# HEAT INACTIVATION OF PORCINE CIRCOVIRUS TYPE 2

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## ABSTRACT

Heat inactivation data for Porcine circovirus 2 (PCV2) could be estimated. The highest reduction of infectivity, ≥6.0 and 5.6 $\log_{10}$, respectively, could be seen after 5 seconds at 95°C, performed in duplicate experiments. For comparison, Porcine parvovirus (PPV) was also studied, and also Porcine circovirus type 1 (PCV1) was compared using old data. The results indicate that PCV2 is even more heat resistant than PPV.

## INTRODUCTION

PCV2 has during the recent years been found in Europe as well as worldwide (Allan et al., 2000). This virus is a definite pathogen, which is associated with PMWS (postweaning multisystemic wasting syndrome), as well as other disease syndromes in the pig (Allan et al., 2003). Since porcine circovirus was first identified as a contaminant of a continuous pig kidney (PK-15) cell line (Tischer et al., 1974), PCV1 has been investigated concerning heat stability, and was shown to be very stable (Allan et al., 1994).

As no heat inactivation data were available for PCV1 or PCV2, we investigated the heat stability of PCV2, since this is a more relevant virus concerning pathogenicity. In two experiments Porcine parvovirus (PPV) was used as a reference virus, as this is a very heat stable virus (Lund et al., 1996). Also in Sweden, PCV2 is shown to be very common and can be present without clinical disease symptoms (Linné et al., 2000).

Swedish full-scale biogas plants use different kinds of biowaste, such as manure and animal by-products. The digested residue from the plants is used as fertilizers and soil improvements. This means that biowaste from apparently healthy animals approved for human consumption, which require pasteurisation at 70 °C for 60 min, as stated by EC 1774/2002, might contain stable microorganisms, such as viruses (Sahlström et al., submitted).

Also in industrial high temperature production processes using porcine raw material, e.g. the production of gelatine, it is of interest to assess heat inactivation data for porcine viruses.

## MATERIALS AND METHODS

Unless otherwise stated, the National Veterinary Institute (SVA), Uppsala, Sweden, supplied material and media used in the experiments.

*Cells and cell culture media*. The porcine kidney cell line PK-15, ATCC (American Type Culture Collection) number CCL-33, obtained from Veterinary Science Division, Department of Agriculture for Northern Ireland, Belfast, UK, was cultivated according to conventional cell culture procedures. The cell line used was designated PK 15 A and was circovirus free (Meehan et al., 1998). Cell culture medium used was Eagle's MEM with non-essential amino acids containing antibiotics and 10 % of foetal calf serum (5% for PPV). This cell line is shown to be very

susceptible for circovirus (Allan et al., 1994) and is used routinely at SVA for PPV isolation.

*Viruses*. Porcine circovirus type 2, "1010-Stoon" (Meehan et al., 1998), was originally obtained from Veterinary Science Division, Department of Agriculture for Northern Ireland, Belfast, UK. The virus batch was propagated by superinfection using glucosamine treatment and carried a titre of about $10^{6.2}$ $TCID_{50}$ /ml, as determined by multiple titrations (Allan et al., 1998). After low speed centrifugation, the virus was aliquoted and stored at -70°C.

Porcine parvovirus, strain PPV 893/76, originally isolated at the Danish Institute for Food and Veterinary Research, Lindholm, Denmark, was obtained in cell culture supernatant, clarified by low speed centrifugation, sterile filtered through 3 µm and 0.45 µm filter, aliquoted and stored at -70°C. The virus batch used carried a titre of about $10^{8.2}$ $TCID_{50}$ /ml as determined by multiple titrations.

*Mouse monoclonal antibodies*. In the immunoperoxidase tests, an α-PPV monoclonal antibody 5B (SVANOVA Biotech, Uppsala, Sweden) and an α-PCV2 monoclonal antibody 2B1 (Allan et al., 1999) were used.

*Heat experiments.* The virus suspensions in glass containers were immersed in a heated shaking water bath. The temperatures (80±0.5°C, 90±0.5°C and 95±0.5°C, respectively) were continuously monitored using a calibrated thermometer, and the pre-heating times recorded. As hold controls, the virus suspensions in glass containers were kept at room temperature. To terminate the heating processes, the virus suspensions were immediately placed on an ice bath.

*Titration of virus and immunoperoxidase (IPX) method*. Freshly trypsinized cells were added to 96-well microplates. As sample titration medium, and as negative controls, cell culture medium without serum, was used. The virus suspensions were titrated to an end-point in 10-fold dilutions, starting with undiluted virus suspension, and added to the microtitre plates, using eight 50-µl replicates per dilution. After 5 days (PCV2) and 7 days (PPV) of incubation at 37°C in 5 % $CO_2$ in a humidified atmosphere the cells of the microtitre plates were fixed using 80% acetone (Merck, NJ, USA), and air dried, followed by an immunoperoxidase (IPX) test (Meyling, 1984) adapted to PPV and PCV2. The mouse monoclonals in conjugate dilution fluid were added to each well, and the plates were incubated for *ca*. 1 h at 37 °C. The cells were then washed twice for 5 minutes with wash fluid and once for 5 minutes with physiological saline, followed by addition of a peroxidase-conjugated rabbit anti-mouse IgG P 0260 (Dakopatts, Denmark), in conjugate dilution fluid. The plates were incubated and washed as above. After pH adjustment with sodium acetate buffer pH 5.0, the virus infected cells were visualised as a brown-reddish colour, by adding a solution consisting of 3-amino-9-ethyl carbazole (Aldrich, St. Louis, USA) dissolved in n,n-dimethyl-formamide mixed with hydrogen peroxide (both Scharlau, Barcelona, Spain) in sodium acetate buffer buffer pH 5.0, and incubating the plates for 20 min at *ca*. 30 °C.

*Calculation of titres and reduction factors.* The virus titres and the reduction factors were calculated according to Kärber (1931) and guideline CPMP/ICH/295/95 (1997), respectively.

## RESULTS AND DISCUSSION

The results from the titrations are shown below in Table 1. Titrations were performed before and after 15(5) minutes (80°C and 90°C) and 5 seconds (95°C). The pre-heating periods were 4-5 minutes.

The titres of all hold controls kept at room temperature were stable, the greatest reduction being 0.2 $\log_{10}$. The reduction factors calculated are shown in Figure 1 below, including the

results of the previous 70°C experiments (Allan et al., 1994).

***Table 1.***

| Virus | Infectivity titre[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 80°C | | 90°C | | 95°C Experiment 1 | | 95°C Experiment 2 | |
| | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| PCV2 | 5.8 | 1.3(1.4) | 5.6 | 0.9(1.2) | 6.4 | 0.8 | 6.4 | ≤0.8[b] |
| PPV | 8.3 | 1.8(2.1) | 7.7 | ≤0.8[b](≤0.8[b]) | n.d. | | n.d. | |

*[a]Titres in log10 issue culture infectious dose (TCID) $_{50}$ per ml; [b]= no virus detected n.d. = not done*

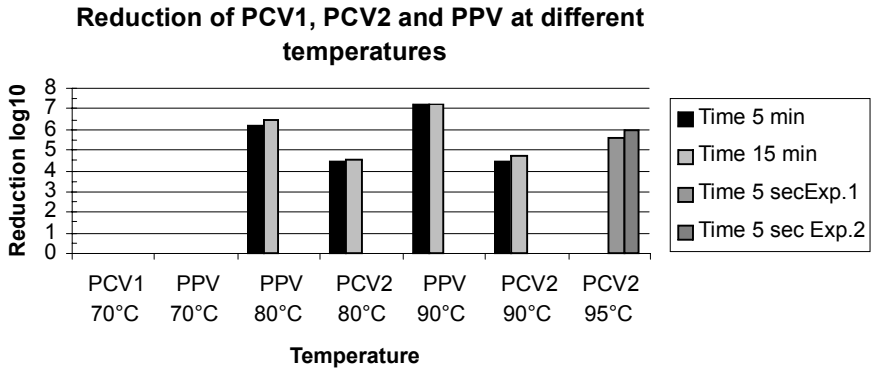


***Figure 1****. PCV1 and PPV at 70 C show no reduction. Reduction of PPV at 90 is ≥7.2. Regarding PCV2, the reduction of the second experiment at 95 is ≥6.0.*

No inactivation of PPV or PCV1 was seen after 15 minutes at 70 °C (Allan et al., 1994). In our experiments consistently higher reduction was found concerning PPV, which seems to be completely inactivated after 5 minutes at 90 °C, which has been shown previously (Charm et al., 1992). Concerning PCV2, some live virus could still be detected at this high temperature. However, after 5 seconds at 95 °C, all detectable PCV2 was inactivated in one of the experiments. This treatment gives reduction factors for PCV2 exceeding 5 logs.

Thus, (depending on the initial virus load of the raw material entering the biogas plant), Porcine circovirus, as well as PPV, must be taken into account as a plausible contaminant of the digested residue from biogas production, if the required pasteurisation temperature is 70 °C for one hour (EC 1774/2002). Regarding PPV this has also been investigated using virus spiked mixed biowaste (Sahlström et al., submitted) and sewage sludge (Lund et al., 1996), which confirm the observations. If this biowaste is used as fertilizer, animals for human consumption and wild animals could be infected, and thereby be a risk for the consumers. There is probable evidence for human infection with PCV1, as antibodies have been found in humans, however, this has to be confirmed (Allan et al., 2000). Also, human cell lines have been infected PCV1 and PCV2, as well as transfected with DNA from these viruses, although no productive infection was seen (Hattermann et al., 2004).

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