Detection of Porcine Circovirus Types 1 and 2 in Serum and Tissue Samples of Pigs with and without Postweaning Multisystemic Wasting Syndrome

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Presence of porcine circovirus type 1 (PCV1) and PCV2 was studied in sera and superficial inguinal lymph nodes from postweaning multisystemic wasting syndrome (PMWS)-affected and non-PMWS-affected pigs by using in situ hybridization and PCR. PCV1 and PCV2 were found in less than 3% and more than 50% of the samples, respectively. The most sensitive technique and site was PCR in superficial inguinal lymph nodes, but in situ hybridization correlated better with presence of characteristic lesions.

Postweaning multisystemic wasting syndrome (PMWS) is a disease that affects late nursery and fattening pigs (3, 18, 24). The disease is associated with porcine circovirus type 2 (PCV2), whereas porcine circovirus type 1 (PCV1) is considered nonpathogenic. Nevertheless, serological surveys have indicated that antibodies to PCV1 are very common in the swine population (1, 3, 7, 21, 22). Other studies indicate that cross-reactions exist between PCV1 and PCV2 by using indirect immunofluorescence (3) and immunoperoxidase monolayer assay (15), therefore overestimating the prevalence of PCV1 antibodies in the field.

Diagnosis of PMWS is based on the presence of compatible clinical signs (6), characteristic histopathological lesions (16), and detection of PCV2 within characteristic lesions (19). However, these three criteria separately are nondiagnostic of PMWS: PCV2 infection does not necessarily imply disease (9, 14), and clinical signs are nonspecific and variable. Usually, a sudden increase in the mortality rate in the early fattening stage is observed (11), and affected pigs show wasting with or without respiratory signs, diarrhea, paleness of the skin, and icterus. Microscopically, the most relevant features are lymphocyte depletion and histiocytic infiltrates in lymphoid organs (16).

Virus can be detected using several techniques, such as immunohistochemistry (4, 16), in situ hybridization (13, 16), indirect immunofluorescence (2), PCR (8, 12, 14), PCR combined with restriction fragment length polymorphism (5), and virus isolation (20). Rapid PCV detection in blood samples is usually performed by PCR, whereas viral detection in tissues can be performed by in situ hybridization, immunohistochemistry, and PCR. Although differences in sensitivity among tests exist, different diagnostic tests and samples are currently used in diagnostic laboratories.

The objectives of this study were (i) to evaluate the presence of PCV1 and PCV2 in porcine sera and tissues by using a type-specific PCR, (ii) to determine the agreement between

the different diagnostic criteria of PMWS (presence of microscopic lesions consistent with PMWS and PCV detection in tissues [in situ hybridization and PCR] and in serum [PCR]), and (iii) to compare PCV detection in tissues by in situ hybridization and PCR, two currently used techniques for the diagnosis of PMWS.

The clinical samples used in this study corresponded to swine submissions to the Veterinary Pathology Diagnostic Service of the Veterinary School of Barcelona from 1997 to 2000 with available serum and lymph node samples. A total of 135 animals from 65 different submissions were received for pathological examination. Pigs were bled at the jugular vein, and 10-ml blood samples were collected in a Vacutainer (Venoject; Terumo Europe, Leuven, Belgium). The sample was allowed to clot and then centrifuged, and the serum was frozen at -80°C until testing. At necropsy, a small piece of the superficial inguinal lymph node was collected in a 1.5-ml Eppendorf tube and immediately frozen at -80°C until DNA extraction. The rest of the lymph node and other tissue samples (including lungs, tonsil, spleen, liver, and kidney) were fixed in 10% buffered formalin, embedded in paraffin, and routinely processed for histopathology. Samples examined microscopically were scored positive when PMWS characteristic lesions were found: lymphocyte depletion with or without histiocytic infiltration in one or more lymphoid tissues.

In situ hybridization for PCV2 DNA detection was performed on lung, lymph node, and tonsil samples by following a previously described protocol (16). In a study performed in our laboratory, in which a total of 17 tissues from 50 PMWS-affected animals were examined by in situ hybridization for PCV2 detection, the inguinal lymph node was among the three tissues where PCV2 detection rate was the highest (17). For that reason, and because of the simplicity of its collection, this lymphoid organ was selected for PCR detection and comparison with the in situ hybridization result.

Serum samples ($n = 135$) and superficial inguinal lymph node samples ($n = 100$) were used for DNA extraction and PCV1- and PCV2-specific amplifications. DNA extraction from serum and superficial lymph nodes was performed using Qiagen DNA blood and Qiagen DNA mini Kit (Qiagen Inc.,

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TABLE 1. Correspondence between PCV2 detection by ISH and PCR and PCV2-characteristic lesions

PMWS lesions	Results for PCV2 detection in:								
	Lymph node						Serum		
	PCR			ISH			PCR		
	+	-	TC ^a	+	-	TC	+	-	TC
Positive	53	2	55	51	11	62	52	10	62
Negative	13	32	45	6	67	73	18	55	73
Total	66	34	100	57	78	135	70	65	135

^a TC, total counts.

Valencia, Calif.), respectively, by following the manufacturer's protocol. Amplification primers and protocol were used as previously described (14). The amplified products were run in a 2% agarose gel and were visualized by staining with 0.5 µg of ethidium bromide/ml.

Agreement between diagnostic tests and also between diagnostic sites was performed using the kappa statistic. The relative risk between different tests and sites was also calculated using EpiInfo software (World Health Organization, Geneva, Switzerland).

PCV2 was amplified from 70 of the 135 (52%) examined serum samples and from 66 of the 100 (66%) examined superficial inguinal lymph nodes. The viral genome was detected by in situ hybridization in 57 of the 135 (42%) examined cases. Characteristic PMWS lesions, from mild to severe in intensity, were found in 62 of these 135 (46%) animals. From the 135 cases examined for PCV1 infection, the virus was detected in three cases. In two, PCV1 genome was detected only in serum, whereas the corresponding tissues were found to be negative. In the third case, PCV1 genome was amplified from both serum and lymph node samples. In all three cases, PCV1 was concomitantly found with PCV2.

Comparison of PCV2 detection in serum (by PCR) with lymph node samples (by PCR and in situ hybridization) was studied. A substantial agreement ($K = 0.72$) between PCR in serum and in situ hybridization in tissues existed, while an almost perfect agreement between both sites existed when PCR was performed on serum and tissue samples ($K = 0.83$).

Table 1 shows correspondence of PCV2 detection in tissues, serum, and PMWS lesions. A substantial agreement was obtained between PCV2 detection in tissue by in situ hybridization ($K = 0.74$) or by PCR ($K = 0.69$) and presence of lesions. The chance of having lesions when PCV2 was detected by in situ hybridization in tissues was 10 times greater than in in situ hybridization-negative tissues. However, PCV2 PCR positivity in tissues was less indicative than in situ hybridization, since the relative risk of having lesions when PCV2 was detected by PCR was 3.34. A moderate agreement between PCV2 detection in serum and presence of lesions ($K = 0.58$) was obtained, and the relative risk of finding lesions in a viremic animal (as detected by PCR) was 3.4. The agreement between in situ hybridization and PCR viral detection in tissues was almost perfect ($K = 0.813$), although PCR was more sensitive than in situ hybridization.

These results indicate that, from the tested techniques and sites, PCR for superficial inguinal lymph nodes is the most

sensitive method for detecting PCV2 infection. However, serum samples are more convenient for epidemiological studies because they are available from live animals. Moreover, PCR with serum has an almost perfect agreement with PCR with the superficial lymph node. In addition, PCR from serum was more sensitive than PCV2 detection in tissue by using in situ hybridization. The high agreement between serum and lymph node detection suggests that viremia and presence in tissue occurs concomitantly in many cases. This finding indicates that viremia is at least as long as the presence of virus in lymphoid tissues.

Although a high agreement between in situ hybridization and PCR detection exists, PCR is more sensitive. On the other hand, in situ hybridization is more associated with the presence of PMWS lymphoid microscopic lesions than PCR with serum or tissues. However, 11 out of 62 cases with PMWS-positive lesions were PCV2 negative by in situ hybridization but positive by PCR. These cases could be interpreted as false-negative in situ hybridization results. Nevertheless, in all of these cases, the PMWS lesions were very mild. Other studies have reported the correlation between the amount of PCV2 nucleic acid or antigen and the severity of microscopic lymphoid lesions (10, 14, 16). Because PCR is more sensitive than in situ hybridization, it is not surprising that in cases with very mild microscopic lesions, PCV2 was detected by PCR and not by in situ hybridization. Recently infected pigs, convalescent PMWS cases, or subclinically infected animals may have very mild or no typical microscopic lesions. PCR is a better tool than in situ hybridization if the latter PCV2 infection conditions need to be diagnosed. However, in situ hybridization should be considered a better technique than PCR to diagnose clinical PMWS cases (19). In almost all cases where a disagreement between microscopic lesions and virus detection by in situ hybridization existed, either the lesions were very mild and no virus was detected or very little virus was detected and no lesions were observed.

This study also points out that PCV1 prevalence in the swine cases received was low, whereas PCV2 was detected in more than 50% of the pigs submitted for diagnostic purposes. PCV1 was found only in PCV2-positive animals, and no differences in history, clinical signs, or lesions were observed when compared with only PCV2-infected animals. These findings support that PCV1 is seldom found in field cases, although the clinical relevance of PCV1 field cases remains to be clarified.

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