Multiple porcine circovirus 2-associated abortions and reproductive failure in a multisite swine production unit

Brendan O'Connor, Henry Gauvreau, Keith West, Jaret Bogdan, Mejid Ayroud, Edward G. Clark, Carrie Konoby, Gordon Allan, John A. Ellis

Abstract — Porcine circovirus type 2 was detected in several stillborn and nonviable neonatal piglets presenting with chronic passive congestion, cardiac hypertrophy, and severe diffuse myocarditis. The presence of the virus in the heart and other tissues of affected piglets was confirmed by polymerase chain reaction, immunohistochemistry, and virus isolation techniques. Other reproductive losses and associated infectious agents in the herd are discussed.

Résumé — Avortements multiples et problèmes de reproduction associés au circovirus porcin 2 dans une unité de production à plusieurs bâtiments. Le circovirus porcin type 2 a été détecté chez plusieurs porcelets morts-nés et non-viables présentant une congestion passive chronique, une hypertrophie cardiaque et une myocardite diffuse sévère. La présence du virus dans le cœur et les autres tissus des porcelets atteints a été confirmée par amplification en chaîne par polymérase, immunohistochimie et par des techniques d'isolation du virus. D'autres déperditions reproductives du troupeau et les agents infectieux associés sont examinés.

(Traduit par Docteur André Blouin)

Can Vet J 2001;42:551–553

Porcine circovirus 2 (PCV2) is a recently recognized virus (1) that has been associated with a variety of disease syndromes in pigs (6), including postweaning multisystemic wasting syndrome (PMWS) in weaned pigs, respiratory disease, and, most recently, myocarditis in stillborn piglets (7). Although the primary means of transmission of PCV2 remains to be determined, the presence of PCV2 infection in neonatal piglets suggests that vertical transmission may be an important means of viral transmission. This mode of transmission could be related not only to reproductive failure, but also to the development of multisystemic disease later in life. In this report, we describe the association of PCV2 infection with a severe episode of reproductive losses in a 3000-sow, gestation and farrowing barn. The possible interactions with other viral infections affecting the reproductive system are discussed.

Porcine circovirus 2 infection was diagnosed in spring 1999 by immunohistochemistry (IHC) (1), virus isolation (VI) (1), and polymerase chain reaction (PCR) (4) in several piglets from the same multisite pork production unit in Alberta. This was a newly established sow facility, containing 3000 F2 gilts that were derived from a single source and were raised in 2, 2000-head, grower barns. The gilts were vaccinated against porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus (PRRSV), *Leptospira* spp., *Erysipelothrix rhusiopathiae,* and *Haemophilus parasuis,* following an established protocol. The gilts were delivered to the barns when ready to breed, starting in April 1998. Farrowing started in mid-August 1998. Subsequently, there was a high rate of reproductive loss. This was due mainly to an increase in the proportion of mummified fetuses farrowed, which approached 15% for an 8-week period, compared with an expected rate of 1.3%. The proportions of stillbirths (8% in the first of these 8 wk) and preweaning mortality (11% for the first of these 8 wk and at least 3 other weeks) was almost double the expected rate during some of this period. Combined piglet losses were elevated for most of the first

Prairie Diagnostic Services, 52 Campus Drive, Saskatoon, Saskatchewan S7N 5B4 (O'Connor, West, Clark); Veterinary Consultant, Bay 2, 55 Wheatland Trail, Strathmore, Alberta T1P 1R7 (Gauvreau); Alberta Agriculture, Food and Rural Development, Postal Bag Service #1, Airdrie, Alberta T4B 2C1 (Ayroud); Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon S7N 5B4 (Bogdan, Konoby, Ellis); Department of Agriculture, Stoney Road, Stormont, Belfast, Northern Ireland BT4 3SD (Allan).

Address correspondence and reprint requests to Dr. John A. Ellis.

Supported by Merial Ltd., Natural Sciences and Engineering Research Council-Collaborative Research Opportunities grant 234281-00, European Union Project QLK2-CT-1999-00445 and The National Pork Producers Council (United States). Mr. Bogdan was supported by a Western College of Veterinary Medicine Summer Student Scholarship.

Figure 1. Transverse ventricular section of myocardium from aborted piglet. Note marked ventricular dilatation and foci of myocarditis (arrow).

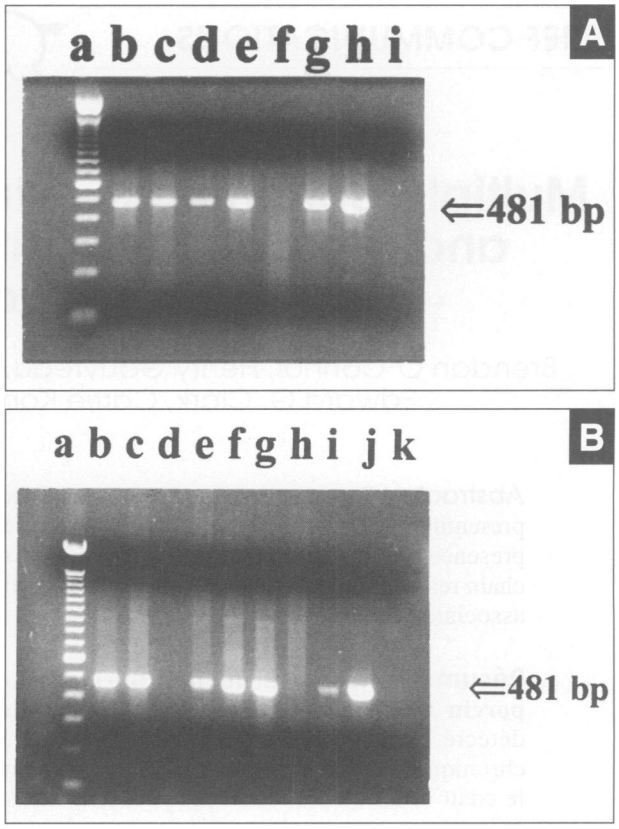

Figure 2. Detection of porcine circovirus 2 (PCV2) in formalin-fixed (A) and fresh frozen (B) fetal porcine tissues by polymerase chain reaction (PCR). A) 100 bp DNA ladder (a); fetus #1 (b); fetus #2 (c); fetus #3 (d); fetus #4 (e); fetus #5 (f); fetus #6 (g); PCV2 PCR using affected liver from pig with PMWS (h); PCV2 PCR using liver from normal pig (i). B) 100 bp DNA ladder (a); fetus #1 heart (b); fetus #1 pooled lung and spleen (c); fetus #2 heart (d); fetus #2 pooled lung and spleen (e); fetus #3 heart (f); fetus #3 pooled lung and spleen (g); fetus #4 heart (h); fetus #4 pooled lung and spleen (i); PCV2 PCR using affected liver from pig with PMWS (j); PCV2 PCR using liver from normal pig (k). PMWS — postweaning multisystemic wasting syndrome.

32 wk of production. The rate of late-term abortions also increased slightly at the start of the outbreak.

Congestive heart failure was a frequent gross finding in stillborn and some of the neonatal piglets that were examined. Piglets from about 40 litters were submitted for necropsy in December 1998 and January 1999. The make-up varied, but most litters included 1 or 2 live and stillborn piglets and several mummified fetuses with sizes varying from 6- to 26-cm crown-rump length. There was excess fluid in the thoracic cavity of several stillborn piglets, and the hearts were dilated and hypertrophied. Pale areas were evident in the myocardium of some (Figure 1). The livers were congested due to chronic passive congestion.

Histologically, there was severe, nonsuppurative myocarditis and, often, myocardial necrosis, with variable fibrosis, indicating possible variation in the duration of lesion development. There were foci of mineralization and scattered intranuclear inclusion bodies in cardiomyocytes. Immunohistochemical staining revealed copious PCV2 antigen in myocardial lesions in the hearts from all 6 of the piglets that were submitted, while 5 of the 6 were positive by PCV2 PCR on fixed tissue (Figure 2A).

Frozen samples from 4 piglets, of which 2 were stillborn and 2 died shortly after birth, were tested by PCR and VI. All 4 piglets had gross evidence of a severe, diffuse myocarditis, cardiac hypertrophy, and chronic passive congestion. The results of PCR for PCV2 were positive in the heart of 2 of piglets and in the pooled lung and splenic tissues of 4 of 4 piglets (Figure 2B). Isolation of PCV2 from affected hearts or pooled lung and splenic tissue was successful in 2 of the 4 cases that were PCV2-positive by PCR.

Porcine parvovirus (PPV) was demonstrated by the fluorescent antibody test in the lungs of 7 out of 10 mummified fetuses examined during the outbreak. Porcine parvovirus was isolated from the tissues of 2 out of 4 neonatal but none of 8 stillborn piglets tested. Encephalomyocarditis virus (EMCV) was not isolated

from the tissues of any of the 8 stillborn or 4 neonatal piglets on which isolation attempts were made using a variety of appropriate cell cultures. However, high antibody titers against EMCV were detected in the thoracic fluid from 6 out of 12 stillborn piglets on virus neutralization tests. The only precolostral serum available was from 1 small piglet without heart lesions and in which no titer against EMCV was detected. Immunohistochemical tests for PRRSV antigen were negative on tissues from all 5 stillborn and 2 neonatal piglets that were tested, although the PCR assay (8) was positive in 2 out of 2 stillborn and 2 out of 3 neonatal piglets tested. Limited serological testing of the gilts for antibodies against PPV, PRRSV, PCV2, EMCV, and *Leptospira* spp. was not helpful in confirming if any active infections had occurred during gestation.

The findings in this study confirm and extend the previous observation that PCV2 can be vertically transmitted and can be present in large amounts within lesions from piglets infected in utero (7). This appears to be a disease manifestation of PCV2 infection, in addition to PMWS (6). Alternatively, it is possible that the PCV2-like viruses isolated from cases of reproductive disease may be genetically and phenotypically different from the PCV-2 associated with PMWS, or have a different tissue tropism or virulence. These possibilities are currently under investigation.

In the one previously reported case of PCV2-associated fetal damage and reproductive failure in swine (7), no other pathogens causally related to reproductive disease in swine were detected in affected piglets, or on serological testing of the swine herd. By contrast, in this multisite operation, the presence of, or previous exposure to, PPV, PRRSV, and EMCV was documented using a variety of techniques applied to affected fetal tissues. These findings are consistent with previous observations that PPV, PRRSV, and other agents may act synergistically with PCV2 to cause a variety of diseases in infected swine (6,9–11). Nevertheless, none of these viruses were directly associated with microscopic lesions in these affected fetuses and piglets. These findings emphasize the importance of associating suspect pathogens directly with lesions in affected animals, before implicating agents that may be endemic in swine populations in a causal relationship with observed disease.

Cofactors that determine whether or not PCV2 infection results in reproductive or other disease in exposed swine are poorly understood. Limited retrospective serological testing (data not shown) of the sow herd in which the abortions occurred suggested that the introduction of PCV2-seronegative gilts may have been an important factor associated with reproductive inefficiency in the herd. Further studies are required to determine how various management factors may affect disease expression associated with PCV2 infection.

Acknowledgment

The authors thank Dr. Susanne Carman, Animal Health Laboratory, Guelph, Ontario, for advice and testing of some of the samples for EMCV and PPV. CVJ

References

1. Ellis J, Hassard L, Clark E, et al. Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. Can Vet J 1998;39:44–51.
2. Tischer I, Rasch R, Tochtermann G. Characterization of papovavirus- and picornavirus-like particles in permanent piglet kidney cell lines. Zentralbl-Bakertiol-Org-A 1974;226:153–167.
3. Dulac GC, Afshar A. Porcine circovirus antigens in PK-15 cell line (ATCC-CCL-33) and evidence of antibodies to circovirus in Canadian piglets. Can J Vet Res 1989;53:431–433.
4. Meehan BM, McNeilly F, Todd D, et al. Characterization of novel circovirus DNAs associated with wasting disease syndromes in pigs. J Gen Virol 1998;79:2171–2179.
5. Allan GM, McNeilly F, Cassidy JP, et al. Pathogenesis of porcine circovirus: experimental infections of colostrum-deprived piglets and examination of piglet foetal material. Vet Microbiol 1995;44: 49–64.
6. Allan GM, Ellis JA. Porcine circoviruses: a review. J Vet Diagn Invest 2000;12:3–14.
7. West KH, Bystrom JM, Wojnarowicz C, et al. Myocarditis and abortion associated with intrauterine infection of sows with porcine circovirus-2. J Vet Diag Invest 1999;11:530–532.
8. Gilbert SA, Larochelle R, Magar R, et al. Typing of porcine reproductive and respiratory syndrome viruses by a multiplex PCR assay. J Clin Microbiol 1997;35:264–267.
9. Ellis JA, Krakowka S, Lairmore M, et al. Reproduction of lesions of postweaning multisystemic wasting syndrome in gnotobiotic piglets. J Vet Diag Invest 1999;11:3–14.
10. Ellis JA, Bratanich A, Clark E, et al. Coinfection by porcine circovirus and porcine parvovirus in pigs with postweaning multisystemic wasting syndrome. J Vet Diag Invest 2000;12:21–27.
11. Ellis JA, Krakowka S, Allan G, et al. The clinical scope of porcine reproductive and respiratory syndrome virus infection has expanded since 1987: an alternative perspective. Vet Pathol 1999;36:262–265.