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Review

Postweaning multisystemic wasting syndrome: a review of aetiology, diagnosis and pathology

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Abstract

This review paper concentrates on the aetiology, diagnosis, and pathological aspects of postweaning multisystemic wasting syndrome (PMWS). PMWS was first recognized in Canada in 1996 as a new emerging disease which caused wasting in postweaned pigs. Since then, PMWS has been recognized in pigs in many countries. The syndrome is caused by a DNA virus referred to as porcine circovirus 2 (PCV2), which is classified in the family Circoviridae. PMWS primarily occurs in pigs between 25 and 120 days of age with the highest number of cases occurring between 60 and 80 days of age.

The diagnosis of PMWS must meet three criteria: (i) the presence of compatible clinical signs, (ii) the presence of characteristic microscopic lesions, and (iii) the presence of PCV2 within these lesions. In order to establish the diagnosis, techniques are required that link virus and tissue lesions, such as immunohistochemistry and in situ hybridization, but not polymerase chain reaction or virus isolation. The three criteria considered separately are not diagnostic of PMWS. For example, the detection of PCV2 alone does not indicate PMWS but merely PCV2 infection. A hallmark of microscopic lesions of PMWS is granulomatous inflammation in the lymph nodes, liver, spleen, tonsil, thymus, and Peyer's patches. Large, multiple, basophilic or amphophilic grape-like intracytoplasmic inclusion bodies are often seen in the cytoplasm of macrophages and multinucleated giant cells.
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1. Introduction

In 1996, a new infectious disease in specific-pathogen-free (SPF) swine herds in Western Canada was identified and reported (Clark, 1997; Harding, 1997). Since then, postweaning multisystemic wasting syndrome (PMWS) has been recognized in pigs in Asia, North and South America, and Europe (LeCann et al., 1997; Segales et al., 1997; Spillane et al., 1998; Choi and Chae, 1999; Hinrichs et al., 1999; Onuki et al., 1999; Allan and Ellis, 2000; Choi et al., 2000; Kiss et al., 2000; Vyt et al., 2000; Wellenberg et al., 2000; Borel et al., 2001; Trujano et al., 2001; Allan et al., 2002; Celer and Carasova, 2002; Sarradell et al., 2002; Saoulidis et al., 2002) (Fig. 1). PMWS is an infectious viral disease of postweaned pigs characterized by progressive weight loss, respiratory signs and jaundice (Clark, 1997; Harding, 1997; Ellis et al., 1998; Allan et al., 1999; Allan and Ellis, 2000; Choi and Chae, 2000). The disease, which occurs in herds that are usually in otherwise in good health, has a low morbidity but a relatively high mortality rate among pigs in the 5- to 12-week-old age group (Harding and Clark, 1997; Allan and Ellis, 2000; Kim et al., 2002; Pallares et al., 2002). PMWS is now endemic in many swine-producing countries and continues to be a major cause of wasting disease in swine. This review concentrates on the aetiology, diagnosis, and pathological aspects of PMWS.

2. Aetiology

The viral families Circoviridae and Nanoviridae are DNA viral pathogens of plants, birds and swine (Lukert

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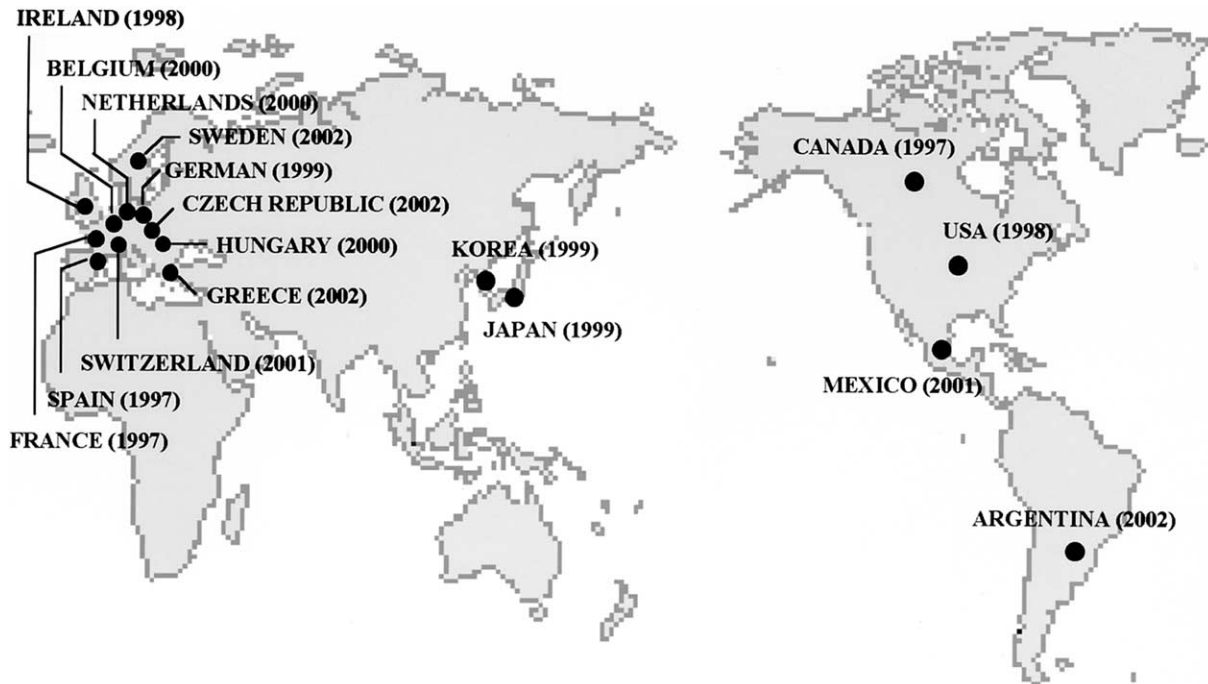 *E-mail address:* swine@plaza.snu.ac.kr (C. Chae).

Fig. 1. Occurrence of PMWS in the world.

et al., 1995). The Circoviridae contain two genera. The *Gyrovirus* genus is represented by chick anaemia virus (CAV) (Todd et al., 1990) which shows similarities to TT virus (TTV) (Miyata et al., 1999; Biagini et al., 2000; Okamoto et al., 2000) and TTV-like mini virus (TLMV) (Takahashi et al., 2000). The *Circovirus* genus contains porcine circovirus (PCV), psittacine beak and feather disease virus and Columbid circovirus of pigeons (Ritchie et al., 1989; Meehan et al., 1997; Niagro et al., 1998; Mankertz et al., 2000). Both genera are icosahedral nonenveloped virions and the characteristic feature of these viruses is the form of virion DNA (Lukert et al., 1995). Several plant circoviruses, now renamed Nanoviridae, include subterranean clover stunt virus, coconut foliar decay virus, banana bunchy top virus, faba bean necrotic yellow virus, and milk vetch dwarf virus (Rohde et al., 1990; Harding et al., 1993; Boevink et al., 1995; Katul et al., 1997; Sano et al., 1998).

PCV is the smallest virus that replicates autonomously in mammalian cells (Mankertz et al., 1997) and shares the distinctive genomic structure of a covalently closed, circular, negative sense, single-stranded DNA molecule (Todd et al., 1991; Studdert, 1993). PCV contains six open reading frames (ORFs) (Mankertz et al., 1997); ORF1 encodes a replication-associated protein essential for replication of viral DNA (Ilyina and Koonin, 1992) and ORF2 encodes major structural proteins (Nawagitgul et al., 2000; Meehan et al., 2001). Two types of PCV have been characterized and subsequently named PCV1 and PCV2 (Meehan et al., 1998). The overall DNA sequence homology between PCV1 and

PCV2 is 68–76% (Hamel et al., 1998; Meehan et al., 1998; Morozov et al., 1998). Between PCV1 and PCV2, ORF1 has more homology than ORF2, with 83% nucleotide homology and 86% amino acid homology. ORF2 is more variable, with nucleotide homology of 67% and amino acid homology of 65% (Morozov et al., 1998).

PCV1 is a persistent contaminant of porcine kidney cell lines, PK-15 (Tischer et al., 1974). Although PCV1 has been recovered from mummified fetuses and one case of wasting disease (Allan et al., 1995; LeCann et al., 1997), experimental infection of neonates with PCV1 has not produced clinical disease and it is considered a nonpathogenic virus (Tischer et al., 1986; Allan et al., 1995; Krakowka et al., 2000). In contrast to PCV1, PCV2 has been consistently detected in pigs with PMWS (Allan et al., 1998; Meehan et al., 1998; Morozov et al., 1998; Choi and Chae, 1999; Choi et al., 2000; Kim et al., 2001, 2002; Pallares et al., 2002). Both PCV1 and PCV2 share at least one common antigen but can be distinguished from each other using virus-specific polymerase chain reaction (PCR) (Larochelle et al., 1999; Ouardani et al., 1999), in situ hybridization (Kim and Chae, 2001a), or monoclonal antibodies (Allan et al., 1995; McNeilly et al., 1999).

Some authors have argued for the necessity of a co-infection (Allan et al., 1999; Choi and Chae, 2000; Harms et al., 2001; Kim et al., 2003), or cofactors (Allan et al., 2000; Krakowka et al., 2001; Kyriakis et al., 2001), for full experimental development of PMWS. Although pigs experimentally infected with PCV2 dis-

played clinical signs and lesions typical of the disease, co-infection with PCV2 and porcine parvovirus (PPV) also appeared to result in wasting disease (Allan et al., 1999; Ellis et al., 1999; Choi and Chae, 2000; Kennedy et al., 2000; Krakowka et al., 2000; Kim et al., 2003). However, histopathological changes characteristic of PMWS were induced with PCV2 alone and not with PPV alone (Allan et al., 1999; Kennedy et al., 2000; Krakowka et al., 2000; Magar et al., 2000), suggesting that PCV2 plays the major role. Furthermore, the consistent identification of PCV2 DNA and/or antigen, closely associated with lesions in a wide range of tissues from pigs with PMWS, has led to the belief that PCV2 is the aetiological agent (Meehan et al., 1998; Morozov et al., 1998; Choi and Chae, 1999; Choi et al., 2000; Kim et al., 2001; Kim et al., 2002).

3. Definition

Generally, diagnosis of viral diseases in swine is based on detection of the virus by culture, PCR, immunohistochemistry or in situ hybridization, and/or detection of virus antibodies by serology. However, diagnosis of PMWS is different from this general diagnostic approach because PCV2 can be detected in normal healthy pigs (Allan and Ellis, 2000; Kim et al., 2001; Calsamiglia et al., 2002). The detection of PCV2 alone does not necessarily confirm a diagnosis of PMWS. Therefore, the diagnosis of PMWS must meet three criteria: (i) the presence of compatible clinical signs, (ii) the presence of characteristic microscopic lesions, and (iii) the presence of the PCV2 within these lesions. These three criteria separately are not diagnostic of PMWS. Since clinical signs of PMWS are nonspecific and variable, the presence of PCV2 DNA or antigen in lymphoid tissues, demonstrated by in situ hybridization and immunohistochemistry (Choi and Chae, 1999; Choi et al., 2000; Kim and Chae, 2001b), together with moderate to severe lymphoid depletion and/or granulomatous lymphadenitis, are used as the criteria for the diagnosis. PMWS cannot therefore be diagnosed if lymphoid tissues are not submitted with the specimens provided.

4. Clinical signs

PMWS primarily occurs in pigs between 25 and 120 days of age, with most cases occurring between 60 and 80 days of age (Kim et al., 2002). In a recent survey in Korea, the prevalence was 8.1% (133 cases of PMWS out of 1634 cases examined), which is very close to the 10.3% (484 cases of PMWS out of 4868 cases examined) reported in the US (Pallares et al., 2002). Clinical signs are nonspecific and variable and those listed below include both field and experimental observations.

In weaned pigs, PMWS is characterized by wasting with or without respiratory signs, diarrhoea, paleness of the skin, or icterus (Clark, 1997; Harding, 1997; Ellis et al., 1998; Allan et al., 1999; Allan and Ellis, 2000; Choi et al., 2000), and a marked increase in mortality from single or multiple concurrent bacterial infections (Madec et al., 2000; Kim et al., 2002) in postweaning and early finishing pigs. Co-infection with single or multiple bacteria, and, less frequently, with other viruses, is a severe complicating factor of PMWS and is clinically important based on observation of increases in postweaning mortality from 1–2% to 10–25%, when all other factors apparently remain unchanged.

PMWS is often seen in combination with other viral and bacterial pathogens such as porcine reproductive respiratory syndrome virus (PRRSV), swine influenza virus, porcine parvovirus (PPV), *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae, Streptococcus suis* and *Mycoplasma hyopneumoniae* (Kim et al., 2002; Pallares et al., 2002). PCV2 infection alone was found in only 15% of PMWS cases. These co-infections may confuse or complicate the clinical presentation. Furthermore, the prevalent co-infecting agents appeared to vary in different countries. For example, in the Republic of Korea, PRRSV and PPV were the most common agents coexisting with PCV2 in cases of PMWS (Kim et al., 2002). However, only one of the 484 cases of PMWS was positive for PPV in the United States (Pallares et al., 2002). *H. parasuis* was the most prevalent bacterial co-infection in Korea and was found in 32.3% of cases, while in the US the most prevalent bacterial co-infection was *S. suis* in 5.5% of the cases. The fact that many different infectious agents are isolated in cases of PMWS strongly supports the view that a variety of pathogens may share a common mechanism in affecting the immune system (Allan et al., 2000; Krakowka et al., 2001; Kim and Chae, 2003a) and allow progression of PCV2 infection to PMWS. Alternatively, PCV2 may initiate lymphoid depletion, resulting in an increased susceptibility to other viral and bacterial infections. Immunosuppression has been confirmed in PMWS-affected swine (Chianini et al., 2003; Darwich et al., 2003).

5. Histopathology

Histopathologically, PMWS has two characteristic lesions; granulomatous inflammation (Fig. 2) and the presence of intracytoplasmic inclusion bodies (Fig. 3). Granulomatous inflammation is seen in the lymph nodes, liver, spleen, tonsil, thymus, and Peyer's patches (Table 1) but occurs consistently in superficial inguinal lymph nodes, and, in our experience, only occasionally in other tissues. This unique lesion is characterized by infiltrates of epithelioid cells and multinucleated giant cells (Fig. 2). The epithelioid cells are activated
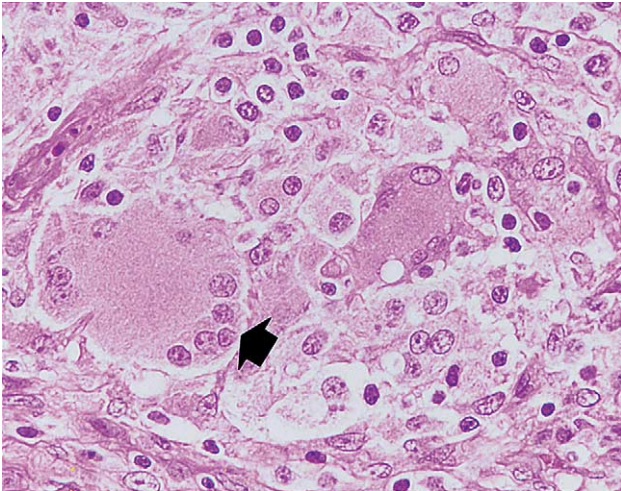
Fig. 2. Pig lymph node with PMWS. A cluster of histiocytes and multinucleated giant cells (Langhans type) (arrow). Haematoxylin and eosin.
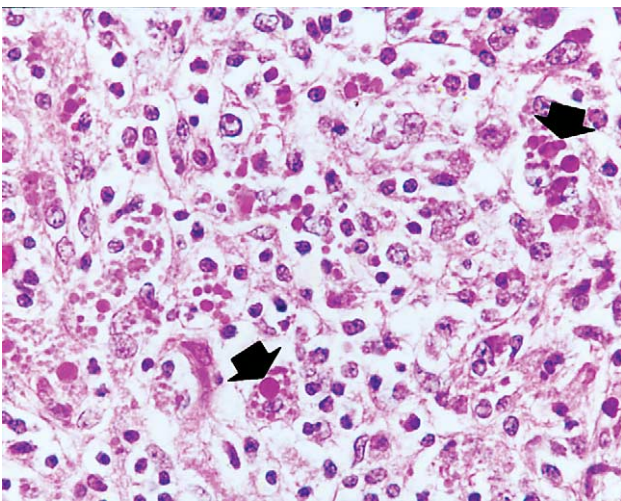

Fig. 3. Pig lymph node with PMWS. Multiple amphophilic grape-like intracytoplasmic inclusion bodies within histiocytes (arrows). Haematoxylin and eosin.

Table 1
Prevalence of characteristic histopathological lesions in tissues from 100 pigs with naturally occurring PMWS

Tissue	Histopathology[a]		
	Lymphoid depletion	Granulomatous inflammation	Inclusion body
Lymph node[b]	98	95	0
Spleen	77	60	15
Tonsil	74	15	5
Thymus	23	1	0
Liver	0	2	0
Peyer's patches	5	5	1

[a] Stained by haematoxylin and eosin.
[b] Superficial inguinal lymph node.

macrophages that appear as large cells with abundant pale, foamy cytoplasm. The multinucleated giant cells are of the Langhans type derived from the fusion of macrophages and characterized by 5–20 nuclei around the periphery of the cells (Fig. 2). Intracytoplasmic inclusion bodies are large, multiple, basophilic or amphophilic grape-like structures often seen in the cytoplasm of histiocytic cells and multinucleated giant cells (Fig. 3).

Lymph nodes may also exhibit depletion and coagulative necrosis of follicular centres and Peyer's patches and tonsils exhibit marked depletion of lymphocytes. Hepatic lesions are characterized by moderate inflammatory cell infiltration of portal areas, some hepatocellular vacuolation and swelling, and sinusoidal collapse. In the kidneys, there can be multifocal lymphohistiocytic interstitial nephritis and pyelitis. Inflammatory foci are surrounded by zones of fibroblast proliferation. The pulmonary lesions are characterized by moderate thickening of the alveolar septa due to the infiltration of mononuclear cells (primarily macrophages and lymphocytes, and occasionally multinucleated giant cells) and type II pneumocyte hyperplasia. Although the grape-like basophilic intracytoplasmic inclusion bodies are pathognomonic of PMWS (Clark, 1997; Kiupel et al., 1998; Kim et al., 2002), these inclusion bodies are not present in all PMWS cases (Choi et al., 2000). Only 27.8% of the PMWS cases examined in Korea had intracytoplasmic inclusion bodies (Kim et al., 2002). In contrast, 97% of PMWS cases examined had granulomatous inflammation in lymphoid tissues (Kim et al., 2002) and the presence of granulomatous inflammation is therefore a more useful indicator of PMWS.

Features of the histopathological lesions suggest that monocyte/macrophage infiltration may be closely related to the pathogenesis and progression of PMWS. The characteristic granulomatous inflammatory lesions are immune mediated (Krakowka et al., 2002). In granulomatous inflammation, monocytes are recruited by a process involving adhesion of circulating monocytes to the vascular endothelium, diapedesis, and migration to a site along gradients of chemotactic substances (Sibille and Reynolds, 1990; Collins, 1999). The recruitment of monocytes into an area of inflammation is a crucial step in the development of granulomatous lesions and chemokines may play an important role in the initiation of granulomatous inflammation.

The chemokine family can be divided into two major classes, CC and CXC, on the basis of a difference in the position of cysteines within a conserved four cysteines motif (Oppenheim et al., 1991; Rollins, 1997). The CC chemokines, such as monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 (MIP-1) have the powerful chemoattractant and acti-

vator properties of monocytes (Wolpe et al., 1988; Oppenheim et al., 1991; Rollins, 1997), whereas the CXC chemokines, such as interleukin-8, are particularly important in the attraction of neutrophils (Oppenheim et al., 1991; Rollins, 1997). It has been suggested that MCP-1 expression may play a role in the pathogenesis of granulomatous inflammation in pigs with PMWS (Kim and Chae, 2003b). The exact mechanisms by which granulomatous inflammation is induced by PCV2 remains unclear. A correlation between the presence of MCP-1 expression and PCV2 in serial sections of lymph node suggests that there is regulated expression of MCP-1 by mononuclear cells in response to PCV2, indicating that PCV2 plays an important role in initiating granulomatous inflammation (Kim and Chae, 2003b). As there is no other virus that produces similar lesions to those of PCV2, the granulomatous inflammation in PWMS provides an excellent animal model for investigating not only the mechanisms of inflammation but also the in vivo role of chemokines.

PMWS caused by PCV2 should be differentiated from porcine reproductive and respiratory syndrome (PRRS) caused by PRRS virus (PRRSV). Both PMWS and PRRS are characterized by lymphohistiocytic interstitial pneumonia (Cheon et al., 1997; Clark, 1997; Harding and Clark, 1997; Cheon and Chae, 1999). Depletion of lymphoid tissues and replacement by macrophages and multinucleated giant cells are the hallmark of PMWS (Clark, 1997; Harding and Clark, 1997; Kiupel et al., 1998; Choi and Chae, 1999; Choi et al., 2000; Kim et al., 2002), whereas PRRSV induces marked follicular hyperplasia of lymphoid tissues (Halbur et al., 1996; Cheon and Chae, 1999).

6. Detection of PCV2

In order to establish the aetiological diagnosis of PMWS, techniques are required that link virus and tissue lesions. Thus techniques such as immunohistochemistry and in situ hybridization, but not PCR or virus isolation, have been employed. The detection of PCV2 does not imply PMWS but PCV2 infection. Immunohistochemistry (Fig. 4) and in situ hybridization (Fig. 5) allow localization of PCV2 in infected tissues or cells. In addition, these methods provide cellular detail and highlight histological architecture so that the presence of PCV2 in lesions may be studied in the same section (Choi and Chae, 1999; Choi et al., 2000; Kim and Chae, 2001a; Kim and Chae, 2003d; Kim et al., 2003). PCV2 DNA or antigen has been identified in macrophages of multiple tissues by immunohistochemistry and in situ hybridization (Choi and Chae, 1999; McNeilly et al., 1999; Choi et al., 2000; Kim and Chae, 2001a; Kim and Chae, 2003d; Kim et al., 2003). In one study, immunohistochemistry for the detection of PCV2

antigen in formalin-fixed, paraffin-wax-embedded tissue was more sensitive than PCV2 DNA detection by in situ hybridization (McNeilly et al., 1999). In that study, however, the hybridization probes used were based on the whole PCV1 genomic sequence. In view of the significant genomic differences now known to exist between PCV1 and PCV2 isolates (Hamel et al., 1998; Meehan et al., 1998; Morozov et al., 1998), probes for the detection of PCV2 should be designed from the PCV2 genomic sequence. Since nucleotide homology of ORF2 between PCV1 and PCV2 is more variable than that of ORF1 (Morozov et al., 1998), the probe designed from ORF2 would be sufficiently heterogeneous to allow the
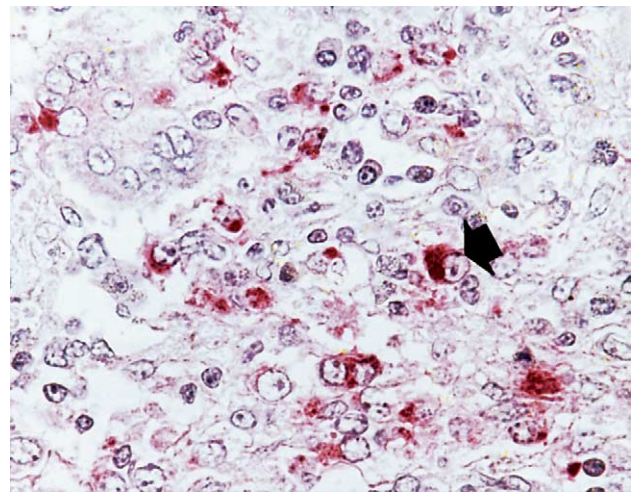

Fig. 4. Pig lymph node with PMWS. PCV2 antigen (red reaction) was detected in macrophages (arrow). Immunohistochemistry; alkaline phosphatase, red substrate, haematoxylin counterstain.
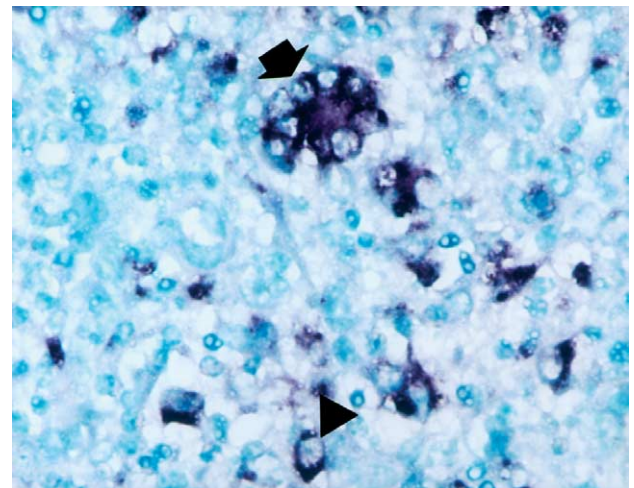

Fig. 5. Pig lymph node with PMWS. PCV2 DNA (black reaction) was detected in multinucleated giant cells (arrow) macrophages (arrow head). In situ hybridization; alkaline phosphatase, nitroblue tetrazolium/5-bromocresyl-3-indolylphosphate, methyl green counterstain.

specific detection of both types of PCV and thus enable their differentiation.

PCV2 antigens have been detected in several swine tissues by monoclonal and polyclonal antibody-based immunohistochemical procedures (Ellis et al., 1998; Choi et al., 2000). However, not all monoclonal and polyclonal antibodies are suitable for use in immuno-histochemistry due to the cross-linking effects of the formalin fixation that renders certain epitopes unde-tectable (Haines and Chelack, 1991). In contrast, in situ hybridization is less susceptible to structural alteration caused by formalin fixation (Choi and Chae, 1999; Kim and Chae, 2001a). Use of both digoxigenin-labelled DNA probes and in situ hybridization would eliminate the possibility for errors that could be caused by anti-genic cross-reactivity or the alteration of binding sites caused by formalin fixation.

In situ hybridization with RNA probes is generally more sensitive than hybridization with DNA probes but requires more exacting conditions (Brown, 1998). If these conditions are not met, false negative results may occur and sensitivity will be reduced (Brown, 1998). However, since PCV is a single-stranded DNA virus (Meehan et al., 1997), DNA probes are not only as sensitive as RNA probes but also more reliable for di-agnostic purpose (Choi and Chae, 1999). In situ hy-bridization can allow the differentiation between PCV1 and PCV2 in formalin-fixed, paraffin-wax-embedded tissues with a nonradioactive digoxigenin-labeled probe (Kim and Chae, 2001a, 2002a).

Low concentrations of PCV2 DNA may escape de-tection in the formalin-fixed, paraffin-wax-embedded tissues. However, thermocycler pretreatment, combined with brief proteinase K digestion can enhance signal intensity and allow detection (Kim and Chae, 2003d). This technical improvement, results in an identical background at the same time as the increased signal.

Although application of in situ hybridization has re-cently become increasingly important in diagnostic procedures, use of the technique remains confined to a few diagnostic laboratories because of its greater tech-nical complexity and expense compared to immunohis-tochemistry. However, the ease of preparation of DNA probes by PCR compared with the generation of monoclonal antibodies and production of ascites fluid, combined with the increasing availability of probes and sequence databases, will ensure the expansion of this technique in both clinical diagnosis and research. The use of a digoxigenin labelled probe avoids handling ra-dioactive materials and renders the technique more easily transferable to those diagnostic laboratories per-forming immunohistochemical procedures. Moreover, in situ hybridization may be adjusted for use with double labelling procedure (Kim and Chae, 2002a,b) or combined for use with immunohistochemistry (Choi and Chae, 2001).

Although PCR is more sensitive than in situ hybrid-ization for the detection of PCV2 in tissues (Calsamiglia et al., 2002), it cannot be accurately used for the diagnosis of PMWS. However, PCR can be used for the detection of PCV2 from formalin-fixed, paraffin-wax-embedded tis-sues containing histopathological lesions of PMWS as described earlier (Kim and Chae, 2001b, 2003c). Patho-logical specimens for microscopic evaluation are rou-tinely processed in this way and form an invaluable resource for molecular studies. The ability to amplify specific regions of DNA from these tissues by PCR has had a profound impact on diagnostic pathology. In comparison to existing identification methods such as immunohistochemistry and in situ hybridization, PCR amplification of viral sequences from formalin-fixed, paraffin-wax-embedded tissue is a simple and sensitive method and should allow the reliable detection of PCV2 DNA in diagnostic pathology (Kim and Chae, 2001b). Moreover, extraction of DNA from these tissues is well documented and is now a routine diagnostic process (Kim and Chae, 2001b; Kim and Chae, 2003c). Several factors may influence the sensitivity of PCR in formalin fixed, paraffin-wax-embedded tissues (An and Fleming, 1991; Kim and Chae, 2001b), but the PCR preparation covers a large portion of the lymph node, thus increasing the sensitivity, whereas a slide of a tissue section for immu-nohistochemistry and in situ hybridization represents only a 5 μm cross-section. However, it should be re-membered that the detection of PCV2 alone is diagnostic, but when interpreted in conjunction with the character-istic histopathological changes in tissues, PCR should provide an alternative confirmatory PMWS diagnostic tool (Kim and Chae, in press).

7. Conclusion

Diagnosticians are constantly seeking better tools for the diagnosis, prevention, and control of infectious agents. Prompt and precise diagnosis would greatly fa-cilitate the management of disease in countries where PMWS is enzootic. Virus isolation is not considered to be the 'gold standard' of PMWS diagnosis. The character-istic histopathological lesions are important criteria for the diagnosis of clinical PMWS. Although PCR can provide an alternative confirmatory PMWS diagnostic tool when interpreted in conjunction with characteristic histopathological changes in tissues, in situ hybridization and immunohistochemistry should be considered better techniques than PCR to diagnose clinical PMWS cases.

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