RESEACH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v18i2.3

Olaniyi *et al*. /*Sokoto Journal of Veterinary Sciences*, **18**(2): 72 - 82.

Retrospective study of swine respiratory diseases in Ogun and Oyo States, Nigeria: Immunohistochemical detection of *Mycoplasma hyopneumoniae*

MO Olaniyi[1], OJ Awoyomi[1], O Akinniyi[1], AA Adebiyi[1], OO Alaka[2]*, OL Ajayi[1], AJ Jubril[2], TA Jarikre[2] & BO Emikpe[2]

1. *Department of Veterinary Pathology, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Nigeria*
2. *Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria*

*Correspondence: Tel.: +2348033520882; E-mail: gbengalaka@yahoo.com

Publication History:
Received: 27-12-2019
Accepted: 19-05-2020

Abstract

Swine respiratory diseases particularly enzootic pneumonia caused by *Mycoplasma hyopneumoniae* (Mhyo) constitutes a significant health problem to pig production in many countries. However, the impact has been underestimated in Nigeria. This study therefore, retrospectively analyzed swine respiratory diseases and the associated pulmonary histopathology. Postmortem records and archival lung samples were obtained from the Departments of Veterinary Pathology University of Ibadan, Ibadan and Federal University of Agriculture, Abeokuta. A total of 98 pig carcasses were presented for necropsy during the period between 2005 and 2017. The diseases presumptively diagnosed using gross morphological criteria were extracted from the postmortem records while, 21 formalin-fixed archival lung samples were used for histopathology and immunohistochemistry using standard techniques. Data were analysed using descriptive statistics while Chi Square was used to test for association between different variables and pulmonary lesions at $\alpha_{0.05.}$ In this study, respiratory diseases had a prevalence of 56.1% with enzootic pneumonia as the most frequently diagnosed at postmortem (49%, 48/98). Only age was identified to be a significant (P = 0.019) predisposing factor in the development of respiratory diseases. Microscopically, hyperplasia of bronchus associated lymphoid tissue (BALT) with formation of lymphoid nodules and thickening of alveolar septa were the most significant changes (38.1%, 8/21). Immunohistochemically, *M. hyopneumoniae* antigen was detected in 13/21 (61.9%) of the lung samples and were immunolabelled as granular brown reactions on the luminal surfaces of bronchial and bronchiolar epithelial cells and intraluminal cellular exudates within the airways. The histopathological findings and the detection of *M. hyopneumoniae* antigen indicated that the organism is primarily involved in the development of enzootic pneumonia in naturally infected pigs and may be central in the pathogenesis. It is concluded that enzootic pneumonia is a serious health issue in pigs in the study area and needs urgent attention.

Keywords: Archival lung samples, Histopathology, Pigs, Pneumonia, Retrospective analysis

Introduction

Pigs have been described as one of the most prolific and fast-growing livestock that can readily convert feed to valuable products (Ajala, 2007; Rahman *et al.,* 2008; Petrus *et al.,* 2011). The numerous inherent potentials in pigs such as high fecundity (Ogunniyi & Omoteso, 2011), high feed conversion efficiency (Rahman *et al.,* 2008; Petrus *et al.,* 2011), early maturity and short generation interval (Ajala, 2007), relatively small space requirement and ability to produce maximally under simple and varied management systems without sophisticated biosafety measures (Ajala, 2007; Muhanguzi *et al.,* 2012); account for the rapid increase in the number of pig farms in recent time (Lekule & Kyvgaard, 2003; Ogunniyi & Omoteso, 2011; Muhanguzi *et al.,* 2012). The pig industry has been reported to thrive well under favourable conditions, particularly in the southern part of this country (Nwanta *et al.,* 2011). However, several disease conditions especially respiratory diseases have been reported to mitigate pig production and proliferation of pig farms in Nigeria (Antia *et al.,* 1981; Shima & Garba, 2014, Olaniyi, 2017).

Porcine respiratory diseases (PRD) have been identified as a major health issue in swine population worldwide (Halbur, 1998; Thacker, 2001; Choi *et al.*, 2003; Martinez *et al.*, 2009; Hansen *et al.*, 2010; Brockmeier *et al.*, 2003; Thacker & Minion, 2012). Pneumonia which is the most prevalent lesion associated with PRD occurs worldwide and is present in most swine herds (Straw *et al.*, 1989; Maes *et al.*, 1996; Christensen & Enoe 1999; Palzer *et al.*, 2008; Fraile *et al.*, 2010), The condition has been reported to be a significant cause of production losses and high mortality in finishing pigs (Noyes *et al.*, 1990; Halbur, 1998; Choi *et al.*, 2003; Fraile *et al.*, 2010) primarily due to reduction in growth performance and feed efficiency (Maes *et al.*, 1996; Ostanello *et al.*, 2007; Fraile *et al.*, 2010). Porcine respiratory disease complex (PRDC) in pigs is rarely caused by a single pathogen; and in most cases is the result of combination of bacterial and viral pathogens (Choi *et al.*, 2003; Palzer *et al.*, 2008; Hansen *et al.*, 2010; Olaniyi, 2017). A variety of respiratory pathogens have been reported to play important roles in the development of PRDC; these include *Mycoplasma hyopneumoniae* (Mhyo) and porcine reproductive and respiratory syndrome virus (PRRSV) which are the most frequently isolated pathogens directly related to PRDC (Palzer *et al.*, 2008). In addition, other potential pathogens reported to be involved in PRDC are *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, β-*haemolytic Streptococcus spp*, *Haemophilus parasuis*, *Bordetella bronchiseptica*, swine influenza virus and porcine circovirus type 2 (Thacker, 2001; Harms *et al.*, 2002; Brockmeier *et al.*, 2003; Kim *et al.*, 2003 Thacker & Minion, 2012). A previous survey of respiratory diseases in intensively managed pig farms in Ibadan, Nigeria reported 60% mortality directly attributable to pneumonia (Antia *et al.*, 1981; Emikpe *et al.,* 2018). Enzootic pneumonia caused by *Mycoplasma hyopneumoniae* is a disease that affects pigs in many countries of the world including Nigeria (Antia *et al.*, 1981; Maes *et al.*, 1996; Van Reeth & Pensaert, 1994; Thacker 2006; Hansen *et al.*, 2010; Thacker & Minion 2012; Olaniyi, 2017). Despite the fact that Nigeria has the second largest population of pigs in Africa with over 6.0 million pigs which account for about 30% of the total pig population in Africa (FAOSTAT, 2015) and approximately 4.45% of the total meat supply in Nigeria (Nwanta *et al.,* 2011), only a few researchers have paid attention to research on swine respiratory diseases, thus leading to rarity of published data on swine respiratory diseases and the associated aetiological agents. Aetiological agents of PRDC have been reported to vary significantly among many farms, production sites, regions and countries, thus making generalization about swine respiratory diseases prevention and control a bit difficult (Thacker, 2001). Critical to effective control and prevention of respiratory diseases in pigs is the determination of pathogens involved. The aim of this study was therefore, to retrospectively study the pathology of swine respiratory diseases and the commonly associated aetiological agents using formalin-fixed lung samples in the study area.

Materials and Methods

Study samples

A retrospective analysis of swine diseases was carried out on the post mortem (PM) records of the pig carcasses submitted for PM examination from 2005-2017 at the Department of Veterinary Pathology Diagnostic Laboratory Units of the Veterinary Teaching Hospital of the University of Ibadan, Ibadan (UI) and Federal University of Agriculture, Abeokuta (FUNAAB). Veterinary Pathology Diagnostic Laboratory receives cases from its immediate environment and referrals from veterinarians from all parts of southwestern Nigeria. Formalin-fixed lung samples obtained from the archives of the Departments of Veterinary pathology, UI and FUNAAB were used to determine the prevalence of

swine respiratory diseases and study the pulmonary histopathology and associated aetiological agents.

Data and sample collection

Data were extracted from the post mortem records of the Department of Veterinary Pathology (UI) and the Department of Veterinary Pathology (FUNAAB). Information relating to the type of lung lesion, age, breed and sex were also extracted from the record. A total of 98 post mortem cases (63 cases from UI, 35 cases from FUNAAB) were examined; only 21 formalin-fixed lung samples (11 from UI, 10 from FUNAAB) were retrieved. Not all the information in relation to the sex and breed were available, so the data was analysed based on the available information.

Gross evaluation of the lungs

Gross examination of the formalinized lungs for changes in consistency, texture and extent of consolidation was carried out as described by Emikpe *et al.* (2015). The extent of consolidation was determined by visual observation and palpation of the lesion.

Histopathological evaluation

Formalin-fixed lung samples were trimmed, dehydrated in graded concentration of alcohol, cleared with xylene, embedded in paraffin wax, sectioned at 4µm and stained with haematoxylin and eosin (H&E) using routine method. Tissues were subsequently examined with light microscope and evaluation was made at various magnifications. Classification of histological lesions followed the semi-quantitative criteria, while BALT hyperplasia was graded as mild, moderate, and extensive according to Hansen *et al*. (2010).

Immunohistochemistry protocol

Twenty-one unstained lung tissue sections were selected and processed for immunohistochemical staining using *Mycoplasma hyopneumoniae* monoclonal antibodies to detect Mhyo-specific antigen. Immunohistochemistry (IHC) test was carried out by the use of heat-induced epitope retrieval technique using citrate base antigen retrieval unmasking solution (Vector Lab., USA). Paraffin-embedded tissue sections were deparaffinized in xylene, rehydrated through graded alcohol, and air dried. Deparaffinized tissue sections were pen-circled using PAP marker (Vector Lab., USA) and placed in antigen retrieval solution (Citra, BioGenex, CA, USA) in a plastic stander and were kept in a microwave oven set at a 212^{o}F for 10 minutes.

Slides were laid on the humid chamber, sections were flooded with 70% methanol with 3% H_2O_2 and incubated at room temperature for 15 minutes (2 times) to quench endogenous peroxidase activity. After washing 3 times (5 minutes each) in phosphate-buffer saline (PBS, pH 7.4, 0.01M) containing 0.1% Tween 20, sections were treated with power block, 1X blocking antibody (Universal Blocking Reagent, BioGenex, CA, USA) for 20 minutes to saturate nonspecific protein-binding sites.

After draining the blocking serum, sections were incubated with primary antibodies (Mhyo monoclonal antibody with identification number D79DI–7 and 100% specificity (Source: Dr Chris F Minion, Iowa State University, Ames, USA) diluted to 1:500 in PBS and kept in a humidified chamber at 4^{o}C overnight.

After washing with PBS 3 times, sections were treated with biotinylated anti-mouse IgG made in goat secondary antibody (Vector Lab. Inc., CA, USA), applied at 1:250 dilution for one hour at room temperature in a humidified chamber.

Sections were washed 3 times with PBS and further treated with a labelled peroxidase-conjugated streptavidin-biotin complex (Vectastain®, Elite ABC, Vector Lab. Inc., CA, USA) for one hour. Preparation was carried out 30 minutes before use by diluting I drop solution A + I drop solution B in 2.5ml PBS.

After another PBS bath (3 times), sections were incubated with 3, 3-diaminobenzidine tetrahydrochloride (DAB) (Vector Lab. Inc., CA, USA). The reaction was stopped after colour change (normally 5-10 minutes). Finally, the sections were washed in running tap water, counterstained with haematoxylin, dried and covered with cover-slip.

Immunohistochemical analysis

The photomicrograph of the Mhyo-positive lung tissues was evaluated using Fiji image J win.32 as previously described (Sachindeim et al., 2012). The mean intensity was measured and optical density (OD) was calculated using the formula below:

$$\text{Optical Density (OD)} = \frac{\text{Maximum intensity}}{\text{Mean intensity}}$$

Where maximum intensity = 255 for 8-bit images. The mean intensity for Mhyo-positive lung tissues was 253.2, while the mean intensity for Mhyo-negative lung tissues was 175.5. Intensity of < 200.0 was taken as negative, 200 – 210.0 as weakly positive, 211.0 – 225.00 as moderate, and 226.0 – 225.0 as strongly positive immunosignal (Sachindeim *et al.,* 2012).

Data analysis

Data were descriptively analysed and presented as percentages. Charts and frequency tables were also used. Chi square was used to test if there was any association between the variables (age, breed, sex and season) and pulmonary lesions. P value < 0.05 was considered statistically significant.

Results

Ninety-eight post mortem cases were considered, 55/98 (56.1%) pigs died of pulmonary related lesions, while 43/98 (43.9%) died of other non-pulmonary lesions. Large White breed had the highest death rate (32.7%); Duroc had (8.2%), while the local breed and Hampshire had the lowest death rate of 2.0%. The summary is presented in Table 1.

Ninety-eight post mortem cases were considered, 55/98 (56.1%) pigs died of pulmonary related lesions, while 43/98 (43.9%) died of other non-pulmonary lesions. Large white breed had the highest death rate (61.5%), Duroc had (53.3%), while the local breed and Hampshire had the lowest (18.2%). The summary is presented in Table 1.

Ninety-two cases were considered (data for two cases were missing). Out of the 92 cases that were examined, adults had the highest mortality rate (35.7%, 35/92) while piglets had the lowest (2.2%, 2/92). Growers had the highest number of pulmonary lesions 26 (76.5%), while weaners and adults had 11/92 (52.4%) and 16/92 (45.7%) pulmonary lesions, respectively. There was a statistically significant (P = 0.019) association between the prevalence of pulmonary lesions and the age group of the pigs (Table 1).

Out of the 98 cases examined, more death was recorded in females (52%, 51/98) than in males (47/98; 48%). Table 1 showed that higher prevalence of pulmonary pathologies occurred in female pigs (31/98; 60.8%) than in males (51.1%, 29/98). However, this association was not statistically significant (P > 0.05). Generally, more pigs' death was recorded during the rainy season (74.2%, 72/98) than in dry season (25.8%, 25/98). There was a higher prevalence of pigs that died with pulmonary pathology during rainy season (45.4%, 44/98) than during dry season (14.4%, 11/98). However, this association was not statistically significant (P>0.05) (Table 1).

Based on gross morphological diagnosis and post-mortem tentative diagnosis, enzootic pneumonia was the most prevalent disease recorded (49.0%, 48/98), followed by pasteurellosis (18.4%, 18/98), African swine fever (15.3%, 15/98) and metatrongylosis (4.1%, 4/98), while other diseases recorded 13.2% (13/98) (Figure 1).

Table 1: Summary of association between different variables and pulmonary pathology in pig carcasses submitted for post-mortem in the Veterinary Teaching Hospitals, University of Ibadan, Ibadan and Federal University of Agriculture, Abeokuta from 2005-2017

Variables	No Pulmonary pathology (%)	With Pulmonary Pathology (%)	Total (%)	p value
Breed				
Duroc	5 (38.5)	8 (61.5)	13 (13.3)	
Hampshire	9 (81.8)	2 (18.2)	11 (11.2)	
Large white	20 (38.5)	32 (61.5)	52 (53)	0.0 95
Mixed breed	7 (46.7)	8 (53.3)	15 (15.3)	
Local breed	2 (28.6)	5 (71.4)	7 (7.1)	
Total	43 (43.9)	55 (56.1)	98 (100)	
Age				
Piglet (< 1 month)	2 (100)	0 (0)	2 (2.2)	
Weaner (1-2months)	10 (47.6)	11 (52.4)	21 (22.8)	
Grower (2-6months)	8 (23.5)	26 (76.5)	34 (37)	0.019
Adult (> 6 months)	19 (54.3)	16 (45.7)	35 (38)	
Total	39 (42.4)	33 (57.6)	92 (100)	
Sex				
Male	23 (48.9)	24 (51.1)	47 (48)	
Female	20 (39.2)	31 (60.8)	51 (52)	0.416
Total	43 (43.9)	55 (56.1)	98 (100)	
Season of the year				
Rainy season (Mar. – Oct)	28 (28.8)	44 (45.4)	72 (74.2)	
Dry season (Nov – Feb)	14 (14.4)	11 (14.4)	25 (25.8)	0.416
Total	42 (43.2)	55 (156.7)	97 (100)	

Histopathological changes

The histological examination of selected 21 cases revealed that varying degrees of lymphoid hyperplasia of bronchus associated lymphoid tissue (BALT) and thickened alveolar septa were the most occurring histopathological changes (38.1%, 8/21) while sub-mucosal gland hyperplasia was the least seen 1/21(4.8%). Summary of the histological changes is presented in Table 2. Microscopic lesions were found in all the lungs with gross pneumonic lesions.

There were varying degrees of BALT hyperplasia which was more pronounced in the chronic stage where partial or complete obliterationof the bronchial or bronchiolar lumen was observed (Plate I). Bronchitis and bronchiolitis were mainly suppurative with concurrent epithelial hyperplasia, intra-luminal cellular exudates and varying degrees of thickening of the alveolar septa, mainly by cellular infiltration consisting of predominantly of neutrophils, lymphocytes and macrophages (Plate II). In some cases, chronic lesions were accompanied by acute lesions; this may represent healing of the existing chronic lesion, or presence of two different disease incidents. Pulmonary congestion

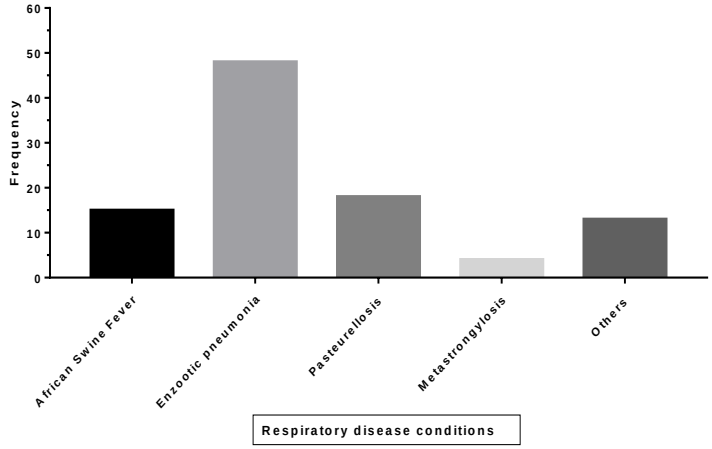

Figure 1: Swine respiratory disease conditions diagnosed at postmortem in the Veterinary Teaching Hospitals of the University of Ibadan, Ibadan and Federal University of Agriculture, Abeokuta from 2005-2017

Table 2: Summary of histopathological changes in formalin-fixed pneumonic lungs (n=21)

Histopathological changes	Frequency	Percentage
Thickening of alveolar septa	8	38.1
Pulmonary congestion and oedema	6	28.6
Epithelial hyperplasia	6	28.6
BALT hyperplasia	8	38.1
Bronchiolitis and bronchitis	6	28.6
Thickening of pleura	3	14.3
Sub-mucosal gland hyperplasia	1	4.8

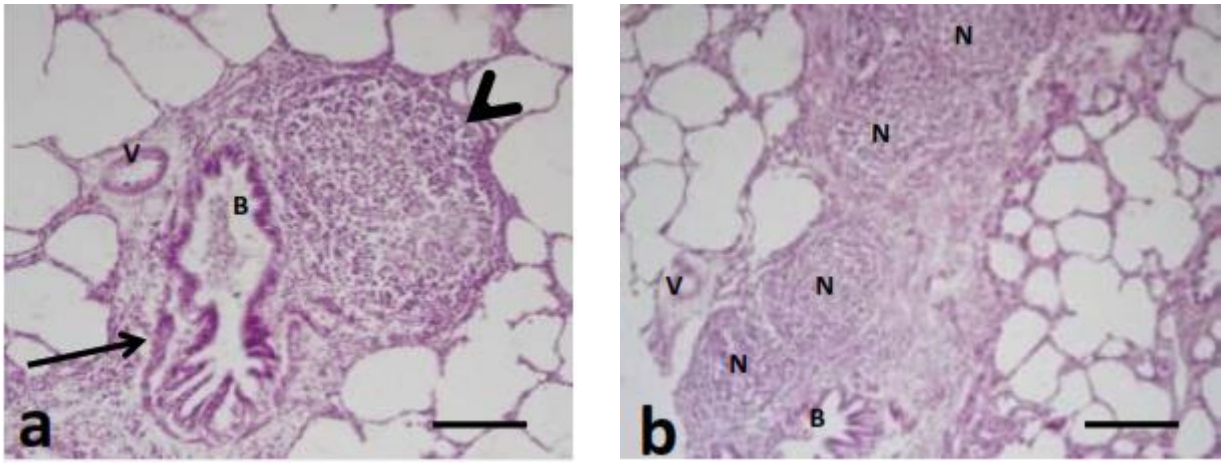

Plate I: Photomicrograph of pig lung sections showing (a) mild BALT hyperplasia (arrow head) with infiltration of lymphocytes into the peribronchiolar tissue including the lamina propria of the airways (arrowed), and a slightly compressed bronchiole (B). (b) Extensive BALT hyperplasia with presence of numerous lymphoid nodules (N) and a markedly compressed bronchiole (B) and blood vessel (V). H&E stain x100, Bar = 100µm

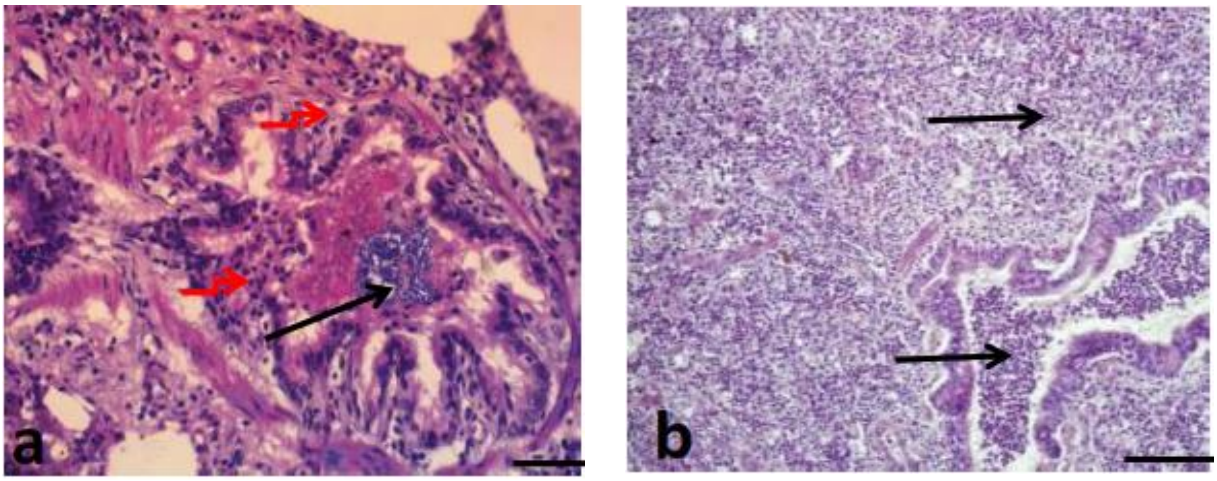

Plate II: Photomicrograph of pig lung sections showing (a) suppurative bronchiolitis (red arrow) with with intraluminal bacterial colony (black arrow), H&E stain, X400. Bar = 40µm (b) extensive area of broncho-interstitial pneumonia and intra-luminal cellular exudates consisting predominantly of neutrophils and cellular debris (arrowed). H&E stain, X100. Bar = 100µm

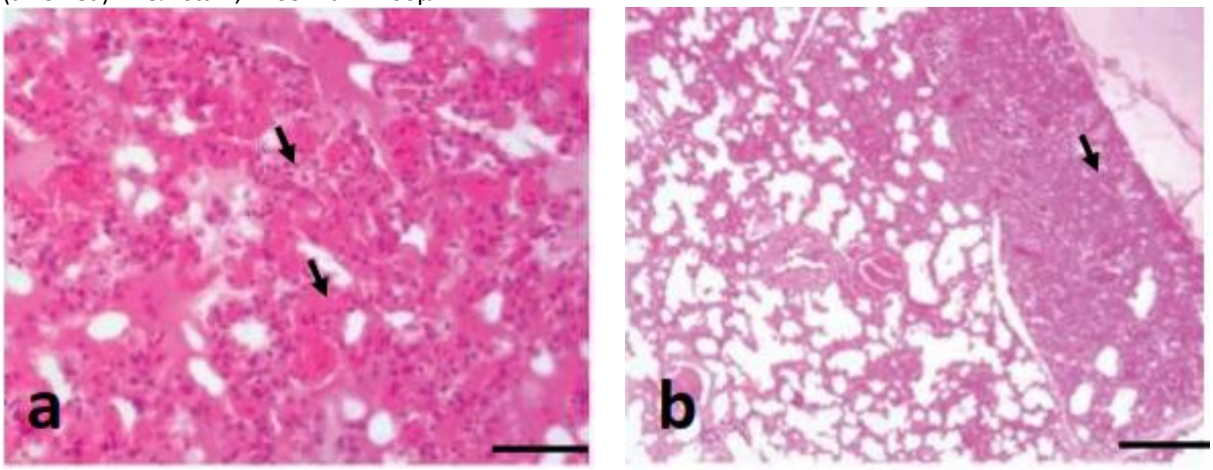

Plate III: Photomicrograph of pig lung sections showing (a) acute broncho-interstitial pneumonia (white arrows) and (b) widespread atelectasis in acute phase of the infection (arrow). H&E stain, x400, Bar = 100µm

(a) while a less intense signal was detected in the cellular exudate (b).

Immunohistochemical analysis of Mycoplasma hyopneumoniae (Mhyo)-positive lung tissues

The result showed that all the 13 IHC slides of lung tissue sections had positive immunolabelling of varying degrees of stain intensity. Strongly positive immunolabelling was recorded in 9/13 (69.2%) of the lung tissues while weak immunolabelling was recorded in 4/13 (30.8%) using Fiji image J win.32. Where maximum intensity = 255 for 8-bit images. The mean intensity for Mhyo-positive lung tissues was 253.2, while the mean intensity for Mhyo-negative lung tissues was 175.5. Intensity of < 200.0 was taken as negative, 200 – 210.0 as weakly positive, 211.0 – 225.00 as moderate, and 226.0 – 225.0 as strongly positive immunosignal (Sachindeim *et al.,* 2012).

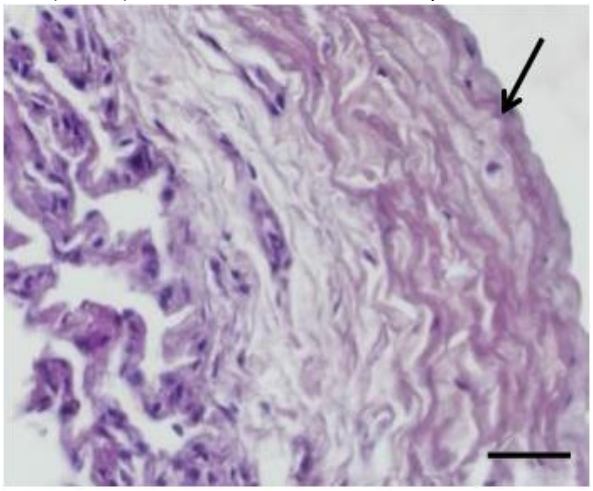

Plate IV: Photomicrograph of lung tissue section showing thickening of the pleura (arrowed) (B). H&E stain x400, Bar = 20µm

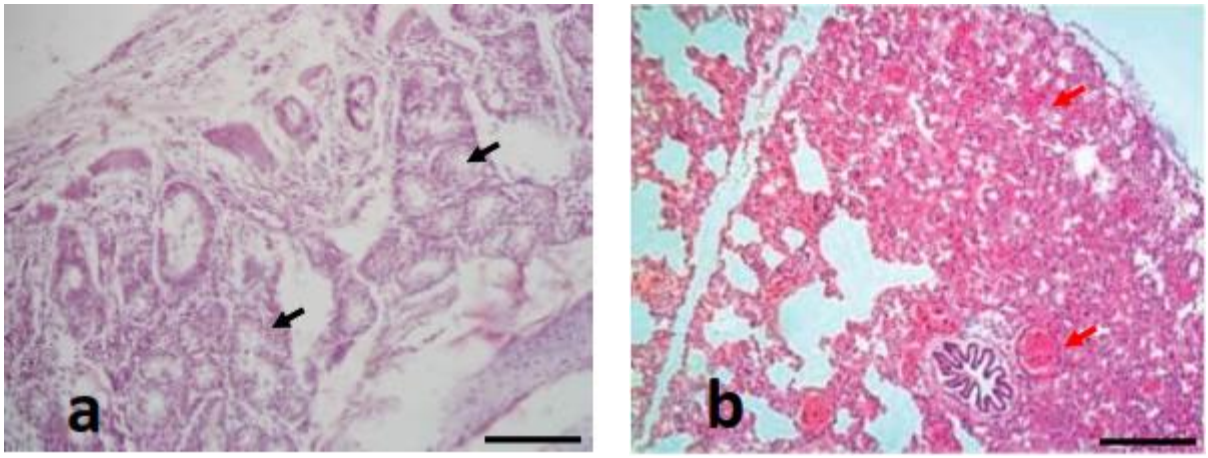

Plate V: Photomicrograph of lung tissue sections showing (a) hyperplasia of bronchial submucosal glands (arrows) and (b) pulmonary congestion (red arrows) and atelectasis. H&E stain, x100, Bar = 100µm

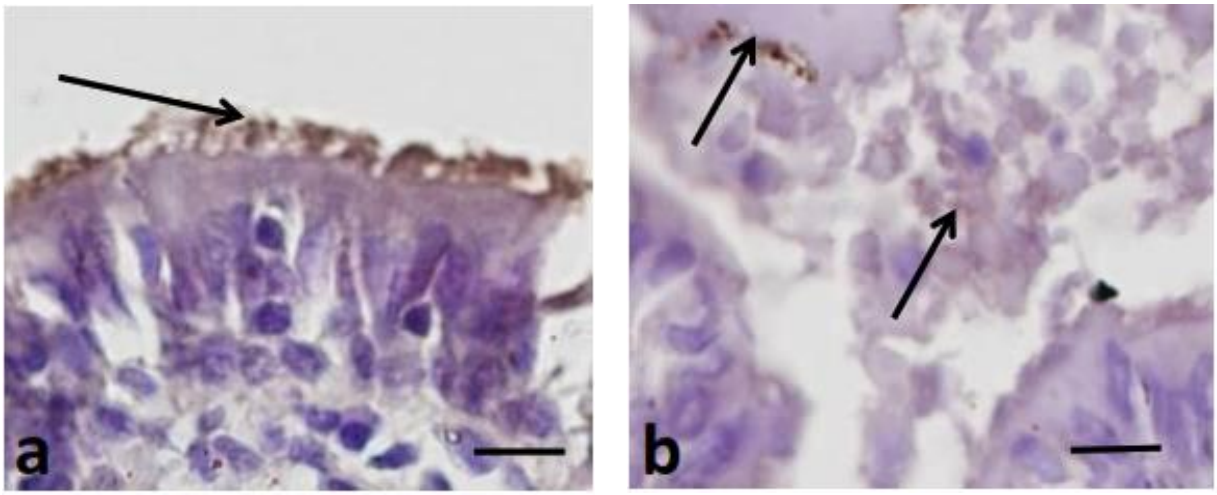

Plate VI: Photomicrograph of lung sections showing (a) immunolabelled *M. hyopneumponiae* antigen on the apical surface of bronchiolar epithelium (arrowed). IHC, Gill's haematoxylin countersta and (b) immunolabelled *M. hyopneumponiae* antigen on the cellular exudate of the airway (arrowed). IHC, Gill's haematoxylin counterstain x400. Bar = 10µm

Discussion

Enzootic pneumonia has long been recognized as a worldwide problem in the pig industry and an important disease factor limiting pig production (Maes *et al.*, 1996; Halbur, 1998; Brockmeier *et al.*, 2003; Choi *et al.*, 2003; Sorensen *et al.*, 2006; Martinez *et al.*, 2009; Hansen *et al.*, 2010; Thacker and Minion, 2012; Emikpe *et al.*, 2015). However, this condition has not been given due attention in the pig in Nigeria.

In the present retrospective study, 56.1% mortalities in pigs were directly attributable to various respiratory related disease conditions, this is similar and close to the findings of Antia *et al.* (1981) and Emikpe *et al*. (2015) who earlier reported about 60% mortality. High prevalence of pneumonia and swine respiratory disease had similarly been reported in many countries. Bahnson *et al. (*1990) reported high prevalence of 70% in Minnesota pig herds; in New Zealand, Christensen (1995) reported a prevalence of 55%; in Switzerland, Wunderli (1993) and Grest *et al.* (1997) reported prevalences ranging from 21% to 24%. In France and Spain, Fablet *et al.* (2012) and Fraile *et al.* (2010) reported prevalences of 69.3% and 55.7%, respectively. Factors that may account for this great variation in the prevalence may include different sampling methods, season of the year when investigation was conducted, age at slaughter, environment and managemental conditions (Noyes *et al.*, 1990; Christensen & Enoe, 1999; Collins *et al.,* 2006; Fraile *et al.*, 2010).

Enzootic pneumonia is a disease that affects pigs in many countries of the world, the high prevalence, coupled with associated losses, makes this disease one of the most important for swine veterinarians and swine producers (Desrosiers, 2001). The results of this study lend credence to this assertion, because aside enzootic pneumonia having the highest prevalence (87.3%) of respiratory diseases, it was also responsible for most deaths in growing/finishing pigs (49%). In contrast to the finding of this study, Shima & Garba (2014) reported high prevalence of parasitic pneumonia; however, this was attributed to lack of deworming regimen and poor management systems. Other pulmonary related disease conditions recorded in this study were pleuropneumonia, metastrongylosis and pasteurellosis, these could also be associated with poor management practices.

Respiratory diseases are one of the costliest diseases affecting growing-finishing pigs raised under confined conditions in intensive systems worldwide (Sorensen *et al.,* 2006). In the present study, there was significant ($P < 0.05$) association between the prevalence of pulmonary lesions and age of the infected pigs; this is an indication that age could possibly be a predisposing/risk factor in development pulmonary lesions and respiratory disease. Growing/finishing pigs had the highest prevalence of death associated with pulmonary lesions; this had earlier been reported to be associated with waning of passively acquired antibodies as this category of pigs lacks sufficient antibodies against respiratory infections (Collins *et al.,* 2006; Pomorska-Mol *et al.,* 2011).

There was no significant ($P > 0.05$) association between the prevalence of pulmonary lesions and breed in this study. However, local breed had the highest prevalence (71.4%) of pulmonary pathology; this may indicate that all breeds of pigs kept under similar conditions are equally predisposed to pulmonary associated diseases. The female pigs in this study exhibited higher prevalence of lung lesions than male pigs. In contrast to the finding of this study, high prevalence of lung lesion was reported in boars than sows, this has been attributed to stress and hormonal changes due to castration (Christensen *et al.,* 1999). The absence of statistically significant ($P = 0.416$) association between the prevalence of lung lesion and sex in this study is an indication that both male and female pigs kept under similar conditions are equally predisposed to respiratory diseases. The result of the study further revealed that pneumonia is more prevalent in the rainy season from March all through October annually compared to dry season. This result agreed with the reports of Stark (2000) and Shima & Garba (2014).

In the present study, chronic lesions were accompanied by acute lesions, this may represent healing of the existing chronic lesion, or presence of two different disease incidents; presence of chronic and acute lesions in the lungs is suggestive of a chronic active inflammatory process; this implies that the antigen is present and persistent, thus continuously triggering an acute inflammatory response with evidence of chronicity. The most frequent histopathological changes observed were thickening of alveolar septa and lymphoid hyperplasia of BALT, pulmonary congestion and oedema and suppurative oedema and suppurative bronchiolitis. while the least frequently associated histopathological changes observed were thickening of pleura and sub-mucosal gland hyperplasia. These histopathological changes have been reported in pigs with enzootic pneumonia caused by *M. hyopneumoniae* and agreed with those previously described (Kwon *et al.,* 2002; Choi *et al.*, 2003; Sarradell *et al.,* 2003; Opriessnig *et al.,* 2004; Lorenzo *et al.,* 2006). Although, thickening of alveolar septa can be seen in infection caused by viral agents (Antia *et al.,* 1981; Hurnik *et al.* 1993, Emikpe *et al.*, 2015), other pulmonary pathology such as degeneration and necrosis of epithelial cells, presence of fibrinous exudate and fibrin strand reported by Hurnik *et al.* (1993) and Emikpe *et al.* (2015) were not observed in this study. Thickening of pleura which was observed in the present study may be associated with pleuritis, this lesion is compatible with *Actinobacillus pleuropneumoniiae* infection as described by Fraile *et al*. (2010). Microscopic lesions associated with proliferative changes such as presence of massive fibrinous exudates, fibrin strands, thickening interlobular septa with fibrinous exudates and proliferation of immature fibroblasts had also been previously reported in cases of porcine respiratory diseases caused by bacterial infection (Emikpe *et al.*, 2015; Emikpe *et al.*, 2018). However, these changes were not observed in the present study.

In this study, Mhyo antigen was detected in the bronchiolar epithelium and cellular exudates in the bronchiolar lumen. The detection and localization of Mhyo antigen in these locations within airways recorded in this study had earlier been reported and may represent local cellular immune response to Mhyo infection (Redondo *et al.,* 2009).

The result of this study showed that different combinations of microscopic lesions recorded in all the lung sections examined were due to fact that

lesions of pneumonia in pigs are broad and overlapping in nature (Hansen *et al.*, 2010). In the present retrospective study of field cases using archival lung samples, the IHC technique demonstrated its usefulness and applicability as a diagnostic tool for the detection of *M. hyopneumoniae* even in archival formalin-fixed lung tissues. This technique is rapid and therefore advantageous compared to isolation of the organism; in which pure isolate is seemingly impossible, because it may involve delay of many days or weeks and concomitant growth of contaminants.

It is concluded that swine respiratory diseases particularly enzootic pneumonia posees a serious health issue and therefore an impediment to swine production and productivity in the study area. This study calls for the need to adopt a good management and housing system, eradication scheme and good bio-security measures including good hygiene as well as vaccination, these are crucial points in the prevention and control of swine respiratory diseases.

Acknowledgments

The authors would like to acknowledge Prof. S.O. Akpavie of the University of Ibadan for excellent review of the manuscript and the staff of the Department of Veterinary Pathology, Federal University of Agriculture, Abeokuta for technical assistance.

Conflicts of Interest

The authors declare no conflict of interest.

References

Adebambo AO (1982). Evaluation of the genetic potential of the Nigerian indigenous pigs. In: *Proceedings Second World Conference Genetic Application.* Madrid, Spain. Pp 133–138.

Ajala MK, Adesehinwa AOK & Mohammed AK (2007). Characteristics of smallholder pig production in southern Kaduna Area of Kaduna State, Nigeria. *American-Eurasian Journal of Agriculture and Environmental Science.* **2**(2): 182−188.

Antia RE Akpavie, SO & Ikede BO (1981). Observations on the pathology of pig pneumonias in Ibadan, Nigeria. *Bulletin Animal Health Production in Africa,* **29**(2): 309−316.

Bahnson PB, Pointon AM, Dial DG & Marsh WE (1990). Prevalence of lesions at slaughter in Minnesota swine herds. Proceedings of the 11th IPVS Congress, Lausanne, Switzerland. Pp 564.

Bochev I (2007). Porcine respiratory disease complex (PRDC). A review. I: Etiology, epidemiology, clinical forms and patho-anatomical features. *Bulgarian Journal of Veterinary Medicine*, **10**(3): 131−146.

Choi YK, Goyal SM & Joo HS (2003). Retrospective analysis of etiologic agents associated with respiratory diseases in pigs. *Canadian Veterinary Journal,* **44**(4): 735–737.

Choi C, Kwon D, Jung K, Ha Y, Lee YH, Kim O, Park HK, Kim SH, Hwang KK & Chae C (2006). Expression of inflammatory cytokines in pigs experimentally infected with *Mycoplasma hyopneumoniae*. *Journal Comparative Pathology,* **134**(1): 40–46.

Christensen G & Enoe C (1999). The prevalence of pneumonia, pleuritis, pericarditis and liver spots in Danish slaughter pigs in 1998, including comparison with 1994. *Dansk Veterinaetidsskrift*, **82**(6): 1006–1015.

Christensen NH (1995). Evaluation of the effects of enzootic pneumonia in pigs on weight gain and days to slaughter under New Zealand conditions. *New Zealand Veterinary Journal,* **43(**4): 146–148.

Clark K (1999). *Mycoplasma hyopneumoniae*: Serology/Vaccinology. In: *Proceedings of the thirtieth annual meeting of the American Association of Swine Practitioners*, St. Louis, Missouri, Pp. 365–369.

Collins JE, Murtaugh, MP & Joo HS (2006). Evaluation of the effects of animal age, concurrent bacterial infection, and pathogenicity of porcine reproductive and respiratory syndrome virus on virus concentration in pigs. *American Journal of Veterinary Research,* **67**(3): 489–493.

Desrosiers R (2001). A review of some aspect of epidemiology, diagnosis and control of *Mycoplasma hyopnuemoniae* infections. *Journal of Swine Health and Production*, **9**(5): 233–237.

Emikpe BO, Jarikre TA, Adediran OA, Olaniyi MO, Dikeogu TC (2018). Haematology, bronchoalveolar cellular changes and pathology of swine pneumonia in Nigeria. *Sokoto Journal of Veterinary Sciences*, **16**(2): 1–9.

Emikpe BO, Adediran OA, Jarkre TA, Olawumi O & Jubril AJ (2015). Evaluation of lung lesions and associated predisposing factors from

slaughtered pigs in Nigeria. *Archives of Basic & Applied Medicine.* **3**(2): 119–122.

Fablet C, Marois-Crehan C, Simon G, Grasland B & Jestin A (2012). Infectious agents associated with respiratory diseases in 125 farrow-to-finish pig herds: A cross-sectional study. *Veterinary Microbiology,* doi: 10.1016/j.vetmic.2011.12.015.

FAOSTAT (2015). Food and Agricultural Organization of the United Nations. Rome, Italy. www.faostat.org, retrieved 13-12-2019.

Fraile L, Alegre A, López-Jiménez R, Nofrarías M and Segalés J & Opriessnig T (2010). Risk factors associated with pleuritis and cranio-ventral pulmonary consolidation in slaughter-aged pigs. *The Veterinary Journal*, doi:10.1016/j.tvjl.2009.03.029.

Grest P, Keller H, Sydler T & Pospischil A 1997). The prevalence of lung lesions in pigs at slaughter in *Switzerland.Schweizer Archiv fur Tierheikunde*, **139**(3): 500–506.

Halbur PG (1998). Porcine Viral Respiratory Diseases. In: *Proceedings of the fourteenth International Pig Veterinary Society*, Birmingham, United Kingdom. Pp 10

Hansen MS, Pors SE, Jensen HE, Bille-Hansen V, Bisgaard M, Flachs EM & Nielsen OL (2010). An investigation of the pathology and pathogens associated with porcine respiratory disease complex in Denmark. *Journal of Comparative Pathology,* **143**(2-3): 120–131.

Harms PA, Halbur PG & Sorden SD (2002). Three cases of porcine respiratory disease complex associated with porcine circovirus type 2 infections. *Journal of Swine Health and Production*, **10**(1): 27-30.

Hurnik D, Hanna PE & Dohoo IR (1993). Evaluation of rapid gross visual appraisal of swine lungs at slaughter as a diagnostic screen for enzootic pneumonia. *Canadian Journal of Veterinary Research*, **57**(1): 37–41.

Kim J, Chung HK & Chae C (2003). Association of porcine circovirus 2 with porcine respiratory disease complex. *The Veterinary Journal*, **166**(3): 251-256.

Kwon D, Choi C & Chae C (2002). Chronologic localization of *Mycoplasma hyopneumoniae* in experimentally infected pigs. *Veterinary Pathology*, **39**(5): 584–587.

Lekule FP & Kyvsgaard NC (2003). Improving pig husbandry in the tropical resource-poor communities and its potential to reduce risk of porcine cysticercosis. Acta Tropica, **87** (1): 111-117.

Lorenzo H, Quesada O, Assuncao P, Castro A & Rodriguez F (2006). Cytokine expression in porcine lungs experimentally infected with *Mycoplasma hyopneuminiae*. *Veterinary Immunology and immunopathology*, doi:10.1016/j.vetimm.2005.07.021.

Maes D, Verdonck M, Deluyker H & De Kruif A (1996). Enzootic pneumonia in pigs. *Veterinary Quarterly*, **18**(3): 104–109.

Martinez J, Peris B, Gomez EA & Corpa JM (2009). The relationship between infectious and non-infectious herd factors with pneumonia at slaughter and productive parameters in fattening pigs. *The Veterinary Journal,* **179**(2): 240–246.

Mousing J, Lybye H, Barfod K, Meyling A, Ronsholt L & Willeberg P (1990). Chronic pleuritis in pigs for slaughter: an epidemiological study of infectious and rearing system-related risk factors*. Preventive Veterinary Medicine,* **9**(1-2): 107–119.

Muhanguzi D, Lutwana N & Mwiine FN (2012). Factors that influence pig production in central Uganda: Case study of Nangabo Sub-country, Wakiso district. *Veterinary World*, **5**(6): 346–351.

Noyes EP, Jacobson L, Mendez A & Pijoan C (1990). Study of immunological parameters of pigs housed with cold air drafts or fluctuating temperatures. *Proceedings of the 11th IPVS Congress, Lausanne*, 25.

Nwanta JA, Shoyinka SVO, Chah KF, Onunkwo JI, Onyenwe IW, Eze JI, IheagwamCN, Njoga EO, Onyema I, Ogbu KI, Mbegbu EC, Nnadozie PN, Ibe EC & Oladimeji KT (2011). Production characteristics, disease prevalence, and herd-health management of pigs in Southeast Nigeria. *Journal of Swine Health Production,* **19**(6): 331–339.

Ogunniyi LT & Omoteso OA (2011). Economic Analysis of Swine Production in Nigeria: A Case Study of Ibadan Zone of Oyo State. *Journal of Human Ecology,* **35**(2): 137–142.

Olaniyi MO (2017). Pathology and Pathogens Associated with Swine Pneumonias in Southwest Nigeria. Ph.D Thesis. Federal University of Agriculture, Abeokuta, Nigeria. Pp 1-186

Opriessnig T, Gimenez-Lirola LG & Halbur PG (2011). Polymicrobial respiratory disease in pigs.

Animal Health Research Reviews, **12**(2): 133–148.

Opriessnig T, Thacker EL, Yu S, Fenaux M, Meng XJ & Halbur PG (2004). Experimental re-production of postweaning multisystemic wasting syndrome in pigs by dual infection with *Mycoplasma hyopneumoniae* and porcine circovirus type 2, *Veterinary Pathology*, doi: 10.1354/vp.41-6-624**.**

Ostanello F, Dottori M, Gusmara C, Leotti G & Sala V (2007). Pneumonia disease assessment using a slaughterhouse lung-scoring method. *Journal of Veterinary Medicine (A) Physiology, Pathology and Clinical Medicine*, **54**(1): 70–75.

Palzer A, Ritzmann M, Wolf G & Heinritzi K (2008). Association between pathogens in healthy pigs and pigs with pneumonia. *The Veterinary Record,* **162**(2): 267–271.

Petrus NP, Mpofu L, Schneider BM & Nepembe M (2011). The constraints and potentials of pig production among communal farmers in Etayi Constituency of Namibia. *Livestock Research for Rural Development,* **23**(7): 159.

Pomorska-Mol M, Markowska-Daniel I, Rachubik J & Pejsak Z (2011). Effect of maternal antibodies and pig age on the antibody response after vaccination against Glasser's disease. *Veterinary Research Communications*, **35**(6): 337–343.

Rahman S, Barthakur SG & Kalita SB (2008). Pig production and management system in Aizawl District of Mizoram, India. *Livestock Research for Rural Development*, **20**(9): 30–38.

Redondo E, Masot AJ, Fernandez A & Gazquez A (2009). Histopathological and Immunohistochemical findings in the lungs of pigs infected experimentally with *Mycoplasma hyopneumoniae. Journal of Comparative Pathology*, 10.1016/j.jcpa.2008.12.008**.**

Sarradell J, Andrada M, Ramirez AS, Fernandez A, Gomez-Villamandos JC, Jover A, Lorenzo H, Herraez P & Rodriguez F (2003). A morphologic and immunohistochemical study of the bronchus-associated lymphoid tissue of pigs naturally infected with *Mycoplasma hyopneumoniae*. *Veterinary Pathology*, doi: 10.1354/vp.40-4-395.

Schindeleim J, Arganda-Carrevas I, Frise E, Kayning V, Longait M, Pietksch T, Preibisch B, Reuben C, Saaifeld S, Schmid B, Tinevez J, White DJ, Hartenstein V, Elicein K, Tomancek P & Cardona A (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, **9**(7): 676–682.

Shima FK & Garba HS (2014). Prevalence of characteristic macroscopic lung pathologies in pigs at slaughter in Makurdi, Benue State, Nigeria*. Bulletin of Animal Health Production in Africa*, **62**(3): 377–385.

Sorensen V, Jorsal SE & Mousing J (2006). Diseases of the respiratory system. In: *Diseases of Swine* (B Straw, JJ Zimmermann, S D'Allaire, DJ Taylor, editors), nineth edition. Iowa State University Press, Ames, Iowa. Pp 149-177.

Stark KDC (2000). Epidemiological investigation of the influence of environmental risk factors on respiratory diseases in swine: A literature review. *The Veterinary Journal*, **159**(1): 37–56.

Statista (2018). Number of pigs worldwide in 2017, by leading country (in million head). *In Statista – The Statistics Portal.* Retrieved 13/12/2-18 https:www.statista.com/statistics/263964/ number-of-pigs-in-selected-countries/#0, retrieved 13-12-2019.

Straw BE, Tuovinen VK & Bigras-Poulin M (1989). Estimation of the cost of pneumonia in swine herds. *Journal of American Veterinary Medical Association*, **195**(11): 1702–1706.

Thacker E (2006). Mycoplasmal diseases. In: *Diseases of Swine* (BE Straw, JJ Zimmerman, S D'Allaire, DJ Taylor, editors), nineth edition*.* Blackwell Publishing Limited, Oxford, United Kingdom. Pp 701–717.

Thacker EL (2001). Porcine respiratory disease complex- What is it and why does it remain a problem? *Pig Journal,* **48**(1): 66–70.

Thacker EL (2004). Diagnosis of *Mycoplasma hyopneumoniae*. *Journal of Swine Health Production,* **12**(5): 252-254.

Thacker EL & Minion FC (2012). Mycoplasmosis. In: *Diseases of Swine* (JJ Zimmerman, A Ramirez, KJ Schwartz, GW Stevenson, editors), tenth edition, Wiley-Blackwell, Ames. Pp 779–798.

Van Reeth K & Pensaert M (1994). Prevalence of infections with enzootic respiratory and enteric viruses in feeder pigs entering fattening herds. *Veterinary Record,* **135**(25): 594–597.

Wunderli F (1993). Macroscopic lung lesions in slaughter pigs. *Swiss Veterinary Journal*, **10**(1): 7–10.