Infection of pigs with porcine circovirus type 2 (PCV2) causes a variety of disorders collectively referred to as porcine circovirus associated diseases (PCVADs). PCV2 isolates can be classified into two major types: PCV2a and PCV2b. In this study, vaccination of piglets against PCV2 was performed using commercially available vaccines. The effectiveness of vaccination against various PCV2 genotypes was also assessed. Among the 16 farms studied, 10 and 6 had been infected with PCV2a and PCV2b, respectively. PCV2a was further subdivided into PCV2a-1 and PCV2a-2. PCV2a-1 and PCV2a-2 prevailed on 6 and 4 farms, respectively, among the 10 farms infected with PCV2a. The PCV2 vaccines were effective in reducing PCV2 infection on commercial pig farms. Mean mortality rates were significantly decreased over 8 months after the start of the PCV2 vaccination program as compared to those before the start of the PCV2 vaccination program on farms infected with PCV2a-2 (20.8% vs. 12.1%) and PCV2b (26.5% vs. 13.7%). On the farms with PCV2a-1 infected pigs, there was no significant difference in the mean mortality rate before versus after the start of the vaccination program (14.7% vs. 14.1%). Mortality rate reduction with the PCV2 vaccination might depend on the genetic types of PCV2. 

KEY WORDS: genotype, mortality rate, porcine circovirus associated disease (PCVAD), porcine circovirus type 2 (PCV2), vaccine.

MATERIALS AND METHODS

All experiments were carried out in strict compliance with Japanese laws concerning the protection and control of animals.

Study sites: The survey was conducted on 16 farrow-to-finish pig farms in various areas in Japan where animals were documented to be PCV2 and PRRS virus (PRRSV) positive; 13 of these farms (farms A to D, F to H and J to O) housed more than 500 sows and the other 3 (farms E, I and P) housed 200 to 500 sows. On each farm, neonatal pigs (up to 3–3.5 weeks old) were kept in a farrow house, and the pigs were then moved and kept as follows: in a piglet house (up to 11–12 weeks old) and feeder pig house (up to 25–26 weeks old). A *Mycoplasma hyopneumoniae* vaccination program, for piglets 1 and 3 weeks of age, had been performed routinely, and the PCV2 vaccination program for piglets at 3 weeks of age started in March of 2008 on each farm.

Experimental design:

**Experiment 1:** The experiment was conducted on farm O in August 2007 before the PCV2 vaccination program had been started. An oil-adjuvanted subunit vaccine containing a recombinant capsid protein of PCV2 (Porcilis® PCV, Intervet International, Boxmeer, The Netherlands) was used in 2 ml doses for this experiment. In total, 997 piglets were divided into three groups; Group A (347 piglets) was vaccinated at 4 and 7 weeks of age, Group B (317 piglets) received a single dose of vaccine at 7 weeks of age, and Group C (333 piglets) was not vaccinated. All groups were housed in equal conditions. Five piglets in each group were identified individually, and sera of these piglets were collected at the ages of 4, 7, 11, 14 and 21 weeks (except Group B which was not sampled at 4 weeks of age). Fecal samples were collected by swabbing the surface of the colo-rectal region of the same piglets weekly from 7 to 19 weeks of age.

**Experiment 2:** This examination assessed the effects of vaccination against various genotypes of PCV2 prevailing on the farms. Serum samples were collected from 25 pigs (5 each from 1-, 2-, 3-, 4- and 5-month old pigs) at each of the 16 pig farms (farms A to P) in December 2007 (before the start of the PCV2 vaccination program for piglets) and in December 2008 (over 8 months after the start of the program). An inactive subunit vaccine (Ingelvac® CircoFLEX™, Boehringer Ingelheim Vetmedica, Ingelheim, Germany) was used in 2 ml doses for 3-week-old piglets.

All samples were kept at –80°C until assayed.

Virological studies: All samples were subjected to polymerase chain reaction (PCR) analysis for detection of PCV2 DNA, and the genotypes of PCV2 isolates were determined by nucleotide sequencing and PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of amplified PCV2 ORF2 as described previously [29]. Briefly, viral DNA was extracted from the serum and fecal swab supernatant immersed in 1 ml of phosphate buffered saline (PBS) using a commercial kit (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany). A fragment including ORF2 of PCV2 was amplified by PCR with two primers, PCV2-f1 (5'-CCA TGC CCT GAA TTT CCA TA-3') and PCV2-r1 (5'-ACA GCG CAC TTC TTG CTT G-3'). PCR was carried out with the following cycling times: 40 cycles at 94°C for 30 sec, 60°C for 30 sec. The RFLP types of the PCV2 isolates were determined by digestion of the PCR products with *AvaII, BamHI* and *ClaI*. The ORF2 nucleotide sequences of the PCR products were determined using two primers, PCV2-sf2 (5'-TGC CGG GAG TGG TAG GAG AA-3') and PCV2-sr2 (5'-CTC TCC CGC ACC TTC G-3'), and analyzed with GENETYX (Genetyx, Tokyo, Japan). The phylogenetic tree of PCV2 based on the nucleotide sequence of ORF2 was constructed by the neighbor-joining method utilizing ClustalW [30] and was visualized with Treeview.

Serological studies: The amounts of anti-PCV2 antibodies in porcine serum samples were quantified by enzyme-linked immunosorbent assay (ELISA) analysis. The ELISA system was established as described below. A fragment including a part of the PCV2 capsid protein was amplified by PCR with 35 cycles at 94°C for 30 sec, 55°C for 30 sec and 68°C for 1 min using following primers: 5'-CAC CGT GGG GCT TCC ACG TGT CTT AT-3' and 5'-TAA AGG GGT AA GTGG GGCTCT-3'. The amplified PCR product was cloned into pENTR/TEV-D/TOPO (Invitrogen, Carlsbad, CA) and the nucleotide sequence was determined. The PCR fragment was then cloned into expression vector pDEST17 using the Gateway® system (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Recombinant protein with an N-terminal 6X histidine tag was expressed in *E. coli* and purified employing an affinity column (Histrap FF crude, GE Healthcare, Buckinghamshire, England).

PCV2-specific IgG in serum samples was detected using the purified recombinant capsid protein with 1:1,000 dilutions of piglet serum samples in a 96-well plate. After 1 hr of incubation, the bound specific antibody was detected using horseradish peroxidase (HRP)-conjugated goat antibody against pig IgG (Bethyl Laboratories, Montgomery, TX).

The PCV2-specific IgG titer of serum samples was estimated by comparing its optical density (OD) with those on the standard curve obtained from a serial dilution of a standard positive serum. One unit was defined as the lower limit of positive in the standard curve.

Mortality rate: Dead pigs and pigs euthanized due to concerns over animal welfare were recorded daily by the farm owner. In experiment 1, from 12 to 24 weeks of age, the number of dead and euthanized pigs was counted for a month in December 2007 and again in December 2008 and the percentage cumulative mortality rate was calculated. In experiment 2, the number of dead and euthanized pigs was counted for a month in December 2007 and again in December 2008 and the percentage mortality rates on each farm were determined.

Statistical analysis: For the mortality rate, the χ²-test and the t-test with Bonferroni’s correction were used to identify and confirm the significance of differences between groups.
in experiments 1 and 2, respectively. Statistical analyses were performed using the STATVIEW statistical program (Abacus Concepts, Berkeley, CA, U.S.A.).

RESULTS

Experiment 1

Viremia and shedding of PCV2: The numbers of piglets tested for viremia and the shedding of PCV2 in feces for each group are shown in Table 1.

Though all piglets in the unvaccinated Group C survived to 11 weeks of age, C1, C2, C3 and C4 died before 13, 14, 17 and 18 weeks of age, respectively. Viremia was not detected at 7 weeks of age, but one piglet was viremic at age 11 weeks. PCV2 was detected in sera from all three surviving piglets at 14 weeks. No deaths were recorded in the piglets of the vaccinated Groups A and B. PCV2 was not detected in the sera of any of these piglets (Table 1-a).

Fecal swabs were taken from the piglets whose blood was sampled. All of the piglets of the unvaccinated Group C excreted PCV2 by the age of 13 weeks. Some piglets excreted the virus for seven consecutive weeks. The genetic type of PCV2 virus excreted by all of the unvaccinated piglets was revealed to be PCV2b (RFLP type 111) by RFLP analysis. PCV2 excretion was not detected in the feces of the piglets in the vaccinated Groups A and B (Table 1-b).

Serologic response to vaccination: The mean PCV2 antibody titers for each group are shown in Fig. 1. At 4 weeks of age before PCV2 vaccination, the mean antibody titer was 2.18 units. At 7 and 11 weeks of age the mean antibody titers in the piglets of the vaccinated Groups A and B increased to 2.0 and 2.4 units, respectively. In the piglets of the unvaccinated Group C, the mean antibody titer had increased to 2.6 units by 14 weeks of age.

Mortality rate: The percentage cumulative mortality rates for each group are shown in Fig. 2. At age 12 weeks, the mortality rate was roughly 7–9% in all groups. Mortality rates at 16 weeks had increased by approximately 3% as compared with those at 12 weeks in the vaccinated Groups A and B, whereas the mortality rate of Group C had increased by approximately 17%. The mortality rate increase in Group C was significantly greater (P<0.001) than those of Groups A and B. The mortality rate differences between the unvaccinated Group C (32% at 24 weeks) and the vaccinated Groups A and B (11% and 13% at 24 weeks, respectively) were significant from 16 to 24 weeks of age (P<0.001).

Experiment 2

Viremia and Genotype: The numbers of pigs with PCV2 viremia at each farm in December 2007 (before the start of the PCV2 vaccination program) and December 2008 (over 8 months after the start of the program) are shown in Table 2. Fewer pigs had PCV2 viremia in 2008 than in 2007, on all of the farms studied. The number of pigs with PRRSV viremia also tended to be lower in 2008, as compared with 2007.

The phylogenetic tree of PCV2, based on ORF2 nucleotide sequences of representative PCV2 isolates on each farm, is shown in Fig. 3. The RFLP types of PCV2 isolates detected on each farm were the same as those of the representative PCV2 isolates on each of the farms (Table 2). According to PCV2 genotypes, PCV2a and PCV2b were detected on 10 and 6 farms, respectively. PCV2a isolates were further subdivided into PCV2a-1 (RFLP type 311) (6 farms) and PCV2a-2 (RFLP type 122) (4 farms). PCV2a-2 corresponded to cluster 2A of PCV2 group 2 of Olvera’s classification.

Mortality rates: Mean mortality rates on farms infected with three PCV2 genotypes (PCV2a-1, PCV2a-2 and PCV2b) were determined in December 2007 (before the start of the PCV2 vaccination program) and again in December 2008 (over 8 months after the start of the program). The results are shown in Fig. 4. Mean mortality rates were significantly decreased in 2008 as compared to those in 2007 on farms infected with PCV2a-2 (20.8% vs. 12.1%) (P<0.05) and PCV2b (26.5% vs. 13.7%) (P<0.01). On the farms with PCV2a-1 infected pigs, there was no significant difference in the mean mortality rate before versus after the start of the vaccination program (14.7% vs. 14.1%).

In 2007, the mean mortality rate for farms with PCV2b or PCV2a-2 infected pigs was significantly higher than that of farms with PCV2a-1 infected pigs (P<0.05). On the other hand, the mean mortality rate among farms housing pigs infected with different PCV2 genotypes did not differ significantly in 2008.

DISCUSSION

PCVADs constitute the most important group of diseases causing huge economic losses to the swine industry. Though several pathogens act as co-factors or triggers of the
There are several vaccines under development to protect against or mitigate the severity of PCV2 infection. The antigens used in these vaccines are recombinant capsid proteins [4, 31], inactivated whole virus [2, 23] or inactivated PCV1-2 chimeric virus [11]. The vaccine generated using the capsid protein of PCV2a as an antigen, vaccination with reduced infection with PCV2 on Farm O.

Increases in PCV2 antibody titers were observed until 4 to 7 weeks of age (ND). Standard error is indicated by error bars.

Mortality rates were similar in vaccinated and unvaccinated groups at 12 weeks old. The mortality rate differences between Group C and the other groups were significant according to the χ² test (P<0.001) at the ages marked with an asterisk.

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Porcilis® PCV was effective in reducing the mortality rate on a commercial farm with a high rate of PCV2b infection. Among the 16 farms studied in Experiment 2, 10 and 6 had been infected with PCV2a and PCV2b, respectively. We previously reported a genotypic change from PCV2a to PCV2b on Japanese commercial pig farms [29]. Those farms were included in this study as farms infected with PCV2b. PCV2a was further subdivided into PCV2a-1 and PCV2a-2 based on RFLP and phylogenetic analysis of the nucleotide sequence of ORF2. PCV2a-2 corresponded to cluster 2A of PCV2 group 2 of Olvera’s classification [20].

We monitored piglets for PCV2 viremia before (December 2007) and after (December 2008) the start of the vaccination program. In 2008, fewer piglets showed PCV2 viremia than in 2007 on all of the farms studied, as shown in Table 2.

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* The RFLP types were determined by the previously described method [29].
the farms tested, irrespective of PCV2 genetic type (Table 2). We also performed monitoring of PRRSV viremia in 2007 and 2008 according to the method of Kono et al. [17]. In addition to this reduction in PCV2 infection, numbers of piglets showing PRRSV viremia were reduced on 10 farms (data not shown). This suggested that vaccination against PCV2 reduced the PCV2 viral load and thereby increased the immune function of vaccinated piglets. Increased immune function might lead to earlier recovery of piglets infected with PRRSV.

Mortality rates were significantly reduced after adoption of the PCV2 vaccination program on farms infected with PCV2a-2 and PCV2b. On the other hand, the mortality rate in 2008 was similar to that in 2007 on farms infected with PCV2a-1 (Fig. 4). PCV2a-2 and PCV2b were suggested to be highly virulent in pigs grown in commercial farms. Therefore, vaccination against PCV2 was effective in reducing the mortality rates on farms infected with PCV2a-2 and PCV2b. Vaccination against PCV2 did not cause significant change in mortality rate in farms infected with PCV2a-1 presumably because increase in the mortality rate attributable to PCV2 was not significant. PCV2a-1 was assumed to be less virulent than other genotypes.

It was recently reported that the virulence of PCV2a and PCV2b did not differ in specific-pathogen-free (SPF) pigs [22]. However, we found that PCV2b can raise the mortality rate on conventional commercial farms infected with various pathogens such as PRRSV and M. hyopneumoniae. Similarly, PCV2a-2, belonging to the PCV2a strain, actually increased the mortality rate. This is the first report of an increase in the mortality rate attributable to PCV2a-2.

In this study, we demonstrated the PCV2 vaccines used (Porcilis® PCV and Ingelvac® CircoFLEXTM) to be effective in reducing PCV2 infection on commercial pig farms infected with PCV2, PRRSV and M. hyopneumoniae. Mortality rate reduction with the PCV2 vaccination might depend on the genetic types of PCV2. Vaccination against PCV2 was effective in reducing the mortality rate on farms infected with PCV2a-2 and PCV2b, while only a marginal effect was observed on farms infected with PCV2a-1.

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REFERENCES


