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# Experimental infection of 3-week-old conventional colostrum-fed pigs with porcine circovirus type 2 and porcine parvovirus

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#### **Abstract**

This report describes an experimental infection with porcine circovirus type 2 (PCV2) in combination with porcine parvovirus (PPV) in 3-week-old conventional colostrum-fed pigs with maternal antibodies to both viruses. Two groups of four pigs each were inoculated with PCV2 and PPV. One of the groups received also a commercial inactivated vaccine against porcine pleuropneumonia to evaluate possible effects of the stimulation of the immune system of pigs on the infection. Another group of four pigs was kept as uninfected control. Clinical signs, rectal temperatures and body weights were recorded. Serum antibody titers to PCV2 and PPV were determined at weekly intervals. Pigs were killed 42 days after inoculation and tissue samples were examined for the presence of gross and microscopic lesions. Tissues were also analyzed for the presence of PCV2 and PPV DNA by PCR, and for the presence of PCV2 antigen by immunohistochemistry (IHC). All the pigs had serum antibodies to PCV2 and PPV at the beginning of the trial. None of them developed clinical symptoms or pathological lesions typical of post-weaning multisystemic wasting syndrome (PMWS), a disease associated to PCV2 infection. However, IHC and/or PCR analyses showed that clinically silent PCV2 infection developed in five of the eight inoculated pigs, regardless of the administration of the vaccine. In particular, PCV2 DNA and/or antigen were detected in most of the tissues examined in the two pigs with the lowest titer of maternal PCV2 antibodies at the beginning of the trial. PPV DNA was not detected in any of the samples examined. The five pigs with PCR and/or IHC evidence of PCV2 infection had a mean weight gain during the experiment lower than that of the inoculated PCR-negative pigs considered together and that of the control pigs. In conclusion, it would appear that passive immunity against PCV2 can play a role in preventing the development of PMWS, but is not able to prevent the establishing of clinically silent PCV2 infections. The dissemination and persistence of the virus in the tissues may depend on the level of PCV2 antibodies at the time of inoculation.

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#### 1. Introduction

Porcine circovirus type 2 (PCV2) infection is now recognized as the major factor in the development of post-weaning multisystemic wasting syndrome (PMWS), a recently described and emerging disease throughout the world, that affects pigs predominantly between 5 and 15 weeks of age. The disease is characterized by growth retardation, dyspnea, enlargement of inguinal lymph nodes, diarrhea, and occasionally jaundice (Allan and Ellis, 2000). At necropsy, the most frequent lesions are enlargement of lymph nodes and non-collapsed, tanmottled lungs (Segalés and Domingo, 2002). The main histological lesions consist of a variable degree of lymphocyte depletion with loss of follicles together with histiocytic and multinucleate giant cell infiltration in the lymphoid tissues, and lymphohistiocytic to granulomatous inflammatory infiltrations in a wide range of tissues (Segalés and Domingo, 2002). Though PCV2 has been consistently detected in pigs with PMWS and has been systematically associated with PMWS microscopic lesions, PCV2 infection does not inevitably lead to clinical symptoms. Antibodies to PCV2 have been demonstrated in a very high percentage of pig sera tested throughout the world, and it has been concluded that sub-clinical infection with PCV2 is common in pig populations.

Several experimental studies using PCV2 alone have resulted in asymptomatic infection with mild to moderate histopathological lesions (Allan et al., 1999; Balasch et al., 1999; Magar et al., 2000), while dual infections of germ free or conventional colostrumsdeprived piglets with porcine parvovirus (PPV) or porcine respiratory and reproductive syndrome virus (PRRSV) increased the PCV2 replication and dissemination and induced clinical disease and more severe histopathological lesions (Allan et al., 1999, 2000,2001a,b; Krakowka et al., 2000; Kennedy et al., 2000; Harms et al., 2001; Rovira et al., 2002). These results are in concordance with field observations of PCV2 co-infection with PPV or PRRSV in PMWS cases (Segalés and Domingo, 2002). Nowadays, PCV2 infection is thought to be necessary but not sufficient to determine the full expression of PMWS, that can be considered a multifactorial disease (Segalés and Domingo, 2002).

The activation of the immune system has also been considered a key component of the pathogenesis of PCV2-associated PMWS. Increase of PCV2 replication, leading to clinical disease, was related to activation of macrophages, virus target cells, in lymphoid tissues following immunostimulation (Krakowka et al., 2001). Krakowka et al. (2001) reproduced severe PMWS in germ free piglets inoculated with PCV2 only and then injected with haemocyanin emulsified in Freund's incomplete adjuvant. Allan et al. (2000) showed that the administration of commercial vaccines directed towards other swine pathogens potentiate PCV2 experimental infection in colostrum-fed piglets, sometimes leading to PMWS. Yet, most of the PCV2 infection experiments that successfully reproduced PMWS have been conducted on germ free or colostrums-deprived piglets (Allan et al., 1999, 2001a; Krakowka et al., 2000; Kennedy et al., 2000; Harms et al., 2001; Bolin et al., 2001), while conventional PCV2-seropositive pigs, which represent the model closest to field conditions, have been rarely used (Allan et al., 2000, 2002; Reynaud et al., 2001; Rovira et al., 2002; Resendes et al., 2004; Roca et al., 2004). The gnotobiotic model cannot be compared to field conditions because of the immature immunological status of gnotobiotes and the great differences in husbandry conditions. Furthermore, under natural condition, PMWS affects 5- to 15-week-old pigs, shortly after weaning. Therefore, the objective of this study was to evaluate the clinical and pathological consequences of an infection with PCV2 in combination with PPV in conventional, 3-week-old pigs with maternal serum antibodies towards both viruses. The effect of an immunostimulation induced by the administration of a commercial inactivated vaccine against porcine pleuropneumonia on the outcome of the infection was also evaluated, together with the effects of the infection on the growth rate of the pigs.

#### 2. Materials and methods

#### 2.1. Pigs

Twelve neutered conventional male pigs were selected 17 days after birth from the litters of two primiparous sows. The sows had been vaccinated with

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