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Pathogenesis of porcine circovirus; experimental infections of colostrum deprived piglets and examination of pig foetal material

G.M. Allan *, F. McNeilly, J.P. Cassidy, G.A.C. Reilly, B. Adair, W.A. Ellis, M.S. McNulty

Department of Agriculture for Northern Ireland, Veterinary Sciences Division, Stoney Road, Stormont, Belfast BT4 3SD, UK

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Abstract

The results of virus and antigen distribution following experimental infection of colostrum deprived pigs with pig circovirus (PCV) by oral/nasal and intravenous routes are reported. PCV and antigen were detected using virus isolation and indirect immunofluorescence on cryostat sections respectively. PCV antigen was detected in tissues throughout the body but primarily in spleen thymus, and lung. No PCV antigen or virus was detected in tissue samples from the central nervous system. Examination of pig foetal material from field cases of abortion/stillbirth resulted in 3 PCV isolates from 2 sera and a spleen sample from 2 groups of stillborn piglets from the same farm. No antibody to PCV alone was detected in 160 foetal sera tested. These results suggest that transplacental infection with PCV does occur, possibly prior to foetal immunocompetance. However, it is probably not a significant cause of reproductive disorders in pigs in Northern Ireland.

Keywords: Porcine circovirus, pathogenesis; Pig

1. Introduction

Recently, a small, icosahedral virus of pigs, designated porcine circovirus, containing a circular, single-stranded DNA genome has been reported (Tischer et al., 1982). It has been proposed by an International Committee on the Taxonomy of Viruses (ICTV) study group that PCV should be included, with chicken anaemia virus (CAV) and psittacine beak and feather disease virus (PBFDV), in a new virus family called *Circoviridae* (Pringle, personal communication, 1993).

* Corresponding author.

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Little is known about the pathogenesis of PCV infections. However, experimental infection of pigs with PCV, demonstrated by seroconversion and recovery of virus from faeces and nasal mucus samples, has been reported (Tischer et al., 1986). Attempts to demonstrate PCV antigen or virus in selected tissue samples from these experimentally infected pigs were unsuccessful. Consequently, nothing is known about the growth of PCV in its natural host or the tissues involved in PCV replication.

Recently Allan et al. (1994b) reported the replication of PCV in porcine and bovine monocyte/macrophage cultures and it is possible that these cells also support replication 'in-vivo'.

In addition, PCV is known to require actively dividing cells for replication in cell cultures (Tischer et al., 1987). This requirement is also necessary for the replication of porcine parvovirus (PPV), a known foetal pathogen. The pig foetus represents a potential source of actively dividing cells and it is possible that PCV, like PPV, can establish a transplacental infection resulting in foetal death or damage. Recently Hines and Lukert (1994) have linked PCV infection with congenital tremor in pigs. These authors reported the isolation of PCV from kidney explants derived from piglets with congenital tremor and transmission of this condition following experimental infection of pregnant sows with the virus isolate.

This paper reports the results of a study undertaken to investigate the distribution of PCV antigen in the tissues of experimentally infected colostrum deprived (CD) pigs and to determine, from examination of foetal bloods and tissue samples submitted to this laboratory, whether transplacental spread of PCV occurs following natural infection of pigs with this virus.

2. Materials and methods

2.1. Experimental animals

CD pigs, from a minimal disease, breeder-finisher unit were used in all experimental infections. Pigs were snatch-farrowed and immediately fed colostrum substitute (Volostrum, Volac Ltd, Ireland) as per manufacturer's instructions. In addition, all snatch-farrowed piglets were immediately injected with 0.2 ml of an antibiotic preparation (Symulux, Smith-Kline Beecham, England) by an intramuscular route, and quickly transferred to clean, previously fumigated isolation houses. Ambient temperature was maintained at 30°C and pigs were bedded on sterilised, dried straw. Three hours after transfer the pigs were again fed Volostrum as per manufacturer's instructions. Four hours later all piglets were bottle-fed with a 50/50 solution of 1% dextrose and full cream evaporated milk (Nestle, England). Following this initial feed, pigs were fed at 4 hourly intervals for a 24 h period and, thereafter, 4 times daily.

2.2. Virus

A pool of virus was prepared from a PCV-persistently infected continuous pig kidney cell line (PK/15/W) and purified as described elsewhere (Allan et al., 1994c). The titre

of this pool was $10^{6.5}TCID_{50}$/0.1 ml as determined by indirect immunofluorescence (IIF) (Allan et al., 1994a).

2.3. Experimental design

Three groups of CD pigs were inoculated with PCV virus.

GROUP A. Ten 1-day-old pigs were each inoculated, by a combined oral/nasal (0.5 ml) and intravenous route (0.5 ml). Age-matched control pigs were held in a separate isolation house and mock-infected with a cell lysate of PCV-free continuous pig kidney (PK/15/H) cell cultures using similar procedures. One pig from the PCV-inoculated group was killed at 1, 3, 5, 7 and 9 days after inoculation. One control pig was killed at 1, 5 and 9 days after inoculation. Samples of tissue as shown in Table 1 were taken from all pigs and processed for virus isolation and immunostaining of cryostat sections as described below.

Table 1
Results of immunofluorescence (IF) staining of cryostat sections for PCV antigen and PCV isolation (VI) following inoculation of 1-day-old CD piglets by a combined intravenous/oral/nasal route

Tissues	Days after inoculation									
	1		3		5		7		9	
	VI	IF	VI	IF	VI	IF	VI	IF	VI	IF
Tonsil	–	–	–	–	–	–	–	–	–	–
Trachea	–	–	–	–	–	–	–	–	–	–
Mes LN.	–	–	+	+	+	+	–	–	+	+
Bronchial LN.	–	–	–	–	–	–	–	–	–	–
Retro Phar LN.	–	–	–	–	–	–	–	–	–	–
Heart	–	–	–	–	–	–	–	–	–	–
Thymus	+	+	–	–	+	+	+	+	+	+
Kidney	–	–	–	–	–	–	–	–	–	–
N Mucosa	–	–	–	–	–	–	–	–	–	–
Lung	+	–	+	+	+	+	+	+	+	+
Liver	–	–	+	+	+	+	+	+	–	–
Spleen	+	–	+	+	+	+	+	+	+	+
Bone Marrow	–	–	–	–	–	–	–	–	–	–
Testes	–	–	NT	NT	–	–	NT	NT	–	–
SI 1[a]	–	–	–	–	–	–	–	–	–	–
SI 2	–	–	–	–	+	+	+	–	–	+
LI[b]	–	–	–	–	–	–	–	–	–	–
Fore Brain	–	–	–	–	–	–	–	–	–	–
Mid Brain	–	–	–	–	–	–	–	–	–	–
Hind Brain	–	–	–	–	–	–	–	–	–	–
Spinal Cord	–	–	–	–	–	–	–	–	–	–
Pancreas	–	–	–	–	–	–	–	–	–	–
Bladder	–	–	–	–	–	–	–	–	–	–

+ = PCV antigen detected or PCV isolated in cell cultures
– = no PCV antigen detected or no PCV isolated in cell cultures
[a] = small intestine
[b] = large intestine

GROUP B. Eight 1-day-old pigs were inoculated with 0.5 ml by an intravenous route only. Age-matched control pigs were also mock-infected with PK/15/H cell lysate. One PCV-inoculated pig was killed on day 1, 3, 5, 9, 11 and 15 after inoculation and 1 control pig on day 1, 9 and 15 days after inoculation. Tissue samples, as described for group A, were taken at post mortem examination for immunostaining of cryostat sections as described below.

GROUP C. Twelve 7-day-old pigs were inoculated by an oral/nasal route only and given, in total, 10 ml of inoculum, The virus pool described above was diluted 1/10 in 0.01 M phosphate buffered saline (pH 7.2) (PBS). Pigs were inoculated on two separate occasions over a 6 h period. Age-matched control pigs, held in isolation from the PCV-inoculated animals, were mock-infected with PK/15/H cell lysate. One PCV-inoculated pig was killed at 1, 2, 3, 5, 7, 9, and 11 days after inoculation and one control pig at 1, 5 and 11 days after inoculation. Tissue samples, as described above, were processed for virus isolation and immunostaining of cryostat sections as described below. In addition, samples of whole blood were taken from all animals in group C at post mortem and the buffy coat separated and processed for virus isolation.

2.4. Virus isolation

Virus isolation was carried out in PK/15/H cell cultures. Briefly, 10% suspensions of tissue material were clarified by centrifugation at 3000 *g* for 30 min and the supernatant fluids adsorbed onto each of 2 semi-confluent coverslip cultures of PK/15/H cells. After 1 h, the inoculum was removed and the cultures refed with Earle's minimal essential medium supplemented with 10% foetal bovine serum, and incubated for 6 h at 37°C. Cultures were then treated with glucosamine as described previously (Tischer et al., 1987) and following incubation for a further 48 h at 37°C, one coverslip culture from each specimen was fixed in acetone and immunostained for PCV antigen by IIF. The second inoculated cell culture was subjected to 3 freeze/thaw cycles and held at −70°C pending the immunostaining result. The resulting cell lysates from PCV IIF-negative cultures were re-inoculated into fresh PK/15/H cells. If no PCV antigen was detected, these cultures were processed as above and again passaged before being discarded as negative.

2.5. Cryostat sectioning and immunostaining

Cryostat sections of tissues were prepared and fixed as described previously (McNeilly et al., 1991).

IIF staining was carried out using a pool of monoclonal antibodies (mAbs) to PCV as a primary antibody. Dialysis sac culture supernatants from 7 mAbs to PCV were prepared as described elsewhere (Allan et al., 1994a). Briefly, PCV-positive hybridoma cells, in 5 ml of RPMI medium supplemented with 10% horse serum (Gibco), were placed in dialysis tubing and the tubing sealed to form a sac. This was then cultured in a 250 ml flask surrounded by 40 ml of the culture media. After 14 days the sac contents were harvested, the hybridoma cells pelleted by centrifugation and the supernatant assayed for PCV-antibody activity. The dialysis sac culture supernatants were diluted 1/10 in PBS and pooled. This pooled primary antibody had a titre of 1/500 as determined by IIF using acetone-fixed PK/

15/W cell cultures, and was used at a dilution of 1/100 for all immunostaining procedures. Initially all sections were incubated with 10% non-immune rabbit serum (Zymed, USA) for 30 min at 37°C. This blocking solution was removed and the primary antibody applied for 1 h at 37°C. All sections were washed in PBS and a 1/80 dilution of FITC-labelled rabbit anti-mouse immunoglobulin (Nordic), in PBS, applied for 30 min at 37°C. Sections were washed again in PBS, mounted in buffered glycerol, and viewed under incident UV illumination. Selected tissue sections were also immunostained with an inappropriate mAb prepared against chicken anaemia virus (McNulty et al., 1990).

Selected cryostat sections were also immunostained using a streptavidin/biotin immunoperoxidase (IP) technique. Initially, endogenous peroxidase activity was inhibited in tissue sections by application of Peroxoblock solution (Zymed, USA) for 17 s. This solution was immediately removed and the sections washed extensively in PBS. Following application of blocking serum and primary antibody as described above for IF, sections were washed in PBS and the procedure completed using a biotinylated anti-mouse Ig, streptavidin peroxidase conjugate, and substrate from a Histostain SP kit (Zymed, USA).

2.6. *Examination of PIG foetal material*

Samples for examination were taken from pig foetuses and stillborn piglets submitted to our institute from commercial breeding herds for routine post mortem examination. The following tests were carried out.

(A) SERUM SAMPLES. Serum samples from 160 pig foetuses from 60 pig herds were tested for PCV antibody and virus. Sera were examined for PCV antibody at a 1/40 dilution, in PBS, and processed by IIF on acetone-fixed, PK/15/W cell cultures. Serum samples were also tested by IIF for antibody to PPV and, selected sera, for group A rotavirus antibody. PPV cell culture preparations were grown in PK/15/H cells and group A bovine rotavirus preparations grown in MA104 cells (McNulty et al., 1976). Cultures were acetone-fixed and IIF carried out as described above, except that, the FITC-conjugated anti-pig Ig used for detection of rotavirus antibody was subjected to 3 cycles of absorption with rotavirus Group A infected cell cultures prior to use (McNulty and Allan, 1984).

Serum samples were also processed for PCV isolation in PK/15/H cell cultures as described above, except that, initially, 0.2 ml of foetal serum was inoculated, without dilution, into each of 2 cell culture preparations.

(B) TISSUE SAMPLES. Cryostat sections of tissues from 160 foetuses from 60 pig herds were tested for the presence of PCV antigen by IIF and selected tissues for infectious virus using the procedures described above. Samples of placenta, lung, kidney, spleen, thymus and, where possible, CNS and bone marrow were taken.

2.7. *mAb staining of field isolates of PCV*

Suspected field isolates of PCV were immunostained with each of 7 PCV-specific mAbs to eliminate the possibility that these isolates may have arisen from laboratory contamination. Acetone-fixed coverslip preparations of suspected PCV field isolates, cultured in PK/15/H cells, and PK/15/W derived PCV were individually immunostained with a panel of mAbs using procedures described previously (Allan et al., 1994a).

3. Results

3.1. PCV distribution in experimentally infected PIGs

The results of PCV antigen detection following IIF on tissue cryostat sections and virus isolation from pigs in group A are presented in Table 1. No immunostaining was detected in sections of tissues from pigs inoculated with PK/15/H cell lysate or in sections from PCV-infected pigs immunostained with the inappropriate mAb.

Cells containing PCV antigen were detected in a number of tissues throughout the experiment. At 1 day after inoculation small amounts of PCV antigen were detected in the cytoplasm of cells in tissue sections of thymus (Fig. 1A). No PCV antigen was detected in any other tissue sections taken from this pig. At day 3 after inoculation, small numbers of PCV antigen-positive cells were detected in tissue sections of spleen (Fig. 1B), liver (Fig. 1C), lung, and mesenteric lymph node. Typically, staining was seen as small plaques with

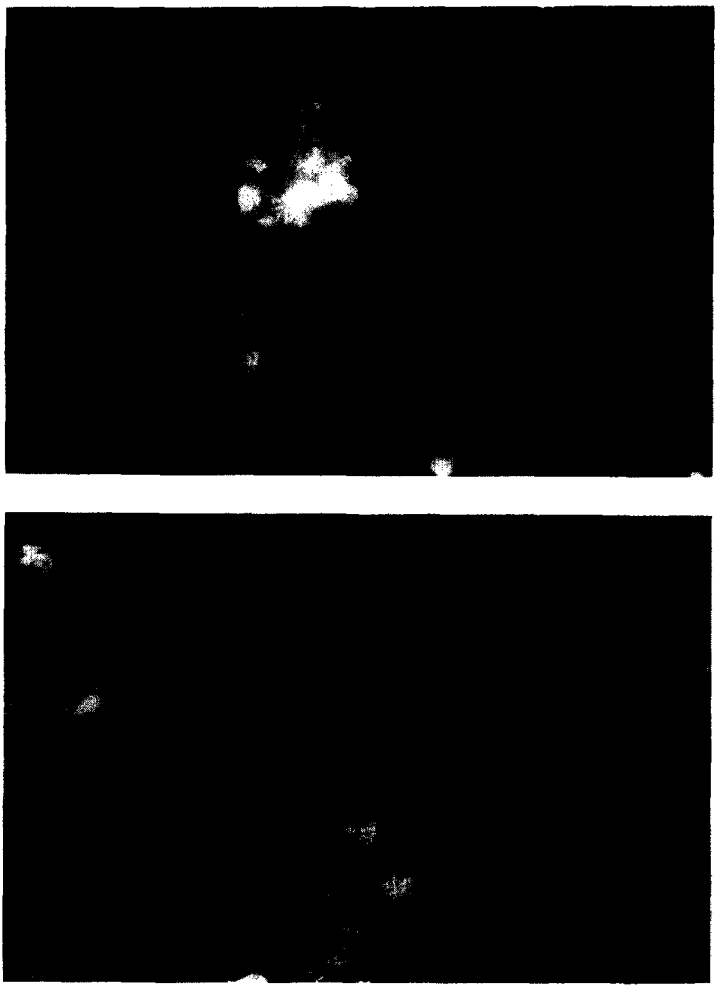


Fig. 1. (A) Immunofluorescent (IF) staining of PCV antigen in cryostat sections of thymus from a pig inoculated with PCV by a combined intravenous/oral/nasal route and killed 1 day after inoculation. IF staining of PCV antigen in tissue section of (B) spleen and (C) liver from a pig inoculated by the same route as (A), killed 3 days after inoculation. IF staining of PCV antigen in tissue section of lung from a pig inoculated by an oral/nasal route only and killed 3 days after inoculation (D).

FITC-labelled pin-like inclusions radiating from the infected cells. The distribution and pattern of staining of PCV antigen in the pigs killed at day 5 and 7 after inoculation was similar to day 3, but in addition, PCV antigen was detected in the thymus and small intestine. Distribution of PCV antigen in the pig sacrificed at 9 days after inoculation was also similar, however the amount of PCV antigen detected in PCV-positive sections from this pig was substantially greater. This was particularly noticeable in sections of thymus where many IIF-positive cells were observed. The results of virus isolation studies on these animals confirmed the IIF studies. With the exception of specimens taken on day 1 after inoculation, little variation was seen (Table 1).

Table 2
Results of immunofluorescence (IF) staining of cryostat sections for PCV antigen and PCV isolation (VI) following inoculation of 7-day-old CD piglets by a oral/nasal route

Tissues	Days after inoculation													
	1		2		3		5		7		9		11	
	VI	IF	VI	IF	VI	IF	VI	IF	VI	IF	VI	IF	VI	IF
Tonsil	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Trachea	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Mes LN	−	−	−	−	−	+	+	+	+	+	−	−	−	−
Bronchial LN	−	−	−	−	+	+	+	+	−	−	+	+	−	
Retro Phar LN	−	−	−	−	−	−	−	−	+	−	+	−	+	−
Heart	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Thymus	−	−	−	−	−	+	+	+	−	−	−	−	−	−
Kidney	−	−	−	−	−	−	−	−	−	−	−	−	−	−
N Mucosa	+	−	+	+	−	−	−	−	−	−	−	−	−	−
Lung	−	−	+	+	+	+	+	+	+	+	+	+	+	+
Liver	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Spleen	−	−	−	−	+	+	+	+	+	+	−	−	−	−
Bone Marrow	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Testes	NT	NT	−	−	−	−	NT	NT	−	−	NT	NT	−	−
SI 1/SI 2[a]	−	−	−	−	−	−	+	−	+	+	+	−	−	−
LI[b]	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pancreas	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Fore Brain	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Mid Brain	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Hind Brain	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Spinal Cord	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Buffy Coat	+	NT	+	NT	−	NT	−	NT	−	NT	−	NT	−	NT
Bladder	−	−	−	−	−	−	−	−	−	−	−	−	−	−

+ = PCV antigen detected or PCV isolated in cell cultures
− = no PCV antigen detected or no PCV isolated in cell cultures
[a] = small intestine
[b] = large intestine

The results of IIF staining of cryostat sections and PCV isolation from tissue samples from pigs in group B, sacrificed at 1, 3 and 5 days after inoculation, were similar to the corresponding results from group A. In group B, however, only small amounts of PCV antigen were detected in spleen tissue and minimal amounts were observed in sections of small intestine and mesenteric lymph node in the pig killed at 9 days after inoculation. All other tissue samples from this animal were negative for PCV antigen. By day 11 and 15 after infection, PCV antigen distribution was confined to a few positive cells in the spleen (day 11), small intestine and large intestine.

PCV distribution in the pigs inoculated by an oral/nasal route only (Group C) was similar to that seen in the other two groups. The results of this experiment are presented in Table 2. In general, less antigen was seen in tissue sections from pigs in this experiment than in tissue sections from groups A and B, with the exception of sections of lung. No PCV antigen was seen in tissue sections from the pig killed 1 day after inoculation, however,

PCV virus was isolated from samples of nasal mucosa. PCV antigen was detected in tissue samples of lung and nasal mucosa taken from the pig killed 2 days after inoculation. The immunostaining pattern and distribution of cells containing PCV antigen was similar to that seen in tissue samples from groups A and B. At day 3 after inoculation, PCV antigen was detected in sections from thymus, lung, spleen and bronchial and mesenteric lymph nodes, however only a single focus of infection was detected in spleen whereas more cells containing PCV antigen were seen in the sections of lung tissue (Fig. 1D). A similar pattern of staining and virus isolation was seen in tissue samples from the pig killed 5 days after inoculation, except that PCV was isolated from small intestine of this animal. PCV antigen was again detected in relatively large amounts in the lung sections from the pigs killed at 7 and 9 days after infection, and also in the tissue samples from small intestine from the pig killed at 7 days after inoculation. At 11 days after inoculation only a few cells containing PCV antigen were seen in sections of lung and retropharyngeal lymph node. PCV virus isolation results again showed good correlation with IIF results. PCV was isolated from the buffy coat samples collected at 1 and 2 days after inoculation.

No cells containing PCV antigen were seen in sections from pigs inoculated with PK/15/H cell culture lysate and no PCV was isolated from tissue samples from these animals.

IP staining of selected PCV antigen containing tissue sections from all three groups of experimental animals confirmed the IIF results. In addition, localisation of PCV antigen was improved and more accurate presumptive assessments of cell types infected with the virus attempted.

PCV antigen seemed to be confined, in most tissue sections, to non-epithelial cell types. In lung tissue, PCV antigen was located mainly in cells with a morphology similar to alveolar macrophages and in thymus tissue, PCV antigen was detected primarily in large cells, some of which appeared to contain processes (Fig. 2A) and in cells located in connective tissue.(Fig. 2B).

3.2. Examination of PIG foetal material

A total of 160 porcine foetal sera were screened for antibody to PCV, PPV and group A rotavirus. Antibody to all three viruses and PCV and group A rotavirus alone was detected in 10 sera. Antibody to PPV alone was detected in 16 sera. Antibody to PCV or Group A rotavirus alone was not detected in any foetal sera.

No PCV antigen was detected in any of the cryostat sections of tissues from pig foetuses or stillborn piglets.

PCV was isolated in cell cultures from 2 foetal serum samples and a spleen sample. These sera were separate pooled samples from 2 litters of stillborn piglets from the same farm. The first submission comprised 4 stillborn piglets, delivered with assistance following a dystocia, and the second a further 6 stillborn piglets delivered normally. The spleen sample was also derived from a stillborn piglet from the second litter. PCV-immunopositive cells were detected in cell cultures inoculated with the serum samples 3 days after inoculation and in the cultures inoculated with the spleen sample 3 days after the first passage of cell lysate. PCV antigen was detected in the nucleus and cytoplasm of a few cells in each culture. Passage of cell lysates from these cultures resulted in an increase in the number of PCV-immunopositive cells and negative contrast electron microscopic examination of cell lysates

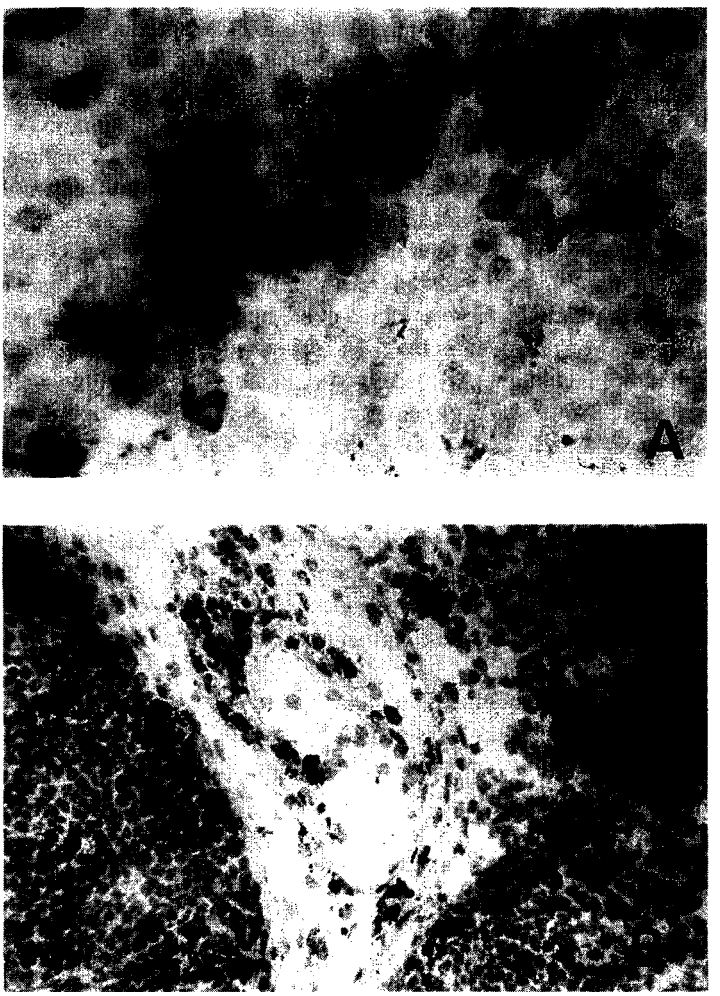


Fig. 2. Immunoperoxidase staining of PCV antigen in thymus of pig inoculated by the combined venous/nasal/oral route and killed 9 days after inoculation. Note; PCV antigen in large, process containing cells (A) and PCV antigen in cells in connective tissue (B).

from these cultures revealed the presence of small (17 nm), non-enveloped, icosahedral virus particles (Fig. 3) with no obvious surface morphology.

3.3. mAb staining pattern of suspected field isolates of PCV

Field isolates of PCV were immunostained with a panel of 7 PCV-specific mAbs and the staining patterns compared to those obtained with PCV derived from the PK/15/W cultures. Immunostaining patterns obtained with the field isolates of PCV were similar, but not identical, to those seen when the PK/15/W derived PCV-infected cultures were examined. Cultures of all 3 isolates immunostained in an identical manner. In general, no pin-like inclusions were seen in any of the cultures infected with the field isolates of PCV. Pin-like

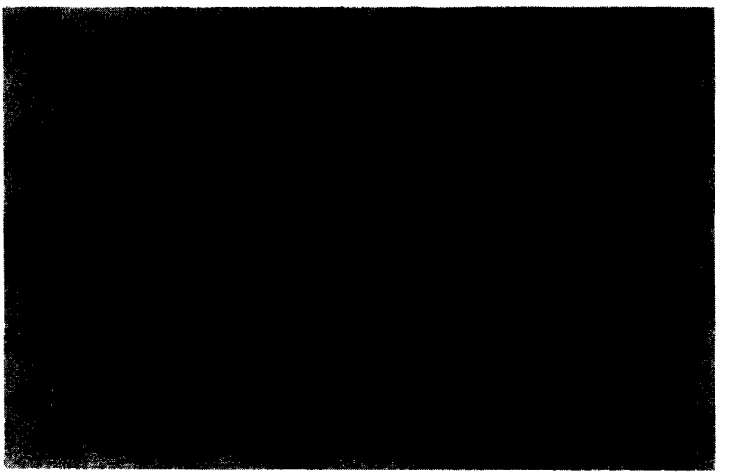


Fig. 3. Electron micrograph of negatively stained cell lysates from cell cultures inoculated with a pooled serum sample from still-born piglets. Note: presence of PCV-like virus particles.

inclusions were seen in the vast majority of cells in cultures derived from the PK/15/W following immunostaining with the mAbs. In addition, distinct differences in nuclear and cytoplasmic staining patterns of PCV antigen were seen following immunostaining with mAb 4B1 (Fig. 4A and B) and mAb 2E10 (Fig. 4C and D).

4. Discussion

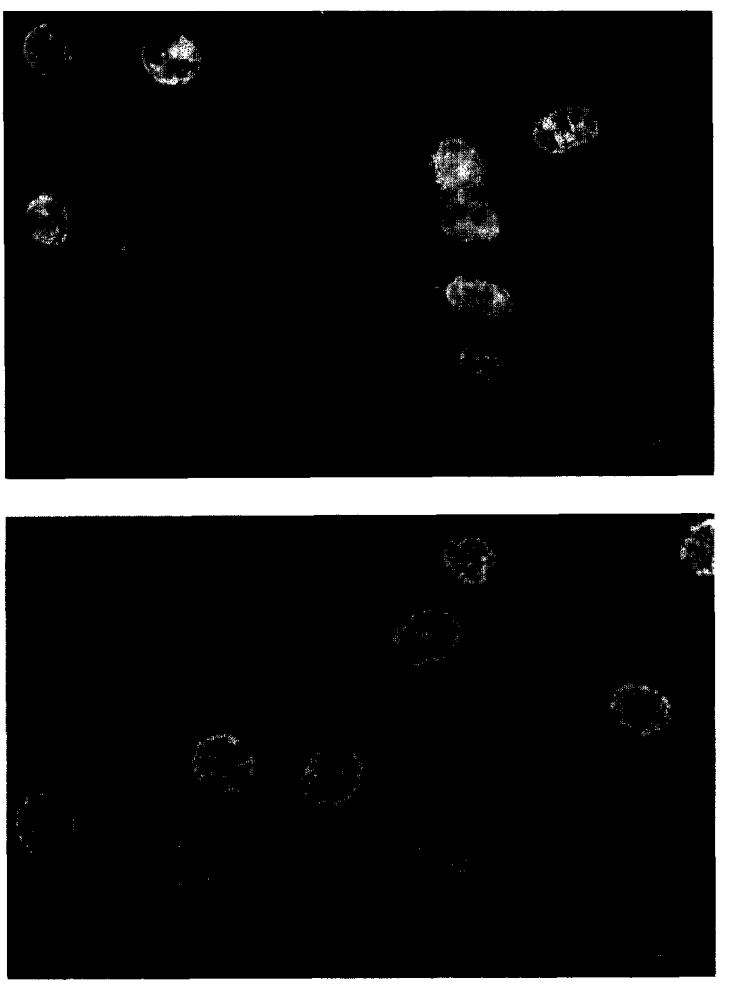
The results of PCV distribution in tissues, following experimental infection of CD pigs, are reported in this paper. In a previous study, Tischer et al. (1986) reported no clinical illness and failed to detect the sites of replication of PCV following experimental infection. In the present study CD pigs were used, primarily because of the inability to locate a PCV-negative antibody herd in N Ireland. No clinical signs, attributable to PCV infection, were seen in any of the pigs inoculated with virus, confirming the results of Tischer et al. (1986), and no gross lesions associated with PCV were seen at post mortem examination. However, in contrast to the results of Tischer et al. (1986), PCV antigen was demonstrated in a range of tissues taken from pigs from all 3 inoculated groups and, in general, virus isolation of PCV agreed with the antigen detection results.

The failure of Tischer et al. (1986) to demonstrate PCV antigen in tissue samples from experimentally infected pigs may have been due to the sampling schedule employed by these workers. Although these workers recovered PCV from faecal and nasal swabs taken from animals between 3 and 13 days after inoculation no animals were sacrificed for virus distribution studies until 6 weeks after inoculation. It is unlikely that PCV antigen could be demonstrated this late in an infection. Cell cultures from kidneys and lungs from some infected animals were also processed for detection of PCV and found negative, indicating the absence of persistent or latent infection with PCV.

In the present study 3 groups of CD pigs were inoculated with PCV using varied inoculation schedules. The combined intravenous/oral-nasal route used for inoculation of piglets

in Group A was chosen to maximise virus access to tissues and cell types susceptible to infection with this virus. The intravenous route used for inoculation of day-old CD pigs in Group B was chosen in an attempt to reproduce the effects of vertical transmission of PCV at a late stage of gestation. The oral/nasal route used for inoculation of piglets in Group C was chosen in an attempt to reproduce the effects of horizontal transmission of PCV under field conditions.

PCV antigen was detected in cells in a range of tissues from the lymphoid system, lung and intestine following experimental infection of piglets in Group A and B. Virus predominated in spleen, thymus and lung tissue. Pathogenicity studies on the other two animal circoviruses, CAV (Smyth et al., 1993) and PBFDV (Latimer et al., 1990) have indicated widespread distribution of viral antigen in tissues following experimental and natural infections. Smyth et al. (1993) reported the detection of CAV antigen in many tissues, but usually within lymphoid tissue therein. Latimer et al. (1990) also reported the detection of PBFDV antigen throughout the body of thirty-five psittacine birds with PBFD, but primarily



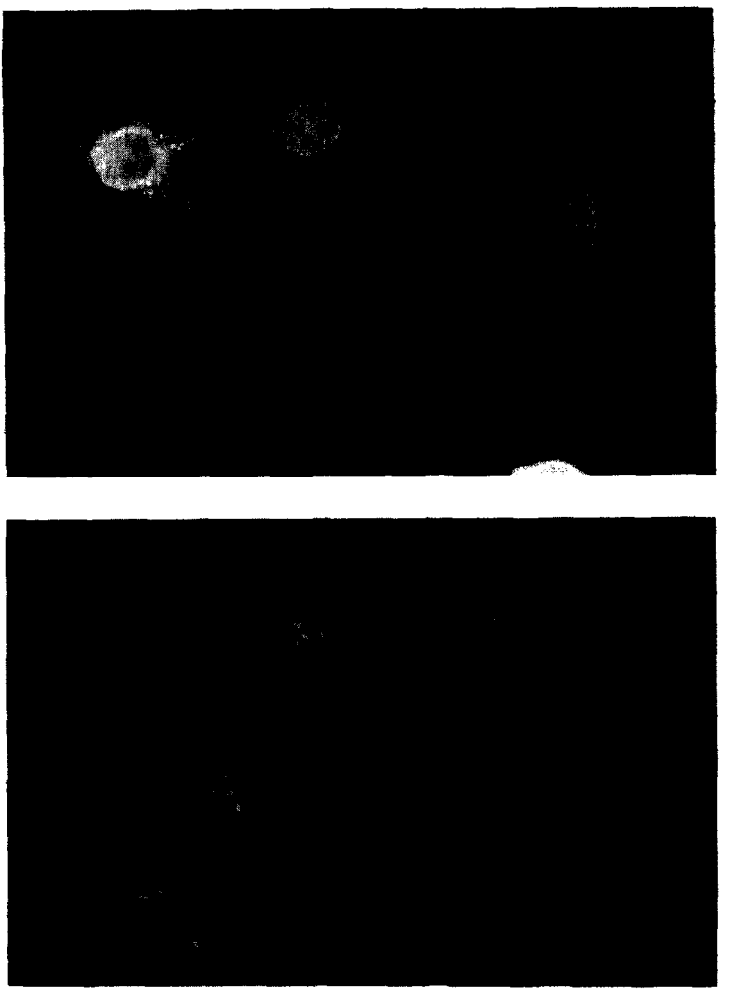


Fig. 4. Immunostaining patterns obtained following application of PCV-specific mAb 4B1 or 2E10 to cell culture preparations of PK/15/W derived PCV (A and C) and a PCV field isolate (B and D). Note: different staining patterns between field isolate and laboratory strain of PCV.

within macrophages in these tissues. This affinity of animal circoviruses for tissues and cells of the immune system appears to be a consistent finding. Although exact interpretation of cell types is difficult using FITC-labelled cryostat sections, it appeared that PCV antigen was located almost exclusively in non-epithelial cell types. No PCV antigen was detected in tissue samples from the CNS. Examination of selected IP-stained sections confirmed this observation, however double immunolabelling of PCV antigen and cell membrane antigens will be necessary to facilitate unequivocal identification of cell types infected with PCV. The distribution and quantity of PCV antigen in tissues of the lymphoid system from the piglet in Group A, killed 9 days after inoculation, merits special mention. These tissues, especially sections from the thymus, contained substantially more PCV antigen than any other tissue samples examined from piglets in Groups A, B or C. Although no histological

changes could be observed in cryostat sections of thymus in these piglets, focal areas of large cells containing PCV antigen were seen. These PCV-infected cells had morphological characteristics similar to those described for macrophages, histocytes, and interdigitating cells or antigen presenting cells (Roitt et al., 1992). The thymus is of crucial importance in the development and maturation of T cells and immune functions. The cell types presumptively identified to contain PCV antigen all play an important function in the defence mechanisms against invasive pathogens and interdigitating cells are also thought to play a role in selection of self antigens during foetal development (Roitt et al., 1992). It is therefore possible that infection of these cells with PCV could severely compromise normal immune function.

The reason for this marked increase in PCV-positive cells in lymphoid tissue from this piglet is unclear. No gross or clinical abnormalities were observed in this animal, however it is possible that a sub-clinical infection with another infectious agent was occuring during this experiment, exacerbating the effect of PCV infection. Increased invasiveness of CAV has been demonstrated following dual infection with other avian viruses (Yuasa et al., 1980; Rosenberger and Cloud, 1989).

When compared with the combined intranasal/venous and intravenous only routes of inoculation, inoculation of CD piglets by the oral/nasal only resulted in a marked decrease in the quantity of PCV antigen detected in sections of tissues of the lymphoid system. However, cryostat section of lung tissue from pigs in group C were shown to contain substantially more PCV-positive cells than sections of lung from piglets in group A or B. Again, PCV antigen was confined to non-epithelial cells in lung tissue and predominated in cells with a morphology similar to alveolar macrophages.

The results of virus isolation studies on serum and plasma samples from the piglets in this group indicated a transient viraemia following infection with PCV, the virus probably being transported in the blood in infected monocytes.

The results presented in this paper on the examination of pig foetal material from reproductive disorders of pigs are the first documented attempt to investigate PCV as a possible foetopathogen. Recently, a conference abstract (Hines and Lukert, 1994) reported the association of PCV infection with congenital tremor in piglets. However, little detail was presented on experimental methods. These authors reported the isolation of PCV from piglets with congenital tremor and experimental transmission of the condition by inoculation of pregnant sows with this virus. No details were given on the time of gestation at inoculation.

The evidence from the present study would suggest that infection of pregnant sows with PCV and subsequent vertical transmission of PCV to immunocompetant pig foetuses does not occur, or, occurs rarely in N Ireland. PCV-specific antibody was not detected in the absence of PPV or group A rotavirus antibody in the 160 foetal sera tested in this study. Antibody to PPV alone was detected in 16 foetal sera, confirming occasional vertical transmission of this virus and, more importantly, the suitability of the serum samples used for testing. Antibody to PCV, PPV and Group A rotaviruses was detected in a number of foetal sera. However the presence of antibody to Group A rotavirus in these foetuses strongly suggests leakage of antibody across the placenta and not transplacental infection.

PCV antigen was not detected in any of the cryostat sections obtained from the 160 foetuses used in this study. However, PCV was isolated from a single spleen sample and 2

pooled serum samples collected from 2 separate submissions of stillborn piglets submitted to our laboratory. This is the first report of PCV isolation from stillborn piglets.

The failure to demonstrate PCV antigen in the tissue sections from these piglets suggests that either minimal amounts of PCV antigen were present in the tissues selected for examination or that inappropriate tissues were collected for immunostaining. The tissues chosen for collection from field material were selected following examination of samples from experimentally infected 1-day-old piglets. It is possible that transplacental infection with PCV at an early stage of gestation (prior to foetal immunocompetance) could result in a persistent infection of the foetus with a different distribution of virus and antigen to that encountered following infection at a later stage of gestation or at 1 day of age. Persistent infections of piglets with PPV, following transplacental infection before immunocompetence, have been indicated (Cartwright et al., 1971; Johnson and Collins, 1971).

The possibility that the field isolates of PCV reported in this present study were the result of laboratory contamination with PK/15/W-derived PCV was eliminated by comparing mAb staining patterns. Although staining patterns were similar with all 7 mAbs used, distinct differences between staining of PK/15/W derived PCV and the field isolates was observed. Extreme variations in staining patterns of PCV-infected Vero cells, compared to persistently infected permanent pig kidney cells, have been reported using these mAbs (Allan et al., 1994a). These changes presumably reflect epitopal changes in the antigenic structure of PCV following growth in a heterologous cell line. The exact nature of these changes and the structural elements recognised by the PCV mAbs are unknown. No other isolates of PCV were recovered from any of the foetuses tested.

The inability to find PCV antibody, in the absence of PPV and group A antibody, in the foetal sera tested in this study combined with the inability to detect PCV antigen in sections of tissues from foetuses strongly suggests that transplacental infection of pig foetuses with PCV is a rare occurrence in pigs in N Ireland. The wide distribution of antibody to this virus in adult pigs in the province and the finding that seroconversion to PCV in pigs in infected herds occurs before 20 weeks of age (Allan et al., 1994c) would suggest that, during pregnancy, the vast majority of sows are protected from an infection with PCV which results in vertical transmission of the virus. However, the isolation of PCV from stillborn piglets reported here and the results reported by Hines and Lukert (1994) on experimental transmission of congenital tremor following inoculation of PCV-seronegative pregnant sows with PCV is evidence that vertical infections of foetuses with PCV can occur and PCV should now be considered as a possible foetopathogen.

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