

Research Article

Bronchiolitis Obliterans Organizing Pneumonia in Swine Associated with Porcine Circovirus Type 2 Infection

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Bronchiolitis obliterans organizing pneumonia (BOOP) is a chronic respiratory disease. Although the pathogenesis of BOOP is still incompletely understood, BOOP is responsive to steroids and has a good prognosis. In our five pigs with chronic postweaning multisystemic wasting syndrome (PMWS), typical BOOP lesions were revealed. All five porcine lungs showed typical intraluminal plugs, and porcine circovirus type 2 (PCV2) was identified. They also exhibited similar pathologic findings such as proliferation of type II pneumocytes and myofibroblasts (MFBs), extracellular collagen matrix (ECM) deposition, and fragmentation of elastic fibers. MFBs migration correlative molecules, for instance, gelatinase A, B and osteopontin, appeared strongly in the progressing marginal area of polypoid intraluminal plugs of fibrotic lesion. These molecules colocalized with the active MFBs. Both gelatinase activity and intercellular level of active MFBs were significantly increased ($P < .05$). Porcine chronic bronchopneumonia leads to BOOP and it is associated with PCV2 persistent infection. Swine BOOP demonstrates similar cellular constituents with human BOOP. Perhaps their molecular mechanisms of pathogenesis operate in a similar way. Thus we infer that the swine BOOP can be considered as a potential animal model for human BOOP associated with natural viral infection. Moreover, it is more convenient to obtain samples.

1. Introduction

Bronchiolitis obliterans organizing pneumonia (BOOP), described firstly in 1901 [1], was clarified as a distinct histopathological and clinical entity in 1985 [2]. It is characterized by the proliferation of fibroblastic tissues, extending as polypoid plugs from lamina propria into the lumen of terminal and respiratory bronchioles. Additional features include a patchy inflammatory process involving mononuclear inflammatory cells in interalveolar septa [3]. Although the pathogenesis of BOOP is still incompletely understood, BOOP is responsive to steroids and has, in the vast majority of cases, a good prognosis.

Postweaning multisystemic wasting syndrome (PMWS) is considered as the essential infection of porcine circovirus type 2 (PCV2). It is a global and multifactorial disease that mainly affects nursing and fattening pigs in almost all farms [4–6]. It thus has great economic impact [7]. Despite that numerous studies have done in PMWS, the affected piglets are regarded as worthless investment and they are destined to be eliminated from farms. Due to this reason, the studies of chronic pathological changes such as pulmonary fibrosis usually have been neglected. Intriguingly, typical histological features of BOOP in swine are not well documented in the past.

Since the constituents of swine BOOP have never been described previously, we assume that they consist of extracellular collagen matrix (ECM), elastic fibrils, and myofibroblasts (MFB), which are identified by α -smooth muscle actin (α -SMA). Matrix metalloproteinases (MMPs), a group of zinc- and calcium-dependent enzymes, are capable of degrading various components of extracellular matrix. They are activated by several proinflammatory agents such as oxidants, elastases, and other MMPs [8, 9]. MMP-2 (gelatinase A) is synthesized by a wide variety of mesenchymal cells, including fibroblasts, endothelial cells, and alveolar epithelial cells. MMP-9 (gelatinase B) is produced mainly by inflammatory cells such as neutrophils, monocytes, macrophages, eosinophils, and lymphocytes [10]. MMP-2 and 9 are involved in tissue remodeling associated with pathological situations such as acute lung injury and acute respiratory distress syndrome [10, 11]. Osteopontin (OPN) is a multifunctional matricellular cytokine abundantly activated upon inflammatory actions of macrophages during inflammation. OPN not only is required for MFB differentiation but also elevates α -SMA expression for MFB identification [12]. In vitro experiments demonstrate that OPN is both chemotactic and mitogenic for fibroblasts. In bleomycin-induced pulmonary fibrosis, OPN acts as a fibrogenic cytokine to promote migration, adhesion and proliferation [13].

We performed the retrospective histopathological analysis of swine nature with BOOP. By comparing histopathological constituents of BOOP samples (ECM, elastic fibrils, fibrotic mediators such as MMP-2,9, and OPN), we observed that persistent virus infection would result in swine BOOP, which shares great similarities with human BOOP. These investigations help widen our knowledge on BOOP fibrogenic pathways, and, hopefully, we will be able to block and even reverse the fibrogenic processes as a means of therapy.

2. Materials and Methods

2.1. Animal. All cases of natural porcine pneumonia were examined in the study. In the farms, affected pigs showed copious coughing, dyspnea, and crackle for approximately four weeks. They were empirically treated with amoxicillin and trimethoprim-sulphamethoxazole for 10 days upon the onset of symptoms. Stratified case samples were drawn from postweaned pigs to grower-finisher pigs. Four serial sections (5–10 mm) were made in each pulmonary lobe. Two aliquots of each lobe were frozen in -80°C until used, and the others were fixed in 10% neutralized formalin. Swine lung tissue blocks were examined for further investigations. All five cases (pigs) were collected from a small farrow-to-finish operation where chronic respiratory syndrome occurs sporadically. Five BOOP pigs and another five specific pathogen-free (SPF) pigs, purchased from Animal Technology Institute Taiwan, were euthanised and necropsied. Fresh samples were stored in -80°C until used.

2.2. Microbiological Examination. The routine microbiological cultures for aerobic and anaerobic bacteria were prepared. In addition, we used PCR method to detect the

common pathogens (specific primers listed in Table 1), such as Porcine reproductive and respiratory syndrome virus (PRRSV) [14], pseudorabies virus (PRV) [14], classical swine fever virus (CSFV) [15], porcine parvovirus (PPV) [14], cytomegalovirus (CMV) [16], porcine enterovirus (PEV) [17], porcine circovirus type 1 (PCV1) [18], *Mycoplasma hyopneumoniae* [19], porcine coronavirus [20], swine influenza virus (SIV) [21], and PCV2 [18].

2.3. Pathological Examination. Routine procedures were performed for histopathological examinations. Serial sections (4–6 μm) of paraffin-embedded samples were deparaffinized and rehydrated. Three of them were stained by haematoxylin and eosin stain and then followed by special stains, picrosirius red stain [22], and orcein-picroIndigocarmine (OPIC) stain [23] for total collagen and elastic fiber determination. The remainder sections were treated with boiling citrate buffer (pH 6.0) for 5 minutes for antigen retrieval and lastly performing modified labeled avidin-biotin (LAB) technique. Modified LAB technique uses specific primary antibodies and Histostain-Plus Bulk kits (Zymed 2nd Generation LAB-SA Detection system, Zymed Laboratories) [24]. The primary antibodies used in this study were anti-PCV2 polyclonal antibody (1 : 200, 210-29-PCV2, VMRD), anti- α -SMA antibody (1 : 200, MU128-UC, Biogenex), anti-MMP-2, 9 polyclonal antibody (1 : 200, sc-8835, sc-6840, Santa Cruz), and anti-OPN polyclonal antibody (1 : 200, ab8448, Abcam). To prepare a negative control, another section was incubated with mouse IgG (8 $\mu\text{g}/\text{ml}$, Vector Laboratories) instead of the primary antibody.

2.4. Zymography. MMP enzyme expression was assayed by SDS-PAGE zymography using gelatin (1.0 mg/ml) as MMP substrates [25]. Equal total protein (20 μg) of samples was subjected to electrophoresis, without boiling or reduction. Enzymatic activities attributed to MMP-2 and MMP-9 can be visualized in the gelatin-containing zymograms where clear bands are revealed against a dark background. Relative MMP-2 and 9 intensities of each sample were quantitated by Image Analysis with a Gel-pro Analyzer.

2.5. Western Blot. MFBs quantification was analyzed by western blot [26]. Frozen lung tissues (6–10 pieces of lung tissue in each case) were, respectively, crushed with a mortar and pestle at liquid nitrogen temperature and then homogenized by sonication in radio-immunoreactive protein extraction assay (RIPA) lysis buffer containing protease and phosphatase inhibitor (100 mM phenylmethylsulfonyl fluoride, 100 mM sodium-orthovanadate, 10 $\mu\text{g}/\text{ml}$ aprotinin, and 10 $\mu\text{g}/\text{ml}$ leupeptin) [26]. The homogenate was centrifuged at $10,000 \times g$ at 4°C for 10 minutes, and the supernatant was collected and stored at -80°C . These preparations were detected by immunoblot analysis. Twenty μg total protein was subjected to SDS-PAGE (12% polyacrylamide) and transferred to a PVDF membrane (Amersham) which was incubated with a 1 : 2,000 dilution of mouse anti- α -SMA antibody (Biogenex). PVDF membrane was then washed and incubated with a 1 : 5 000 dilution of goat antimouse IgG

TABLE 1: Sequence of primers used for these pathogens detection by PCR analysis.

Pathogen	Primer sequence
Porcine reproductive and respiratory syndrome virus (PRRSV)	Forward primer: 5'-CCC GGG TTG AAA AGC CTC GTG T-3' Reverse primer: 5'- TGT AAC TTA TCC TCC CTG AAT CTG-3'
Pseudorabies virus (PRV)	<i>gD</i> , forward primer: 5'-CAC GGA AGA GAT GGG GCT-3' Reverse primer: 5'-GTC GAC GCC CGC TTG AAG CT-3'
Classical swine fever virus (CSFV)	Forward primer: 5'-TTA AAR ATA GCC CCA AAA GAG CAT G-3' Reverse primer: 5'-CTG GCG TCC ATC ATY CCG YGT AT-3'
Porcine parvovirus (PPV)	<i>VP2</i> , forward primer: 5'-GCA GTA CCA ATT CAT CTT CT-3' Reverse primer: 5'-TGG TCT CCT TCT GTG GTA GG-3'
Cytomegalovirus (CMV)	<i>gB</i> , forward primer: 5'-CCC TGA TCT TAA ATG ACG AGG ACG TGA C-3' Reverse primer: 5'-ACC GTC TGA GAG AGA CTG AAC TTC TCT GAC AC-3'
Porcine enterovirus (PEV)	Talfan-3D, forward primer: 5'-CAA AGA CTG GTC CTT CAT TG-3' Reverse primer: 5'-ATA CGC CGA GCG CGG AAG AT-3'
Porcine circovirus type 1 (PCV1)	Forward primer: 5'-ATA CGG TAG TAT TGG AAA GGT AGG G-3' Reverse primer: 5'-ACA CTC GAT AAG TAT GTG GCC TTC T-3'
<i>Mycoplasma hyopneumoniae</i>	Forward primer: 5'-GAG CCT TCA AGC TTC ACC AAG A-3' Reverse primer: 5'-TGT GTT AGT GAC TTT TGC CAC C-3'
Porcine coronavirus	Forward primer: 5'-GCC ATT GAT TTA TGG AGA CA-3' Reverse primer: 5'-GTA TAA AAC CTC CTG GCT GT-3'
Swine influenza virus (SIV)	Forward primer: 5'-AGT ATA CAG CCT AAT CAG AC-3' Reverse primer: 5'-AGT AGA AAC AAG GGT ATT TTT C-3'
Porcine circovirus type 2 (PCV2)	ORF-2, forward primer: 5'-GGT TTG TAG CCT CAG CCA AAG C-3' Reverse primer: 5'-GCA CCT TCG GAT ATA CTG TCA AGG-3'

conjugated to horseradish peroxidase (SC-2005, Santa Cruz). Bands were visualized by the Amersham ECL-Plus detection reagents (Amersham) and were quantitated by Image Analysis with a Gel-pro Analyzer.

2.6. Statistical Analysis. All data were expressed as mean \pm SD. Descriptive statistic was first used for analysis of normality. Student *t*-test or Mann-Whitney Rank Sum Test was used to check if the data normality value is within accepted range. The mean values of two groups were considered significantly different if * $P < .05$, ** $P < .01$, or *** $P < .001$.

3. Results

3.1. Microbiological Examination and Pathogen Confirmation. In microbiological examination, the common culture for bacteria such as *Pasturella multocida*, *Streptococcus* sp., *Salmonella* sp., and *Actionbacillus pleuroneumoniae*, acid-fast organism, and fungus was negative. The results of PCR detections, PRRSV, PRV, CSFV, PPV, CMV, PEV, PCV1, *M. hyopneumoniae*, Porcine coronavirus, and SIV were all negative; only PCV2 was detectable in BOOP lungs (Figure 1). Furthermore, sequencing the amplicons resulted in hundred percent identical to PCV2 Taiwan strain (NCBI Accession AF166528 nt1169-1190). In addition, PCV2 antigen signals were revealed strongly positive in the foamy alveolar macrophage (Figure 2, arrow head) and intraluminal plugs of BOOP lesions by immunochemistry (Figure 2).

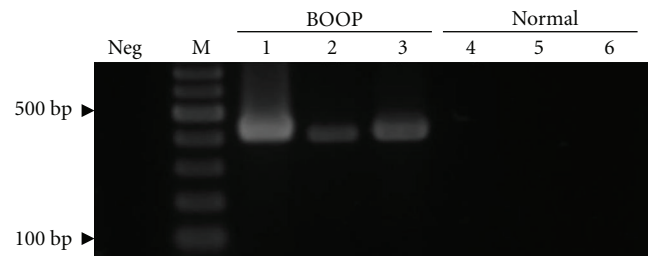


FIGURE 1: Detection of porcine circovirus type 2 (PCV2) with PCR method. The primers designed on the basis of the ORF2 of the PCV2 virus genome to produce a 416 bp long amplicon. The BOOP lung tissues from five pigs were positive. In the agarose gel, three representative cases were shown. Neg: negative control. M: leader. Lanes 1–3: BOOP lung samples. Lanes 4–6: normal lung samples from SPF pigs ($n = 5$, six replicates in each individual BOOP or normal pig).

3.2. Histopathological Constituents of BOOP Lesion. These lungs showed paleness and were noncollapsed and tan-mottled macroscopically. The alveolar septa appeared widened with proliferation of type II pneumocytes and mononuclear inflammatory infiltrates. The features of chronic pulmonary fibrosis showed grey hepatization including patchy distribution, intraluminal plugs of loose connective tissues that occlude bronchioles, alveolar ducts, and

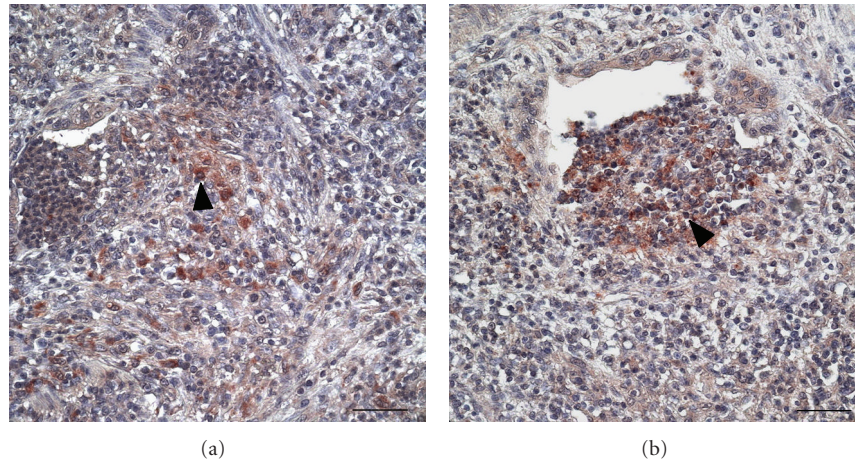


FIGURE 2: Confirmation of PCV 2 antigen in BOOP lesion. PCV 2 antigen signals appeared strong positive in the foamy alveolar macrophages ((a)-(b), arrow head) and intraluminal plugs of two representing BOOP lesions. The other lung sections in five individual BOOP pigs revealed the similar results ($n = 5$, six replicates in each individual pig; Bar = 50 μm).

surrounding alveoli (Figure 3(a)). Collagen and elastin, the main composition of extracellular matrix, are presented in picro-sirius red and OPIC stains. The intima of proliferated alveolar septa is full of large collagen fascicles, stained cherry in picro-sirius red stain (Figure 3(b)). Large accumulation of collagen also appeared dark blue in OPIC stain in evaginated BOOP lesion (Figure 3(c)). However, elastic fibrils (Figure 3(c), arrow head) were found disrupted and fragmented in peribronchial alveolar septa as compared to normal condition (Figure 3(c), upright panel). Most cells of the proliferative intima and evaginated BOOP lesion were identified as OPN positive (Figure 3(e)) and α -SMA positive cells (Figure 3(d)), which were further referred to as active MFBs. The OPN expression and the remodeling of collagens and elastines were remarkably colocalized with the distribution of active MFBs.

3.3. MMPs Activity in BOOP Tissues. In chronic pulmonary fibrosis of viral pneumonia, MMP-2 (Figure 3(f)) and MMP-9 (Figure 3(g)) were expressed actively near the margin of intraluminal plugs of bronchioles as macrophage and MFBs. The MMP activity was assessed by the intensity of gelatinolytic bands in zymography shown in (Figure 4(a)), and the quantified active MMPs were presented in bars (Figure 4(b)). Both pro- and active types of MMP-2 and MMP-9 were detectable in normal and BOOP lungs. All of them were significantly increased in BOOP group as compared to normal groups ($P < .05$, Figure 4(b)).

3.4. MFBs Quantification in BOOP Tissues. The expression level of α -SMA protein was used for MFBs quantification. The Western blotting picture revealed significant elevation of α -SMA expression in representative BOOP cases (pigs) (Figure 5(a)). The quantitative data were assayed from five cases (six lung sections in each case). The α -SMA expression was increased significantly in BOOP group as compared to the normal group ($P < .05$) (Figure 5(b)).

4. Discussion

BOOP is defined as granulation tissue plugs within lumens of small airways, sometimes results in complete obstruction and may have permanent effects on airway ventilation. The additional pathologic features include the following: (1) proliferation of connective tissue that forms intraluminal polyps (proliferative bronchiolitis obliterans), (2) fibrinous exudates, (3) alveolar accumulations of foamy macrophages, (4) inflamed alveolar walls, and (5) evenly spaced, rounded balls of myxomatous connective tissue [3]. Of note, all findings of our swine cases correlate with these definitions.

BOOP may be caused by radiotherapy, drug treatments, or response to viral infection. There are a variety of infectious etiologies associated with human BOOP, including *Serratia marcescens* [27], *Legionella pneumophila* [28], *Nocardia asteroides* [29], *Cryptococcus neoformans* [30], *Parainfluenza* type 3 [31], *Human immunodeficiency virus* (HIV) [32, 33], *Chlamydia pneumoniae* [34], *M. pneumoniae* [35], *Pneumocystis carinii* [36], *Human Herpesvirus-7* [37], *Coxiella burnetii* [38], severe acute respiratory syndrome coronavirus [39], *Mycobacterium avium intracellulare* complex [40], and parasite infection such as malaria *Plasmodium vivax* [41].

To date, several etiologies have also implicated in the pathogenesis of animal BOOP. Experimentally infected dogs with adenovirus [42–44], *Mycoplasma* [45], or cattle clinical infected bovine respiratory syncytial virus pneumonia occasionally develop BOOP [46]. Accidental intra-airway exposure of dog with a noninfectious agent oleic acid also induced BOOP [47]. Majeski et al. had developed a respiratory reovirus-infected mice model of BOOP which is T (CD4⁺ or CD8⁺) cells and interferon- γ dependent [48]. To our knowledge, BOOP has not been previously reported occurring naturally in pigs.

In this study, affected pigs showed copious coughing, dyspnea, and crackle for approximately four weeks. They were empirically treated with amoxicillin and trimethoprim-sulphamethoxazole for 10 days upon the onset of symptoms.

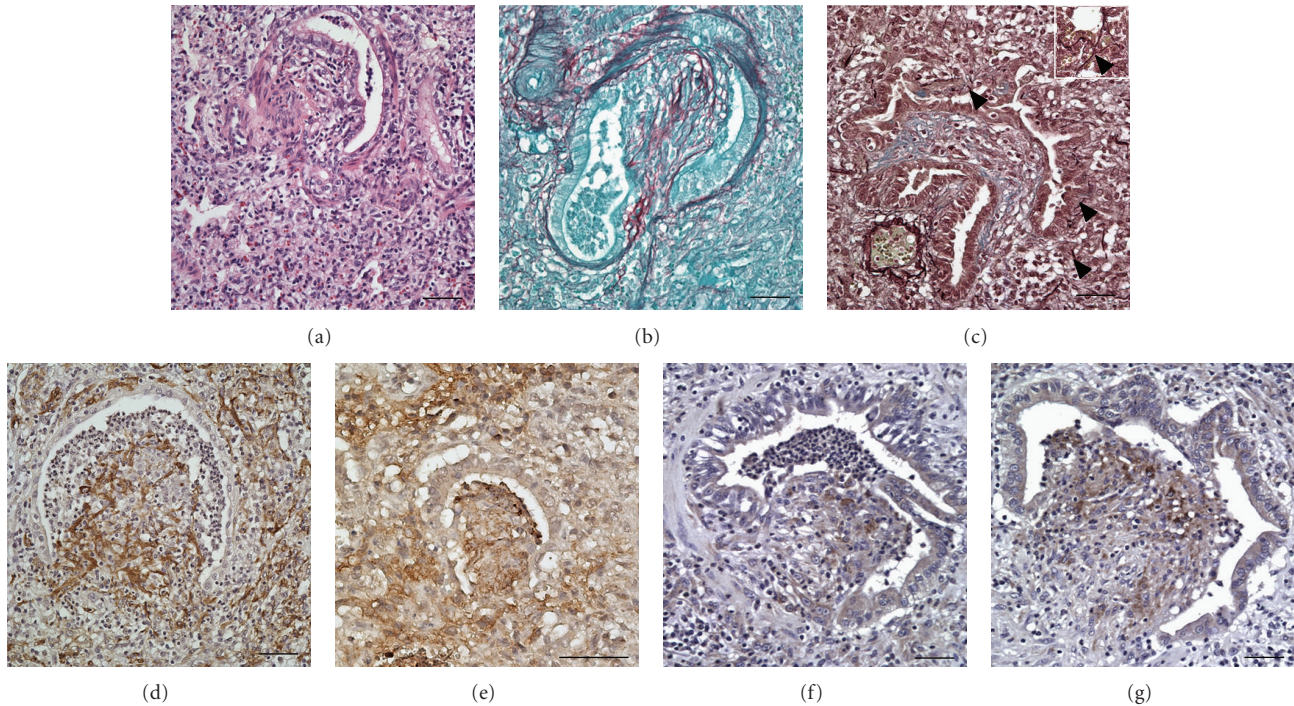


FIGURE 3: Histopathological constituents of BOOP lesion in specific staining. The representative picture showed the intraluminal plugs of loose connective tissue that occlude bronchioles in H&E stain (a). A large amount accumulation of collagen matrix was shown in picrosirius red stain (b) and strongly blue in OPIC stain (c) at the evaginated BOOP lesion. The elastic fibril revealed disruption and fragmentation in peribronchial alveolar septa ((c), arrow head) as compared to normal lung ((c) upright panel, arrow head). The proliferative cells of the intraluminal plugs and alveolar septa were identified as α -SMA positive MFBs (d) and OPN positive cells (e). MMP-2 (f) and MMP-9 (g) seemed to be expressed actively at the margin of intraluminal plugs of bronchioles as macrophage and myofibroblasts. The results shown represent one of six replicates in each independent experiment demonstrating similar results ($P < .01$ versus normal group). (Bar = 50 μ m).

However, there was no symptomatic relief. Several coinfecting pathogens associated with PMWS had been identified. They were PRRSV, PRV, *P. multocida*, *Bordetella bronchiseptica* [49], PPV [50], SIV, *M. hyopneumoniae* [51, 52] and coinfection of PCV2, PRRSV, and PPV in PMWS [50, 53–55]. Those lung specimens of our BOOP cases revealed negative for common bacterial isolation and PCR detection of most swine pathogens were only positive for PCV2 (Figure 1), which was subsequently confirmed by sequencing the 416 nucleotide PCR amplicons and immunohistochemical staining of PCV2 antigen in the intraluminal plugs of BOOP lesion (Figure 2). The pronounced neutrophil predominance in some tissue sections of our samples may suggest infectious origin especially in bacteria. However, there was no evidence of other infections aside from PCV-2 at the time of diagnosis of swine BOOP. But we cannot exclude the possibility that PCV-2 may not be the sole factor of swine BOOP, especially after drug treatment.

PMWS, caused by PCV2, has worldwide distribution [56–58] including Taiwan [59]. Clinically, the affected pigs are common when aging from 5 to 12 weeks old. The mobility and motility vary case by case. Motility may reach 10% in acute outbreak. It is characterized by progressive weight loss, dyspnea, cough, diarrhea, and occasionally

icterus [60, 61]. At necropsy, tan-mottled pulmonary consolidation and enlarged lymphoid organs are mostly observed [62]. They usually presented typical lymphohistiocytic to disseminated granulomatous interstitial pneumonia [50, 62], even that necrosis and sloughing of airway epithelium may progress to chronic stage and bronchiolitis fibrosa obliterations may be present [63, 64]. Furthermore, porcine respiratory disease complex (PRDC), pigs with PCV2 and other coexisting pathogens, may show moderate to marked multifocal peribronchial and peribronchiolar fibrosis and often extend into the airway lamina propria [52]. In this study, typical BOOP lesions were found in about 16-week-old growing and finishing pigs with chronic respiratory syndrome. These lungs were non-collapsed and hepatized in macroscopy. Interstitial fibrotic pneumonia, intraluminal organizing granulation tissue plugs, and connective tissue deposition extended into the alveolar ducts and covered the epithelium cells or type 2 pneumocytes in microscopy (Figure 3(a)). PCV2 nucleic acid and antigens were detectable in the multisystemic organs of affected pigs, mainly present in the monocyte/macrophage lineage and antigen presenting cells [62]. Besides positive response of PCV2 nucleic acid in our chronic PMWS cases, we also displayed conspicuous PCV2 antigen in the intraluminal

plugs of BOOP lesion by immunochemical staining as Figure 2.

Although many studies of common worldwide disease such as PMWS have been done and various infectious or noninfectious agents have been implicated as possible participants in different animals, the pathogenesis of PMWS is still not completely well understood. Nowadays, no literature review documented that swine BOOP and PCV2 infection that associated. It is highly probable that pigs with chronic bronchiopneumonia are usually obsolete from the economic aspect, thus these histopathological changes in lung were neglected. Through positive PCV2 antigen in the intraluminal plugs of BOOP lesions as shown in Figure 2, it strongly meant that PCV2 was associated with swine BOOP or intraluminal organization. After PCV2 was inoculated in previous studies, the levels of TNF- α , monocyte chemoattractant protein-1 (MCP-1), Interleukin (IL)-1 β , 8, 10, and Interferon- γ (IFN- γ) were significantly raised [51, 65–67]. However, future study is needed to elucidate the cytokine effects by which the PCV2 induces BOOP.

MMPs have been proposed to play a pivotal role in the pathogenesis of pulmonary fibrosis, but the exact mechanisms are not well characterized. There are several interrelated processes in ECM remodeling, such as collagens and elastins. MMP-2 and 9 are mark prolong degraded fibrillar gelatin and substrate affinity for type IV collagen, which is the key structural component of basement membrane. Furthermore, MMPs also participate in the regulation of other fibrotic mediators. MMP-2, 7, 9 and TIMP-1, 2, 3, 4 are upregulating in pulmonary fibroblastic foci [65]. MMP7 (matrilysin) is another regulator of pulmonary fibrosis in human and mice [66]. To examine whether gelatinases involve in this BOOP, both MMP-2 and 9 seemed to be expressed actively at the margin of intraluminal plugs of bronchioles as macrophage and MFBs (Figures 3(f) and 3(g)). Indeed, the activities of pro- and active form gelatinases were significantly increased in the swine BOOP compared to normal group by zymography (Figure 4).

Abundant MFBs presented as activated type (α -SMA positive) which invaded and migrated into bronchial lumen to form the intraluminal plugs (Figure 3(d)), and the expression level of α -SMA was also elevated significantly compared to the control by Western blot (Figure 5). The fibroblast and MFB play a central role in ECM synthesis and deposition. Remodeling of ECM and elastic fibrils (Figures 3(b) and 3(c)) presented at the evaginated BOOP lesions remarkably colocalized with the distribution of α -SMA positive MFBs. These findings included not only elevation of ECM deposition but also disruption and fragmentation of elastic fibrils in the peribronchial alveolar septa.

OPN was strongly expressed at the peribronchial and intraluminal plugs of swine BOOP (Figure 3(e)), which has not been described previously. OPN is a multifunctional matricellular cytokine abundantly expressed during inflammation. It is both chemotactic and mitogenic for fibroblasts [13] and required for fibroblast differentiated to MFB [12]. It could upregulate MMP-2,9 expression [67, 68]. OPN deficient transgenic mice demonstrated a reduction

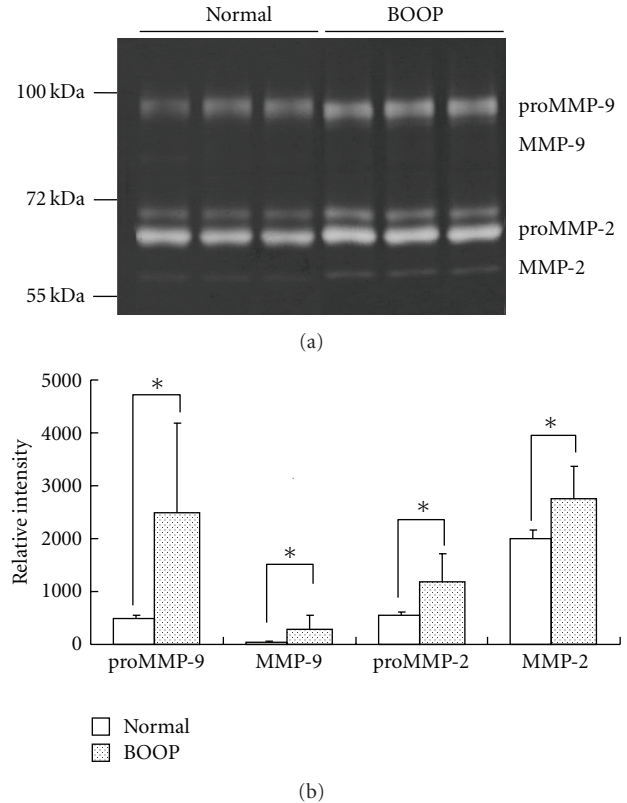


FIGURE 4: Metalloproteinase activity in BOOP tissues. The gelatin zymography revealed MMPs activity from three representative BOOP and normal pigs (a). For each individual BOOP or normal pig, six sample lung sections were evaluated for MMP activity and averaged. Then, the averages from the BOOP and normal animals were pooled from the five different pigs to generate the data presented (b). The activities of proMMP-9, MMP-9, proMMP-2, and MMP-2 were all significantly increased in BOOP group ((b), $P < .05$). * $P < .05$ versus normal group ($n = 5$, six replicates in each individual pig).

production of type I collagen and the level of activated MMP-2 and TGF- β 1 [69]. Although the expression of OPN has been demonstrated in different human unusual interstitial pneumonia and murine bleomycin-induced pulmonary fibrosis, OPN is still be elucidated in BOOP. MMP-2,9 bind to CD44, CD44 act as a transmembrane platform, latent TGF- β 1 is recruited and bound on which is activated by CD44-associated MMP-2,9 [70]. Based on previous observation, active TGF- β 1 induces pulmonary fibrosis and OPN promotes significant migration and proliferation in both epithelium and fibroblast/MFB in the fibrotic process [71]. Responding to lung damage, induced MMP-2 and MMP-9 promote the migration of fibroblast/MFB; meanwhile, MMP-2 activating MMP-9 and MMP-9 increases fibroblast proliferation and collagen synthesis [71].

In this study, identified BOOP of domestic pigs within natural PCV2 pneumonia shared critical features. They are temporal heterogeneity with fibrosis, differentiation and migration markers of MFBs, and ECM remodeling in human BOOP. Since there is no information about mediators and

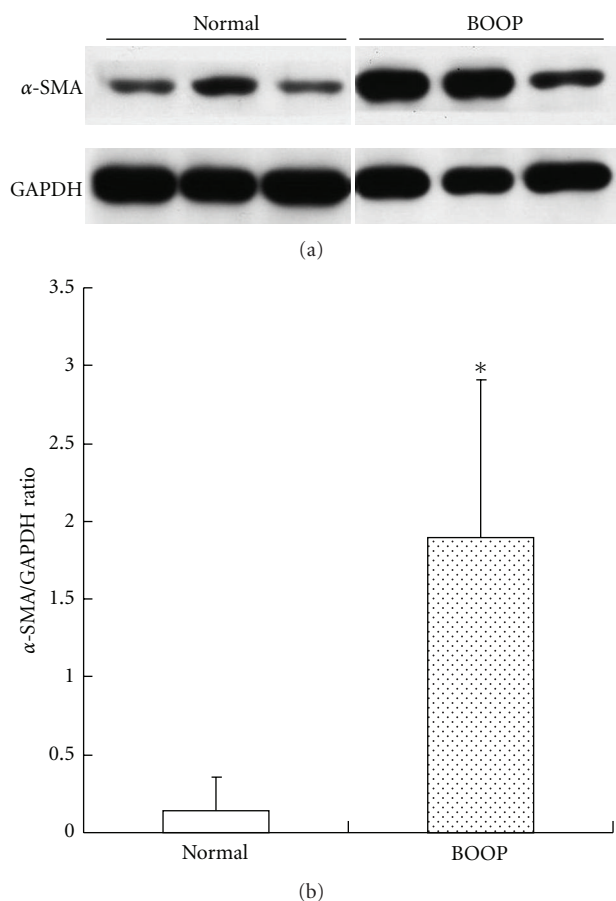


FIGURE 5: Quantitation of MFBs by alpha-SMA expression level. The Western blotting picture revealed that alpha-SMA expression level was increased in three representative BOOP cases (pigs) (a). The quantitative data were assayed from five cases (six lung sections in each case). Significant elevation of α -SMA expression in BOOP group was revealed ($P < .05$) (b). * $P < .05$ versus normal group ($n = 5$, six replicates in each individual BOOP or normal pig).

signal transduction pathway of swine BOOP, future studies must be conducted which may help prevent the sick pigs from the irreversible end stage of fibrotic pneumonia with worse prognosis. Most importantly, it is easier to get samples in swine BOOP than that in human so as to compare the pathological changes and pathogenesis with human BOOP. This can lead to pursuing the antifibrotic targeting and rational strategy of therapy.

Abbreviations Used

BOOP: Bronchiolitis obliterans organizing pneumonia
 PMWS: Postweaning multisystemic wasting syndrome
 ECM: Extracellular collagen matrix
 MFB: Myofibroblast
 α -SMA: α -smooth muscle actin
 G3PDH: Glyceraldehyde-3-phosphate dehydrogenase
 MMP: Matrix metalloproteinase
 OPN: Osteopontin.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

C.-C. Cheng conducted the experiments, analysed the data, and participated in the preparation of the first draft of the paper, Y.-F. Lee performed microbiological examination and helped with zymography, N.-N. Lin assisted in immunohistochemistry, C.-L. Wu and K.-C. Tung cared for the project and read the manuscript, and Y.-T. Chiu is the principal investigator who participated in the study design, data analyses, and paper preparation. The contribution of K.-C. Tung and Y.-T. Chiu is equal.

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