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Review

Discovery and evolving history of two genetically related but phenotypically different viruses, porcine circoviruses 1 and 2

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ABSTRACT

Porcine circoviruses (PCVs) belong to the genus *Circovirus*, family *Circoviridae*, and are the smallest non-enveloped, single stranded, negative sense, circular DNA viruses that replicate autonomously in mammalian cells. Two types of PCV have been characterised, PCV1 and PCV2 and these two viruses show 83% sequence identity at open reading frame (ORF) 1 and 67% identity at ORF2. PCV1 is a non-pathogenic virus of pigs. In contrast, PCV2 has emerged as a major pathogen of swine around the world. The discovery of PCV1 and how the subsequent studies on this virus eventually led to the recognition and characterisation of PCV2, and the disease scenarios associated with PCV2, serve as a model of how multidisciplinary collaboration among field veterinarians, diagnosticians and researchers can lead to the rapid characterisation and control of a globally important emerging disease.

Contents

1. Introduction

Porcine circovirus (PCV) was first described by Tischer et al. (1974) as a picornavirus-like contaminant of the permanent pig kidney cell culture PK/15 (ATCC-CCL 33). No further publications on this picornavirus-like virus were forthcoming until the same group of researchers reported the isolation and characterisation of a new small, non-enveloped icosahedral porcine circovirus containing a circular, single-stranded DNA genome (Tischer et al., 1982). Following publication of this important paper a small body of research accumulated on what we now recognise as the non-pathogenic porcine circovirus type 1 (PCV1). The isolation and characterisation of a "new" PCV, now known as PCV2, was first reported by Ellis et al. (1998) and heralded a new era of research into porcine circoviruses that continues today. This short review article will highlight the research efforts that led to the "discovery" of both PCV1 and PCV2 and evaluate the impact this research has had on swine health and production over the last 10 years.

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2. PCV 1

2.1. First identification of a contaminant of PK/15 cells

PCV was first encountered by Tischer et al. (1974) during an investigation into the occurrence of viral-like particles in uninoculated cultures of the permanent porcine kidney cell line PK/15 (ATCC-CCL 33). These workers reported the characterisation of parvovirus-like and picornavirus-like particles in some PK/15 cell lines. It is of interest to note that, at the time and using the technologies available, what was initially described as a picornavirus-like virus with an RNA genome eventually was identified as porcine circovirus with a DNA genome. The nature of the genomic nucleic acid of the picornavirus-like contaminant was determined by radioactive labelling followed by centrifugation on a CsCl gradient. Subsequent to this publication, and notably 8 years later, the same group of researchers published the seminal paper on the discovery and characterisation of a small, non-enveloped, icosahedral virus with a circular single stranded DNA genome, proposing the name porcine circovirus (Tischer et al., 1982). In this study characterisation of the viral nucleic acid using [Me-^{3H}]-thymidine-labelling, buoyant density and alkali stability demonstrated that the nucleic acid of PCV was DNA. This was confirmed by the ability of DNase-1 to completely abolish the infectivity of viral nucleic acid used in transfection studies and further clarified as being single-stranded by the observation that the single-stranded-specific nuclease S^1 degraded purified viral nucleic acid. Electron microscopic examination of PCV DNA revealed that more than 85% of the molecules were circular and, from contour length measurements, a genomic size of 1.76 kb was estimated. Sedimentation behaviour and the lack of detectable linearization following heat denaturation confirmed that PCV DNA was a covalently closed, circular DNA molecule. The complete nucleotide sequence (1759 nt) of the circular genome of PCV1 was first published in 1985 (Buhk et al., 1985) and later confirmed by others (Meehan et al., 1997; Niagro et al., 1998). Since this first isolation and characterisation of what we now know as PCV1, two further isolates have been recovered from pig foetal material and cultured *in vitro* (Allan et al., 1995), a PCV1 virus has been isolated from pigs with a wasting disease in France (LeCann et al., 1997) and a PCV1 PCR product has been recovered from wild boar (Csagola et al., 2006). A PCV1 virus was isolated from a commercial human rotavirus vaccine (Victoria et al., 2010).

2.2. First period of research forms a foundation for future discoveries

Following the first isolation and characterisation of the PCV contaminant of PK/15 cells (Tischer et al., 1982) a small body of research findings on this virus accumulated over the next 15 years. Serum antibodies to PCV1 were demonstrated in pigs in Germany (Tischer et al., 1995), Canada (Dulac and Afshar, 1989), New Zealand (Horner, 1991), Great Britain (Edwards and Sands, 1994) and Northern Ireland (Allan et al., 1994), indicating that infection of pigs with this virus was widespread. However, in hindsight, much of the seropositivity to PCV1 in these tests, especially those detected by indirect immunofluorescence (IIF) and immunoperoxidase (IIP) assays, was probably the result of a cross reactivity of high titre PCV2 antibodies with PCV1 antigen, since the apparent prevalence of PCV1 in pigs in the field is low (Ellis et al., 2000). In one study, serum antibodies to PCV1 were not detected in cattle, sheep, chickens, turkeys, goats, mice, rabbits or humans (Allan et al., 1994). In another study PCV1 antibodies were detected in humans (30.2%), mice (12–69%), and cattle (35%) in Germany (Tischer et al., 1995); however, this analysis was done using tests with questionable ability to discriminate between PCV1 and PCV2. Examination of the photographs in this manuscript (Tischer et al., 1995) would suggest that some of the reactors detected in serum from the cattle and humans in this study may not have been specific for PCV1.

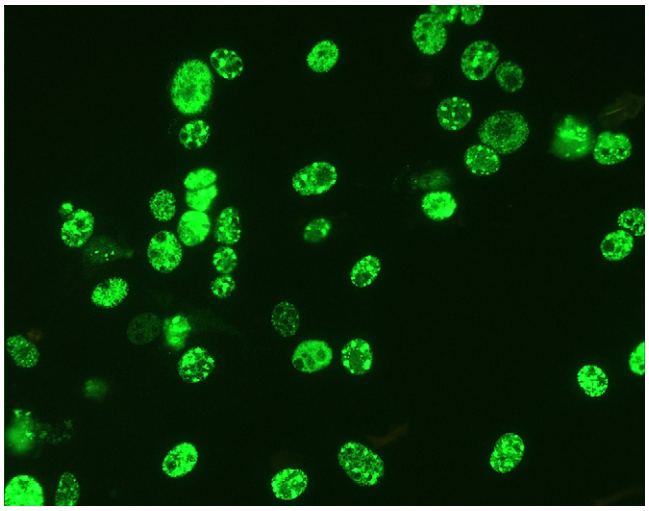

Fig. 1. Immunofluorescent staining of PCV1 antigen in acetone-fixed PK/15 cell cultures with a monoclonal antibody to PCV1. Note immunostaining of PCV1 antigen in the nucleus of infected cells.

PCV1 is now generally accepted to be a non-pathogenic virus. Evidence for this was gleaned from field survey work on porcine foetal material (Allan et al., 1995) and experimental infection studies of minipigs and 1-day old piglets (Tischer et al., 1986) and 2-day-old colostrum-deprived pigs (Allan et al., 1995). Experimental infection of gnotobiotic pigs with PCV1 failed to produce lesions and/or clinical disease (Krakowka et al., 2000). The suggested association of PCV1 with congenital tremors (type A2) (Hines and Lukert, 1994) has not been supported by any other studies.

Monoclonal, polyclonal antibodies and in situ hybridization (ISH) probes to PCV1 were prepared and characterised (Allan et al., 1994, 1995), these reagents were used to confirm the isolation of field strains of PCV1 from porcine foetal material (Fig. 1) by Allan et al. (1995) and, more importantly, were critical tools in the characterisation and differentiation of the first isolate PCV2.

Studies on the replication strategies of PCV1 were also performed after the initial isolation of the PK/15 contaminant. The virus replicates via a double stranded replicate form (RF) (Tischer and Buhk, 1988). Both strands of this RF are transcribed and encode proteins (Meehan et al., 1997). Seven open reading frames (ORFs) for PCV1 with the potential to encode proteins greater than 5 kDa, encoded on either strand of the PCV RF were recognised (Mankertz et al., 1993). PCV1 DNA replication was shown to be dependent on cellular enzymes expressed during S phase of growth of pig kidney cell cultures and it was suggested that PCV DNA replication starts only when the cells have passed mitosis (Tischer et al., 1987). However the same authors demonstrated that the treatment of infected cell cultures with 300 mM D-glucosamine-HCL reduced the requirement for mitotic activity and enhanced viral growth.

The importance of the relatively small body of research on PCV1 outlined above should not be underestimated. Without the seminal studies by Tischer and her group in Germany and the preparation of PCV1 specific reagents (antibodies and ISH probes) the foundations and expertise would not have been in place to facilitate the discovery of PCV2.

3. PCV2

3.1. First descriptions of an emerging wasting disease in swine

The first known outbreak of an undiagnosed wasting disease in swine was seen in western Canada in 1991 on a 40 sow farrow to