Porcine respiratory disease complex (PRDC) in Indian pigs: a slaughterhouse survey

Jigarji Chaturji Thakor[1], Monalisa Sahoo[1,5*], Karam Pal Singh[2**], Rajendra Singh[1], Salauddin Qureshi[3], Ajay Kumar[4] , Pradeep Kumar[1], Sagar Patel[1], Rohit Singh[2], Nihar Ranjan Sahoo[5]

[1]*Division of Pathology, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, India.*
[2]*ICAR-CADRAD, Indian Veterinary Research Institute (IVRI), Izatnagar, India.*
[3]*Division of Biological Standardization, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, India.*
[4]*Division of Biochemistry, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, India.*
[5]*ICAR-DFMD-International Centre for Foot and Mouth Disease, Arugul, Jatni, Bhubaneswar, Odisha.*

**Corresponding author at: Division of Pathology, Indian Veterinary Research Institute (IVRI), Izatnagar-243112 India.*
E-mail: vety.lisa@gmail.com.
***Corresponding author at: ICAR-CADRAD, Indian Veterinary Research Institute (IVRI), Izatnagar, India.*
E-mail: karam.singh@rediffmail.com.

Veterinaria Italiana 2023, **59** (1), 23-39. doi: 10.12834/VetIt.2935.20591.2
Accepted: 03.08.2022 | Available on line: 31.03.2023

Keywords
Bacterial pathogens,
India,
PRDC,
Pigs,
Pathology,
PCR,
Slaughterhouse,
Viruses,
Pakistan.

Summary
Porcine Respiratory Disease Complex (PRDC) is an unequivocally leading cause of economic losses to the pig industry. To investigate the pathogens associated with PRDC, a total of 900 lungs with gross lesions and 125 lungs with no appreciable gross lesions were collected from the abattoirs and subjected to pathological investigation for distribution of lesions / and types of exudates, as well as to molecular confirmation of bacterial and viral pathogens by PCR. The pneumonic lungs showed the higher prevalence of *Mycoplasma* spp. (31.22%), with evidence of *M. hyorhinis*, *P. multocida* (21.33%), *S. suis* (18.66%), *B. bronchiseptica* (16.77%), and viral pathogens as porcine circovirus type 2 (PCV2) (28.11%), porcine reproductive and respiratory syndrome virus (PRRSV) (2.7%) and swine influenza virus (SIV) (1.2%). On histopathological examination, high prevalence of bronchopneumonia (37.88%) followed by enzootic pneumonia-like lung lesions (11.44%), and interstitial pneumonia (7.44%) was recorded in the majority of affected pigs. The winter season was found to be more conducive for highest prevalence of pneumonia as compared to other seasons. The present study reports the high prevalence of PRDC in slaughtered pigs of India. *M. hyorhinis* showing the EP-like lesions, PCV2 and their combination were likely to be the prime contributors of PRDC in Indian pigs.
Keyword: Bacterial pathogens, India, PRDC, pigs, pathology, PCR, slaughterhouse, viruses.

Introduction

Respiratory diseases are the major obstacle in pig production worldwide. This is due to complex interaction of multiple aetiologies *Mycoplasma*, bacterial and viral pathogens, host, environment, and management practices, leading to overlapping lesions, hence the term porcine respiratory disease complex (PRDC) is commonly used (Opriessnig *et al*. 2011, Chae 2016, Li *et al*. 2021). The PRDC accounted for up to 40% morbidity and more than 50% mortality in young pigs and piglets and 60% in growers / finishers (Sorensen *et al*. 2006). The highest rate of incidence in pigs compared to other domestic animals is related to high density rearing practices in pigs over extended period, which favours easy transmission and persistence of pathogens in small to medium sized pig farms (Opriessnig *et al*. 2011).

Please refer to the forthcoming article as: Jigarji *et al*. 2023. PRDC in Indian slaughtered pigs. Vet Ital. doi: 10.12834/VetIt.2935.20591.2

The PRDC often accounts for 14% of total economic losses in the pig production system (Sorensen *et al*. 2006), along with the reduction in daily weight in infected pig around 6–10% (Donko *et al*. 2005). Pneumonic lungs in pigs are most frequently sighted at slaughter, with reported prevalence ranging from 19% to 79% (Fablet *et al*. 2012). Moreover, a recent investigation reported the economic loss of $6.55 per animal with lung lesions at slaughter (Ferraz *et al*. 2020). It is an established fact that the PRDC is a leading cause of economic losses as a consequence of lung condemnation (3%), condemnation of the diseased animals (37%), and 60% reduced weight gain (Sorensens *et al*. 2006). In Brazil, condemnations of pig carcasses (about 30%) are mainly due to the pulmonary lesions (Coldebella *et al*. 2017). Among the various causes of pneumonic lesions in PRDC, bacterial causes-like *Mycoplasma* spp, *Pasteurella multocida* (*P. multocida*), *Actinobacillus pleuropneumoniae*, *Streptococcus suis* (*S. suis*), *Bordetella bronchiseptica*, and *Haemophilus parasuis* top the list in several reports (Hansen *et al*. 2010, Lyutskanov *et al*. 2010, Zha *et al*. 2013). Among the viral pathogens, porcine circovirus type 2 (PCV2), porcine reproductive and respiratory syndrome virus (PRRSV) and swine influenza virus (SIV) are frequently reported to be associated with respiratory lesions in slaughtered pigs (Galdeano *et al*. 2019, Zhu *et al*. 2021, Tonni *et al*. 2022). The pathological alterations of lungs are highly dependent on the type of pathogens involved, passing through no visible lesions to nearly 100% multifocal tan mottling or consolidation of the lung. In India, the detailed information on the prevalence of the PRDC with reference to aetiology of various forms of pneumonia is not available. The published literature on respiratory affections in pigs encompasses isolated case studies, outbreaks, and some fallen / slaughter cases. Therefore, the present study was undertaken to investigate the issue with a wider coverage, by using the 5 major pork rearing states of India, to figure out the prevalence of six targeted bacterial pathogens (*P. multocida, Mycoplasma* spp, *B. bronchiseptica*, *S. suis*, *A. pleuropneumoniae*, and *H. suis*), and 3 major viral pathogens (PCV2, PRRSV, and SIV). Moreover, associated pathological alterations of lungs in pigs were investigated, so that effective strategies could be considered to minimize the PRDC prevalence.

Materials and methods

Field study area

During the period from August 2020 to April 2022, a total of 1300 lungs were screened. Out of which, 900 lungs showing appreciable gross lesions were randomly collected from adult pigs of either gender at different slaughterhouses, namely at Bareilly (115), Lucknow (61), Kanpur (98) of Uttar Pradesh, Deonar (231) of Maharashtra, Guwahati (165) of Assam, Haringhata (125) of West Bengal, and Ranchi (105) of Jharkhand. In addition, a total of 125 apparently healthy lungs were also collected randomly from these abattoirs for comparison purposes. The above places were selected because of the high density of pig population.

As per Statista Research Development, the pig population in Uttar Pradesh, Maharashtra, Assam, West Bengal and Jharkhand are 0.41 million, 0.16 million, 2.1 million, 0.54 million, and 0.34 million, respectively. The numbers of pigs slaughtered by electrocution method on a daily basis at each slaughterhouse varied from 40 to 200. For the detailed investigation of respiratory lesions, the lungs after evisceration were visually examined for change in colour, presence of rib imprints, inflation/ deflation; and on palpation for texture, exudates, and distribution of lesions, etc.

Sample collection

Representative lung tissue pieces of approx. < 5 mm thickness from the interface of lesions were collected in 10% neutral buffered formalin (NBF) as well as on ice for histopathological and molecular investigation, respectively. The similar procedure was adopted for the 125 uninfected lung samples.

Pathological investigation

After overnight fixation of the tissues in the NBF, the NBF was refreshed and left out for additional time to ascertain complete fixation of tissues. The fixed tissue pieces were subjected to paraffin embedding procedure. The paraffin embedded tissue cassettes were cut into 4-5 µm thick sections using Semiautomatic Microtome (Leica RM2245) followed by staining with routine hematoxylin and eosin stain. The dominant histopathological lung lesions were captured digitally using upright microscope (Olympus, BX3, Japan and 5 Mega PixJenoptic Camera). The lesions scoring of lungs showing cranioventral consolidations was based on the previously score proposed by Pallares *et al*. (2021). The scoring of enzootic–pneumonia (EP) like lesions was done as per Przyborowska-Zhalniarovich *et al*. (2021).

Molecular investigation

DNA isolation from lung tissues

Normal and pneumonic / affected lung pieces were processed for DNA isolation by using DNeasy

Blood and Tissue Mini Kit (Qiagen, USA) as per manufacturer's instructions.

Molecular detection of bacterial pathogens by PCR

The PCR was carried out in standard 25 µl reaction volume in 0.2 ml eppendorf tubes using DNA thermal cycler (Applied Biosystems). For the genomic detection of the six targeted bacteria (*P. multocida*, *Mycoplasma* spp, *A. pleuropneumoniae*, *S. suis*, and *B. bronchiseptica*, and *H. parasuis*), and 3 viral pathogens (PCV2, PRRSV, and SIV) published primers were used (Table I).

The amplified products were detected in 1.5% agarose gel prepared in 1X TBE buffer containing ethidium bromide (final concentration 0.5 µg/ml). Amplified product (8 µl) of each sample was loaded and gels were run at 100V for 45 min. The products were observed under UV trans-illuminator (UVP, USA), documented using Gel Documentation System (BioRad).

Statistical analysis

Chi square test was used for the comparison of different types of respiratory affections between the seasons. Data were analysed by Graphpad Prism software 9.0 and $P<0.05$ was considered as statistically significant.

Results

Out of 1300 lungs screened, 900 (69.23%) cases were selected showing different types of gross pulmonary lesions. A total of 900 pneumonic lungs and 125 apparently healthy lungs were tested for the six targeted bacterial pathogens and three major viral pathogens by polymerase chain reaction (PCR). The PCR amplification of *P. multocida*, *Mycoplasma* spp., *M. hyorhinis*, *S. suis*, *B. bronchiseptica*, *A. pleuropneumoniae*, *H. parasuis*, PCV2, PRRSV and SIV showed on agarose gel the amplicons sizes of 460 bp, 460 bp, 604bp, 688 bp, 164 bp, 342bp, 821bp, 481 bp, 803 bp, and 244 bp, respectively (Figure 1A-J). Among the bacterial pathogens, *Mycoplasma* spp. (31.22%) was the most prevalent followed by *P. multocida* (21.33%), *S. suis* (18.66%), *Bordetella bronchiseptica* (16.77%), *A. pleuropneumoniae* (1.88%), and *H. parasuis* (0.81%).

Among 31.22% *Mycoplasma* spp. positive cases, *M. hyorhinis* (247 lungs, 27.44%) were detected in higher frequency, whereas remaining 34 cases

Table I. *List of PCR primers used for the detection of bacterial respiratory pathogens of pigs.*

Pathogen	Gene	Primer sequence (5'–3')	PCR product size (bp)	Reference
Actinobacillus pleuropneumoniae	*dsbE*	F: GATAAACCTTTTCCGGAATT R: TACCACACCGTGTTTATCAA	342	Chiers *et al*. 2001
Mycoplasma sp.	*16s r-RNA*	F:GGCGAATGGGTGAGTAACACG R:CGGATAACGCTTGCGACCTATG	460	Wong-Lee and Lovett 1993
M. hyorhinis	*16s r-RNA*	FP:AACGGGATGTAGCAATACATTC RP:AGCGGACTGAAGTTGAGCTTCAG	604	Timenetsky *et al*. 2006
M. hyopneumoniae	*P36*	F:CCGATTAGTGTCTCCCGTTATG R:GGGCCGATGAAACCTATTAAAATAGCT	853	Caron *et al*. 2000
P. multocida	*KMT17 and KMT1SP*	F: TATTTA GGTGACACTATAG R: TAATACGACTCACTAT AGGG	460	Townsend *et al*. 1998
S. suis	*gdh*	F: GCAGCGTATTCTGTCAAACG R: CCATGGACAGATAAAGATGG	688	Okwumabua *et al*. 2003
Bordetella bronchiseptica	*fla*	F :CCCCCGCACATTTCCGAACTTC R : AGGCTCCCAAGAGAGAAAGGCTT	164	Hozbor *et al*. 1999
Haemophilusparasuis	*16s r-RNA*	F: GTGATGAGGAAGGGTGGTGT R: GGCTTCGTCACCCTCTGT	821	Oliveira *et al*. 2001
***(Porcine circovirus 2)* PCV2**	*ORF2*	F: CGGATATTGTAGTCCTGGTCG R: ACTGTCAAGGCTACCACAGTCA	481	Ellis *et al*. 1999
***Porcine reproductive and respiratory syndrome Virus* (PRRSV)**	*Orf 5*	F: TGACACCTGAGACCATGAGG R: GTGCAGAAGCCCTAGCAGTC	803	Rajkhowa *et al*. 2016
Swine Influenza Virus (SIV)	*Matrix (M) Protein*	F: CTTCTAACCGAGGTCGAAACG R: AGGGCATTTTGGACAAAG/TCGT CTA	244	Schmidt *et al*. 2016

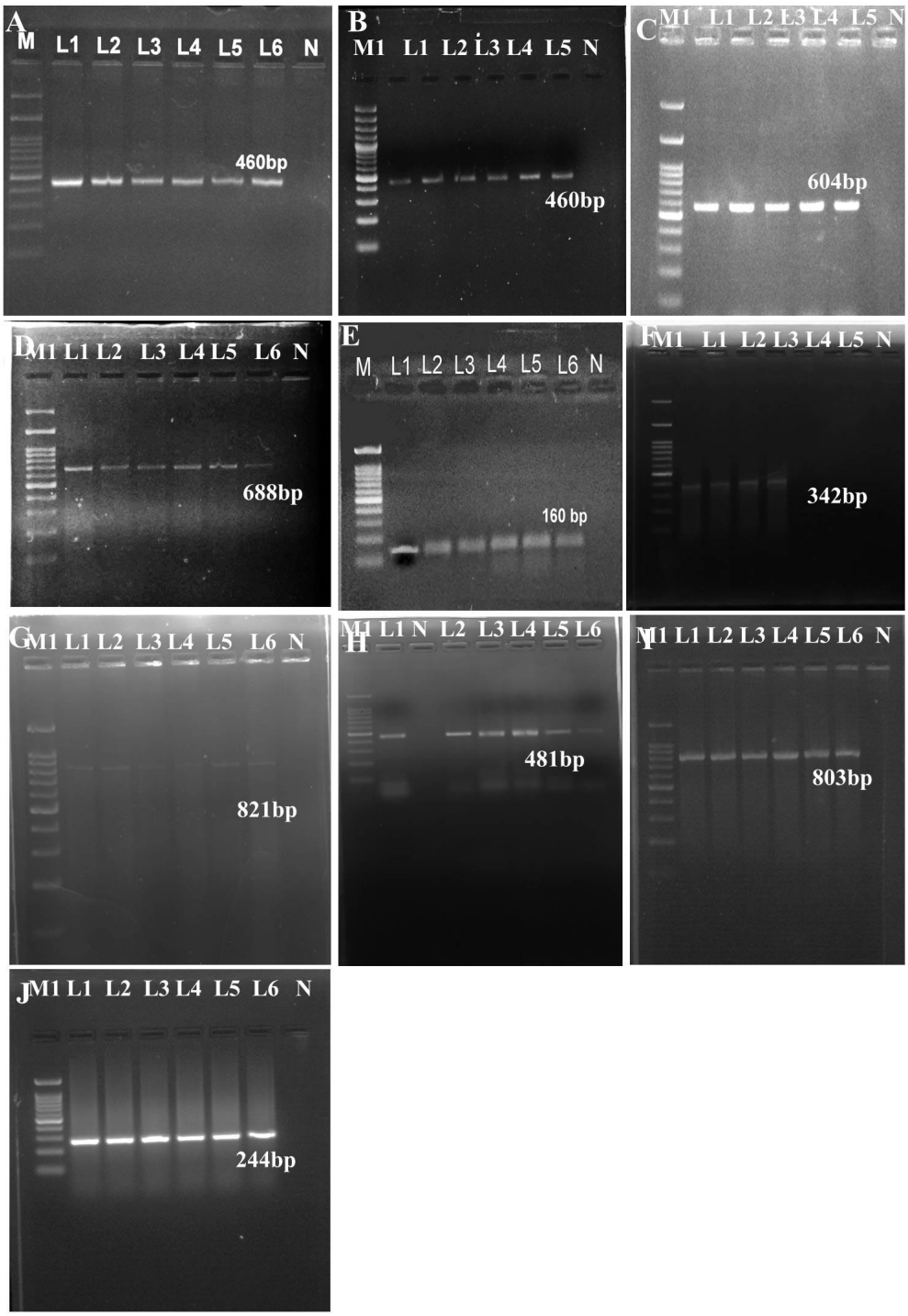

Figure 1. *Molecular detection of bacterial pathogens in PRDC affected pigs.* ***A****) M- marker, L1: Positive control, l2-6: positive PCR products of P. multocida (460bp), N-negative control.* ***B****) M-marker, L1: Positive control, L2-5: positive PCR products for* Mycoplasma *spp. (460bp), N-negative control.* ***C****) M-marker, L1: Positive control, L2- positive PCR products (604bp) for* Mycoplasma hyorhinis*, N-negative control.* ***D****) M-marker, L1: positive control, L2-5: PCR products for* S. suis *(688bp), N-negative control.* ***E****) M-marker, L1: positive control, L2-5: positive PCR products for* B. bronchiseptica *(160bp), N-negative control,* ***F****) M-marker, L1: Positive control, L2-5: positive PCR products for* A. pleuropneumoniae *(342bp), N-negative control.* ***G****) M-marker, L1: Positive control, L2-5: PCR products for* H. parasuis *(821bp), N-negative control.* ***H****) M-marker, L1: Positive control, L2-6: PCR products for PCV2 (481bp), N-negative control.* ***I****) M-marker, L1: Positive control, L2-6: PCR products for PRRSV (803bp), N-negative control.* ***J****) M-marker, L1: Positive control, L2-6: PCR products for SIV (244bp), N-negative control.*

failed to amplify for either *M. hyorhinis* or *M. hyopneumoniae*. In apparently healthy lung samples (125 cases), *Mycoplasma* spp, *M. hyorhinis*, and *S. suis* were detected in 4 (3.2%), 1(2.4%) and 7 (5.6%) cases, respectively.

The contributions of the microbiome and microaspirates towards development of pneumonia, either singly or in combination were 50.81% and 49.18%, respectively. In the mixed infections, the predominant coinfection was observed in case of *Mycoplasma* spp. in combination with *P. multocida* (60.81%) followed by in combination with *S. suis* (18.77%), and with B. *bronchiseptica* (16.73%).

Out of 900 samples tested for the detection of three major viral pathogens, the PCV2 was detected at the higher rate (28.11%), followed by PRRSV (2.7%), and SIV (1.2%). PCV2 was also detected in the apparently healthy lungs (18 cases). The combination of PCV2 with PRRSV was detected in 12 cases of diseased lungs. The coinfection of PCV2, and *Mycoplasma* spp. (68 cases) was the predominant one followed by the mixed infections of PCV2, *Mycoplasma* spp., and *P. multocida* (21 cases), and concomitant infections of PCV2, *Mycoplasma*, PRRSV, and *S. suis* in four cases. In apparently healthy lungs, mixed infection of PCV2 and *Mycoplasma* spp. were only detected in seven cases. On exploration of respiratory passages and the lung parenchyma, different types of lesions were noted with respect to infectious and non-infectious causes. Cranioventral pulmonary consolidation was the most frequent lung lesion (543 lungs; 60.33 %), followed by enzootic pneumonia-like (EP-like) lesions (210 lungs; 23.33%), EP-like lesions with pleuritis (92 lungs; 10.22 %), pleuritis alone (17 lungs; 1.88 %), dorsocaudal infarct (26 lungs; 2.88%), and dorso-caudal infarct with pleuritis (12 lungs, 1.33%). The lungs scoring for cranioventral consolidation are presented in Table II.

Table II. *Lung gross lesion scoring in pigs naturally affected with PRDC.*

Gross lung lesions	Number of lungs (percentage)
Cranioventral pulmonary consolidation	543 (60.33%)
Score 1	114 (12.66%)
Score 2	187 (20.77%)
Score 3	162 (18.00%)
Score 4	72 (8.88%)
Score 5	8 (0.88%)
Enzootic pneumonia-like lung lesions	210 (23.33%)
Dorsocaudal infarcts with pleurisy	26 (2.88%)
Pleurisy alone	12 (1.33%)

The lesions scores 2 and 3 were the most frequent with 20.77 % (187 lungs) and 18.00% (162 lungs), respectively.

The pulmonary lesions in 900 affected lungs were arbitrarily classified as bronchopneumonia in 341 (37.88%) cases, EP-like lesions in 103 (11.44%) cases, interstitial pneumonia in 67 (7.44%), parasitic pneumonia in 14 (1.55%) cases, and miscellaneous lesions, like vascular changes (congestion/ hemorrhage/edema), emphysema, and atelectasis in 92 (10.22%) cases. Further, the prevalence of the bronchopneumonia was 37.88%, with varying degree and duration. Depending on the composition of the cellular exudates, the bronchopneumonia was further described as acute suppurative bronchopneumonia, sub acute hemorrhagic pneumonia and necrotizing bronchopneumonia.
Acute bronchopneumonia was diagnosed in 110 cases (12.22%), characterized by congestion, reddish brown-to-grayish discoloration, and firmer consolidation of cranio-ventral lobes (Figure 2A). Microscopically, the respiratory air passages packed with heavy infiltration of neutrophilic exudates, RBCs, and cellular debris (Figure 2B).

Sub-acute bronchopneumonia was observed in 81 (9.0%) cases, revealing gray-pink discoloration, firmer consistency, consolidated apical and intermediate lobes with little involvement of diaphragmatic lobes (Figure 3A). The interlobular septa were more prominent and edematous. Histopathologically, the bronchi, bronchioles, and alveoli were filled with moderate numbers of neutrophils, macrophages, and cellular debris (Figure 3B); the respiratory epithelium degenerated and sloughed into the lumen, with the peribronchial connective tissue infiltrated with neutrophils and macrophages.
Chronic bronchopneumonia was diagnosed in 17 (1.88%) cases in which lungs were gray-to-pink in color with diffused hepatization of cranio-ventral lobes, involving the apical, cardiac, and intermediate lobes (Figure 4A). Microscopically, there were varying degrees of mononuclear cells (macrophage, lymphocytes, and fibroblast) infiltration in the lumen of air passages and in the parenchyma. The fibro-vascular proliferation forming concentric plugs were seen in the airways, causing bronchiolitis obliterans with associated atelectasis, emphysema, and abscess formation. The abscesses were walled off with thick connective tissue infiltrated with many neutrophils, a few macrophages, round cells, and fibroblasts (Figure 4B).

The fibrinous bronchopneumonia was observed in 88 cases (9.77%), depicting cranioventral lobes with deep red discoloration due to congestion and hemorrhages; yellowish-to-tan fibrin covering the pleura (Figure 5A). Microscopically, there was

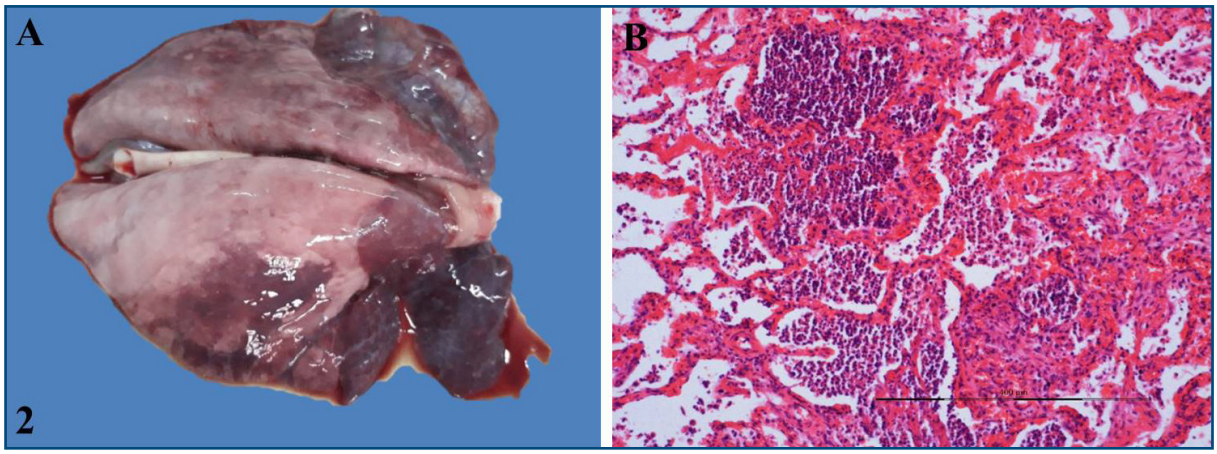

Figure 2. Acute suppurative bronchopneumonia in pig. ***A****) Cranio-ventral lobes and part of diaphragmatic showing consolidation.* ***B****) Alveoli packed with inflammatory cellular exudates, mainly constituted by neutrophils (H&E; x200).*

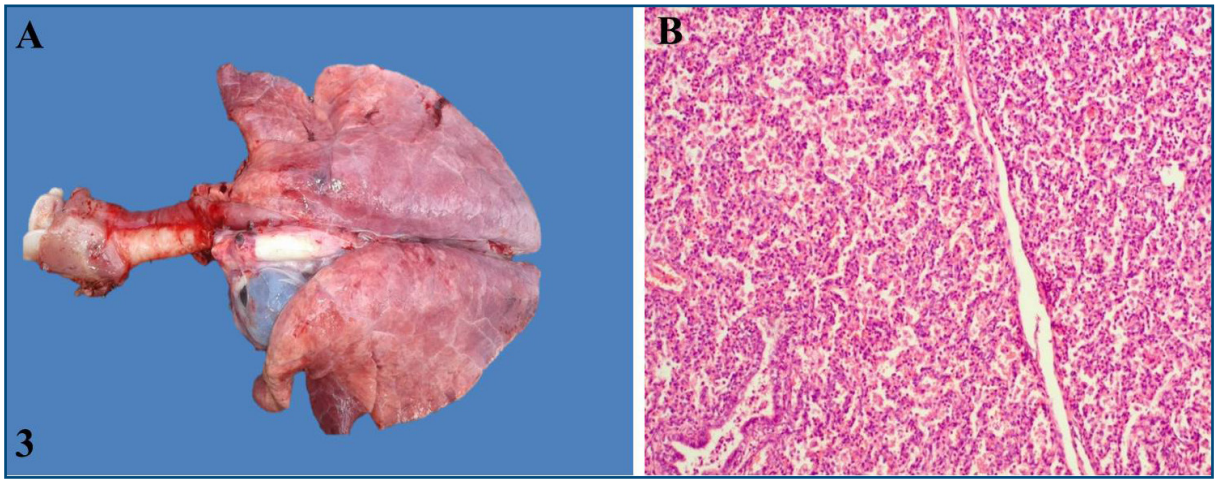

Figure 3. Sub-acute bronchopneumonia in pig. ***A****) Consolidation of apical and cardiac lobes and marbling of the lung.* ***B****) Heavy mix infiltration of mononuclear cells and neutrophils within the alveolar spaces (H&E staining; x100)..*

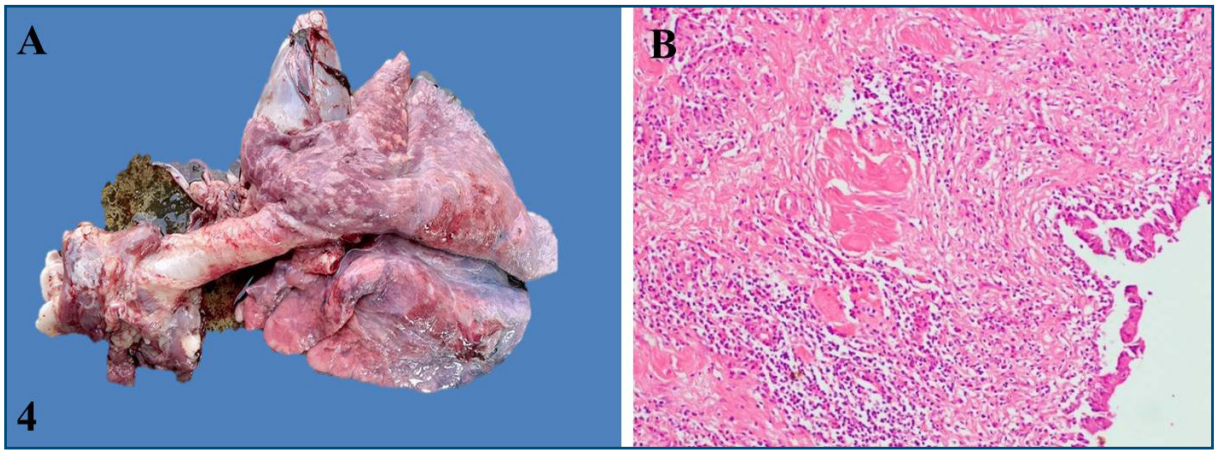

Figure 4. Chronic bronchopneumonia in pig: ***A****) Cranio-ventral consolidations of both lobes of lungs with pleural adhesions.* ***B****) Extensive fibrous tissue proliferation with huge infiltration of mononuclear cells (H&E; x100).*

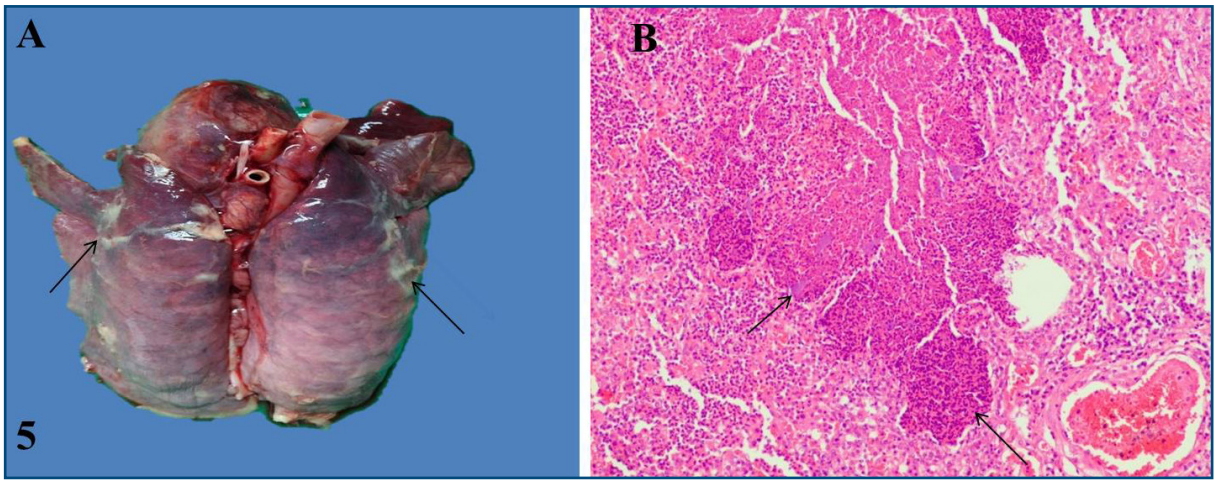

Figure 5. Fibrinous bronchopneumonia in pig. ***A****) Deposition of the fibrin (arrow) on the pleura of lung lobes.* ***B****) Many alveoli packed with oat shaped cells (arrow) (H&E staining; x100).*

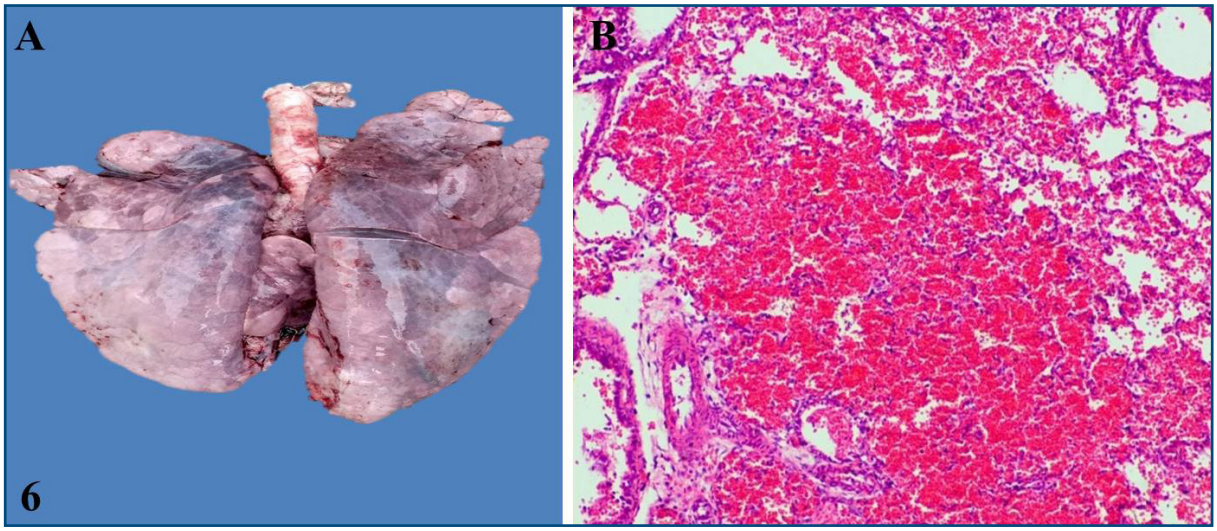

Figure 6. Haemorrhagic pneumonia in pigs. ***A****) Bilateral involvement of marked congestion with haemorrhages.* ***B****) Marked congestion and hemorrhages within the alveoli (H&E staining; x100).*

fibrino-cellular reaction, comprising predominantly macrophages (oat cells), fibrin, edema fluid, bluish bacterial colonies, and occasionally neutrophils in the respiratory airways and alveolar spaces. The leukocytes especially macrophages in the form of basophilic spindle-shaped cells or oat-shaped cells, often arranged in a streaming pattern, surrounding multifocal areas of necrosis and obliterating the alveolar spaces at various locations (Figure 5B).

In 19 cases (2.11%), lungs showed acute haemorrhagic pneumonia, with marked congestion. Moderate to severe petechiae along with some echymotic haemorrhages were present in multifocal areas of the lungs (Figure 6A). Histopathologically, marked congestion and haemorrhages were present in the entire lungs tissue section (Figure 6B). In some cases, marked infiltration of lymphocytes and macrophages were observed in the interlobular septa and pleura.

Necrotizing bronchopneumonia was noticed in 26 (2.88%) lungs examined during the present study. Grossly, all the lobes of lungs were dark in colour and haemorrhagic. A large necrotic mass was present in the anterior half of the diaphragmatioc lobe (Figure 7A). Microscopically, large areas of coagulative necrosis causing considerable destruction of pulmonary architecture were noticed. In some cases, numerous bacterial colonies were found at the sites of necrosis (Figure 7B). Infiltration of large number of polymorphs and mononuclear cells was

conspicuous in the lobules adjacent to the necrosed areas.

A total of 103 cases (11.44%) were histologically diagnosed as EP-like lesions, out of which 98 cases were positive for *M. hyorhinis* by PCR. Grossly, the bilateral apical and cardiac lobes were consolidated, and sharply demarcated from the healthy area (Figure 8A). Histologically, the bronchial mucosa showed hyperplasia and infiltration of neutrophils in the wall and the lumen. Lymphoid aggregates were present at multi-locations in the areas of the parenchyma (Figure 8B), perivascular space, and interalveolar septa, obliterating the alveolar spaces in the lung sections. The average value of EP-like lesion scoring was 2.98 for all the lungs and 3.75 for the affected lungs. The EP-like lesion score in the slaughtered pigs ranged from 0.42 to 3.56.

A total of 67 (7.44%) samples showed microscopic lesions of interstitial pneumonia, which were further subdivided into acute and chronic interstitial pneumonias, and broncho-interstitial pneumonia. Thirty six (4.00%) cases of acute interstitial pneumonia were observed showing pale, heavy, rubbery, and non-collapsible lungs with multifocal areas of congestion (Figure 9A). The cut surfaces of lungs revealed meaty appearance and scanty exudate on squeezing. Histopathologically, the alveolar spaces contained scanty eosionophilic fluid mixed with few cells; mild to moderate thickening of interstitium of alveolar walls with mononuclear cells (lymphocytes, macrophages) (Figure 9B).

Fourteen (1.55%) samples showed the lesions of chronic interstitial pneumonia in the dorsocaudal parts. The lungs were pale, heavy, swollen, reddish-grey in color, meaty/rubbery in texture, and airways were clean (Figure 10A). Histopathologically, the alveolar walls were thickened with fibro-cellular tissue characterized by the infiltration of mononuclear cells (lymphocytes, macrophages), fibrosis, and type II pneumonocytes hyperplasia. Peribronchial and perivascular connective tissue had fibro-cellular infiltration as a contiguous change (Figure 10B).

Broncho-interstitial pneumonia was detected in 17 (1.88%) cases; lesions of both bronchopneumonia and interstitial pneumonia were evident. The lungs shared lesions of interstitial pneumonia such like- heavy, edematous, reddened, swollen lungs with bronchopneumonia lesions of grayish, firm, consolidation distributed in all the lobes (Figure 11A). Microscopically, bronchi and bronchiolar mucosa were infiltrated with moderate numbers of neutrophils, epithelium desquamated, and lumen filled with heavy neutrophilic exudate. The pulmonary interstitium was infiltrated with mononuclear cells (lymphocytes, macrophages, and fibroblasts, respectively). The alveolar lumen contained a few neutrophils and mononuclear cells, and the wall of the alveoli and the bronchioles was thickened and infiltrated with moderate to severe infiltration of mononuclear cells (Figure 11B).

Parasitic pneumonia was detected in 14 cases (1.55%). Grossly, many small grey nodules were observed in the caudo-ventral borders of the diaphragmatic lobes.
On cut section of the lesions, several adult white, thread-like worms exuded along with mucous exudates.

Microscopically, the cut sections of the parasites and the eggs were present in association with scant catarrhal cellular exude in the lumen of the bronchioles (Figures 12A&B).

The parasites were identified as *Metastrongylus* spp. in all the cases. Hydatidosis were found in 8 (0.88%) cases, characterized by multiple clear fluid filled sterile cysts protruding on different lobes of lungs. Histopathological examination showed extensive inflammatory reactions at the interface of cysts with the tissues and an acellular lamellar cyst wall, germinal membrane, and a surrounding foreign body granulomatous reaction (Figures 13A & B).
Other miscellaneous lesions were detected in 92 cases, and included vasculitis (25, 2.77%), bronchitis (34, 3.77%), pulmonary oedema (19, 2.11%), and bronchial smooth muscle hypertrophy (15, 1.66%). Pleuritis was observed in 12 cases, and characterized by the thickened pleura with infiltration of inflammatory cells and with proliferation of connective tissue, congestion, oedema, and mesothelial hyperplasia.

The most frequent combination of microscopic patterns was acute bronchopneumonia + broncho-interstitial pneumonia + EP-like lesions in 121 lungs (13.44 %) and the same combination accompanied with pleuritis in 51 lungs (5.66%), followed by the EP-like lesions with interstitial pneumonia in 113 cases (12.55%), and by combination of subacute bronchopneumonia + acute interstitial pneumonia + fibrinous bronchopneumonia in 47 lungs (5.2%).

The presence of *Mycoplasma* spp. was detected in lungs showing bronchopneumonia (121 cases, 13.44%), EP-like lesions (103 cases, 11.44%), and interstitial pneumonia (57 cases, 6.33%). *P. multocida* was detected in lungs lesions mainly associated with bronchopneumonia (acute bronchopneumonia (82 cases, 9.11%); fibrinous bronchopneumonia 63 cases, 7%); necrotizing pneumonia- (14 cases, 1.56%); subacute bronchopneumonia- (22 cases, 2.44%), and interstitial pneumonia- (11 cases, 1.22%). *S. suis* was detected in lesions associated with the following diseased conditions: acute bronchopneumonia

(78 cases, 8.67%); subacute bronchopneumonia (13 cases, 1.44%); chronic bronchopneumonia (5 cases, 0.56%); fibrinous pneumonia (27 cases, 3%); EP-like lesions (31 cases, 3.44%); broncho-interstitial pneumonia (14 cases, 1.56%). *B. bronchiseptica* was found in higher association with subacute pneumonia (51 cases, 5.67%), fibrinous pneumonia (29 cases, 3.22%) acute bronchopneumonia (27 cases, 3%), necrotizing pneumonia (21 cases, 2.33%), acute hemorrhagic pneumonia (19 cases, 2.11%), and chronic bronchopneumonia (4 cases, 0.44%). *A. pleuropneumoniae* was detected predominantly in lesions associated with haemorrhagic pneumonia (17 cases, 1.89%). *H. parasuis* was detected in acute bronchopneumina (6 cases, 0.67%) followed by acute interstitial pneumonia (1 case, 0.11%). Similarly, PCV2 detected in the lungs showing the lesions in following order: EP-like lesions (89 cases, 9.89%), interstitial pneumonia (67 cases, 7.44%), miscellaneous lesions (57 cases, 6.33%), and acute bronchopneumonia (22 cases, 2.44%), and chronic bronchopneumonia (17 cases, 1.89%). PRRSV was detected in higher frequency with lungs showing interstitial pneumonia (24 cases, 2.67%). SIV cases were detected in lungs showing broncho-interstitial pneumonia (7 cases, 0.78%) followed by chronic interstitial pneumonia (4 cases, 0.44%).

The seasonal variation of pulmonary diseases is summarized in Table III. The highest prevalence of pneumonia was observed in winter, but these differences were not statistically significant when compared to the other seasons.

Discussion

Respiratory diseases are the most challenging health problems in pigs to deal with, compared to other livestock. The recording of pulmonary lesions is widely used in many major pork producing countries for estimating the prevalence and severity of respiratory diseases (Sanchez *et al*. 2021, Przyborowska-Zhalniarovich *et al*., 2021). In the present study, respiratory lesions were recorded in 69.23% (900/1300) cases. The high prevalence of respiratory lesions was reported in slaughtered pigs from different countries (Conti *et al*. 2021, Pallares *et al*. 2021, Paz-Sánchez *et al*. 2021).

Body of literature on PRDC describes most importantly *Mycoplasma* spp. in association with bacterial microbiome, following viral infections to cause pneumonia (Opriessnig *et al*. 2007, Fachinger *et al*. 2008). The highest detection rate of *Mycoplasma*s spp. (31.28%) in the diseased lungs might suggests its primary role in PRDC which was congruent with the earlier observations (Maes *et al*. 2021, Sonalio *et al*. 2022). The prime involvement of *Mycoplasma* spp. in slaughtered pigs is worldwide reported with the prevalence ranging from 23.85 % to 72.60 % (Galdeano *et al*. 2019, Pallares *et al*. 2021, Przyborowska-Zhalniarovich *et al*. 2021). The high prevalence of *M. hyorhinis* associated with EP-like lesions is an interesting finding in the present study considering that *M. hyopneumoniae* is instead frequently associated with EP-like lesions in pigs (Pallares *et al*. 2021, Przyborowska-Zhalniarovich *et al*. 2021). Recent studies suggest the involvement

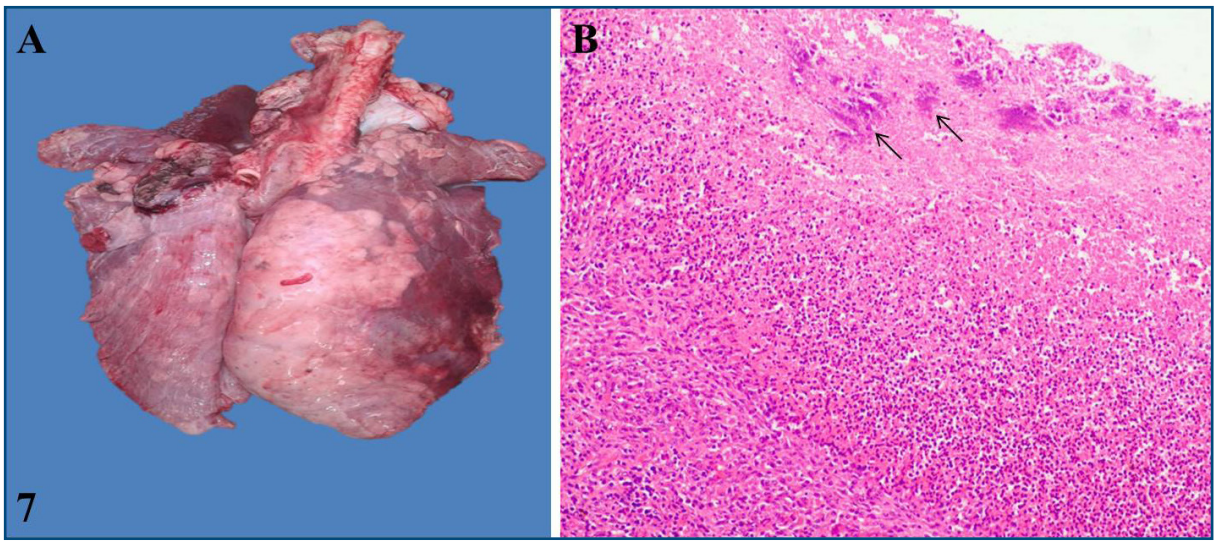

Figure 7. Necrotizing pneumonia in pig. ***A****) Circumscribed area of necrosis in the anterior half of the diaphragmatic lobe with consolidation in the apical, cardiac and diaphragmatic lobe of lungs.* ***B****) Large area of necrosis filled with huge infiltration of inflammatory cells mixed with numerous bacterial colonies (arrow) (H&E staining; x100).*

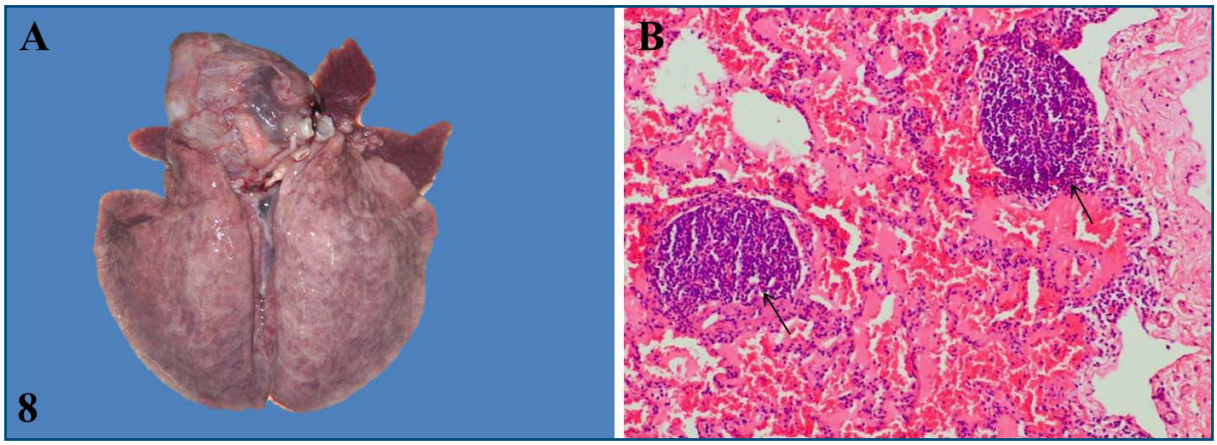

Figure 8. Enzootic pneumonia in pig. A) *Clearly demarcated bilateral consolidations of apical and cardiac lobes. B) Bronchial associated lymphoid tissue (BALT) hyperplasia (arrow) with oedema and congestion in multifocal areas of lungs (H&E staining; x100).*

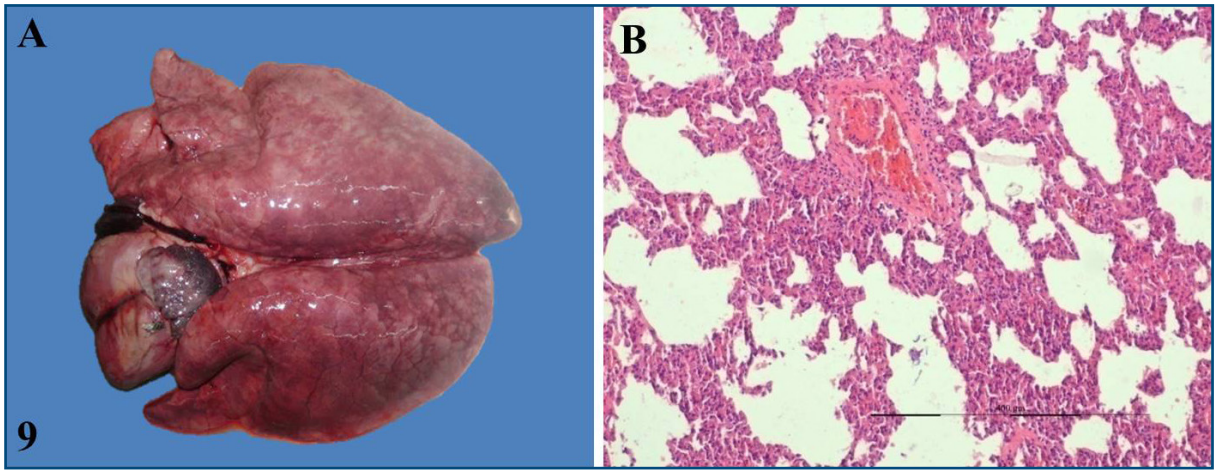

Figure 9. Acute interstitial pneumonia in pig. A) *Heavy and non-collapsed lungs with rubbery texture showing multi focal reddish areas. B) Thickened alveolar and bronchiolar walls with mild to moderate infiltration of mononuclear cells, ruptured alveoli devoid of any exudate (H&E staining; x100).*

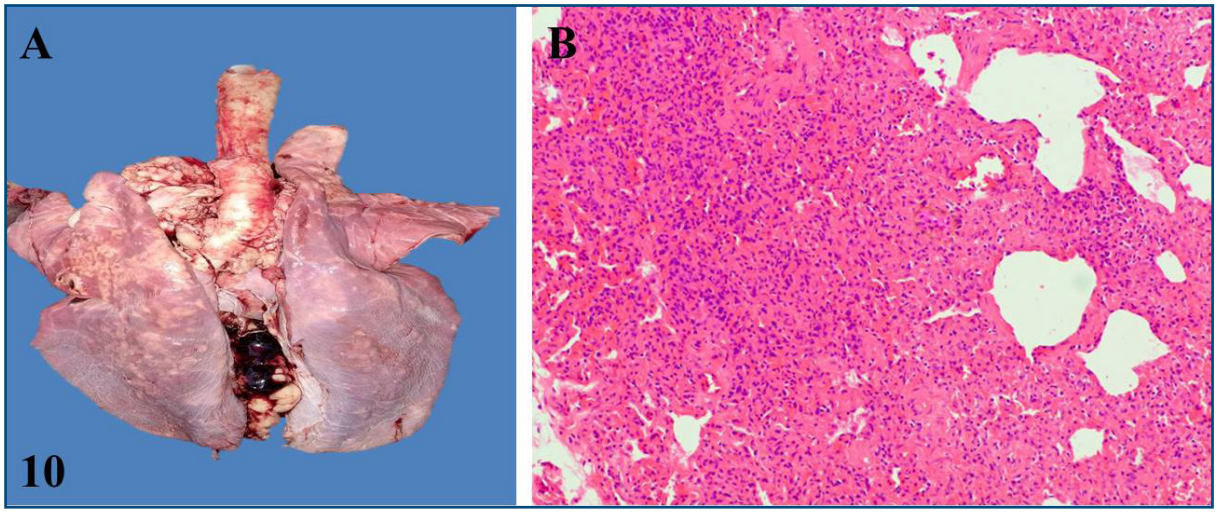

Figure 10. Chronic interstitial pneumonia in pig. A) H*eavy and non-collapsed lungs with rubbery texture showing multi focal reddish areas. B) Thickened pulmonary interstitium showing severe infiltration of mononuclear cells (H&E staining; x100).*

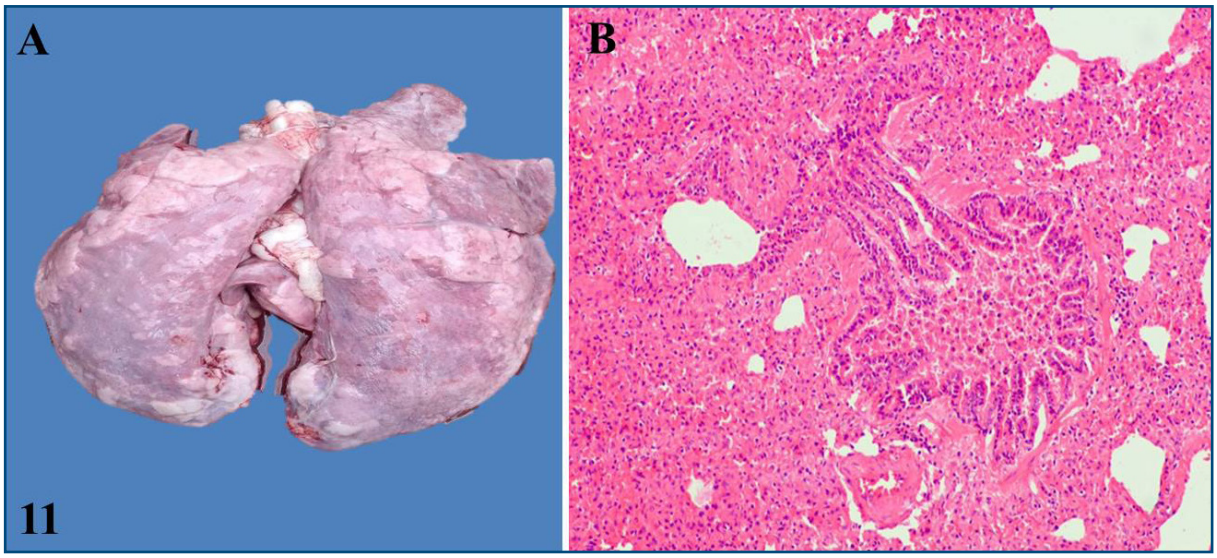

Figure 11. Bronchointerstitial pneumonia in pigs. A) *Heavy, swollen, edematous lungs with consolidation throughout in a lobular pattern. B) Both bronchioles and the pulmonary interstitium showing severe infiltration of mononuclear cells (H&E staining; x100).*

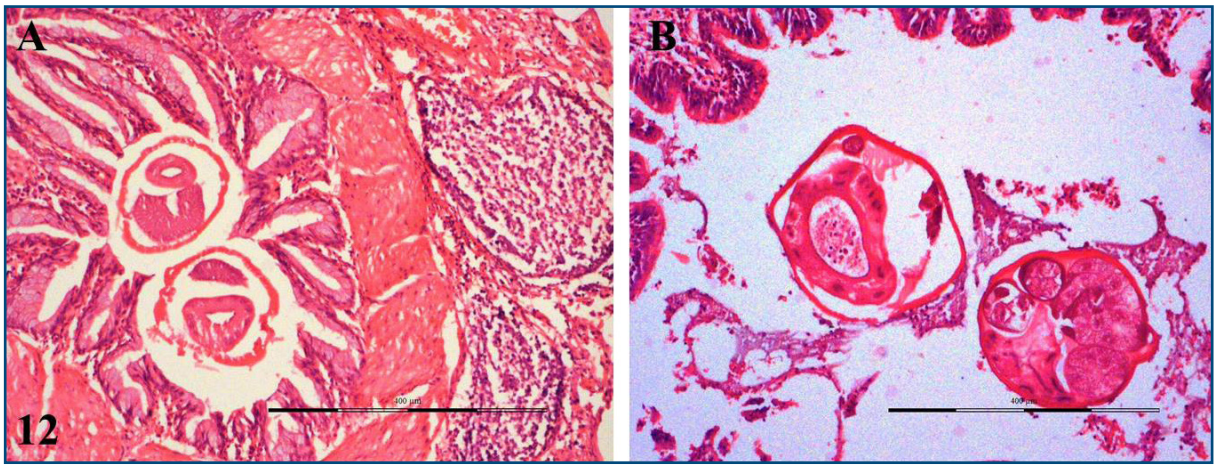

Figure 12. Parasitic pneumonia in pig. A) *Presence of parasite within the lumen of bronchiole surrounded by lymphoid hyperplasia. B) Cross-section of adult Metastrongyle nematode within the lumen of bronchiole (H&E staining; x100).*

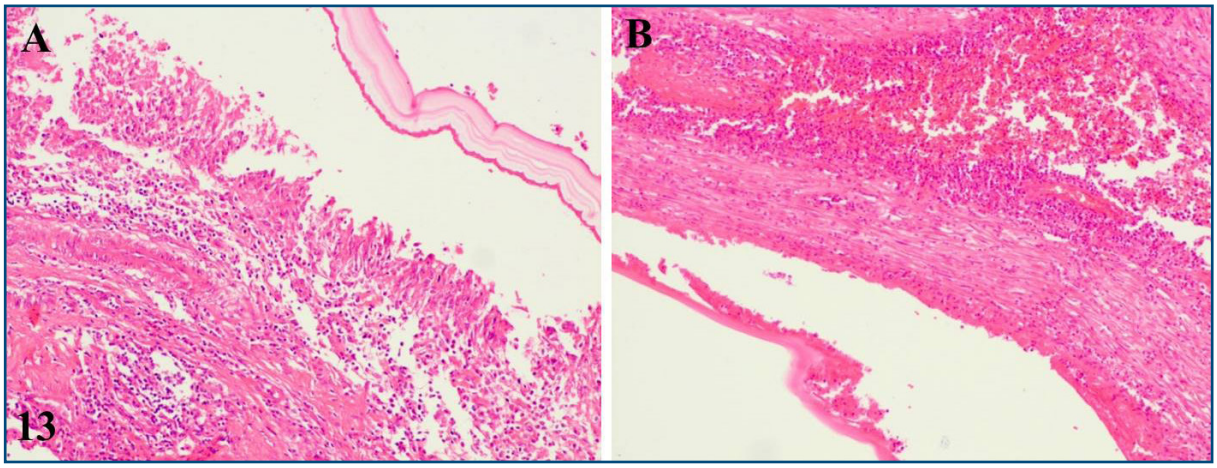

Figure 13. Pulmonary hydatidosis. *A&B) Histological sections of lung showing laminated membrane of the parasite and fibro-cellular reaction (H&E staining; x100).*

Table III. *Gross and histopathological diagnosis of spontaneous respiratory affections in pigs*.

Lesion		Winter		Summer		Monsoon	
Pneumonia	**Sub-types of pneumonia**	**Number of affected Lungs (%)**	**Prevalence (%) (n=300)**	**Number of affected Lungs (%)**	**Prevalence (%) (n=300)**	**Number of affected Lungs (%)**	**Prevalence (%) (n=300)**
Bronchopneumonia (341)	Acute bronchopneumonia	41 (15.76)	13.16	32 (20.25)	10.66	37 (18.59)	12.33
	Sub-acute bronchopneumonia	32 (12.30)	10.66	21 (13.29)	7.0	28 (14.07)	9.33
	Chronic bronchopneumonia	8 (3.07)	2.66	3 (1.89)	1.0	6 (3.01)	2.0
	Fibrinous pneumonia	28 (10.76)	9.33	25 (15.82)	8.33	35 (17.58)	11.66
	Acute haemorrhagic Pneumonia	7 (2.69)	2.33	8 (5.06)	2.66	4 (2.01)	1.33
	Necrotizing Pneumonia	11 (4.23)	3.66	6 (3.79)	2.0	9 (4.52)	3.0
Enzootic pneumonia (BALT hyperplasia) (103)	Mycoplasmal associated pneumonia	47 (18.07)	15.66	24 (15.18)	8.0	32 (16.08)	10.66
Interstitial Pneumonia (67)	Acute interstitial pneumonia	13 (5.0)	4.33	10 (6.32)	3.33	13 (6.53)	4.33
	Chronic interstitial Pneumonia	7 (2.69)	2.33	4 (2.53)	1.33	3 (1.50)	1.0
	Broncho-interstitial pneumonia	8 (3.07)	2.66	4 (2.43)	1.33	5 (2.51)	1.66
Parasitic pneumonia (14)	Metastrongyle spp. Pneumonia (6)	3 (1.15)	1.0	1 (0.63)	0.33	2 (1.0)	0.66
	Pulmonary hydatidosis (8)	5 (1.92)	1.66	0	0	3 (1.50)	1.0
Other lesions (92)	Vasculitis	13 (5.0)	4.33	7 (4.43)	2.33	4 (2.01)	1.33
	Bronchitis	17 (6.53)	5.66	8 (5.06)	2.66	9 (4.52)	3.0
	Pulmonary oedema	9 (3.46)	3.0	5 (3.16)	1.66	5 (2.51)	1.66
	Bronchial smooth muscle hypertrophy	11 (4.23)	3.66	0	0	4 (2.01)	1.33
	Total	**260**	**86.09**	**158**	**51.29**	**199**	**66.28**

of *M. hyorhinis* in EP-like lesions in pigs (Lin *et al*. 2006, Luehrs *et al*. 2017). The coinfection of *Mycoplasma* spp. with other bacterial pathogens (*P. multocida*, *S. suis*, and *B. bronchoseptica*), and viral pathogens (PCV2 and PRRSV) recorded in the present study suggest the role of *Mycoplasma* spp. in aggravating the lung lesions, thereby facilitating the other bacterial/viral pathogens (Pieters & Maes, 2019, Conti *et al*. 2021). Next to *Mycoplasma*, high prevalence of *P. multocida* (21.41%), and *S. suis* (18.77%) suggest their important role in pneumonia development. Similar findings were reported in several studies (Zha *et al*. 2013, Desjardins *et al*. 2014, Conti *et al*., 2021, Dinesh *et al*. 2022). The prevalence of *B. bronchiseptica* was observed to be 16.73%. Our findings corroborated with the findings of Zhao *et al*. (2011) who reported the similar isolation rate (18.12%) in the pigs suffering from clinical respiratory diseases. *Actinobacillus pleuropneumoniae* has been widely reported in several countries for causing pleuropneumonia in slaughtered pigs. Characterised by high morbidity, it is responsible for severe economic losses to the swine industry (Fablet *et al*. 2012, Watt *et al*. 2020). In our case, *A. pleuropneumoniae* was detected in 2.01% cases, which suggests a possible involvement in swine respiratory disease.

Among the viral pathogens, PCV2 was detected at a higher frequency (28.11%) which suggests its association with swine respiratory disease. Published literature report the cranioventral consolidation of lungs in PCV2 affected pigs (Wellenberg *et al*. 2010, Opriessnig and Langohr 2013). Next to PCV2, the recorded prevalence of 2.7% for PRRSV cases may also suggest his involvement. The detection

of PRRSV has been documented in pneumonic lungs of slaughtered pigs (Hansen *et al*. 2010, Przyborowska-Zhalniarovich *et al*. 2021). In the present investigation, SIV was found at the lowest frequency (1.2%).

The association of SIV with PRDC has been documented in earlier reports (Thacker *et al*. 2001, Schmidt *et al*. 2016). Among the various histopathological lesions, highest prevalence of bronchopneumonia, followed by EP-like lesions and interstitial pneumonia were observed. The similar findings were documented in the earlier reports (Hansen *et al*. 2010, Galdeano *et al*. 2019). Bronchopneumonia is the predominant lesion in 37.88% cases.

This might be caused by bacterial and *Mycoplasma* sp. infections, which reachedthe lower part of the respiratory system, compromising the pulmonary defenses by breaching the broncho-alveolar junction. The gross and microscopic lesions of bronchopneumonia were similar to previous reports (McGavin and Zachary 2007, Pallares *et al*. 2021).

In fibrinous pneumonia, the exudation of plasma and fibrin takes place due to disruption of blood-air barrier by highly virulent bacteria and *Mycoplasma*, along with the inflammatory process (McGavin and Zachary 2007). In haemorrhagic pneumonia, vascular changes might be due to the toxins and super antigens liberated by haemolytic bacteria which might damage the lungs defense system. The association of *S. suis* and *B. bronchiseptica* was reported in acute haemorrhagic pneumonic cases (Madsen *et al*. 2002). The gross and microscopic lesions in necrotizing bronchopneumonia were similar to previous reports (Liljegren *et al*. 2003). The association of *A. pleuropneumonia, P. multocida*, and *S. suis* was documented in necrotizing, necrohaemorrhagic and fibronecrotic pneumonic cases (Reams *et al*. 1994, Rao *et al*. 2002).

The gross and microscopic appearance of interstitial pneumonic lesions in the present study were previously reported by several workers (Rao *et al*. 2002, Palleres *et al*. 2021). Published reports have shown the association of *Mycoplasma* spp. (Rao *et al*., 2002), *Pasteurella* spp. (Dutta *et al*. 2007) and *Salmonella* spp. (Maxie and Miller 2015) with interstitial pneumonia. This might be due to the primary damage in lungs caused by viral pathogens like PRRS, PCV and SVI, which might have caused immune suppression, thus favouring the growth of this opportunistic pathogen for the development of the lesions (Berthelot-Herault *et al*. 2001, Meyns *et al*. 2011).

In the present investigation, EP-like lesions (11.44%) were observed in several pigs. Lungs showing bronchial associated lymphoid tissue (BALT) hyperplasia with perivascular and peribronchiolar lymphoid hyperplasia are suggestive of *Mycoplasma* spp. infections (Conti *et al*., 2021). These lesions might be due to the cytotoxic protein and other noxious agents such as hydrogen peroxide produced by *Mycoplasma* or mitogenic protein on the bacterial membrane favouring the massive lymphoid hyperplasia around airways and blood vessels (Hansen *et al*. 2010). The association of *Mycoplasma* with *P. multocida*, and *S. suis* in lungs of slaughtered pigs was described in previous reports (Madsen *et al*. 2002, Charlebois *et al*. 2014).

The gross and histopathological lesions of 14 lungs affected with parasitic pneumonia were in conformity with the lesions explained previously by Kumar *et al*. (2000) and Rao *et al*. (2002).

The miscellaneous lesions in the present study included bronchitis, pulmonary oedema, and bronchial smooth muscle hyperplasia that were well within the range described in previous studies (McGavin and Zachary, 2017).

In conclusion, the results of this investigation report a high prevalence of PRDC in slaughtered pigs in India. To best of our knowledge, this is the first systematic investigation of the pathology and pathogens associated with PRDC in slaughtered pigs of India. *M. hyorhinis* showing the EP-like lesions, PCV2 and their combination were likely to be the prime contributors of PRDC in Indian pigs. Besides, the prevalence of zoonotic pathogens like *S. suis*, *B. bronchiseptica* and *P. multocida* may pose public health risk.

Thus, designing of vaccination and treatment programmes against these pathogens will help to control PRDC in pigs, which will reduce the economic losses for the swine industry.

Acknowledgments

The authors are thankful to the Director, ICAR-Indian Veterinary Research Institute, Izatnagar, India for providing necessary facilities to carry out this work.

References

Caron J., Ouardani M. & Dea, S. 2000. Diagnosis and differentiation of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* infections in pigs by PCR amplification of the p36 and p46 genes. *J Clin Microbiol*, **38**, 1390-1396.

Chae C. 2016. Porcine respiratory disease complex: Interaction of vaccination and porcine circovirus type 2, porcine reproductive and respiratory syndrome virus, and *Mycoplasma hyopneumoniae*. *Vet J*, **212**, 1-6.

Charlebois A., Marois-Créhan C., Hélie P., Gagnon C.A., Gottschalk M. & Archambault M. 2014. Genetic diversity of *Mycoplasma hyopneumoniae* isolates of abattoir pigs. *Vet microbiol*, **168**, 348-356.

Chiers K., Van Overbeke I., Donné E., Baele M., Ducatelle R., De Baere T. & Haesebrouck F. 2001. Detection of *Actinobacillus pleuropneumoniae* in cultures from nasal and tonsillar swabs of pigs by a PCR assay based on the nucleotide sequence of a dsbE-like gene. *Vet Microbiol*, **83**, 147-159.

Coldebella A., Kich J.D., Albuquerque E.R.& Buosi R.J. 2017. Reports of Brazilian federal meat inspection system in swine slaughterhouses. In Embrapa Suínos e Aves-Artigo em anais de congresso (ALICE). In: international symposium on the epidemiology and control of biological, chemical and physical hazards in pig and pork, 12, Proceedings Book. Concórdia: Embrapa Suínos e Aves, 2017. p. 251-254.

Conti E.R.D., Takeuti K.L., Schwertz C.I., Bianchi R.M., Driemeier D. & de Barcellos D.E. 2021. Agents of pneumonia in slaughtered pigs in southern Brazil. *Pesqui Vet*, **41**, e06669.

Dinesh M., Thakor J.C., Singh K.P., Singh R., Anbazhagan S., Chauhan R., Qureshi S., Sahoo R. & Sahoo M. 2022. Patho-epidemiological study of *Streptococcus suis* infections in slaughtered pigs from North and North-Eastern Region, India. *Indian J Vet Pathol* **46**, 26-32.

Donko T., Kovacs M. & Magyar T. 2005. Association of growth performance with atrophic rhinitis and pneumonia detected at slaughter in a conventional pig herd in Hungary. *Acta Vet Hung*, **53**, 287 – 298.

Dutta B., Rahman T., Baruah G.K. & Sarmah R.K. 2007. Incidence and pathology of pneumonia in piglets. *Indian Vet J*, 84, 202-203.

Ellis J., Krakowka S., Lairmore M., Haines D., Bratanich A., Clark E., Allan G., Konoby C., Hassard L., Meehan B. & Martin K. 1999. Reproduction of lesions of postweaning multisystemic wasting syndrome in gnotobiotic piglets. *J Vet Diagn. Investig*, **11**, 3-14.

Fablet C., Marois C., Dorenlor V., Eono F., Eveno E., Jolly J.P., Le Devendec L., Kobisch M., Madec F. &Rose N. 2012. Bacterial pathogens associated with lung lesions in slaughter pigs from 125 herds. *Res Vet Sci*, **93**, 627-630.

Fachinger V., Bischoff R., Jedidia S.B. Saalmüller A. & Elbers K., 2008. The effect of vaccination against *porcine circovirus* type 2 in pigs suffering from porcine respiratory disease complex. *Vaccine*, **26**, 1488-1499.

Ferraz M.E.S., Almeida H.M.S., Storino G.Y., Sonálio K., Souza M.R., Moura C.A.A., Costa W.M.T., Lunardi L., Linhares D.C.L. & de Oliveira L.G. 2020. Lung consolidation caused by *Mycoplasma hyopneumoniae* has a negative effect on productive performance and economic revenue in finishing pigs. *Prev Vet Med*, **182**, 105091.

Galdeano J.V.B., Baraldi T.G., Ferraz M.E.S., de Souza Almeida H.M., Mechler-Dreibi M.L., Costa W.M.T., Montassier H.J., Mathias L.A. & De Oliveira L.G. 2019. Cross-sectional study of seropositivity, lung lesions and associated risk factors of the main pathogens of Porcine Respiratory Diseases Complex (PRDC) in Goiás, Brazil. *Porc Health Manag*, **5**, 1-10.

Goyette-Desjardins G., Auger J.P., Xu J., Segura, M. & Gottschalk M. 2014. *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent—an update on the worldwide distribution based on serotyping and sequence typing. *Emerg Microbes Infect*, **3**, 1-20.

Hansen M.S., Pors S.E., Jensen H.E., Bille-Hansen V., Bisgaard M., Flachs E.M. & Nielsen O.L. 2010. An investigation of the pathology and pathogens associated with porcine respiratory disease complex in Denmark. *J Comp Pathol*, **143**, 120–131.

Hozbor D., Fouque F. & Guiso N. 1999. Detection of *Bordetella bronchiseptica* by the polymerase chain reaction. *Res Microbiol*, **150**, 333-341.

Kumar G.B.A., Nalini T.S. & Vijayasarathi S.K. 2000. Pathology of parasitic pneumonia in pigs. *Indian J Vet Pathol*, **24**, 127-128.

Li Z., Wang X., Di D., Pan R., Gao Y., Xiao C., Li B., Wei J., Liu K., Qiu Y. & Ma Z. 2021. Comparative analysis of the pulmonary microbiome in healthy and diseased pigs. *Mol Genet Genom*, **296**, 21-31.

Liljegren C.H., Aalbaek B., Nielsen O.L. & Jensen H.E. 2003. Some new aspects of the pathology, pathogenesis, and aetiology of disseminated

lung lesions in slaughter pigs. *Apmis*, **111**, 531-538.

Lin J.H., Chen S.P., Ye, K.S. & Weng C.N. 2006. *Mycoplasma hyorhinis* in Taiwan: diagnosis and isolation of swine pneumonia pathogen. *Vet Microbiol*, **115**, 111-116.

Luehrs A., Siegenthaler S., Grützner N., Grosse Beilage E., Kuhnert P. & Nathues H. 2017. Occurrence of *Mycoplasma hyorhinis* infections in fattening pigs and association with clinical signs and pathological lesions of Enzootic Pneumonia. *Vet Microbiol*, **203**, 1-5.

Lyutskanov M., Urumova V., Marutsov P. & Zhelev G. 2010. Investigation On The Microbial Factor Of Porcine Respiratory Disease Complex (PRDC) In Industrial Pig Farming Conditions. *Trakia J Sci*, **8**, 89-94.

Madsen L.W., Svensmark B., Elvestad K., Aalbaek B. & Jensen H.E. 2002. *Streptococcus suis* serotype 2 infection in pigs: new diagnostic and pathogenetic aspects. *J Comp Pathol*, **126**, 57-65.

Maes D., Boyen F., Devriendt B., Kuhnert P., Summerfield A. & Haesebrouck F. 2021. Perspectives for improvement of *Mycoplasma hyopneumoniae* vaccines in pigs. *Vet Res*, **52**, 1-20.

Maxie M.G. & Miller M.A. 2015. Introduction to the diagnostic process. Jubb, Kennedy & Palmer's Pathology of Domestic Animals: 3-Volume Set: 3-Volume Set, p.1, 6th ed. Academic press, China 167-175

McGavin M.D. & Zachary J.F. 2007. Pathologic basis of veterinary disease, Mosby. 5th ed. Academic press, China, 118-119.

Meyns T., Van Steelant J., Rolly E., Dewulf J., Haesebrouck F. & Maes D. 2011. A cross-sectional study of risk factors associated with pulmonary lesions in pigs at slaughter. *Vet J*, **187**, 388-392.

Okwumabua O., O'Connor M. & Shull E. 2003. A polymerase chain reaction (PCR) assay specific for *Streptococcus suis* based on the gene encoding the glutamate dehydrogenase. *FEMS Microbiol Lett*, **218**, 79-84.

Oliveira S., Galina L. & Pijoan C. 2001. Development of a PCR test to *diagnose Haemophilus parasuis* infections. *J. Vet Diagn. Investig*, **13**, 495-501.

Opriessnig T. & Langohr I. 2013. Current state of knowledge on porcine circovirus type 2-associated lesions. *Vet Path*, **50**, 23-38.

Opriessnig T., Giménez-Lirola L.G. & Halbur P.G. 2011. Polymicrobial respiratory disease in pigs. *Anim Health Res Rev*, **12**, 133-148.

Opriessnig T., Meng X.J. & Halbur P.G. 2007. Porcine circovirus type 2–associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. *J Vet Diagn Investig*, **19**, 591-615.

Pallares F.J., Añón J.A., Rodríguez-Gómez I.M., Gómez-Laguna J., Fabré R., Sánchez-Carvajal J.M. Ruedas-Torres I. & Carrasco L. 2021. Prevalence of *Mycoplasma*-like lung lesions in pigs from commercial farms from Spain and Portugal. *Porc Health Manag*, **7**, 1-8.

Paz-Sánchez Y., Herráez P., Quesada-Canales Ó., Poveda C.G., Díaz-Delgado J., Quintana-Montesdeoca M.D.P., Plamenova Stefanova E.& Andrada M. 2021. Assessment of Lung Disease in Finishing Pigs at Slaughter: Pulmonary Lesions and Implications on Productivity Parameters. *Animals*, **11**, 3604.

Pieters M. & Maes D. 2019. Mycoplasmosis. Diseases of Swine. In: Zimmerman J., Karriker L., Ramirez A., Schwartz K., Stevenson G. & Zhang J. (Eds), 11th ed. Academic press, Wiley-Blackwell, Ames, 863-883.

Przyborowska-Zhalniarovich P.A.U.L.I.N.A., Zhalniarovich Y.A.U.H.E.N.I. & Wasowicz K. 2021. Estimation of the prevalence of respiratory diseases in pigs in north-eastern Poland: Survey of pulmonary lesions in pigs at a slaughterhouse. *Vet Med*, **66**, 242-247.

Rajkhowa T.K., Jagan Mohanarao G., Gogoi A. & Hauhnar L. 2016. Indian porcine reproductive and respiratory syndrome virus bears discontinuous deletion of 30 amino acids in nonstructural protein 2. *Virus disease*, **27**, 287-293.

Rao A.N., Paliwal O.P., Sharma A.K. & Ramkumar. 2002. Pulmonary lesions in pigs. *Indian J Vet Pathol*, **26**, 53-56.

Reams R.Y., Glickman L.T., Harrington D.D., Thacker H.L. & Bowersock T.L. 1994. *Streptococclcs suis* infection in swine: a retrospective study of 256 cases. Part II. Clinical signs, gross and microscopic lesions, and coexisting microorganisms. *J Vet Diagn Investig*, **6**, 326-334.

Sanchez-Vazquez M.J., Strachan W.D., Armstrong D., Nielen M. & Gunn G.J. 2011. The British pig health schemes: Integrated systems for large-scale pig abattoir lesion monitoring. *Vet Rec*, **169**, 413.

Schmidt C., Cibulski S.P., Andrade C.P., Teixeira T.F., Varela A.P.M., Scheffer C.M., Franco A.C., de Almeida L.L. & Roehe P.M. 2016. Swine influenza virus and association with the porcine respiratory disease complex in pig farms in southern Brazil. *Zoonoses Public Health*, **63**, 234-240.

Sonalio K., Almeida H., Mechler-Dreibi M.L., Storino G.Y., Haesebrouck F., Maes D. & de Oliveira L.G. 2022. Influence of *Mycoplasma hyopneumoniae* natural infection on the respiratory microbiome diversity of finishing pigs. *Vet Res*, 53, 1-12.

Sorensen V., Jorsal S.E. & Mousing J. 2006. Diseases of the respiratory system. In: Diseases of Swine, 9th Edit., BE Straw, JJ Zimmerman, S D' Allaire, DJ Taylor, Eds., Blackwell Publishing, Oxford, 149 -e177.

Thacker E.L. 2001. Immunology of the porcine respiratory disease complex. *Vet Clin North Am Food Anim Pract*, **17**, 551-565.

Timenetsky J., Santos L.M., Buzinhani M. & Mettifogo E. 2006. Detection of multiple *Mycoplasma* infection in cell cultures by PCR. *Braz. J. Med. Biol*, **39**, 907-914.

Tonni M., Formenti N., Boniotti M.B., Guarneri F., Scali F., Romeo C., Pasquali P., Pieters M., Maes D. & Alborali G.L. 2022. The role of co-infections in *M. hyopneumoniae* outbreaks among heavy fattening pigs: a field study. *Vet Res*, **53**, 1-10.

Townsend KM., Boyle D. O., Phan T.T., Hanh T.X., Wijewardana T.G., Wilkie I., Trug N.T. & Frost A.J. 1998. Acute septicaemic pasteurellosis in Vietnamese pigs. *Vet Microbiol*, **63**, 205-215.

Watt A.E., Browning G.F., Markham P.F. & Marenda M.S. 2020. Detection of naturally aerosolized *Actinobacillus pleuropneumoniae* on pig farms by cyclonic air sampling and qPCR. *Vet Microbiol*, **250**, 108856.

Wellenberg G.J., Bouwkamp F.T., Wolf P., Swart W.A.J.M., Mombarg M.J. & De Gee A.L.W. 2010. A study on the severity and relevance of porcine circovirus type 2 infections in Dutch fattening pigs with respiratory diseases. *Vet Microbiol*, **142**, 217-224.

Wong-Lee J.G. & Lovett M. 1993. Rapid and sensitive PCR method for identification of *Mycoplasma* species in tissue culture. Diagnostic Molecular and Microbiology Principles and Applications. Washington DC: *ASM*, 257-260.

Zha Y., Xie J., Chen Y., Wei C., Zhu W., Chen J., Qi H., Zhang L., Sun L., Zhang X. & Zhou P. 2013. Microbiological identification and analysis of swine lungs collected from carcasses in swine farms, China. *Indian J Microbiol,* 53, 496-498.

Zhao Z., Wang C., Xue Y., Tang X., Wu B., Cheng X., He Q. & Chen H. 2011. The occurrence of *Bordetella bronchiseptica* in pigs with clinical respiratory disease. *Vet J*, **188**, 337-340.

Zhu M., Li X., Sun R., Shi P., Cao A., Zhang L., Guo Y. & Huang J. 2021. The C/EBPβ-dependent induction of TFDP2 facilitates porcine reproductive and respiratory syndrome Virus proliferation. *Virologica Sinica*, **36**, 1341-1351.