mean corpuscular hemoglobin	15		17–22	pg
Mean corpuscular hemoglobin concentration	338		300–341	g/l
Platelets	766		325–700	$\times 10^9$/l
Differential	%	Absolute		
Bands	0	0	0.0–0.6	$\times 10^9$/l
Neutrophils	60	7	4.0–9.5	$\times 10^9$/l
Lymphocytes	34	4	6.0–12.6	$\times 10^9$/l
Monocytes	6	0.7	0.3–2.2	$\times 10^9$/l
Eosinophils	0	0	0.0–2.2	$\times 10^9$/l
Basophils	0	0	0.0–0.3	$\times 10^9$/l

[a]Provided by the Animal Health Laboratory, University of Guelph, Ontario, Canada.

neutrophils were scattered throughout the parenchyma, with occasional single-cell necrosis of hepatocytes. In addition, necrotizing vasculitis was present in the spleen and consisted of segmental necrosis and inflammation of small arterioles.

Immunohistochemistry findings. Immunohistochemistry staining for PCV2 demonstrated scattered, strongly positive staining for PCV2 antigen in affected areas of kidney and spleen (Figure 1 F). Positive staining for PCV2 was not observed in sections of heart, lung, and small intestine. All slides were negative for PRRSV antigen. In light of the clinical assessment and gross and histologic findings, a diagnosis of PDNS was made.

Discussion

We describe a case of PDNS in a 60-kg Yorkshire pig purchased from a commercial farm for use in an animal research facility. PDNS is an important emerging syndrome in North America that predominantly occurs in pigs between 11 to 14 wk of age, although all age groups and both sexes can be affected.[17,18] The syndrome was first recognized in the United Kingdom in 1993 as a sporadic condition affecting individual finisher pigs and since then has been described in Europe, North and South America, Oceania, and Africa, suggesting a worldwide distribution.[15,17] The disease is an important problem in Europe but, until recently, has been reported infrequently in Canada and the United States.[8] PDNS is characterized by severe, necrotizing vasculitis affecting the dermis and subcutis and is often accompanied by lesions in the kidneys, lymph nodes, and spleen with variable lung involvement.[18] Other clinical signs include pyrexia, anorexia, lethargy, and lameness and, less commonly, respiratory disease and scouring. The disease can be fatal, and severely affected surviving pigs may experience weight loss and wasting. Mildly affected animals may remain afebrile and recover spontaneously.

The pathogenesis of PDNS is unknown; however, an immune-mediated mechanism is thought to be responsible for the underlying vasculitis associated with the condition.[6] Microscopic features and the presence of immunoglobulin and complement complexes in damaged vessels and glomeruli suggest a type III hypersensitivity reaction as the possible pathogenic mechanism.[9] Potential initiating antigens involved in this immune complex-mediated disorder include drugs, chemicals, and food and endogenous allergens.[6] Infectious agents including *Pasteurella multocida*, PCV2, and PRRSV also have been implicated in the pathogenesis of PDNS.[5,10,13,18,19] Accurate diagnosis of PDNS is based on a combination of clinical assessment and pathology findings. In this case, the clinical presentation and microscopic lesions were not consistent with *Pasteurella multocida* coinfection; however, it would be useful in similar cases to collect and submit lymphoid tissue and specimens for bacteriology. Immunohistochemistry staining was positive for porcine circovirus and negative for porcine reproductive and respiratory syndrome virus.

Porcine circovirus was first isolated from a line of porcine kidney cells used for laboratory research purposes in 1974 and was later typed as porcine circovirus type 1.[20] To date, the clinical significance of porcine circovirus type 1 has not been established, and it does not appear to cause disease. Another distinct porcine circovirus, PCV2, has been associated with several disease syndromes in pigs, including postweaning multisystemic wasting syndrome, reproductive failure, porcine respiratory disease complex, granulomatous enteritis, necrotizing lymphadenitis, and potentially exudative epidermitis.[4,15] Although PCV2 has been consistently detected in cases of PDNS, the syndrome has yet to be reproduced experimentally. The demonstration of PCV2 antigens and nucleic acids, closely associated with skin and renal lesions, supports a circumstantial link between PCV2 and PDNS.[4,13]

The diagnosis of clinical PDNS within a research facility may have serious implications for researchers. Animals with PDNS generally are euthanized for humane reasons, resulting in lost time and research data and the need for replacement animals. There is no known specific treatment for PDNS, and supportive care is symptomatic.[1] Because PDNS may be associated with PCV2 infection, it is unknown whether there are immunomodulatory effects caused by viral interactions that could affect research data. Although the precise mode of transmission of the virus is not known, pigs that have been experimentally infected with PCV2 may shed virus in their feces and nasal secretions.[16,22]

Emerging diseases, such as those associated with PCV2, present unique diagnostic challenges to laboratory animal veterinarians. The value of routine serologic testing for PCV2 is questionable because the virus is considered endemic in North America, including 'specific pathogen-free' and 'high-health-status' herds. In Canada, the extent of exposure of

Table 3. Urinalysis parameters from a female Yorkshire pig

Sample appearance	Yellow, slightly turbid
Specific gravity	1.012
PH	5.0
Urobilinogen	Normal
Blood	++++
Urine bilirubin	Negative
Glucose	Trace amount
Ketones	Negative
Protein	++
Microscopy	
Sediment	
Red blood cell count	3 to 5 per high-power field
White blood cell count	Occasional
Crystals	
Amorphous urate	Large amount

normal, healthy pigs to PCV2 was determined serologically to be 82.4% at slaughter.[11] Sensitive polymerase chain reaction assays distinguishing between the 2 circoviruses are available, but the value of these assays may be limited because whether animals infected solely with PCV2 will develop clinical disease is unknown.[7,12,16] Polymerase chain reaction assays to screen and remove PCV2-infected animals may be useful to consider for pigs entering immunosuppression or transplantation studies. PCV2 can be eliminated from swineherds but requires Caesarian section rederivation and strict barrier-rearing.[21]

Prevention of PDNS and PCV2 infection in swine at research facilities is important but not always achievable. Some measures can be taken to minimize the risks and include careful inspection of the animal, with special attention to skin lesions, upon arrival. Crusty skin lesions may be readily mistaken for dirt by the inexperienced eye. Pigs that are received at research facilities from different commercial suppliers or in different shipments should be housed separately to minimize opportunities for pathogen transmission between animals. As a nonenveloped DNA virus, PCV2 is very hardy and potentially can be spread by fomites, clothes, and shared equipment. Complete cleaning and disinfection of facilities between groups of pigs may be helpful in preventing disease transmission as well as lessening the severity of infections. Agents useful against naked DNA viruses should be used, such as chlorine-releasing disinfectants, iodophors, and quaternary ammonium products.[14] Control of the environment to ensure good ventilation, and maintenance of appropriate room temperature and humidity also may help to minimize the impact of PCV2 infection.

It is important for laboratory animal veterinarians to be aware of the diseases affecting commercial swine and of disease trends within the commercial swine population. At the diagnostic laboratory of the Animal Health Laboratory in Guelph, PCV2-associated diseases in commercial swine submissions, including cases of PDNS, increased markedly in 2005 compared with the previous 7 y, and swine submissions are 14 times more likely to have PCV2-associated disease now than in the past.[3] Porcine dermatitis and nephropathy syndrome associated with PCV2 infection should be included in the differential diagnosis for acutely developing skin lesions in laboratory swine.

Acknowledgments

L Phaneuf is cosupported by graduate scholarships from the Ontario Veterinary College and Pfizer Global Research and Development (Ann Arbor, MI).

References

1. **Baird FJ, Brown P, Pearson RB, Whitehead AW.** 2000. Possible treatment options for PMWS/PDNS. Porcine multisystemic wasting syndrome/porcine dermatitis and neuropathy syndrome. Vet Rec **146:**619–620.
2. **Canadian Council on Animal Care.** 1993. Guide to the care and use of experimental animals. Vol 1, 2nd ed. Ottawa, Ontario, Canada: Canadian Council on Animal Care.
3. **Carman S, McEwen B, Delay J, van Dreumel T, Lusis P, Cai H, Fairles J.** 2006. Porcine circovirus-2 associated disease in swine in Ontario (2004 to 2005). Can Vet J **47:**761–762.
4. **Chae C.** 2005. A review of porcine circovirus 2-associated syndromes and diseases. Vet J **169:**326–336.
5. **Choi C and Chae C.** 2001. Colocalization of porcine reproductive and respiratory syndrome virus and porcine circovirus 2 in porcine dermatitis and nephrology syndrome by double-labeling technique. Vet Pathol **38:**436–441.
6. **Drolet R, Thibault S, D'Allaire S, Thompson J, Done S.** 1999. Porcine dermatitis and nephropathy syndrome (PDNS): an overview of the disease. J Swine Health Prod **7:**283–285.
7. **Hamel AL, Lin LL, Sachvie C, Grudeski E, Nayar GP.** 2000. PCR detection and characterization of type-2 porcine circovirus. Can J Vet Res **64:**44–52.
8. **Harding JC.** 2004. The clinical expression and emergence of porcine circovirus 2. Vet Microbiol **98:**131–135.
9. **Helie P, Drolet R, Germain MC, Bourgault A.** 1995. Systemic necrotizing vasculitis and glomerulonephritis in grower pigs in southwestern Quebec. Can Vet J **36:**150–154.
10. **Lainson FA, Aitchison KD, Donachie W, Thomson JR.** 2002. Typing of *Pasteurella multocida* isolated from pigs with and without porcine dermatitis and nephropathy syndrome. J Clin Microbiol **40:**588–593.
11. **Liu Q, Wang L, Willson P, O'Connor B, Keenliside J, Chirino-Trejo M, Melendez R, Babiuk L.** 2002. Seroprevalence of porcine circovirus type 2 in swine populations in Canada and Costa Rica. Can J Vet Res **66:**225–231.
12. **Ouardani M, Wilson L, Jette R, Montpetit C, Dea S.** 1999. Multiplex PCR for detection and typing of porcine circoviruses. J Clin Microbiol **37:**3917–3924.
13. **Rosell C, Segales J, Ramos-Vara JA, Folch JM, Rodriguez-Arrioja GM, Duran CO, Balasch M, Plana-Duran J, Domingo M.** 2000. Identification of porcine circovirus in tissues of pigs with porcine dermatitis and nephropathy syndrome. Vet Rec **146:**40–43.
14. **Royer RL, Nawagitgul P, Halbur PG, Paul PS.** 2001. Susceptibility of porcine circovirus type 2 to commercial and laboratory disinfectants. J Swine Health Prod **9:**281–284.
15. **Segales J, Rosell C, Domingo M.** 2004. Pathological findings associated with naturally acquired porcine circovirus type 2 associated disease. Vet Microbiol **98:**137–149.
16. **Shibata I, Okuda Y, Yazawa S, Ono M, Sasaki T, Itagaki M, Nakajima N, Okabe Y, Hidejima I.** 2003. PCR detection of porcine circovirus type 2 DNA in whole blood, serum, oropharyngeal swab, nasal swab, and feces from experimentally infected pigs and field cases. J Vet Med Sci **65:**405–408.
17. **Smith WJ, Thomson JR, Done S.** 1993. Dermatitis/nephropathy syndrome of pigs. Vet Rec **132:**47.
18. **Thibault S, Drolet R, Germain MC, D'Allaire S, Larochelle R, Magar R.** 1998. Cutaneous and systemic necrotizing vasculitis in swine. Vet Pathol **35:**108–116.
19. **Thomson JR, MacIntyre N, Henderson LE, Meikle CS.** 2001. Detection of *Pasteurella multocida* in pigs with porcine dermatitis and nephropathy syndrome. Vet Rec **149:**412–417.
20. **Todaro GJ, Benveniste RE, Lieber MM, Sherr CJ.** 1974. Characterization of a type C virus released from the porcine cell line PK(15). Virology **58:**65–74.
21. **Tucker AW, McNeilly F, Meehan B, Galbraith D, McArdle PD, Allan G, Patience C.** 2003. Methods for the exclusion of circoviruses and gammaherpesviruses from pigs. Xenotransplantation **10:**343–348.
22. **Yang JS, Song DS, Kim SY, Lyoo KS, Park BK.** 2003. Detection of porcine circovirus type 2 in feces of pigs with or without enteric disease by polymerase chain reaction. J Vet Diagn Invest **15:**369–373.