

## STUDY ON THE VIRULENCE AND CROSS-NEUTRALIZATION CAPABILITY OF RECENT PORCINE PARVOVIRUS FIELD ISOLATES AND VACCINE VIRUSES IN EXPERIMENTALLY INFECTED PREGNANT GILTS

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### ABSTRACT

The pathogenicity of two recent German field isolates of *Porcine parvovirus* (PPV-27a and PPV-143a) and two vaccine viruses [PPV-NADL-2 and PPV-IDT (MSV)], which are used for the production of inactivated vaccines, was investigated by inoculation of pregnant sows at day 40 of gestation. Post-infection sera of these sows as well as antiserum prepared in rabbits by immunization with the four above-mentioned PPV isolates and with the virulent strain PPV-Challenge (Engl.) were tested for their homologous and heterologous neutralization activities. All antiserum had high neutralization activities against the vaccine viruses, the PPV-Challenge (Engl.) and PPV-143a, but much lower activity against PPV-27a. These results suggest that PPV-27a represents a new antigenic variant or type of PPV and vaccines based on the established vaccine viruses may not be fully protective against this field isolate. PPV-27a has been characterized based on the amino acid sequences of the capsid protein as a member of a new and distinct PPV cluster. Interestingly, the homologous neutralizing antibody titres of the sera of all three pigs and both rabbits inoculated or immunized with PPV-27a were 100- to 1000-fold lower than the heterologous titres against any of the other viruses. The low homologous neutralizing antibody titres suggest a possible, yet undefined, immune escape mechanism of this PPV isolate.

### INTRODUCTION

*Porcine parvovirus* (PPV) is a member of the family *Parvoviridae*. PPV is widespread in swine herds, despite vaccination. The virulent strains cause reproductive failures in swine, represented by stillbirth, embryonic death, infertility (SMEDI-syndrome) and delayed return to oestrus. The manifestation of clinical disease depends on the pathogenicity of the virus and on the stage of gestation. Fetuses infected before day 70 of gestation usually die, whereas fetuses infected at a later time point develop antibodies against PPV, eliminate the virus and survive the infection.

PPV strains can be distinguished by their different pathogenicity. Substitution of only a few residues in the VP2 capsid protein is thought to be responsible for distinct biological properties.

Phylogenetic analysis of the VP1/VP2 protein gene revealed that there is a relatively weak sequence similarity between PPV-NADL-2 and recent field isolates from Germany.

The aim of the study was to examine two of these recent field isolates, one from each cluster, under experimental conditions for their pathogenicity (*in vivo*) and antigenicity (*in vitro*), particularly in comparison to the vaccine viruses PPV-NADL-2 and PPV-IDT (MSV).

## MATERIAL AND METHODS

**Animal experiment.** Twelve specific-pathogen-free Pietrain x Large White sows, 11 months of age, were randomly assigned to four groups. Groups were kept separately throughout the experiment. At day 40 of gestation the sows were inoculated with the respective viruses by both the intranasal (i.n.) and intramuscular (i.m.) route. Clinical signs (general performance, respiratory activity, food and water intake, and rectal temperature) were recorded daily for 50 days postinoculation. Blood samples were taken in intervals and analysed for antibodies against PPV. At day 90, about three weeks before term, all gilts were euthanized and the fetuses were aseptically delivered via Caesarean and euthanized. Blood and tissue samples were collected from the sows and all fetuses.

**Polyclonal sera.** To prepare virus-specific sera for cross-neutralization tests with the selected field isolates, vaccines viruses and PPV-Challenge (engl.), rabbits were immunized with CsCl-density-purified virus. The resulting sera were heat-inactivated and stored frozen at  $-20^{\circ}\text{C}$ .

**Serology.** Hemagglutination inhibition test for detection of PPV-specific antibodies and serum neutralization test for checking cross-neutralization activity are described in detail in Zeeuw, E. J. et al. (2007).

**Virus detection.** SPEV cells were used for virus reisolation from lung and kidney of the fetuses. Viral DNA was detected by real-time PCR as described by Wilhelm, S. et al. (2006).

## RESULTS

**Clinic.** All sows remained clinically healthy. Fetal mummification was significantly ( $P < 0.05$ ) higher in the gilts infected with PPV-27a as compared to the other groups (85% vs. 5–18%). Almost all fetuses of the gilts of the group 2 infected with PPV-27a showed various ranges of fetal mummification. In contrast, only single mummified fetuses were found in litters of the gilts of groups 1 (PPV-143a), 3 (PPV-IDT [MSV]), or 4 (PPV-NADL-2).

**Serology.** Gilts infected with PPV-143a, PPV-27a and PPV-NADL-2 developed a significant ( $p < 0.05$ ) higher serological response at 2 weeks p.inf. compared to PPV-IDT (MSV). Umbilical cord blood of the non-mummified fetuses from all groups revealed HI antibody titre (Table 2), indicating transplacental infection of all PPV-isolates examined. Neutralizing antibody titres were determined in the post infection sera of the sows and rabbit sera raised against the various PPV-isolates. The neutralizing antibody titre in sera raised against PPV-143a, PPV-IDT (MSV), PPV-NADL-2 and PPV-Challenge (Engl.) against the PPV-Isolate 27a were generally very low, with SN titres ranging from 0.5–0.69, but high against PPV-143a, PPV-IDT (MSV), PPV-NADL-2 and PPV-Challenge (Engl.). Sera raised against PPV-27a neutralized all heterologous PPV-isolates with high titres ranging from 2.99–3.99 (overall geometric mean titre), the homologous virus, however, was less efficiently neutralized (0, 69–1, 19, see table 4).

Virtually identical results were obtained with rabbit sera raised against the PPV isolates, with SN titres of antiserum raised against PPV-27a ranging from 2.29–3.99 against all heterologous viruses, but only titres of 0.69–1.39 against the homologous virus.

**Virus detection.** After two passages, no evidence for virus replication was observed in the fetuses of group 1 (PPV-143a), group 3 (PPV-IDT [MSV]) and group 4 (PPV-NADL-2). In contrast, virus could be readily isolated from fetuses of group 2 (PPV-27a). Viral DNA could be detected by PCR in virtually all mummified and non-mummified fetuses of the PPV-27a inoculated sows, and in single non-mummified piglets of the other groups. However the viral

loads differed dramatically (by a factor of  $10^9$ ) between PPV 27a piglets and those of the other groups.

## DISCUSSION

The fact that in this study antibody and viral DNA could be detected in fetuses of all four groups provides indirect evidence for transplacental infection of both the PPV isolates and the vaccine viruses PPV-IDT (MSV) and PPV-NADL-2. This is in contrast to previous reports where it was postulated that PPV-NADL-2 is not able to cross the placental barrier. But the direct proof for transplacental transmission, the virus reisolation of infectious virus is still missing.

A difference in virulence of PPV-27a to members of the other cluster (PPV-143a, PPV-IDT [MSV], PPV-NADL-2) was indicated by the high mortality of the fetuses. PPV spreads inside the uterus from fetus to fetus. Virus spread was probably more slowly between the fetuses of the groups PPV-143a, PPV-IDT (MSV) and PPV-NADL-2 than between those of the group PPV-27a.

In the present study we investigated in two independent cross-neutralization tests post infection sera of pigs and antiserum of rabbits immunized with the respective viruses. Cross-neutralization of the sera rose against the vaccine viruses PPV-NADL-2 and PPV-IDT [MSV], against the field isolates PPV-143a and PPV-27a as well as against the PPV-Challenge (Engl.) revealed low neutralization activity (0.5–0.69) against PPV-27a, indicating an incomplete protection. Therefore, if PPV-27a is representative for current PPV-isolates in the population, this indicates that vaccines, which are used since 30 years, may no longer be fully protective.

The phylogenetic cluster containing the German isolate PPV-27a is defined by three amino acid substitutions (Q228→E, E419→Q and S436→T) in VP2 (Simpson, A. A. et al., 2002b ; Soares, R. M. et al., 2003 ; Zimmermann, P. et al., 2006 ). All three residues are located in accessible regions on the capsid's surface and position 228 was identified to be part of one of the nine known linear epitopes on VP2 (Kamstrup, S. et al., 1998 ; Simpson, A. A. et al., 2002a ). To what extent the capsid structure will be altered by changing amino acid 228 from Gln to Glu and amino acid 419 from Glu to Gln, and whether they are even involved in the apparent immune escape, needs to be further investigated.

## CONCLUSIONS

In conclusion, our results indicate that possible antigenic variation represented by PPV-27a may influence the effective vaccination against PPV. Further studies and animal inoculation experiments using PPV-27a mutants will be required to address this important issue.

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