ABSTRACT

Interest in porcine circovirus has been stimulated by the recent emergence of postweaning multisystemic wasting syndrome (PMWS) in pigs and the potential use of pig organs for xenotransplantation in humans. Porcine circovirus type 1 (PCV1) is considered to be widespread in pigs but nonpathogenic. Circovirus type 2 (PCV2) is a similar virus but has been differentiated only recently as a separate type. High tissue concentrations of PCV2 are associated with lesions in PMWS cases, but the etiological role of this agent in the disease remains unclear. The presence of PCV1 in New Zealand pigs has been previously reported based on serological data. PMWS has been recently recorded in New Zealand pigs. The epidemiology of PCV2 in New Zealand pigs has not been examined. The purpose of the study was to look for evidence of circoviruses in New Zealand pig herds. Pig circovirus DNA was sought in various tissues using the polymerase chain reaction. Circovirus type 2 was found in New Zealand pig herds, without any evidence that PMWS has ever occurred in these herds. Newborn piglets were shown to have infection, suggesting vertical transmission of the virus.

CIRCOVIRUSES HAVE BEEN DESCRIBED for pigs (porcine circovirus, PCV), chickens (chicken anemia virus, ChAV), psittacines (psittacine beak and feather disease virus, PBFDV), and pigeons. ChAV, PBFDV, and pigeon circovirus are all associated with disease in their specific hosts.1,2 Circovirus type 1 is ubiquitous in pigs but does not appear to cause disease in adult pigs. Porcine circovirus type 2 has been putatively associated with the recently recognized syndrome of poor growth and wasting in weaned pigs known as postweaning multisystemic wasting syndrome (PMWS).3 In some circumstances the disease porcine dermatitis and nephropathy syndrome (PDNS) has occurred contemporaneously with PMWS, and it has been proposed that they share the same etiological agent. Both types of virus can produce silent infection in pigs,3 and thus infection may not be recognized.

The epidemiology of circoviruses in New Zealand is unclear. A high prevalence of PCV type 1 antibody has been reported in New Zealand pigs.4 Epidemiological studies that are based on serology now need to differentiate between circovirus types 1 and 2. It appears that both types are widely distributed in pig populations, but the situation needs further clarification now that two distinct strains of circovirus have been characterized.5

Our study looked for virological evidence of PCV types 1 and 2 in New Zealand pigs. Pigs were examined from three herds representing a spectrum of origins: a high health status (HHS) herd maintained under strict biosecurity and health surveillance, a commercial herd, and a group of feral pigs that had been transferred to New Zealand from the Auckland Islands after living in isolation from commercial pigs for about 200 years.

Testing in this study was based on direct detection of PCV2 nucleic acid using PCR.

MATERIALS AND METHODS

Animals and Tissues

The following tissues were examined. In the HHS herd, four lungs, peripheral blood mononuclear cells (PBMC), from 1-week-old piglets; 14 fecal samples from 14 to 16-week-old pigs; two semen samples from boars were examined. In the commercial herd, 14 fecal samples from 14- to 16-week-old pigs were examined. And in the Auckland Islands pigs, 20 blood samples from adult pigs (more than 6 month of age); 20 fecal samples from adult pigs; and tissues from 1-week-old piglets were collected during surgery and snap-frozen in liquid nitrogen. Tissues awaiting analysis were stored at −70°C. Blood from adult pigs was collected in EDTA tubes from the jugular vein.
DNA Extraction

DNA from tissues, PBMC, and feces was extracted using Puregene DNA Isolation Kit (Gentra) in accordance with the manufacturer’s recommendations.

PCR

All PCR were performed using the Perkin Elmer GeneAmp PCR System 2400 thermocycler. Two sets of primers were used.6 One set of primers (CFs:5'-TAGGGTAGGCTGCCCT-3'; CRs:5'-CCGCACCCTCTGCCATACTG-3') was designed to amplify a fragment of 263 nt from the open reading frame (ORF) 2 of the PCV type 2, and the other set of primers (PF2:5'-TTGCTGAGCCTGACGACACC-3'; reverse primer was 5'-CAGGCTACCAGTCACAC-3'). For the PCR reaction, 2 μL of DNA was added to a PCR mixture with a final concentration of 1.5 mmol/L MgCl₂, 0.2 mmol/L (each) dNTP, 1.00 μmol/L of each primer, and 2.5 U of Taq DNA polymerase (Life Technologies) per 50 μL. PCR was performed in 35 cycles of denaturing at 95°C for 1 minute, annealing at 65°C for 1 minute, and extension at 72°C for 1 minute. The PCR products were analyzed by gel electrophoresis. A PCV1 isolate from PK15 (porcine kidney) cell line was used as a positive control in PCV1 amplification. No positive control for PCV2 was used initially in order to exclude the possibility of laboratory contamination. The identities of all amplifiers were confirmed by sequence analysis.

Sequence Analysis of PCR Products

Amplified products were separated on a 1.8% agarose gel and examined for the presence of PCR products of the appropriate size. PCR products were purified using the Highly Purified PCR Product Purification Kit (Boehringer) according to the manufacturer’s protocol and sequenced utilizing an ABI 373A sequencer (Centre for Gene Technology, Auckland University). Computer analysis was performed by BLASTN and DNASTAR programs.

RESULTS

PCV2 DNA was amplified from the fecal samples and PBMC of the 14- to 16-week-old pigs and sows from both the HHS and the commercial herds (Table 1). Phylogenetic analysis of the sequenced products showed 98% homology with an already described PCV2 strain (U49186). Four lungs and fecal samples from 1-week-old piglets of the HHS herd were also positive for virus DNA (Table 1). No piglet tissues showed any histological abnormality (data not shown).

Farm records indicated that stillborn piglets per litter typically ranged from 1.5% to 6%, with losses up to 10% in occasional litters. Regular clinical surveillance showed no evidence of wasting disease consistent with PMWS.

None of the tested Auckland Island samples were positive for PCV2.

No evidence of PCV1 was found in any of the tissues tested.

DISCUSSION

It appears that PCV infection is ubiquitous in pigs throughout the world. PCV1 was first discovered in 1974 as a contaminant of the continuous porcine kidney cell line, PK15.7 Subsequent serological studies in pig sera from Germany, Canada, New Zealand, Great Britain, Northern Ireland, and the United States have shown 25% to 98% positivity for PCV1 antibodies in fattening and adult pigs.8–10

Recently, a new strain of porcine circovirus, named porcine circovirus type 2, has been found in pigs with PMWS.3 PMWS most commonly affects 5- to 12-week-old piglets and is characterized by progressive weight loss, jaundice, and respiratory signs. In experimental settings piglets developed PMWS or mild to severe lesions consistent with this disease after injection of PCV2 or recombinant PCV2.11–14 Severe clinical disease and death with multiple lesions, typical of PMWS, were seen in pigs inoculated with a combination of PCV2 and pig parvovirus (PPV).15,16 Other dual infections, such as PCV2 and porcine reproductive and respiratory syndrome virus (PRRSV),17,18 PCV2 and Suid herpesvirus 1,19 and PCV2 with porcine epidemic diarrhoea virus in neonatal pigs, have been reported.20 Recently, PCV2 has been associated with myocarditis in stillborn piglets.21 A study on gnotobi-
otic piglets infected with PCV2 alone and with injection of keyhole limpet hemocyanin in incomplete Freund’s adjuvant (KLH/ICFA) showed that none of the piglets infected with PCV2 alone developed PMWS, whereas all immunized piglets developed moderate to severe PMWS.22

It has been suggested that PCV2 may be a primary immunosuppressive agent, which may initiate various disease conditions.

There is so far no substantive evidence to suggest that PCV has zoonotic potential, although the issue is not definitively resolved. Antibodies reacting with PCV type 1 have been detected in people, mice, and cattle.23 About 20% of healthy adults and 30% of hospitalized patients in Germany and 24% of hospitalized patients in Canada were seropositive to PCV-like antigen. However, neither virus nor viral genome has been detected in any mammalian species (including humans) other than pigs. The PCV1 antibody reactivity found in humans and other species may be nonspecific. Other studies did not show antibodies to PCV1 in serum samples from a range of domestic animal species, nor were antibodies found in human sera.24,25 Bovine circovirus with 99% similarity to porcine circovirus has been amplified from cases of bovine respiratory disease and from bovine fetuses.26 However, the absence of antibody to PCV2 in bovine sera collected from mixed-species farms suggests that PCV2 does not readily infect cattle under field conditions.24 In man, PCV2 antibody has not been found in sera of healthy blood donors nor in population groups occupationally exposed to pigs.24 However, it has been reported recently that PCV type 2 can infect human cells in vitro and BALB/c mice in the experimental setting.26

Several categories of pig herds were investigated in this study in order to obtain maximum data on circovirus epidemiology in New Zealand. The HHS herd has been established and maintained in isolation from other pig herds. The commercial herd “farrow-to-finish” is representative of commercial farm pigs. The Auckland Islands pigs are a unique herd that has been genetically isolated for 200 years, subsequently transferred to New Zealand in 1999, and kept in quarantine facilities, which include bird and rodent control.

Two HHS herds and a commercial herd showed the presence of PCV type 2. The HHS and the commercial herd showed extremely high prevalence of the virus (100% positivity in the fecal 14- to 16-week-old pigs both in HHS herd and commercial herd). Neither of these herds has at any stage shown signs of disease, which might be a consequence of PCV2 infection. PCV2 nucleic acid was also found to be present in lung and PBMC in very young piglets (7 to 10 days old), which might indicate possibility of vertical transmission. There was no increase in numbers of stillborn or mummified piglets. These results on newborn piglets were the most unexpected. To avoid any risk of contamination with PCV type 2, DNA analysis of fecal and tissue samples from newborn piglets was done in the human virology laboratory at the Auckland Hospital. Positive control was intentionally omitted.

No evidence of PCV2 was found in boar semen. Auckland Island pigs were free from PCV2, and PCV1.

Our study has shown widespread infection with PCV type 2 in New Zealand pigs. Contrary to expectation, there was no evidence of infection with PCV type 1.

REFERENCES

27. Hatterman K, Mankertz A: Infection studies on human cell-lines with porcine circovirus type 1 and type 2. Paper presented at The Word of Microbes; 27th July to 1st August, 2002; Paris