The Recombinant Nonstructural Polypeptide NS1 of Porcine Parvovirus (PPV) as Diagnostic Antigen in ELISA to Differentiate Infected from Vaccinated Pigs

L. Qing, J. Lv, H. Li, Y. Tan, H. Hao, Z. Chen, J. Zhao and H. Chen

Laboratory of Animal Virology, College of Veterinary Medicine, and The National Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, P. R. China

*Correspondence: E-mail: chenhch@hotmail.com; hzauvet@public.wh.hb.cn

Qing, L., Lv, J., Li, H., Tan, Y., Hao, H. Chen, Z., Zhao, J. and Chen, H., 2006. The recombinant nonstructural polypeptide NS1 of porcine parvovirus (PPV) as diagnostic antigen in ELISA to differentiate infected from vaccinated pigs. Veterinary Research Communications, 30(2), 175–190

ABSTRACT

To differentiate pigs infected with porcine parvovirus (PPV) from those vaccinated with inactivated whole-virus vaccine, an enzyme-linked immunosorbent assay (ELISA) based on detection of a nonstructural polypeptide 1 (NS1) was developed. A threshold of 0.23 optical density units was established and the assay had high specificity (100), sensitivity (88), accuracy (90) and positive predictive value (100) using haemagglutination inhibition as the standard method. A reproducibility test revealed that the coefficients of variation of sera within-plates and between-run were less than 10.

The assay showed no cross-reactivity with antibodies to porcine reproductive respiratory syndrome virus, pseudorabies virus, foot and mouth disease virus, Actinobacillus pleuropneumoniae, Toxoplasma or Chlamydia. Sera obtained from pigs infected with PPV reacted with recombinant NS1 protein in the ELISA. Sera from pigs vaccinated with inactivated whole virus did not recognize this protein in the ELISA. In contrast, antibodies against PPV whole virus were present in both PPV-infected and vaccinated animals. Serum conversion against NS1 was first detected 10 days after infection and NS1-specific antibodies were detectable up to half a year post infection. In conclusion, the PPV-NS1 ELISA can differentiate PPV-infected versus inactivated PPV-vaccinated pigs and could be applied in disease diagnosis and surveillance.

Keywords: differentiation diagnostic method, ELISA, nonstructural polypeptide NS1, porcine parvovirus (PPV)

Abbreviations: ADV, Aleutian mink disease parvovirus; APP, Actinobacillus pleuropneumoniae; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; FA, fluorescent antibody; FMDV, foot and mouth disease virus; HA, haemagglutination; HI, haemagglutination inhibition; HRP, horseradish peroxidase; IgG, immunoglobulin G; IPTG, isopropyl-β-D-thiogalactopyranoside; MVM, minute virus of mice; NS, nonstructural; NT, neutralization test; OD, optical density; ORF, open reading frame; PCR, polymerase chain reaction; PCV2, porcine circovirus 2; PMWS, postweaning multisystemic wasting disease; PPV, porcine parvovirus; PRRSV, porcine reproductive and respiratory syndrome virus; PRV, pseudorabies virus; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; TCID₅₀, tissue culture infective dose 50%; TMB, 3,3,5,5-tetramethylbenzidine
INTRODUCTION

Infection with porcine parvovirus (PPV) is of economic concern to pig breeders worldwide (Jenkins, 1992). It is a major cause of stillbirth, mummification, embryonic death and infertility (SMEDI) syndrome (Soares et al., 1999). Co-infection of PPV and porcine circovirus 2 (PCV2) has been shown to be responsible for a significant proportion of field cases of porcine postweaning multisystemic wasting syndrome (PMWS) (Kim et al., 2003; Kim and Chae, 2004; Opriessnig et al., 2004). Experiments showed that dual infection of pigs with PCV2 and PPV caused severe clinical signs and lesions of PMWS, while inoculation with PCV2 alone produced only mild to moderate histological lesions (Nawagitgul et al., 2002). Some types of inactivated PPV vaccines are available. Vaccination with inactivated PPV is generally considered to offer good protection against PPV and is used to control reproductive failure due to PPV (Westenbrink et al., 1989; Jenkins et al., 1992).

In farms where vaccination is carried out, the majority of susceptible pigs should be seropositive. Various serological tests are available for the detection of antibodies against the virus, including haemagglutination inhibition (HI), neutralization test (NT), immunodiffusion test, complement-fixation test and whole-virus ELISA, etc. (Hohdatsu et al., 1988; Westenbrink et al., 1989). However, current serological techniques cannot determine whether PPV, the causal agent of the disease, is present. Diagnosis of the virus or the viral antigen may be important in determining the outcome of the infection. The diagnosis can be made by means of fluorescent antibody (FA) test, haemagglutination (HA) techniques, virus isolation or polymerase chain reaction (PCR) assay (Soares et al., 1999; Prikhodko et al., 2003). All of these techniques require a considerable degree of expertise on the part of the operator, particularly with regard to reading and interpreting the results. In addition, the FA and HA tests have the drawbacks of non-specificity and low sensitivity. As isolation of virus and PCR assay were reported to be unsuitable for large-scale routine diagnostic operation (Soares et al., 1999), it is necessary to develop a simple and easy-to-operate serological test that differentiates infected pigs from vaccinated pigs.

PPV is a member of the family Parvoviridae. It has a single-stranded DNA comprising 5000 nucleotides with two open reading frames (ORFs). The left ORF encodes for nonstructural (NS) proteins and the right ORF encodes structural proteins VP1, VP2 and VP3 (Soares et al., 1999). NS1 (86 kDa) is the major NS protein produced during PPV infection (Cotmore and Tattersall, 1989). It was reported to be a phosphorylated polypeptide, and to be highly conserved among the parvoviruses (Molitor et al., 1985). It is produced during the early phase of replication, before the onset of viral structural protein synthesis (Molitor et al., 1984; Molitor et al., 1985). Among the parvoviruses studies, NS1 proteins of minute virus of mice (MVM) and the Aleutian mink disease parvovirus (ADV) have been reported to have important functions during infection in vitro (Legendre and Rommelaere, 1992; Christensen et al., 1995). Detection of parvovirus B19 NS1 specific antibody is useful in determining the level of protection from a capsid-based vaccine, as the emergence of anti-NS1 antibodies indicates parvovirus infection (Heegaard et al., 2002). Since the inactivated PPV vaccines that consist of semi-purified, chemically inactivated virus can elicit only