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

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Abstract

Swine respiratory diseases particularly enzootic pneumonia caused by *Mycoplasma hyopneumoniae* (Myho) 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 α₀.₀₅. 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 & Omotoso, 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 & Omotoso, 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 aetiologic 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 212°F for 10 minutes. Slides were laid on the humid chamber, sections were flooded with 70% methanol with 3% H2O2 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°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 1 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 metatrongoysis (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 obliteration of 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

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        |

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

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 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
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).

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. hyopneumoniae antigen on the apical surface of bronchiolar epithelium (arrowed). IHC, Gill’s haematoxylin countersta and (b) immunolabelled M. hyopneumoniae antigen on the cellular exudate of the airway (arrowed). IHC, Gill’s haematoxylin counterstain x400. Bar = 10µm
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 pleuroneumonia, 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; Opiressnig 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 pleuropneumoniae 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, Myho antigen was detected in the bronchiolar epithelium and cellular exudates in the bronchiolar lumen. The detection and localization of Myho antigen in these locations within airways recorded in this study had earlier been reported and may represent local cellular immune response to Myho 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 poses 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.

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Conflicts of Interest
The authors declare no conflict of interest.

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