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Exudative epidermitis and porcine circovirus-2 infection in a Swedish SPF-herd

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Abstract

An outbreak of exudative epidermitis (EE) among piglets in a Swedish SPF-herd initiated a survey for indications as to the cause of disease.

The herd was established by caesarean section and has been closed to all new animal material, with the exception of semen for artificial insemination (AI). The study comprised serum samples from the SPF-herd over a 10-year period \((n = 109)\) and a close monitoring of animals in the herd during the period after the EE outbreak. Serum samples from conventional boars at the AI-station servicing the herd were also included \((n = 9)\). All serum samples were tested for antibodies to porcine circovirus-2 (PCV-2). In addition, 3-week-old piglets from three litters \((n = 24)\) farrowed close after the initial EE outbreak were closely monitored for clinical signs of skin disease, sampled for \textit{Staphylococcus hyicus}, tested for antibodies to porcine parvovirus and in sequentially collected serum samples tested for interferon-\(\alpha\) (IFN-\(\alpha\)) and interleukin-6.

The PVC-2 serology showed that animals in the herd were sero-negative at least until 2 months prior to the EE outbreak. During the period close after the EE outbreak the animals showed varying levels of antibodies to PCV-2 but all the tested animals had sero-converted 4 months later. The AI boars were also sero-positive to PCV-2 at the time of the EE outbreak. Animals in the SPF-herd remained sero-positive to PCV-2 during the following 7 years. In the monitored litters, one piglet had clinical EE and 15 piglets displayed defined erythemas on the abdomen. Fourteen of the piglets also had IFN-\(\alpha\) in serum on one or more occasions during the study, indicating viral activity among the animals. \textit{S. hyicus} was isolated from all of the piglets from the earliest sampling point (3 days of age)

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and onwards, irrespective of clinical signs. PCV-2 was isolated from lymphnode tissue collected from one of the EE affected pigs.

Further, increases in the number of stillborn piglets, small litters (<6 piglets) and repeat breeders could be correlated to the time of PCV-2 sero-conversion. Coincidence of active viral infection and sero-conversion to PCV-2 points to the virus as the cause of the EE outbreak and reproductive disturbances. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Porcine circoviruses (PCVs; family *Circoviridae*) are small, non-enveloped, DNA viruses containing an unique single-stranded circular genome (for reviews, see Lukert, 1999; Allan and Ellis, 2000). PCV-1 is considered apathogenic and ubiquitous throughout the world. PCV-2 on the other hand, has been associated with various disease syndromes in pigs, including congenital tremors, post-weaning multisystemic wasting syndrome (PMWS) and recently also dermatitis/nephropathy syndrome (Allan et al., 2000b; Rosell et al., 2000; Thomson et al., 2000). The syndrome PMWS was first identified in 1991 in a Canadian SPF-herd and PCV-2 was subsequently associated with the disease (Allan and Ellis, 2000). PCV-2 has since been identified in association with cases of PMWS from other countries in Northern America, Europe and Asia (Allan et al., 1999; Choi et al., 2000; Fenaux et al., 2000; Mankertz et al., 2000; Mori et al., 2000; Wellenberg et al., 2000) but to date information on sero-prevalence and modes of virus transmission is limited. However, serum antibodies to PCV-2 seem to be widespread in the swine populations of those countries where testing has been carried out (Allan and Ellis, 2000). In the case of PMWS, other viruses such as porcine parvovirus (PPV) or porcine reproductive and respiratory syndrome virus (PRRSV; Allan and Ellis, 2000) as well as non-specific activation of the immune system (Allan et al., 2000a; Krakowka et al., 2001) have been demonstrated as cofactors in the full expression of disease syndromes.

Exudative epidermitis (EE) is a generalised skin infection with greasy exudation and exfoliation, affecting mainly suckling piglets (reviewed by Wegener and Skov-Jensen, 1999). Exfoliative-toxin producing strains of *Staphylococcus hyicus* are considered the causative agents of EE, but predisposing factors are probably necessary for the disease (Andersen et al., 1993; Tanabe et al., 1996; Aarestrup and Wegener, 1997). For instance PPV has been suggested to be involved in the pathogenesis of EE (Whitaker et al., 1990).

In April 1993, a Swedish SPF-herd was inexplicably struck by an outbreak of EE. An extensive survey was initiated to determine the cause of the disease and possible mode of entry into the herd, but at the time no plausible explanation was found. In 1999, a retrospective sero-survery for antibodies to PCV-2 among Swedish pigs, revealed that the SPF-herd in question was sero-negative to the virus early in 1993 but later this year animals in the herd had sero-converted to PCV-2. Therefore, samples from the outbreak of EE were re-analysed with respect to PCV-2 and the results are presented herein.
2. Materials and methods

2.1. Description of the SPF-herd

The SPF-herd was established in June 1988 by hand-rearing of colostrum deprived Swedish Yorkshire piglets delivered by caesarean section. Once established, the herd has been completely closed to new animal material, but semen collected from conventional boars free from Aujeszky’s disease virus (Quality Genetics, Kävlinge, Sweden) has continuously been used for artificial insemination (AI). A teaser boar was kept and this boar was occasionally also used for coverings. Due to the risk of PPV transmission via AI semen, sows were vaccinated against this virus and due to conventional handling of feed and straw sows were also vaccinated against the bacteria *Erysipelothrix rhusiopathiae*. The herd is tested free from a number of pathogens¹ (Wallgren and Vallgård, 1993; Wallgren et al., 1999) and the overall pathogen load is considered to be low indicated by a high weight gain, a low incidence of medical treatments and a low mortality (Wallgren, 1994, 2000). In 1993 this farrow to finish herd comprised 25 sows and the production system was continuous, but with batchwise farrowings. During 1997 the herd increased to 50 sows and an age segregating system was introduced. The herd subsequently increased further and since 1999 it has comprised around 70 sows.

2.2. Reproductive parameters and piglet mortality

Reproductive parameters in the SPF-herd were documented since its establishment. Repeat breeders within an interval of 18–24 days were defined as having a normal oestrous cycle, whereas returns at other intervals were regarded as abnormal. Due to the housing of dry sows, untied in deep litter, aborted foetuses are seldom discovered and therefore no distinction between abortion and non-pregnant in late gestation was made. Piglet mortality was expressed as deaths pre-weaning (days 0–35) and post-weaning (days 35–60, corresponding to 25 kg body weight).

2.3. Animals and sample collection

The present study comprised a retrospective survey of serum samples from pigs in the SPF-herd during the period 1990–2000 and from AI boars servicing the herd in 1993 (Table 1). In addition, a closer survey was performed on the first sows to farrow after the initial observation of EE among piglets, i.e., 2 weeks after the outbreak. Three sows (nos. 1, aged 1 year; 2, aged 2 years and 3, aged 3 years) and their offspring were monitored clinically and with serum samples. Piglets in litter 1 were numbered 4–9; litter 2, 10–17 and litter 3, 18–27, however, piglets nos. 5 and 9 were the offspring of sow no. 2 and received

¹The SPF-herd is free from the following exotic micro-organisms: African swine fever virus, Aujeszky’s disease virus, foot and mouth disease virus, hog cholera virus, Japanese B encephalitis virus, porcine epidemic diarrhoea virus, PRRSV, rabies virus, swine vesicular disease virus, transmissible gastro-enteritis virus and *Brucella abortus*, and the indigenous: *Mycoplasma hyopneumoniae*, *Actionobacillus pleuropneumoniae*, toxin producing strains of *Pasteurella multocida*, *Brachyspira* spp., *Leptospira* spp., *Haemophilus suis*, *Salmonella* spp., *Mycobacterium* spp., porcine respiratory coronavirus, swine influenza virus and *Sarcoptes scabiei*. 
Table 1
Description of animals included in the retrospective survey for antibodies to PVC-2 in the SPF-herd

| Year            | Number of pigs | Age         |
|-----------------|----------------|-------------|
| 1990            | 2              | 5 months    |
| 1992            | 10             | 4–5.5 months|
| February 1993   | 10             | 10–17 weeks |
| April 1993      | 3+8h           | 5 weeks     |
| April 1993      | 7c             | Unknown     |
| April 1993      | 9a             | Unknown     |
| August 1993     | 5              | 5–7 months  |
| 1994            | 10             | 1 to >2 years|
| 1996            | 9              | 6 months–2 years |
| 1997            | 7              | 8–10 months |
| 2000            | 11             | 6–7 months  |

* All serum samples, apart from those collected in April 1993, were part of a continuous sero-monitoring of the SPF-herd.

h Suriving piglets in the two litters affected by the initial EE outbreak.

c A representation of clinically healthy young pigs in the herd.

a Conventional boars at the AI-station.

colostrum from this sow. Sows and piglets were sampled 3 days after farrowing (day 3) and piglets were sampled daily from the day of the first clinical signs of EE (day 17) until day 23. Seven of these piglets remained in herd at the age of 21 weeks and were sampled at that age.

2.4. Detection of S. hyicus

Skin swabs for isolation of *S. hyicus* were collected from sows 1–3 and their offspring on day 3. In addition, samples were collected from piglets with and without clinical signs between days 17 and 23. All sampled pigs, irrespective of symptoms, were swabbed on an area of 5 × 5 cm² on at least three different sites. The fibre swabs were placed in modified Stuart medium for transport (Culturette®). Samples were cultured within hours of sampling on blood-agar (5% horse blood, v/v) and on a selective and elective medium modified after Devriese (1977). Briefly, the latter medium consisted of a blood-agar base (Oxoid no. 2) supplemented with polymyxin B (Sigma Chemicals), 125 mg/l, polysorbate 80, 10 ml/l, and CaCl₂·2H₂O, 100 mg/l. Both agar plates were incubated for 24 h at 37 °C. Colonies with morphology consistent with *S. hyicus* were subcultured and tentatively identified according to Barrow and Feltham (1993).

2.5. Detection of antibodies to PCV-2 and PPV in serum

All serum samples were analysed for presence of antibodies to PCV-2 by ELISA (Walker et al., 2000) and indirect immuno-fluorescence (IIF) technique (Allan et al., 1994) was also applied to some samples. A sonicated preparation of PCV-2 infected cells was used as antigen in the ELISA and results were expressed as percent inhibition (PI), where <33% was considered negative, >44% considered positive and 33–44% considered
intermediate. The IIF technique detects antibodies binding to a PCV-2 infected cell-line and the results were graded as: (+++) strong positive to (+) weak positive and (−) indicating a negative result.

Serum samples collected from animal nos. 4–27 were also analysed for presence of antibodies to PPV, using a competitive ELISA technique (Svanovir® PPV-Ab, Svanova Biotech, Uppsala, Sweden).

2.6. Detection of IFN-α and IL-6 in serum

IFN-α was detected with a DELFIA technique described by Artursson et al. (1995). In this assay the detection of ≥0.1 Units IFN-α/ml serum was considered a positive result.

IL-6 activity was measured in a bioassay using the murine B9 cell-line as described by Fossum et al. (1998). Serial dilutions of a porcine recombinant IL-6 preparation (Endogen, Woburn, MA, USA) was included on every test plate and used as positive control.

2.7. Detection and isolation of PCV-2

Some surviving pigs from the litters affected by EE were kept under SPF conditions and included in another study which also comprised pigs born in the SPF-herd the following year, i.e., 1994 (Watrang et al., 1997). Cryopreserved (liquid N₂) samples of lymphnodes collected at euthanasia, from one of the EE pigs at 8 months of age and from one pig born in 1994 aged 10 months, were used for detection of PCV-2 DNA and virus isolation according to (Allan et al. (2000c).

3. Results

3.1. Anamnestic history of the outbreak

In April 1993, piglets in two litters in the same farrowing batch, unexpectedly displayed exudative skin disease with blackening crusts, i.e., signs of EE. Both dams of the affected litters were 3 years of age and the litters were fairly big, 16 and 17 piglets, respectively. Most piglets in the litters were affected by clinical EE and were treated with antibiotics (Fucidic acid locally; Fucidin, Leo, Denmark, and/or penicillin parentally, Penovet, Boehringer Ingelheim, Germany). However, due to the EE 10 and four piglets, respectively, died before weaning in these litters. The first batch of sows to farrow after this outbreak was therefore monitored closely for clinical signs of EE, and samples collected, as described below.

3.2. Clinical signs

Four sows were due to farrow in the monitored batch (see above), one gave birth to three stillborn piglets while the other sows gave birth to four (sow 1) and 10 (sows 2 and 3) live
Table 2
Clinical signs of EE or erythemas and sample collection for isolation of *S. hyicus* among piglets from three SPF-litters

| Litter | Pig | Age       |       |       |       |       |       |
|--------|-----|-----------|-------|-------|-------|-------|-------|
|        |     | 17 days   | 18 days | 19 days | 20 days | 21 days | 22 days | 23 days |
| 1      | 4   | –         | –      | ?      | ?      | –      | –      |
|        | 5<sup>b</sup> | –         | –      | –      | ?      | ?      | –      |
| 6      | –   | –         | –      | ?      | –      | –      | –      |
| 7      | –   | –         | –      | –      | –      | –      | –      |
| 8      | –   | –         | –      | –      | –      | –      | –      |
| 9<sup>b</sup> | –     | –         | –      | +      | (+)    | –      | –      |
| 2      | 10  | EE*       | EE*    | EE     | EE     | –      | –      | –      |
|        | 11  | ?         | +      | +      | +      | (+)    | (+)    |
| 12     | –   | –         | –      | –      | –      | –      | –      |
| 13     | –   | –         | –      | –      | –      | –      | –      |
| 14     | –   | –         | ?      | ?      | +      | +      | +      |
| 15     | +   | +         | (+)    | –      | –      | –      | –      |
| 16     | +   | +         | (+)    | –      | –      | –      | –      |
| 17     | +   | +         | –      | –      | –      | –      |+      |
| 3      | 18  | +         | +      | (+)    | –      | –      | –      | ?      |
| 19     | –   | –         | +      | (+)    | (+)    | –      | –      |
| 20     | +   | +         | (+)    | –      | –      | –      |+      |
| 21     | –   | –         | –      | –      | –      | –      | –      |
| 22     | ?   | +         | +      | (+)    | (+)    | +      | –      |
| 23     | ?   | +         | (+)    | (+)    | +      | +      | (+)    |
| 24     | –   | –         | –      | –      | –      | –      | ?      |
| 25     | –   | –         | +      | (+)    | (+)    | –      | –      |
| 26     | –   | –         | –      | +      | –      | –      | ?      |
| 27     | –   | –         | –      | –      | –      | –      | –      |

<sup>a</sup> EE: exudative epidermitis (clinical); +: defined erythemas on abdomen; (+): remission of erythemas; ?: tendencies to/suspected erythemas and –: no clinical signs.

<sup>b</sup> Piglet nos. 5 and 9 were the offspring of sow no. 2.

<sup>*</sup> Indicates samples collected for isolation of *S. hyicus*. The bacteria were demonstrated in all of these animals.

piglets, respectively. When the piglets in the three remaining litters were 16 days of age, blackening crusts where noticed on the abdomen of piglet no. 10 in litter 2. This initiated the close survey of the piglets regarding general health and monitoring of clinical signs of EE (Table 2). Piglet no. 10 had clinical EE and was treated with penicillin from the age of 17 days (after sample collection) until remission 4 days later. None of the other piglets monitored showed clinical EE, but on close examination some piglets showed defined red erythemas on the abdomen. When piglets showed very small or undefined reddish skin lesions, these were recorded as tendencies to/suspected erythemas. In total, 15 piglets displayed defined erythemas during the study, two in litter 1, five in litter 2 and eight in litter 3. Apart from the skin symptoms all piglets were generally bright and healthy apart from piglet no. 11 which showed lethargy and pyrexia on day 20 and piglet no. 14 which was treated with penicillin for suspected septic arthritis on day 18.
3.3. Isolation of *S. hyicus*

Among the swab samples collected when the piglets were 3 days old, *S. hyicus* was isolated from at least one of the sites sampled on each of the sows as well as on each of their offspring. Over the 1-week period when the piglets were closely monitored for signs of EE, 12 piglets displaying varying degrees of clinical signs and four piglets with no clinical signs, were swabbed for bacteriology on one or more occasions (in total 22 sets of swabs, Table 2). *S. hyicus* was isolated from all of these individuals regardless of time point or clinical signs. Thus, the bacteria were present on the skin of all pigs prior to the onset of EE and no clear correlation to clinical signs could be made.

3.4. Antibodies to PCV-2

All tested serum samples from the SPF-herd collected in 1990, 1992 and February 1993, i.e., up to 2 months prior to the EE outbreak, were negative for antibodies to PCV-2 both in ELISA (*n = 22*) and IIF (*n = 10*, February 1993 samples).

At 5 weeks of age, piglets in one of the litters initially affected by EE were either clearly sero-negative or intermediate for antibodies to PCV-2 (PI ranging 20–40%, *n = 3* out of 6 survivors). Pigs in the second of these litters were all sero-positive to PCV-2 (PI ranging 45–86%, *n = 8* out of 13 survivors). At this time point, other young pigs in the herd ranged from intermediate to sero-positive (PI 37–87%, *n = 7*). Thus, after being sero-negative to PCV-2 earlier in 1993, pigs in the SPF-herd had varying levels of antibodies to this virus 2 weeks after the first outbreak of EE. Further, all other serum samples collected from pigs in the SPF-herd after April 1993, i.e., August 1993–2000 (*n = 42*) were positive for antibodies to PCV-2 with titers ranging from 1:125 to 1:78,125 (median 1:3125).

Among the adult animals, the three sows farrowing 2 weeks after the first outbreak of EE showed different levels of antibodies to PCV-2 at farrowing. Sow no. 1 was negative both in ELISA and in IIF (PI 15%, IIF −), sow no. 2 showed a strong positive reaction (PI 97%, IIF ++ +), and sow no. 3 a weak positive reaction (PI 38%, IIF +). The boars at the AI-station servicing the SPF-herd were all clearly sero-positive to PCV-2 (PI 57–100%) at this time point. The serological status to PCV-2 among the dams was also reflected by the transfer of maternal antibodies to their offspring (Table 3). In litter 1, the offspring of sow no. 1 were all sero-negative to PCV-2 at 3 days of age, while the offspring of sow no. 2 raised in this litter (piglet nos. 5 and 9) showed the same high antibody levels as their siblings in litter 2. The offspring of sow no. 1 remained sero-negative at least until the age of 23 days but were strongly sero-positive to PCV-2 at the age of 21 weeks. The offspring of sow no. 2 were all strongly sero-positive to PCV-2 both during suckling and at the age of 21 weeks. Piglets in litter 3 were also sero-positive to PCV-2 at 3 days of age but had lower levels of antibodies compared to the offspring of sow no. 2. The maternal antibodies to PCV-2 in litter 3 quickly declined and by the age of 23 days all but one of the piglets were sero-negative. However, at the age of 21 weeks pigs from litter 3 were again strongly sero-positive to PCV-2. Thus, these observations show that maternal antibodies to PCV-2 were transferred to the offspring. The seven piglets remaining in the herd until 21 weeks of age were all strongly sero-positive to PCV-2 at that time, regardless of their antibody status during their
Table 3
Antibodies to PCV-2 in serum samples from three SPF-litters collected at the age of 3, 17 and 23 days and at 21 weeks

| Litter | Piglet | IIF, 3 days | ELISA (% inhibition) |
|--------|--------|-------------|----------------------|
|        |        | 3 days      | 17 days  | 23 days | 21 weeks |
| 1      | 4      | –           | 4        | 0       | 0        | 97      |
| 5b     | ++     | 97          | 97       | 96      | na       |
| 6      | –      | 38          | 0        | 0       | na       |
| 7      | –      | 14          | 0        | 0       | na       |
| 8      | –      | 13          | 1        | 4       | 98       |
| 9b     | +++    | 98          | 96       | 94      | na       |
| 2      | 10     | na          | na       | 66      | 58       | na      |
|        | 11     | ++          | 98       | 99      | 92       | 93      |
|        | 12     | +++         | 100      | 95      | 94       | 88      |
|        | 13     | +++         | 99       | 88      | 88       | na      |
|        | 14     | +           | 96       | 98      | 93       | na      |
|        | 15     | +++         | 98       | 98      | 94       | na      |
|        | 16     | na          | na       | 94      | 82       | na      |
|        | 17     | na          | na       | 94      | 83       | na      |
| 3      | 18     | ++          | 51       | 13      | 8        | na      |
|        | 19     | ++          | 72       | 46      | 21       | na      |
|        | 20     | ++          | 77       | 37      | 43       | 99      |
|        | 21     | +           | 53       | 15      | 0        | na      |
|        | 22     | +           | 57       | 27      | 7        | na      |
|        | 23     | +           | 45       | 14      | 14       | na      |
|        | 24     | +           | 58       | 26      | 10       | 99      |
|        | 25     | ++          | 72       | 42      | 22       | na      |
|        | 26     | +           | 49       | 6       | 9        | na      |
|        | 27     | +           | 51       | 15      | 9        | 94      |

*a Antibodies to PCV-2 were detected with ELISA (expressed as PI where <33% is negative, >44% is positive) and indirect immuno-fluorescence (IIF; +++ strong positive to (+) weak positive and (−) negative), for details, see Section 2. na: no sample available.

b Piglet nos. 5 and 9 were the offspring of, and received colostrum from, sow no. 2.

new-born period. Taken together, these results point to an introduction of infectious PCV-2 into the herd in 1993 and a subsequent persistence of the virus in the herd.

3.5. Antibodies to PPV

Among the 24 piglets tested for antibodies to PPV, all were sero-positive to this virus at three days of age. The offspring of sows nos. 2 and 3 all had similar levels of antibodies to PPV at this time point ($A_{450}$: 0.94 ± 0.01, $n = 19$), while the offspring of sow no. 1 showed lower antibody levels ($A_{450}$: 0.78 ± 0.03, $n = 4$). The PPV antibodies then constantly declined at days 17 and 23 for all of the animals. At 21 weeks of age, the seven pigs remaining in the herd were all sero-negative to PPV. Thus, transfer of PPV antibodies induced by vaccination of dams to the piglets occurred but there were no indications of an active infection with PPV in the SPF-herd.
Table 4
Production and fertility parameters recorded in the SPF-herd during 1990–2000*

| Year | Farrowings (n) | Live piglets (per litter ± S.D.) | Stillborn (per litter ± S.D.) | Deaths pre-weaning (per litter ± S.D.) | Small litters (%) | Matings (n) | Repeat breeders, 18–24 days (%) | Repeat breeders abnorm interval (%) | Non-pregnant or abortions (%) |
|------|---------------|---------------------------------|--------------------------------|----------------------------------------|-------------------|-------------|--------------------------------|----------------------------------|------------------------------|
| 1990 | 45            | 10.9 ± 2.7                      | 0.7 ± 1.2                      | 1.3 ± 1.3                              | 2.2               | 46          | 10.9                           | 2.2                              | 8.7                          |
| 1991 | 48            | 10.5 ± 2.9                      | 0.9 ± 1.2                      | 1.5 ± 1.4                              | 6.3               | 77          | 7.8                           | 1.2                              | 5.2                          |
| 1992 | 57            | 11.2 ± 2.4                      | 0.7 ± 1.0                      | 1.4 ± 1.6                              | 0.2               | 60          | 5.0                           | 0                                | 1.7                          |
| 1993:1b | 13          | 12.5 ± 2.3                      | 0.5 ± 0.5                      | 1.6 ± 1.4                              | 0.0               | 20          | 5.0                           | 5.0                              | 25.0                         |
| 1993:2b | 10          | 11.1 ± 4.4                      | 1.4 ± 1.1                      | 2.6 ± 3.8                              | 20.0              | 16          | 0                             | 0                                | 18.8                         |
| 1993:3b | 16          | 10.1 ± 4.0                      | 0.7 ± 1.1                      | 1.1 ± 1.2                              | 6.3               | 12          | 0                             | 0                                | 25.0                         |
| 1993:4b | 10          | 10.8 ± 3.6                      | 0.8 ± 1.1                      | 1.0 ± 1.5                              | 10.0              | 13          | 23.1                          | 0                                | 0                            |
| 1994 | 55            | 12.1 ± 3.1                      | 0.7 ± 1.1                      | 1.3 ± 1.4                              | 3.6               | 68          | 8.8                           | 0                                | 0                            |
| 1995 | 60            | 11.7 ± 3.4                      | 0.8 ± 1.2                      | 1.4 ± 1.5                              | 5.0               | 60          | 5.0                           | 0                                | 0                            |
| 1996 | 72            | 11.2 ± 3.0                      | 1.1 ± 1.3                      | 1.3 ± 1.2                              | 4.2               | 91          | 5.5                           | 0                                | 0                            |
| 1997 | 98            | 11.5 ± 2.6                      | 0.9 ± 1.1                      | 1.4 ± 1.7                              | 1.0               | 124         | 4.8                           | 0                                | 1.6                          |
| 1998 | 118           | 11.1 ± 3.0                      | 1.0 ± 1.3                      | 0.9 ± 1.1                              | 5.9               | 149         | 8.1                           | 0.7                              | 2.6                          |
| 1999 | 153           | 11.4 ± 3.2                      | 0.9 ± 1.4                      | 1.2 ± 1.1                              | 6.5               | 168         | 4.2                           | 1.2                              | 0.7                          |
| 2000 | 135           | 11.5 ± 3.4                      | 1.3 ± 1.8                      | 1.2 ± 1.4                              | 6.7               | 151         | 4.6                           | 0                                | 1.3                          |

* For details on the definition of production and fertility parameters, see Section 2. Due to the sale of pregnant animals the number of matings may occasionally exceed the number of farrowings.

b The results for 1993 are given quarterly.
3.6. IFN-α and IL-6 in serum

IFN-α in serum was used as an indicator of ongoing viral infections and serum IL-6 as an indicator of acute bacterial infections. The piglets were tested at 3 days of age and daily during the period of clinical signs of EE (days 17–23). Regarding serum IFN-α, none of the piglets in litter 1 displayed this cytokine at any of the time points tested. In litter 2, four out of the eight piglets had IFN-α in serum at 3 days of age, and three piglets had IFN-α in serum on one or more occasions during the rest of the study. All of the 10 piglets in litter 3 were positive for IFN-α in serum at 3 days of age and five of them also had IFN-α in serum on one or more occasions during the rest of the study. In total, 14 out of the 18 piglets in litters 2 and 3 were positive for IFN-α at 3 days of age, one on day 17, one on day 20, five on day 21, five on day 22 and three on day 23. The sows were tested for IFN-α in serum when the piglets were 3 days old and sows nos. 1 and 2 were negative while sow no. 3 showed 0.27 Units IFN-α/ml serum. None of the piglets displayed IL-6 in serum at any of the time points tested.

3.7. Detection and isolation of PCV-2

PCV-2 immuno-histochemistry was negative for the virus when applied on the cryo-preserved lymphnodes. However, using PCR technique, the sample from the EE affected pig was positive for PCV-2 DNA. PCV-2 was also isolated at the third passage of tissue homogenates from the EE pig through cell cultures. The sample from the pig born in 1994 was negative for PCV-2 in all tests.

3.8. Reproduction parameters and piglet mortality data

Reproduction parameters and pre-weaning piglet mortality for 1990–2000 are summarised in Table 4. During this time period no piglet mortality was observed in post-weaning. With some exceptions during the years immediately after the establishment of the herd, piglet production remained at an even and high level with approximately 10.3 weaned piglets per litter. The percentage of small litters (<6 piglets) and repeat breeders were also on a fairly low and uniform level. During the second quarter of 1993 pre-weaning piglet mortality increased due to the EE outbreak. The number of stillborn piglets and the percentage of small litters were also unusually high during this quarter. Further, aborting sows and sows found non-pregnant during late stage gestation was increased for animals covered during the first three quarters of 1993. Despite a previous normal fertility of the heard teaser boar, none of the sows covered by him during the first two quarters of 1993 (n = 5) farrowed. Taken together, these results are indicative of a fertility disturbance occurring around the second quarter of 1993.

4. Discussion

An unexpected outbreak of EE in a SPF-herd initiated an extensive survey into possible causes of the disease. The causative agent of EE, i.e., S. hyicus, is a natural part of the porcine skin flora (Aarestrup and Wegener, 1997) and was indeed also present in this SPF-herd.
It is well known that even virulent strains of *S. hyicus* may be present on healthy as well as diseased pigs (Devriese, 1977; Tanabe et al., 1996; Wegener and Skov-Jensen, 1999). It is, however, also believed that predisposing factors causing skin trauma, disrupting the epidermis and exposing the dermis to *S. hyicus*, are needed for the development of EE (Aarestrup and Wegener, 1997; Wegener and Skov-Jensen, 1999).

Among viruses, PPV has been put forward as causing skin lesions (Kresse et al., 1985; Choi et al., 1987; Whitaker et al., 1990), but this virus was not present in the herd in question. Interestingly, PCV-2 has also been associated with skin lesions in the porcine dermatitis and nephropathy syndrome (Allan et al., 2000b; Rosell et al., 2000; Thomson et al., 2000). The PCV-2 serology revealed that the SPF-herd was sero-negative at least until February 1993. Two months later, around the EE outbreak, the pigs showed varying levels of antibodies to PCV-2. All the monitored piglets sero-converted later that year and the herd has remained sero-positive since. The serological evidence thus reveal an active infection with PCV-2. Further, the presence of serum IFN-α in the young pigs also indicates viral activity amid the piglets. We therefore hypothesise that PCV-2 replicating in the naïve piglets caused the skin lesions necessary for *S. hyicus* to gain access to the dermis, and thereby predisposed for the EE outbreak. Indeed, PCV-2 was also isolated from one of the EE affected piglets which is the first time this virus has been isolated in Sweden. Moreover, the introduction of PCV-2 in the herd coincided with the most pronounced reproductive disturbance ever recorded in the herd. Indeed, PCV-2 has been associated with reproductive problems (West et al., 1999), and the observed increases in number of stillborn piglets, percentage of small litters and repeat breeders may therefore have been caused by the introduction of the virus into the herd. Further, it is likely that the fertility problems were due to PCV-2 infection of naïve or insufficiently immune pregnant sows and gilts as this type of reproductive signs at least became less evident when PCV-2 became established in the herd.

The SPF-herd is closed to live animals, geographically isolated and practices strict management routines. Therefore, the sero-positive boars at the AI-station point out a plausible route of how PCV-2 was introduced into the herd. Indeed, PCV-2 DNA has been detected in semen of experimentally infected boars (Larochelle et al., 2000). Further, transmission of virus via semen has been shown for chicken anaemia virus (Hoop, 1993) which is another *Circoviridae* family member.

No cases of PMWS have to date been reported in Sweden. This disease has been closely linked to PCV-2, but it has also been emphasised that other pig husbandry practices and/or infectious agents such as PPV or PPRS may be required for a full expression of the syndrome (Allan and Ellis, 2000). The present SPF-herd is free from both PPV and PPRS, and the overall pathogen load is low. This may well explain why the presence of PCV-2 in this SPF-herd has passed relatively unnoticed.

Taken together, these data indicate that the introduction of PCV-2 into the SPF-herd coincided in time with the EE outbreak. Hence, this newly introduced virus may well have been the predisposing factor initiating the *S. hyicus* skin lesions.

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