

PMWS/PCV2: Experimental Reproduction of Disease and Field Studies

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A Brief History of PCV1/PCV2

- **PCV1**

- **1974**; picornavirus-like virus reported in pig cell cultures
- **1982**; first report of PCV1 in pig cell cultures
- **1983-95**:
 - Antibody in pigs worldwide
 - No disease association
 - Virus “grows” *in -vitro* in actively dividing cells and pig mononuclear cells
 - Virus has some slight effect on pig immune system
 - Work on PCV1 virtually stops

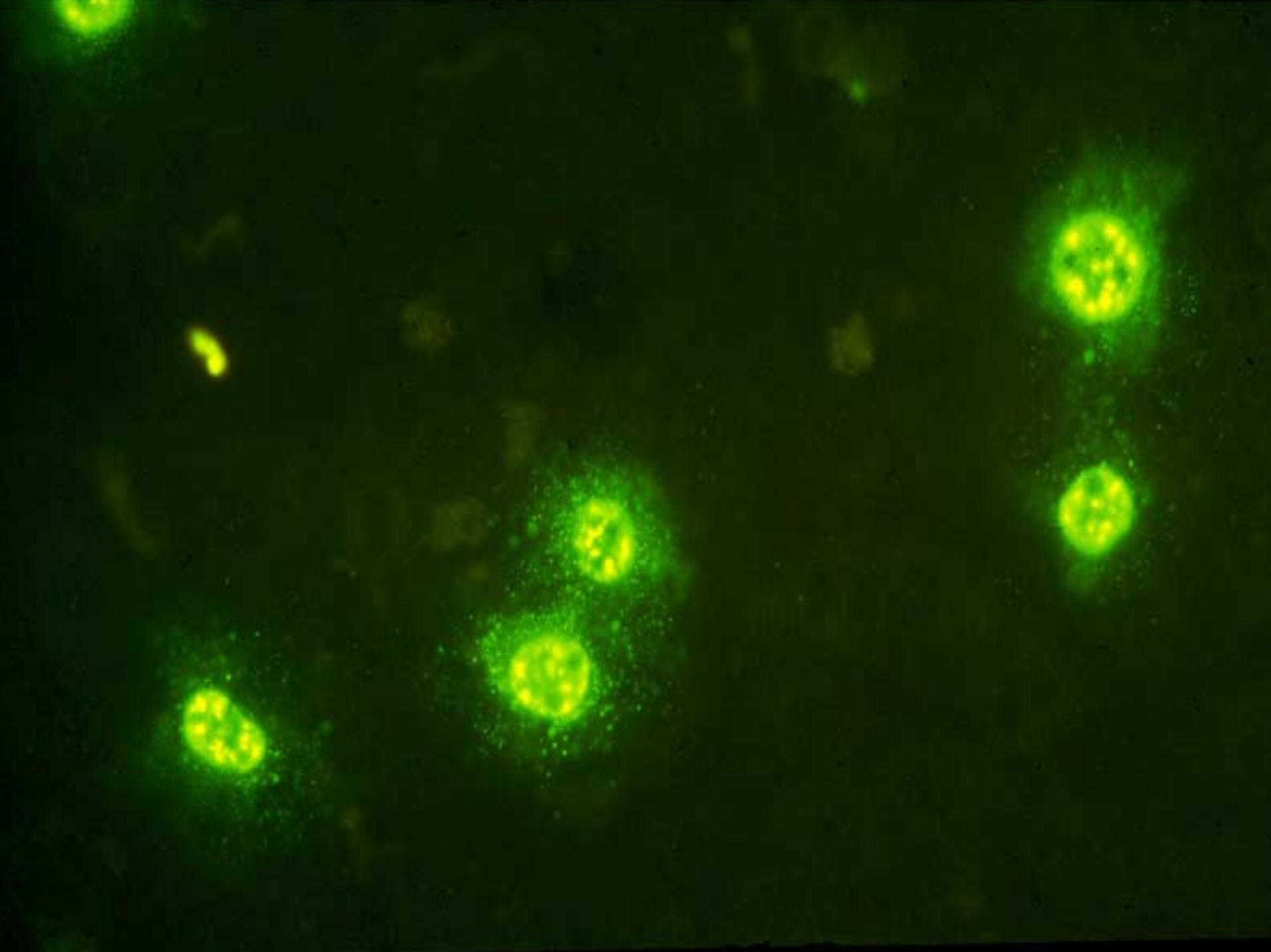
- **PCV2**

- **1996-97**; Wasting disease in pigs reported in Canada, France, Spain, USA
- **1997**; Named postweaning multisystemic wasting syndrome (PMWS)
- **1997-**; “New” PCV isolated from PMWS cases in Canada, USA, France, Spain, Denmark USA (PCV2).
- PCV2 antibody demonstrated world wide
- Experimental infection with PCV2 = PMWS
- **1998/9**; PCV2-specific reagents and tests available for diagnostics

Tests and Reagents Available for Study of PCV/PMWS (1997-2001)

- Quantitative PCV1 and PCV2-specific **antibody detecting** ELISAs (Walker et al 200)
- Quantitative PCV1 and PCV2-specific **antigen detecting** ELISAs (McNeilly et al., 2001)
- Monoclonal and polyclonal antibodies to PCV1 and PCV2 for **immunostaining** of tissue sections (Allan et al., 1999)
- Serum neutralisation assay for PCV2 to **differentiate serotypes**
- Real-time, **quantitative PCR** tests for PCV1 and PCV2 (Meehan et al., 2000)
- Probes for in-situ hybridization (McNeilly et al., 2000)
- Pools of purified PCV **cell culture grown isolates** from N America, Europe and the Far East (Allan et al., 1998).





Is PCV2 a “New” Virus?

- PCV2 is antigenically and genomically distinct from PCV1 (Meehan et al., 1998)!
- Antibodies to a PCV2 virus have been found in pigs in UK and elsewhere from at least 1974 (Walker et al., 2000)
- PCV2-associated PMWS-like diseases has now been reported retrospectively in Spain and UK from at least 1986 (Higgins et al., 2001)
- **PCV2 is not a new virus and PMWS is not a new disease!** We (UK) did not “import” this virus from Canada in the late 1990’s
- PCV2 is a newly recognised virus!
- PCV2-associated diseases in pigs have exploded in the last 3 years. Why?

PCV2 and Field Disease

- Wasting syndromes (PMWS). ✓ ✓ ✓
 - Dermatitis nephropathy syndrome (PDNS). ?
 - Proliferative necrotizing pneumonia (PNP). ?
 - Reproductive disorders. ✓
 - Post weaning PRRS. ✓?
 - Type A2 congenital tremor (CT). ?
 - Exudative epidermatitis (greasy pig disease). ?
 - SAMS ?
 - Myocarditis ✓?
- Diagnosticians should consider PCV2 in differential diagnosis of a wide range of conditions. We need MORE information!

PCV2/PMWS: Experimental Infections. Is PCV2 the Causal Agent of PMWS?

- Koch's Postulates

- To establish the causative relation of PCV2 to a given disease.

- 1) PCV2 must be present in **every case** of the disease
 - 2) PCV2 must be isolated and cultivated in pure culture
 - **3) *Inoculation of PCV2 pure cultures must produce the disease in susceptible animals***
 - **4) *PCV2 must be observed in, and recovered from, the experimentally diseased animal***

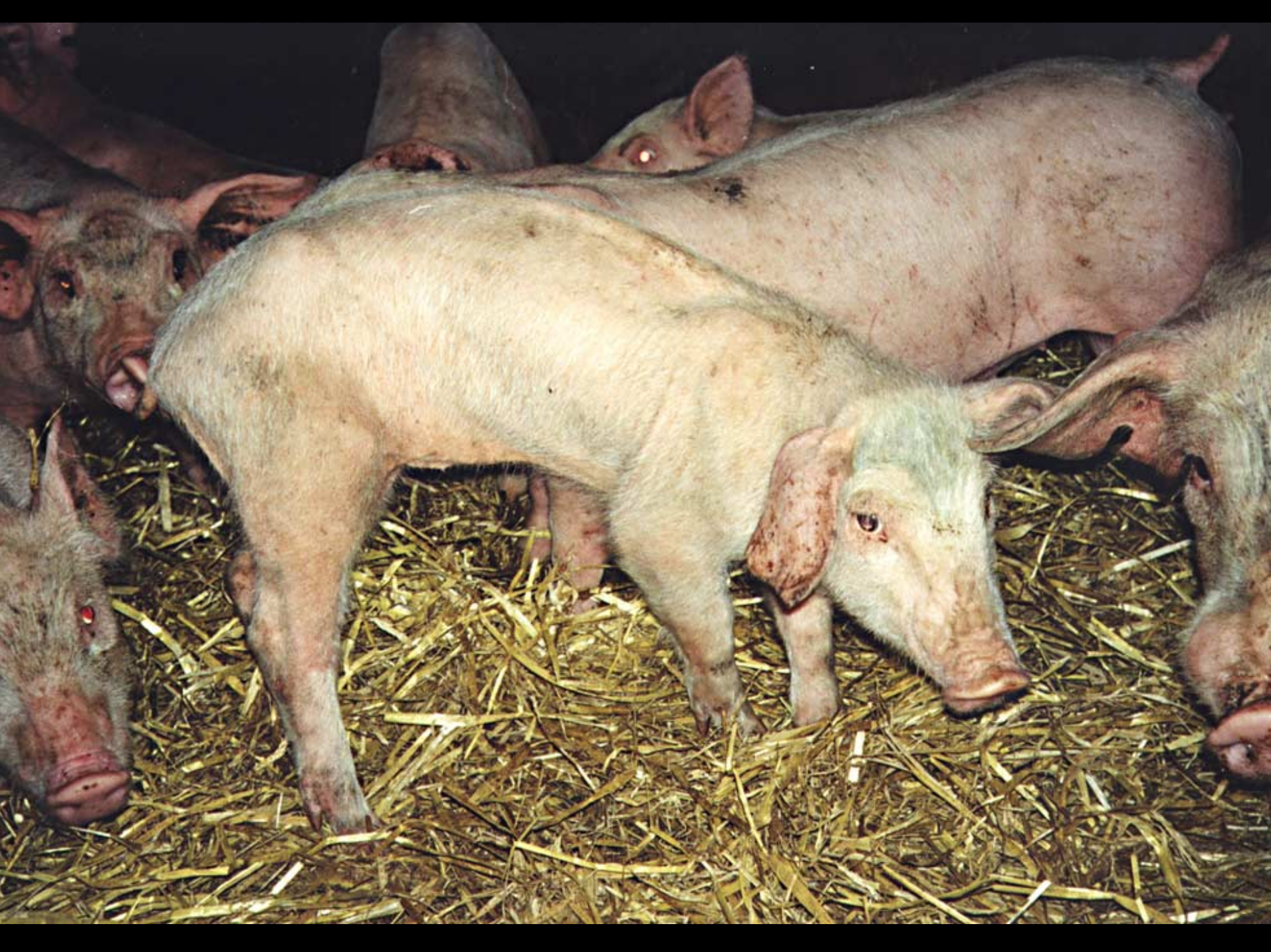
Note: Koch's Postulates DO NOT STATE that the infectious agent under investigation should be absent from clinically normal animals. Example; porcine parvovirus

PCV2 and Experimental Infections (Belfast/Saskatoon/OSU) 1997-2000

- PCV2 alone in colostrum-deprived (CD) and gnotobiotic pigs
- PCV1 alone in CD and gnotobiotic pigs
- PCV1+PCV2 in CD and gnotobiotic pigs
- PRRSV alone in CD pigs
- PCV2 + PRRSV in CD pigs
- PPV alone in CD and gnotobiotic pigs
- PCV2 + PPV in CD and gnotobiotic pigs
- PCV1 + PPV in CD and gnotobiotic pigs
- PCV2 alone in immunosuppressed gnotobiotic pigs
- ***PCV2 alone in immunostimulated gnotobiotic pigs***
- PCV2 alone in pregnant sows
- PCV2 alone in pig foetuses
- PCV2 alone in colostrum-fed seronegative and seropositive conventional pigs.

Results of Experimental Infections (1997-2000)

- PCV2 alone (cell culture grown and purified) in gnotobiotic pigs = **no clinical disease and mild lesions**
- PCV2 + PPV in gnotobiotic pigs = **clinical PMWS and severe lesions in all inoculates**
- PCV2 alone in CD pigs = **clinical disease and lesions in 15-25%** of inoculates. Mild lesions in 75% of inoculates and no clinical disease
- PCV2 + PPV in CD pigs = clinical disease and severe lesions in 100% of inoculates
- PCV2 + PRRSV in CD pigs = moderate to **severe histological lesions and clinical disease**
- PRRSV/PPV/PCV1 alone or in combination in gnotobiotic and/or CD pigs = no clinical diseases and minimal lesions.
- Lesions in diseased animals full of PCV2 antigen. Little or no PPV/PRRSV antigen!
- **IMMUNOSUPPRESSION????**



Results* of immunofluorescent staining for porcine parvovirus (PPV) antigen in cryostat sections of tissues from pigs dually infected with porcine circovirus 2 (PCV2) and PPV at 2 days of age.

	Days after inoculation													
	3		6		10		14		17		21		26	
<i>Pig number -</i>	142	145	113	114	117	118	127	128	150	151	155	156	160	
Trachea	-	-	-	-	-	-	NT	-	-	-	-	-	-	
Lung	-	-	-	-	-	-	+	-	-	+	-	-	-	
Tonsil	-	-	-	+++	-	++	NT	-	-	-	-	-	-	
Thymus	-	-	-	NT	-	-	+	-	-	+	-	+	-	
Spleen	-	+	+	++	+	-	+	-	-	-	-	-	-	
Mesenteric lymph node	+	+	+++	+	+	+	+	-	-	+	+	+	-	
Parotid lymph node	-	-	-	NT	-	-	+++	+	+	+	-	+	-	
Salivary gland	-	+	-	-	-	-	+++	++	-	-	-	-	-	
Liver	-	-	-	+	-	-	-	+	+	+	-	++	+	
Kidney	-	-	-	-	-	-	+	-	+	+++	+	++	++	
Adrenal	-	+	-	+	+	-	+	NT	+	+	-	+	+	
Pancreas	-	-	-	-	-	-	-	+	+	+	-	-	+	
Cecum	-	+	-	+++	+	+	+	-	+	+	-	+	+	
Colon	-	-	-	-	+	++	+	+	-	-	-	-	+	
Small Intestine	-	-	+	+++	++	NT	++	+	-	+	-	+	+	
Brain	-	-	-	-	-	-	-	-	-	-	-	-	-	

*- = no PPV antigen detected; the number positive of cells in each tissue section is scored from a single + indicating low levels of PPV antigen detected increasing to a +++ indicating moderate levels of PPV antigen detected; NT = not tested

Results* of immunofluorescent staining for porcine circovirus 2 (PCV2) antigen in cryostat sections of tissues from pigs dually infected with PCV2 and porcine parvovirus (PPV) at 2 days of age.

<i>Pig number -</i>	Days after inoculation												
	3		6		10		14		17		21		26
	142	145	113	114	117	118	127	128	150	151	155	156	160
Trachea	-	-	-	-	-	-	NT	-	+	+	+	+	++
Lung	-	-	-	-	+	-	+	+	++	++++	++++	++++	++++
Tonsil	-	-	-	-	-	-	-	-	-	++	++	+++	+
Thymus	-	-	-	-	-	-	+	-	++++	+++	++++	++	++++
Spleen	-	-	-	-	-	-	+	+	+++	++++	+++	+++	+++
Mesenteric lymph node	-	-	-	-	+	-	+	-	++++	++++	+++	+++	+++++
Parotid lymph node	-	-	-	-	-	-	+	+	++	++++	+++	+	+++++
Salivary gland	-	-	-	-	-	-	+++	+	+	++	++++	+++	++
Liver	-	-	-	-	-	-	+	+	++	+++	+++	++	++++
Kidney	-	-	-	-	-	-	+	-	++	++++	+++	++++	++++
Adrenal	-	-	-	-	-	NT	-	NT	+	+	+	+	++
Pancreas	-	-	-	-	-	-	-	+	+	++	+++	+	+
Cecum	-	-	-	-	-	-	-	-	+	+++	++	+++	++
Colon	-	-	-	-	-	-	-	-	+	+++	++	+	+++
Small Intestine	-	-	-	-	-	-	+	-	+++	+++	++	+	++++
Brain	-	-	-	-	-	-	-	-	-	+	+	+	+

*- = no PCV2 antigen detected; the number of positive cells in each tissue section is scored from a single + indicating low levels of PCV2 antigen detected increasing to a +++++ indicating high levels of PCV2 antigen detected; NT = not tested

Results of immunofluorescent staining for porcine circovirus 2 (PCV2) and porcine reproductive and respiratory virus (PRRSV) antigens in cryostat sections of tissues from pigs experimentally infected with PCV2/PRRSV

	154		166		171		177		178	
	PRRSV	PCV2	PRRSV	PCV2	PRRSV	PCV2	PRRSV	PCV2	PRRSV	PCV2
Spleen	- [#]	++	-	-	-	+	-	+++	-	+
Trachea	-	+	-	-	-	-	-	-	-	-
Lung	+	++++	-	+	-	+++	-	++	-	+++
Tonsil	-	+	-	+	-	+++	-	+++	-	++
Thymus	-	-	-	-	-	++	-	++	-	+
Ing lymph node	-	ND	-	ND	-	+++	-	+++	-	+++
Mes lymph node	-	+++	-	+	-	++++	-	++++	-	+++
Par lymph node	-	+++	-	+	-	++++	-	+++	-	+++
Salivary gland	-	++	-	-	-	-	-	+	-	+++
Liver	-	+	-	-	-	+	-	+	-	+
Kidney	-	+	-	-	-	+	-	+	-	+
Adrenal	-	+	-	-	-	+	-	+	-	+
Pancreas	-	-	-	-	-	-	-	+	-	-
Cecum	-	-	-	-	-	-	-	+	-	-
Colon	-	-	-	-	-	-	-	+	-	-
Small Intestine	-	+++	-	+	-	++	-	+++	-	++
Brain	-	-	-	-	-	-	-	-	-	-
Bone marrow	-	+	-	-	-	-	-	+	-	+