

5. STUDY III: Immunohistochemical characterization of PCV2 associated lesions in lymphoid and non-lymphoid tissues of pigs with naturally occurring postweaning multisystemic wasting syndrome (PMWS)

5.1. Materials and methods

5.1.1. Animals and sampling

Ten 2.5-month-old pigs suffering from acute PMWS-like clinical signs were selected from a conventional, 1300 sow, farrow-to-finish operation. The farm had a previous diagnosis of PMWS, and was seronegative to PRRSV, ADV, and PPV. In this farm, sows were regularly vaccinated against ADV (aqueous live vaccine), PPV, and erysipelas (combined killed vaccine). Fattening pigs were vaccinated against ADV at 2 and 3 months of age. PRRSV vaccination was never performed in this farm.

Pigs were killed by intravenous sodium pentobarbital injection and a complete necropsy was performed on all animals. Samples of the following tissues were collected: lung, thymus, tonsil, lymph nodes (mesenteric, superficial inguinal and submandibular), spleen, liver, kidney and ileum. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 3 μm , and stained with HE for microscopic study. For IHC and ISH, serial 4 μm -thick sections of all tissues were cut and placed on silane-coated slides.

5.1.2 PCV2 detection

ISH and IHC to detect PCV2 were performed in formalin-fixed, paraffin-embedded tissue, following previously published procedures (Rosell et al., 1999). Sections from a previous case of PMWS diagnosed in Spain

were used as positive controls (Segalés et al., 1997). Negative controls consisted of archive samples of lung and lymphoid tissues of pigs from an experimental farm with no history of PWMS or PCV2 infection.

5.1.2.1. ISH

For ISH a PCV2 specific oligonucleotide was used (Rosell et al., 2000a). Briefly, sections were placed on Probe-On-Plus glass microscope slides (Fisher Scientific, Pittsburgh, USA). A workstation was used to control the temperature of the hybridisation reactions and various incubations, and to minimise reagent consumption. Tissue sections were deparaffinised, and rehydrated through graded alcohols to Automotion Buffer (Biømeda Corp., CA, USA). Proteolytic digestion with 0.3% pepsin and incubation with 100% formamide for 5 min. at 105 °C was done. Hybridisation was performed for 5 min. at 105 °C and 30 min. at 37 °C. Stringent washes with saline sodium citrate buffers, an anti-digoxigenin antibody conjugated with alkaline phosphatase was applied. Colour was developed with nitroblue tetrazolium dye. The sections were counterstained with fast green stain, dehydrated and mounted.

5.1.2.2. IHC

IHC was performed using a polyclonal anti-PCV2 antibody and a protocol previously described (Sorden et al., 1999). Briefly, tissue sections were

deparaffinised with xylene and rehydrated through graded alcohols. Endogenous peroxidase activity was blocked by incubation with hydrogen peroxide 3% in distilled water for 30 min. Then, tissue sections were rinsed in 0.1 M Tris-buffered saline (pH 7.6) and incubated with 20% normal goat serum solution in 0.1 M Tris-buffered saline for 1 hour at room temperature. PCV2 antibody was incubated overnight at 4 °C. Biotinylated goat anti-mouse (1/200) and biotinylated goat anti-rabbit (1/400) were used as secondary antibodies, for 1 hour at room temperature. An ABC complex (Pierce, IL, USA) diluted 1/100 in 0.1 Tris-buffered saline was applied for 1 hour at room temperature. Sections were finally incubated in diaminobenzidine (DAB)-hydrogen peroxide solution for 10 min., counterstained with Harris's haematoxylin, dehydrated, covered and examined microscopically.

5.1.3. Immune system cells characterisation

Cell markers used to classify the different cell phenotypes present in the lymphoid tissues are shown in table 4. T cells were identified using an anti-human CD3 antibody. B cells were labelled with an anti-human CD79 (HM57) and CD45RA (3C3/9) antibodies. Macrophages were detected with lysozyme. Antigen presenting cells (APCs) were identified with a SLA-II-DQ (BL2H5) antibody. Polymorphonuclear granulocytes were labelled with MAC387. All the antibodies were used with a standard avidin-biotin peroxidase (ABC) method following procedures described in the second study. Briefly, tissue sections were deparaffinised in xylene

and rehydrated in graded alcohols to distilled water. Endogenous peroxidase activity was blocked by incubation with hydrogen peroxide 3% in distilled water for 30 min. After pronase, trypsin or citrate buffer treatment, depending on the antibody used, tissue sections were rinsed in 0.1 M Tris-buffered saline (pH 7.6) and incubated with 20% normal goat serum solution in 0.1 M Tris-buffered saline for 1 hour at room temperature. The slides were then incubated overnight at 4 °C with one of the primary antibodies. Biotinylated goat anti-mouse (1/200) and biotinylated goat anti-rabbit (1/400) were used as secondary antibodies, for 1 hour at room temperature. An ABC complex (Pierce, Illinois, USA) diluted 1/100 in 0.1 Tris-buffered saline was applied for 1 hour at room temperature. Sections were finally incubated in diaminobenzidine (DAB)-hydrogen peroxide solution for 10 min., counterstained with Harris's haematoxylin, dehydrated, covered and examined microscopically. As negative controls, irrelevant primary antibodies at the same dilution were used in substitution of the specific antibodies.

Table 4. Details of the primary antibodies used in the immunohistochemical study

Specificity	pAb/mAb (clone)	Host origin	Treatment	Dilution	Source
CD3	pAb	Human	Pronase ^a	1/150	Dako (Denmark)
CD79	mAb (HM57)	Human	Microwave ^b	1/25	Dako (Denmark)
L1	mAb (MAC387)	Human	Pronase	1/200	Dako (Denmark)
Lysozyme	pAb (A099)	Human	Trypsin ^c	1/100	Dako (Denmark)
CD45RA	mAb (3C3/9)	Swine	No treatment	Undiluted ^d	INIA Lab ^e
SLA-DQ	mAb (BL2H5)	Swine	Pronase	Undiluted ^d	INIA Lab

^a Incubation with 0.1% pronase for 10 min at 37°C

^b Incubation with citrate buffer (pH 6) 20 min at 100°C

^c Incubation with 0.1% trypsin for 2 hours at 37°C

^d culture supernatant

^e Kindly provided by Dr. Javier Domínguez, INIA, Valdeolmos, Madrid (Spain).

5.2. Results

5.2.1. Macroscopic lesions

At necropsy, cranioventral pulmonary consolidation (9 out of 10 pigs), and enlargement of inguinal and mesenteric (jejunal) lymph nodes (8/10) were the most obvious lesions. Other lesions observed in the PMWS affected pigs were non-collapsed lungs (3/10), parakeratosis of the oesophageal part of the stomach (3/10), slight atrophy of nasal turbinates (3/10), and multifocal white spots in the superficial renal cortex (2/10). No gross lesions were observed in the control pigs.

5.2.2. Microscopic lesions

The main lesions observed in the lymphoid organs were depletion of lymphocytes in B- and T-cell areas and infiltration of lymphoid tissues by histiocytic cells. Based on the intensity of these lesions, three stages of the infection were established: stage I that included animals showing mild lesions (2 animals out of 10), stage II, animals showing moderate lesions (3/10), and stage III, animals showing severe lesions in the lymphoid organs (5/10). A complete description of each stage is included in the next sections. A summary of these results is shown in Table 5.

5.2.2.1. Stage I

Slight depletion of B-cell areas and mild infiltration of macrophages and sporadic multinucleate giant cells (MGCs), mainly in the germinal centres of follicular areas of lymph nodes, tonsil, spleen, and Peyer's patches, were observed in all animals at this stage. Furthermore, in lymph nodes, mild infiltration of the medulla-like tissue by macrophages was also noted. Scattered circulating granulocytes, mainly eosinophils, and occasional mitotic and apoptotic figures were found in all lymphoid tissues.

Slight, multifocal thickening of the alveolar septa due to the presence of mononuclear cells mainly lymphocytes and scattered macrophages was observed in the lung of both pigs. In one of them, , randomly distributed, multifocal aggregates of lymphocytes and plasma cells were noted in the hepatic and renal parenchyma.

5.2.2.2. Stage II

The main histopathological findings in lymph nodes, tonsil, spleen and Peyer's patches of animals at this stage were moderate lymphocyte depletion of B-cell areas, mild lymphocyte depletion in T-cell areas (Fig 6c), and a variable number of infiltrating macrophages in follicular and interfollicular areas. MGCs were usually present in lymphoid organs, especially in the lymph nodes of two animals. In one animal, small, round nucleated cells and degenerated granulocytes were found in the cytoplasm of MGCs. Large single or smaller multiple spherical

basophilic inclusions were present in the cytoplasm of scattered macrophages in the tonsil and mesenteric lymph node of one animal. In the tonsil of the same animal, a large number of eosinophils infiltrated the tissues and a tonsillar crypt. In the ileal Peyer's patches and submandibular lymph node of two animals many mitotic figures were observed. Apoptotic figures were occasionally observed in all lymphoid tissues.

In lung, mild exudation of neutrophils in bronchi, bronchioli and alveoli, and slight, multifocal thickening of the alveolar septa due to the presence of lymphocytes and scattered macrophages were observed. Few macrophages in the alveolar lumen and alveolar oedema were detected in one animal.

Mild multifocal or periportal lymphoplasmacytic infiltrations were sporadically observed in the liver, while in one pig, multifocal lymphoplasmacytic infiltrations were found in the kidney.

5.2.2.3. Stage III

In lymph nodes of animals at this stage, follicles were drastically reduced or disappeared and subcapsular and medulla-like tissue and interfollicular areas were invaded by a large number of macrophages (Fig. 6b, d) Large single or smaller multiple cytoplasmic spherical, basophilic inclusions were occasionally observed in the macrophages invading the lymphoid tissue. Sporadic MGCs were also found in the cortical areas of lymph nodes. Activated high endothelial venules were observed in the lymph nodes of one animal. Occasional mitotic and

apoptotic figures were mainly apparent in follicular areas of all animals. Furthermore, a large number of circulating polymorphonuclear granulocytes, mainly eosinophils, were constantly seen, except in submandibular lymph nodes. Changes in tonsil were dominated by marked loss of lymphocytes in B and T cell areas and macrophage infiltration. Numerous eosinophils were constantly found in follicular and interfollicular tissue, as inside the crypts. A large number of mitotic figures were observed, whereas apoptotic cells and MGCs were sporadically present. Spleen changes were characterised by diminution of lymphoid cells and infiltration of histiocytic cells in PALs and follicles. Occasionally, scattered MGCs and eosinophils in the red pulp were seen.

Changes in Peyer's patches consisted in lymphocytic depletion in B and T cell areas associated to macrophage infiltration. Small multiple basophilic cytoplasmic inclusion bodies were observed in macrophages infiltrating follicular areas. Scattered mitotic and apoptotic figures were also seen. Thymic changes were characterised by diminution of thymocytes, especially in the medulla, and macrophage infiltration in the cortex and medulla.

In all pigs at this stage, macrophage infiltration and diminution to loss of BALT was prominent in lung. Furthermore, neutrophilic exudation in bronchi, bronchioli and alveoli was a constant finding, associated with moderate to severe thickening of the alveolar septa due to the presence of mononuclear cells, mainly lymphocytes and scattered macrophages.

Lymphoplasmacytic multifocal or perilobular infiltration, and diffuse hydropic degeneration of hepatocytes were the more frequent lesions observed in the liver. Slight to moderate, multifocal lymphoplasmacytic infiltration was observed in the renal cortex of one animal.

5.2.3. PCV2 detection

PCV2 nucleic acid and antigen were systematically detected in the lymphoid tissues of all animals, whereas in non-lymphoid tissues, detection of PCV2 was variable. In follicular lymphoid tissues, PCV2 nucleic acid and antigen were labelled in the cytoplasm of macrophages, follicular dendritic cells, interdigitating cells and MGCs; however, the nuclei of scattered macrophages, follicular dendritic cells and lymphocytes were also occasionally stained. In lymphoid tissues, the intensity of PCV2 detection correlated with the severity of the histological lesions. Results are summarised in Table 5.

5.2.3.1. Stage I

PCV2 nucleic acid and antigen in follicular lymphoid tissues were mainly confined in infiltrating macrophages and dendritic cells of follicular areas and in scattered interdigitating cells and lymphocytes in T cell areas. Furthermore, in Peyer's patches, PCV2 was labelled in histiocytic cells in the lamina propria and in intraepithelial lymphocytes. In thymus, PCV2 was observed in thymic corpuscles and

in scattered macrophages of the medulla and cortex. No PCV2 nucleic acid and/or antigen were detected in lung, liver, and kidney.

5.2.3.2. Stage II

PCV2 nucleic acid and antigen was observed in infiltrating macrophages, MGCs and interdigitating or dendritic cells in the cortex of lymph nodes. In tonsil, PCV2 was mainly located in macrophages and dendritic cells of follicular areas, around the crypt and in few scattered lymphocytes in interfollicular areas. In spleen, PCV2 was observed in histiocytic cells of PALs and in circulating cells in the red pulp. In lung, a variable amount of PCV2 positive histiocytic cells were found in the BALT. Sporadic PCV2 positive Kupffer cells were observed in liver. In one case, PCV2 was detected in the cytoplasm of macrophages and MGCs in an inflammatory focus located in the kidney.

5.2.3.3. Stage III

PCV2 distribution in lymphoid organs was very similar to that of stage II (Fig. 8a); however viral nucleic acid and antigen were also detected in the cytoplasm of macrophages infiltrating the medulla-like area of lymph nodes. In lung, a discrete amount of PCV2 was located in BALT macrophages, in alveolar septa, peribronchiolar and peribronchial macrophages, and in the macrophages of intrabronchiolar exudates. In liver, PCV2 antigen and, in a smaller number of cases, nucleic acid

were found in Kupffer cells and in perilobular and periportal macrophages. In one animal, PCV2 was demonstrated in a small focus of macrophages located in the kidney.

5.2.4. Immune system cells characterisation

The most consistent findings were reduction to loss of follicular CD79 and 3C3/9 positive cells, diminution in T cell areas of CD3 positive lymphocytes, increase of subcapsular and peritrabecular lysozyme positive macrophages, and partial loss and redistribution of BL2H5 positive antigen presenting cells throughout lymphoid tissues. A varying number of circulating MAC387 stained granulocytes were constantly observed in lymphoid and non-lymphoid tissues of all animals. These results are summarized in table 5. Normal distribution of the cell markers used in this study is described in the study II.

5.2.4.1. Stage I

In follicular organs, a decreased number of CD79 stained lymphocytes were observed in germinal centres and mantle zones of slight affected cases. The 3C3/9 antibody stained germinal centres of all follicular immune system organs, similarly than in control cases. In spleen, a moderate number of 3C3/9 stained cells were observed in the marginal zones, as in control animals. The majority of T cell areas were visibly delimited resembling control animals, and CD3 stained lymphocytes were only slightly diminished. In Peyer's patches, the

distribution of CD3 positive cells was comparable to control animals, being more numerous in the lamina propria and in an intraepithelial localisation than in domes, and germinal centres.

In follicular lymphoid organs, lysozyme positive cells were depicted in the germinal centre of the follicles. In lymph nodes, a slightly increased number of lysozyme positive histiocytic cells were found in medulla-like tissues and peritrabecular zone. In tonsil, the majority of lysozyme positive cells were detected in follicular zones and around the crypts, which epithelium resulted also occasionally stained. In spleen, lysozyme stained cells were detected in follicular and marginal zones. In the Peyer's patches, lysozyme stained histiocytes were found in the lamina propria.

The BL2H5 antibody diffusely stained the surface and occasionally the cytoplasm of histiocytic cells and many endothelial cells in all lymphoid organs. In follicular lymphoid organs, the BL2H5 antibody stained follicular dendritic cells in germinal centres, similarly than in control animals, whereas lymphocytes in B-cell areas were not stained with this antibody. In thymus, a larger number of BL2H5 positive cells were found in the medulla compared to control animals.

The MAC387 antibody stained a moderate to high amount of circulating polymorphonuclear granulocytes in the red pulp of the spleen and in lower number in the peritrabecular sinus of the lymph nodes. In tonsil, a variable number of scattered circulating granulocytes were depicted with MAC387. In the Peyer's patches positive granulocytes were found in the lamina propria of the intestinal villi. Very few circulating granulocytes were seen in the medulla and

cortex of the thymus; in some cases thymic corpuscles were faintly stained with this antibody.

In lung, circulating CD3 lymphocytes were constantly observed, as well as in the BALT, and in peribronchiolar and peribronchial areas. Lysozyme stained a variable number of histiocytic cells in the BALT, and in the alveolar septa. BL2H5 positive cells were mainly located in the BALT and in the alveolar septa, but frequently found also in peribronchiolar and peribronchial areas.

In one animal, CD3-stained lymphocytes, lysozyme- and BL2H5-stained macrophages, and MAC387-stained polymorphonuclear granulocytes were observed in randomly distributed multifocal inflammatory infiltrates throughout the hepatic parenchyma and renal pelvis.

The BL2H5 antibody stained a larger number of endothelial cells of glomerular and interstitial vessels, compared to control animals.

5.2.4.2. Stage II

In some lymph nodes, only a few number of CD79 stained cells were observed in the mantle zone of the follicles, whereas in the other lymph nodes no staining was observed with the CD79 antibody. In follicular lymphoid organs, 3C3/9 antibody only stained lymphocytes in marginal zone and follicles of spleen. In lymph nodes, tonsil, and spleen, T cell areas were sometimes disorganised and showed a slight to moderate diminution of CD3 stained cells. In T cell areas of Peyer's patches and spleen, CD3 positive cells were mildly reduced in number.

An increase of lysozyme positive histiocytes in lymph nodes was observed in subcapsular areas and medulla-like tissues. In tonsil, many lysozyme stained cells were seen diffusely distributed throughout the tissue. In two animals, the cytoplasm of numerous MGCs strongly stained with lysozyme (Fig. 8d). Several lysozyme positive histiocytic cells showed a perinuclear granular staining in lymphoid organs. A large number of scattered tingible body macrophages were abundantly depicted with lysozyme in thymic cortex, and tonsil and in lower number in spleen and lymph nodes.

The BL2H5 antibody stained the surface and sometimes the cytoplasm of histiocytes, MGCs (Fig. 8c), and endothelial cells in all lymphoid tissues studied. In lymph nodes, the majority of BL2H5 positive cells were observed in the medulla-like and peritrabecular areas, whereas scattered positive stained cells were observed in the cortical area. In thymus, the BL2H5 antibody stained cells in the medulla, similarly to animals in stage I.

MAC387 positive cells had a similar pattern of distribution than in animals from stage I, being more numerous especially in the red pulp of the spleen and in the sinus of the lymph nodes. Occasionally, positive granulocytes were found in the cytoplasm of MGCs.

In lung, CD3, BL2H5 and lysozyme antibodies showed a similar staining pattern than in animals from stage I. The MAC387 antibody stained strongly polymorphonuclear granulocytes and faintly macrophages inside bronchi, bronchioli and alveoli, as well as in alveolar septa.

In liver, CD3, lysozyme and BL2H5 positive cells were observed in mild inflammatory infiltrates localised in periportal areas or multifocally distributed.

In kidney, one animal had a large number of CD3 stained cells and BL2H5 stained cells in the renal pelvis, and associated with interstitial inflammatory foci. Circulating MAC387 stained polymorphonuclear granulocytes were constantly observed, sometimes associated with small foci of inflammation.

5.2.4.3. Stage III

In follicular lymphoid tissues no stained CD79 or 3C3/9 cells were found in B cell areas, and only scattered 3C3/9 positive cells were observed in the marginal zone of the spleen. Reduction of CD3 positive cells was especially relevant in lymph nodes (Fig. 7a) and spleen. The majority of lymph nodes and spleens, showed no delimited T cell areas and, in lymph nodes, CD3 positive lymphocytes showed a diffuse disorganised pattern of distribution, invading subcapsular and peritrabecular areas (Fig. 7c) In spleen, moderate depletion of CD3 stained cells was observed in PALs. T cell dependent interfollicular zones were maintained in tonsil, showing a moderate to severe degree of CD3 lymphocytic depletion. In Peyer's patches, only a slight decrease of CD3 stained cells was observed. In thymus, a slight reduction of medullar mature CD3 positive thymocytes was occasionally observed.

An increased number of lysozyme positive macrophages was relevant, and followed the distribution of infiltrating histiocytes observed in the histological study (Fig. 8b) In lymph nodes, lysozyme positive cells were constantly observed in medullar and peritrabecular zones, and infiltrating the cortex (Fig. 7b). In tonsil, lysozyme-stained macrophages were found uniformly distributed, but especially numerous around the crypts which epithelium resulted also occasionally positive. Positive lysozyme inclusion bodies and MGCs were also observed. In spleen, the lysozyme antibody stained infiltrating macrophages in PALs and scattered macrophages in the marginal zones and red pulp. In Peyer's patches, lysozyme positive macrophages were mainly observed in follicular areas and domes. A variable number of tingible body macrophages were stained with the lysozyme antibody in thymic cortex, and in lower number in Peyer's patches, lymph node and spleen.

The surface and occasionally the cytoplasm of histiocytic and MGCs and many endothelial cells stained with the BL2H5 antibody. The positive cells had a diffuse pattern of distribution in all lymphoid tissues (Fig. 7d). The largest number of stained cells was observed in the peritrabecular areas of the lymph nodes and in the PALs of the spleen. In the Peyer's patches, stained cells were mainly observed in the follicular zones, and in a variable number in the lamina propria of the intestinal villi. In thymus, similarly than in moderate affected cases, the BL2H5 antibody stained the surface of interdigitating cells and macrophages in the medulla, and in the medullar cortical junction, and cytoplasmic processes in the cortex.

In this group of animals, MAC387 stained polymorphonuclear cells with the same distribution than in animals of stage II.

In lung, only circulating CD3 lymphocytes were observed in these cases. The lysozyme antibody stained a variable number of histiocytic cells in the alveolar septa and less frequently in the bronchi and bronchioli. Positive BL2H5 cells were mainly located in the alveolar septa, but frequently found also in peribronchiolar and peribronchial position. In very few cases, histiocytic BL2H5 stained cells were observed inside bronchi or bronchioli. Large numbers of MAC387 positive polymorphonuclear granulocytes and few macrophages were seen inside bronchi, bronchioli and alveoli, as well as in alveolar septa.

In liver and kidney of the pigs at this stage, all tested antibodies stained the same cellular types with the same pattern of distribution than in animals of stage I.

Table 5. Histological and immunohistochemical results

Stages	Microscopic lesions				PCV2 detection ^c					Cell markers ^e			
	Lymphocyte depletion ^a	Histiocytic cells ^b	MGC ^b	ICIB ^b	IP	HIS	CD79	3C3/9	CD3	Lysozyme	BL2H5	MAC387	
Controls	-	+	-	-	-	-	+++	+++	+++	+	+++	+	
I	+	+	+	-	+	+	++	++	+++	+	+++ ^d	+	
II	++	++	+++	+	++	++	-	+	++	++	+++ ^d	++	
III	+++	+++	+	++	+++	+++	-	-	+	+++	++	++	

^a Lymphocyte depletion degree: (-) = absence, (+) = slight, (++) = moderate, (+++) = severe.

^b Presence of histiocytic cells, MGC and intracytoplasmic inclusion bodies (ICIB) has been graded as: (-) = absence, (+) = low numbers, (++) = moderate numbers, (+++) = large numbers.

^c Staining has been graded as: (-) = no stained cells, (+) = low number of stained cells, (++) = moderate number of stained cells, (+++) = high number of stained cells

^d Different distribution compared to control cases

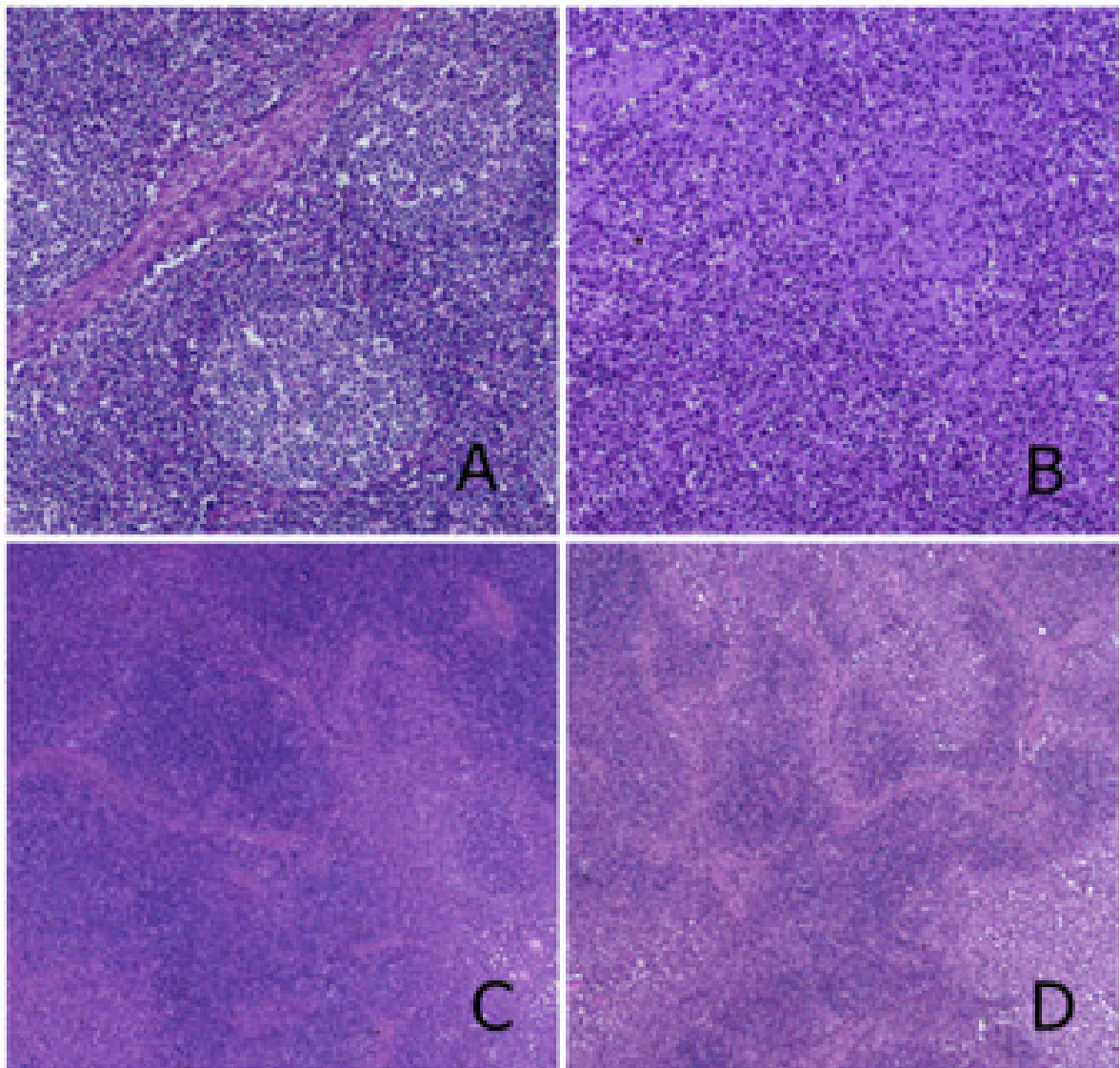


Fig. 6. Haematoxylin and eosin staining of formalin-fixed, paraffin-embedded porcine lymph node sections from a healthy (A) and PMWS (B, C and D) affected pigs. Normal lymph node showing secondary follicles, x50. Lymph nodes classified as stages II (x5, C) and III (x50, B and x5, D).

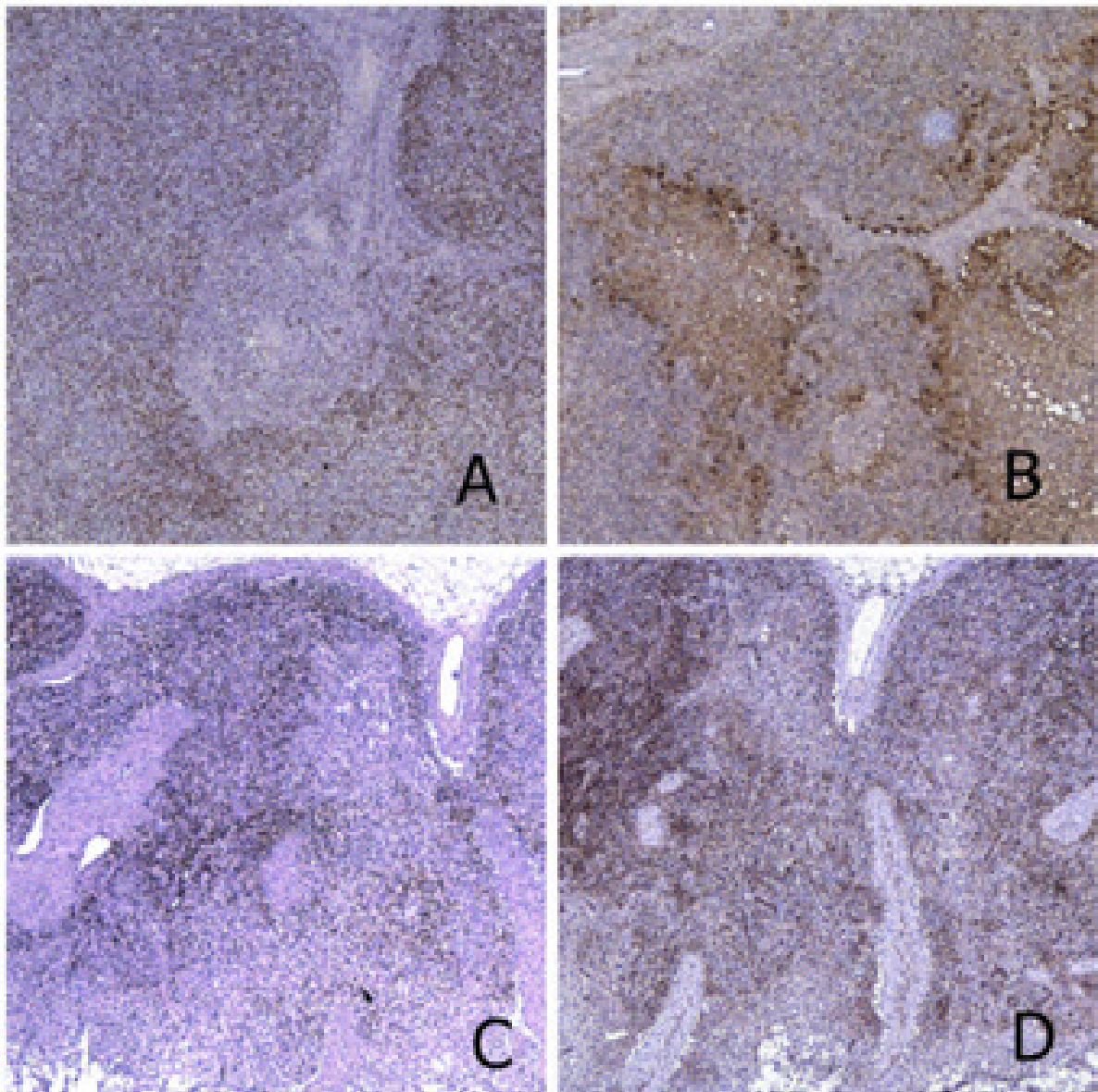


Fig. 7. Immunohistochemical staining of formalin-fixed, paraffin-embedded porcine lymph node sections from pigs suffering from PMWS with anti-CD3 (A and C), anti-Lysozyme (B) and anti-SLAII-DQ (BL2H5) (D) antibodies using the ABC method. Mayer's haematoxylin counterstain, x10. Marked reduction of T cells (A). Massive infiltration of macrophages in medullar and peritrabecular areas, and cortex (B). CD3 lymphocytes showing a diffuse disorganised pattern of distribution invading subcapsular and peritrabecular areas (C). Diffuse pattern of distribution of APC (D).

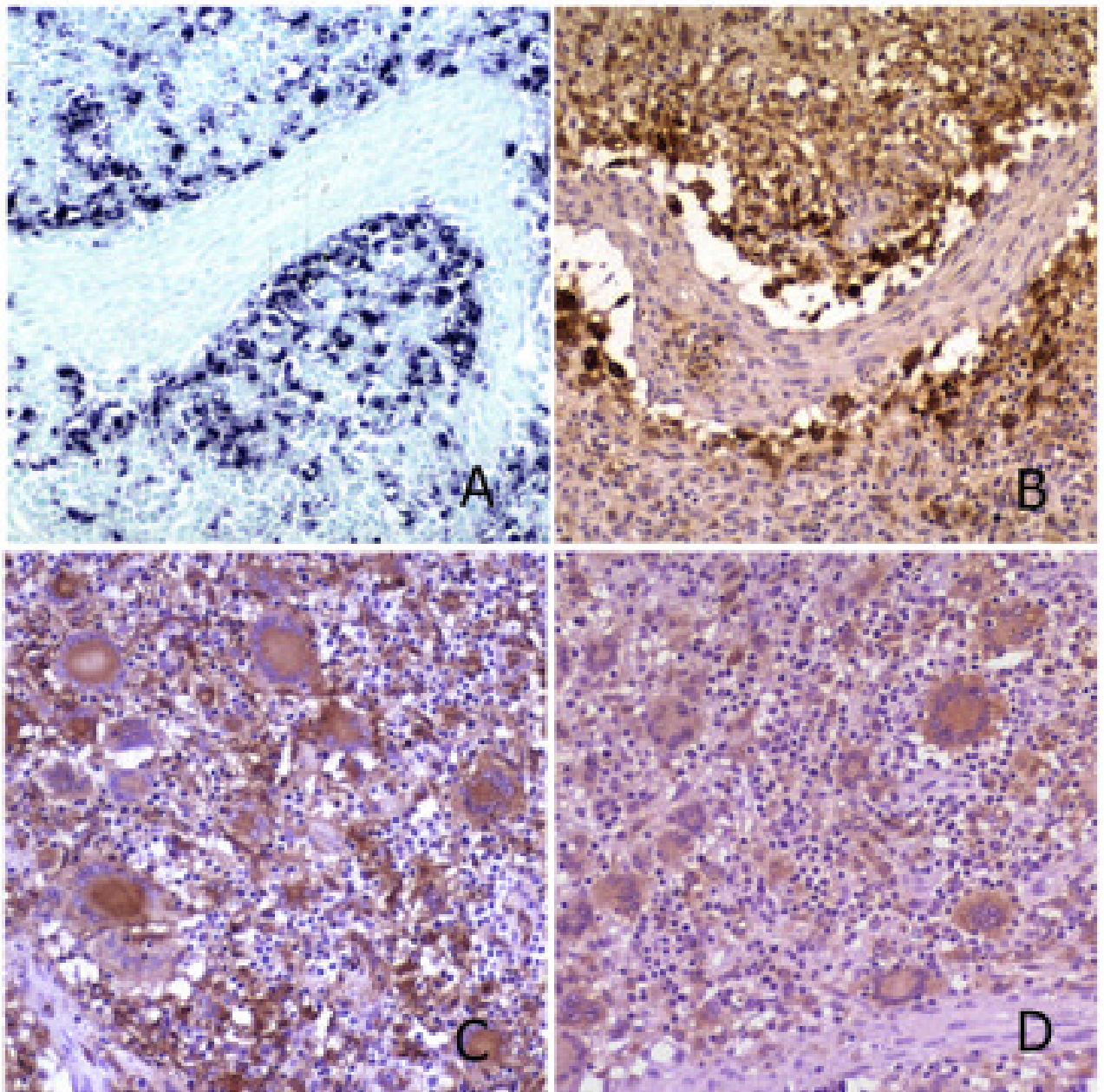


Fig. 8. *In situ* hybridisation technique to detect PCV2 in a lymph node of a PMWS affected pig; note the massive viral nucleic acid amount within the cytoplasm of macrophages located in the peritrabecular area, x50 (A). Immunohistochemical staining of formalin-fixed, paraffin-embedded lymph nodes from a PMWS affected pig with anti-Lysozyme (B and D) and anti-SLAII-DQ (BL2H5) (C) antibodies using the ABC method. Mayer's haematoxylin counterstain, x50. High number of macrophages infiltrating a peritrabecular area of a pig with lesional stage III (B). Marked MGCs and macrophage infiltration in peritrabecular and follicular areas of a pig with lesional stage II (C and D).