RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

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

http://dx.doi.org/10.4314/sokjvs.v21i4.4

Olaniyi *et al.*/*Sokoto Journal of Veterinary Sciences*, **21**(4): 185 – 193.

Granulomatous pneumonia due to metastrongylus species associated with *Mycoplasma hyopneumoniae* and *Pasteurella multocida* in slaughtered pigs

MO Olaniyi[1], FA Akande[2], MI Takeet[2], EO Omoshaba[3], OA Akintuotu[2], TA Jarikre[4]*, AO Sonibare[5], OE Ojo[3] & BO Emikpe[4,6]

1. *Department of Veterinary Pathology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria*
2. *Department of Veterinary Parasitology and Entomology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria*
3. *Department of Veterinary Microbiology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria*
4. *Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan. Nigeria.*
5. *Department of Veterinary Medicine, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria*
6. *Department of Veterinary Pathobiology, School of Veterinary Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

*Correspondence: Tel.: +2348062602408; E-mail: get2theo@yahoo.com

Publication History:
Received: 23-05-2023
Revised: 22-08-2023
Accepted: 25-08-2023

Abstract

Pneumonia has been identified as one of the limiting factors to pig production. Some of the pneumonia-inducing agents include *Mycoplasma hyopneumonia*e (MHYO), the primary cause of enzootic pneumonia and *Metastrongylus* species. which are widely distributed lungworms commonly found in indigenous free-range pigs. This paper describes the pathological findings of 6 cases out of 204 lungs randomly collected from slaughtered pigs in Southwest Nigeria. Samples of the lungs were collected from the cases for bacterial culture, histopathology and detection of MHYO antigens using immunohistochemisty. Gross lesions were severe acute lobular bronchopneumonia (104/204, 50.98%) and greyish discrete nodules in the lungs. Microscopically, there were varying degrees of lymphoid hyperplasia of bronchial-associated lymphoid tissue (BALT) (82.2%), suppurative bronchiolitis with widespread bronchiolar epithelial cells necrosis (57.4%) and granulomatous bronchopneumonia with presence of *Metastrongylus* spp. and bronchiolar intraluminal cellular exudate consisting predominantly of eosinophils (2.9%). *Pasteurella multocida* was the most isolated bacterial pathogen (49.0%) either as a single pathogen or in combination with other pathogens from the infected lung samples. Immunohistochemical labelling showed strong MHYO antigens on the surface of bronchial epithelial cells in infected lungs (86/204). This is the first report of granulomatous bronchopneumonia due to *Metastrongylus* spp. associated with a co-

infection of MHYO and *Pasteurella multocida* in Nigerian indigenous pigs. It is suggested that metastrongylosis may be more common than reported in this study. The detection of respiratory pathogens such as *Mycoplasma. hyopneumoniae, Metasrongylus* spp. and *Pasteurella multocida* suggest that they are potential contributors to bronchopneumonia observed in this study.

Keywords: Granulomatous bronchopneumonia, *Metastrongylus* species, *Mycoplasma hyopneumoniae*, *Pasteurella multocida,* Pig

Introduction

The pig industry in Nigeria has recorded remarkable growth, especially in the South (Nwanta *et al.,* 2011). Pig production is mainly based on commercial and small semi-commercial systems in peri-urban and rural areas, where it contributes significantly to the urban food supply, and economic returns (Adetunji & Adeyemo, 2012). Pneumonia has been identified as one of the limiting factors to swine husbandry (Alawneh *et al.,* 2018; Galdeano *et al.,* 2019; Olaniyi *et al.,* 2020c). The condition has been reported to be a significant cause of production losses and high mortality in finishing pigs (Choi *et al.,* 2003; Fraile *et al.,* 2010; Shima *et al.,* 2014; Asenso *et al.,* 2015). In Nigeria, it was reported that about 60% of mortality in pigs was directly attributable to pneumonia (Olaniyi *et al.,* 2020c).

Pneumonia associated with co-infections in pig farms involving multiple organisms is well documented and is more frequently encountered than single infections in pig farms (Opriessnig, *et al.*, 2011; Saade *et al.,* 2020). The term porcine respiratory disease complex (PRDC) is often used to describe co-infection involving viruses and bacteria (Pieters & Maes 2019; Saade *et al.,* 2020). Marruchella *et al.* (2012) reported coinfection of porcine circovirus type 2 and *Metastongylus elongatus*. *Mycoplasma hyopneumoniae* which causes porcine enzootic pneumonia occurs worldwide and has been recognized as a serious impediment to global swine production (Garcia-Morante *et al.*, 2016; Raymond *et al.,* 2018; Ferraz *et al.,* 2020; Olaniyi *et al.,* 2020c). It is important in PRDC, usually in association with pathogenic bacteria (Amass *et al.,* 1994; Reams *et al.,* 1994; Opriessnig *et al.,* 2011; Pieters & Maes 2019; Saade *et al.,* 2020). PRDC may manifest clinically in more than 70% of the pigs as poor feed conversion, reduced weight gain, and coughing (Maes *et al.,* 2018; Surendran *et al.,* 2019; Pallarés *et al.,* 2021).

Porcine lungworms belong to the genus *Metastrongylus* (roundworm) with the intermediate host being an earthworm (Taylor *et al.,* 2016). The adult lungworm has a predilection for the bronchi and bronchioles (Nssien & Adesehinwa, 1999, Taylor *et al.,* 2016). Different stages of *Ascaris suum* have also been observed in the airway of pigs (Wallgren & Pettersson, 2022). Grossly, lungworm lesions were reported to be usually mild or even absent; however, presence of larval stages, adult worm and eggs may cause verminous or nodular inflammation of the airway and pneumonia (Marruchella *et al.,* 2012) which may cause obstruction of the bronchi and bronchioles (Poglayen *et al.,* 2016) resulting in bronchitis and bronchiolitis, respectively (Stockdale, 1976). These can be compounded by management, environmental factors and nutritional deficiencies (Marruchella *et al.,* 2012), as well as some pathogenic bacteria such as *Pasteurella multocida* (Wallgren & Pettersson, 2022). Although there were previous reports on the prevalence of metasrongylosis in Nigeria (Stockdale 1976; Nssien and Adesehinwa, 1999) and other countries (Sibila *et al.,* 2010; Oba *et al.,* 2021; Wallgren & Pettersson, 2022). Report of co-infection associated with *Metastrongylus* spp., *M. hyopneumoniae* and *Pasteurella multocida* is scanty in literature, only a few reports such as those of Wallgren & Pettersson (2022) in Sweden, Oba *et al.* (2021) in Northern Uganda and Sibila *et al.* (2010) in wild boars and had been documented. In Nigeria, despite high prevalence of pneumonia and metastrongylosis, co-infection associated with MHYO and *Metastrongylus* spp. has not been previously reported. This paper presents six cases of granulomatous bronchopneumonia in slaughter-age pigs due to *Metastrongylus* spp. associated with a co-infection of *M. hyopneumoniae*, and *P. multocida.*

Materials and Methods

Ethical statement

Ethical approval was obtained from the Animal Care, Use and Research Committee of the College of Veterinary Medicine, Federal University of Agriculture, Ogun State, Nigeria (Reference number: FUNAAB-ACURC/20/0013). Consent of the pig owners and butchers was sought prior to the commencement of the study.

Sample collection

Two hundred and four (204) lungs were sampled randomly comprising 144 pneumonic and 60 grossly normal lungs at the Ibadan Municipal central abattoir, Bodija, Ibadan and Oke-Aro pig farm, Lagos, Lagos State and slaughter slabs at Edo, Ekiti, Ondo and Osun States, all in Southwestern Nigeria. The selection was based on high daily slaughter capacity ranging from 20-30 pigs. Lung samples were collected and kept in an ice pack prior to transportation to the laboratory. Fresh samples of the lungs were submitted for bacterial culture and some portions were fixed in 10% neutral buffered formalin for at least 48 hours and thereafter, they were processed for histopathological and immunohistochemical staining. Histopathology was carried out at histopathology laboratory, Department of Veterinary Pathology, Federal University of Agriculture, Abeokuta, Nigeria while immunohistochemistry was performed at the Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, USA.

Histopathology

The formalin-fixed tissues were trimmed and routinely processed before being embedded in paraffin wax, Sections (3µm) were stained with haematoxylin and eosin (H&E) staining as previously described (Bancroft & Gamble, 2014). Sections were examined carefully with the light microscope (Olympus, CX21FS1) at X10 and X40 objective lens to evaluate the airways, lymphoid aggregates, air spaces and interstitium. Hyperplasia of the bronchial-associated lymphoid tissue (BALT) was scored as absent (0); mild (+); moderate (++); marked (+++) and extensive (++++) (Hansen *et al*., 2010). Parasite identification was according to Bowman (2014) and Lopez (2016).

Bacteriology

Lung samples were cultured for bacterial pathogens on appropriate media (Barrow & Feltham, 1993; Cheesbrough, 2006). Briefly, samples were placed in sterile buffered peptone water (BPW) incubated at 37^{o}C for 14 hours (overnight) before inoculation on MacConkey Agar, Chocolate Agar and Blood Agar (Oxoid® Basingstoke, England). Morphology and biochemical tests were used for identification of specific bacteria

Immunohistochemistry

To confirm the infection with MHYO in the infected lung tissues, an immunohistochemical (IHC) test using monoclonal antibody directed against MHYO-specific antigen was performed. Immunohistochemical test was carried out using a heat-induced epitome retrieval technique using citrate base antigen retrieval unmasking solution to detect MHYO-specific antigen as previously described (Olaniyi *et al.,* 2020b). Briefly, paraffin-embedded lung tissue sections were deparaffinized by microwaving for 20 minutes and treated with antigen retrieval unmasking solution (Citra, BioGenex, CA, USA) using heat-induced method. Non-specific binding was prevented by blocking with hydrogen peroxide and blocking serum (Fisher scientific®, UK). Sections were incubated with the primary antibody (1:500 dilution) (MHYO monoclonal antibody with identification number D79DI-7) and kept overnight at 4^{o}C. After washing with phosphate buffer saline (PBS, pH 7.4) 3 times, sections were treated with biotinylated anti-mouse IgG (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 and further treated with peroxidase-congugated streptavidin-biotin complex (Vectastain®, Elite ABC, Vector Lab. Inc., CA, USA) for one hour. 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, sections were washed in running tap water, counterstained with Gill haematoxylin (Vector Lab. Inc., CA, USA), air-dried and covered with VWR micro cover glass (VWR®, USA).

Results

One hundred and four lungs (104/204, 50.98%) showed gross pneumonic lesions of various morphological patterns. Out of 104 lungs, 91 lungs (87.50%) showed lesions consistent with acute lobular bronchopneumonia. Thirteen lungs (12.50%) had lesions ranging from mild congestion to hepatization in the diaphragmatic lobes, while 3 lungs (2.9%) had multifocal grayish discrete nodules containing creamy viscid exudate, measuring 1-2 cm in diameter in the left caudal lobe (Plate 1).

Section of lungworm (*Metastrongylus* spp.) and several worm larvae were found in the bronchial lumina admixed with mucus and inflammatory cells predominantly of eosinophils in 6 lung samples (2.9%) (Plate IIa, b, c). There was hyperplasia of BALT with formation of lymphoid nodes and compression of the airway (Plate IIIa, b). Severe thickening of the alveolar septa with concurrent granulomatous reaction in the lung parenchyma was also observed (Plate IVa, b). Two of the cases showed severe suppurative

bronchiolitis with concurrent degeneration and necrosis and acute inflammatory cells consisting predominantly of neutrophils with a few lymphocytes and macrophages in the bronchiole and airspaces (Plate Va, b).

Six bacterial pathogens were identified. *Pasteurella multocida* was the most isolated pathogen 51 (49.0%) from grossly pneumonic lungs and was isolated either as single pathogen or in association with other pathogens especially β-haemolytic *Streptococcus* spp. 33 (31.7%. There was moderate growth of *Haemophilus* spp. from 24 (23.1%) lung tissues and low *Staphylococcus* spp. 5 (4.8%) lung tissues. From the other lung samples, a few mixed non-pathogenic bacteria including *Escherichia coli* and *Proteus* spp. were isolated (Table 1).

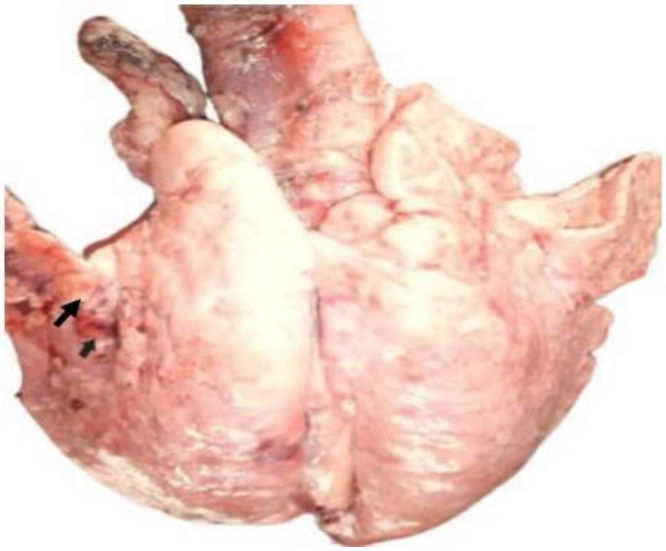

Plate I: Pig lung with two discrete nodules in the left caudal lobe of the lung (arrows)

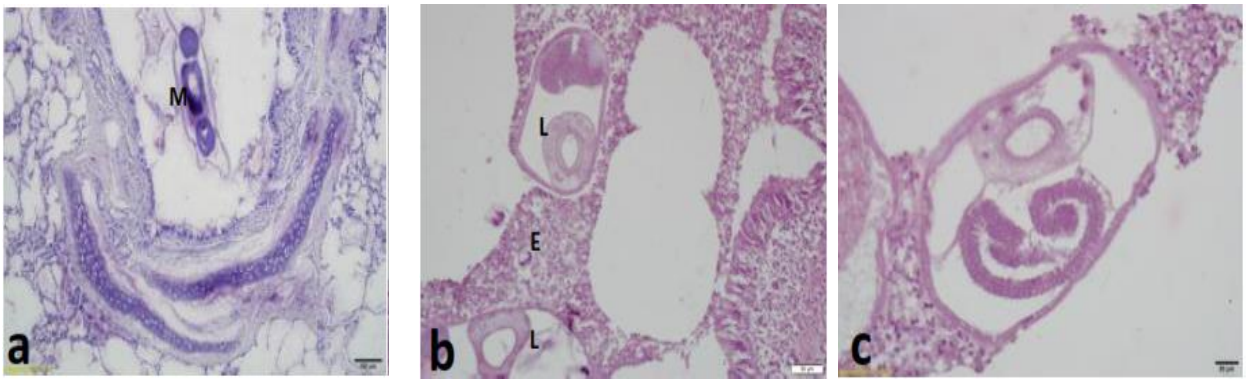

Plate II: Bronchus sections of the pig lungs showing (a) *Metastrongylus* spp. (M) in the bronchial lumen (b) worm larvae (L) admixed with mucus and intraluminal cellular exudate (E). H&E stain, Bar = 100 µm. (c) Higher magnification of a worm larva from (b). H&E stain, Bar = 10 µm

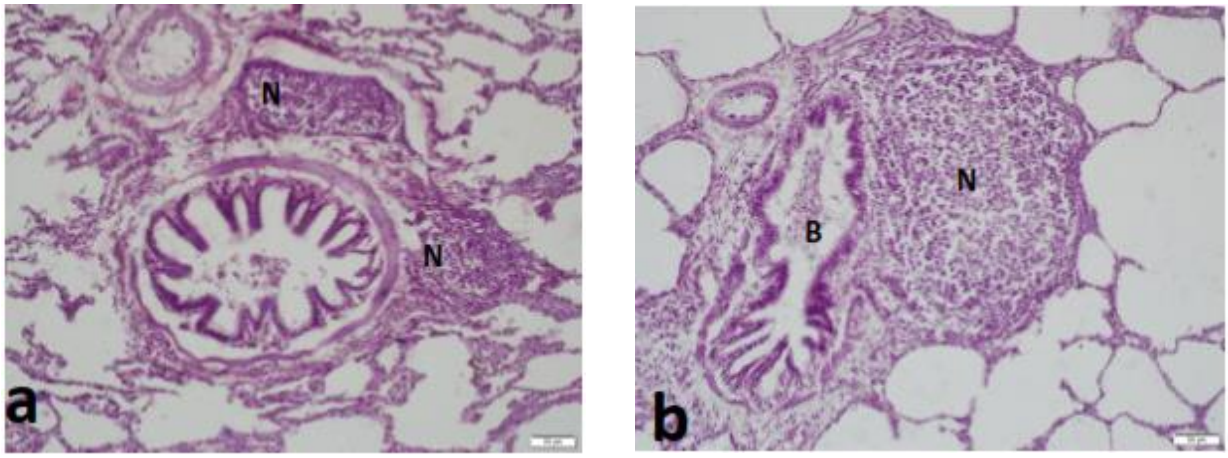

Plate III: Lung sections showing (a) mild lymphoid hyperplasia of BALT with formation of lymphoid nodes (N) (b) severe lymphoid hyperplasia of BALT with formation of a bigger lymphoid node which compreseds the bronchiole (B). H&E stain, Bar = 100µm

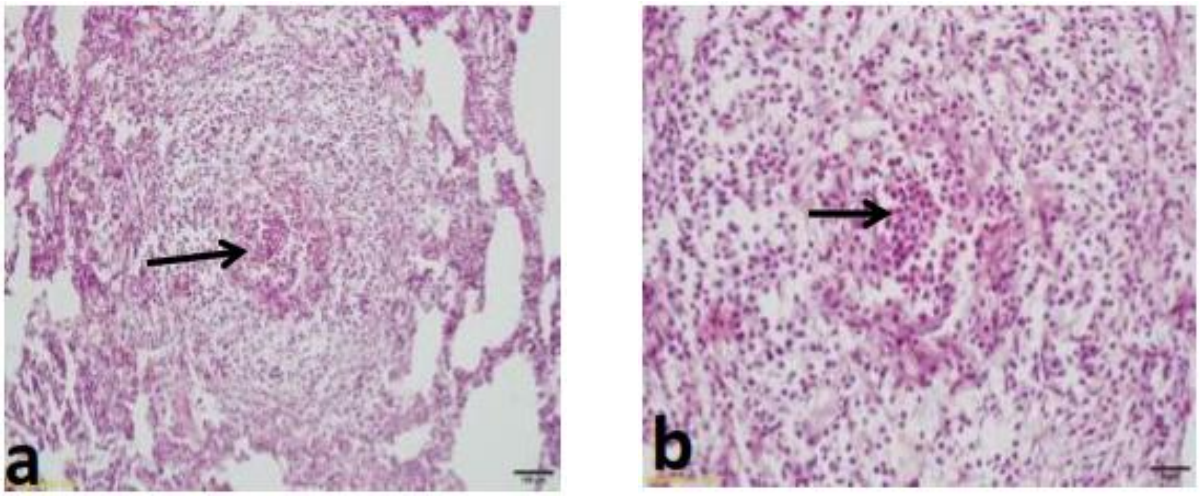

Plate IV: Lung sections showing (a) granulomatous reaction within the lung tissue with eosinophils in the centre (arrow). H&E stain, Bar = 100µm. (b) Higher magnification of (a). H&E stain, Bar = 10µm

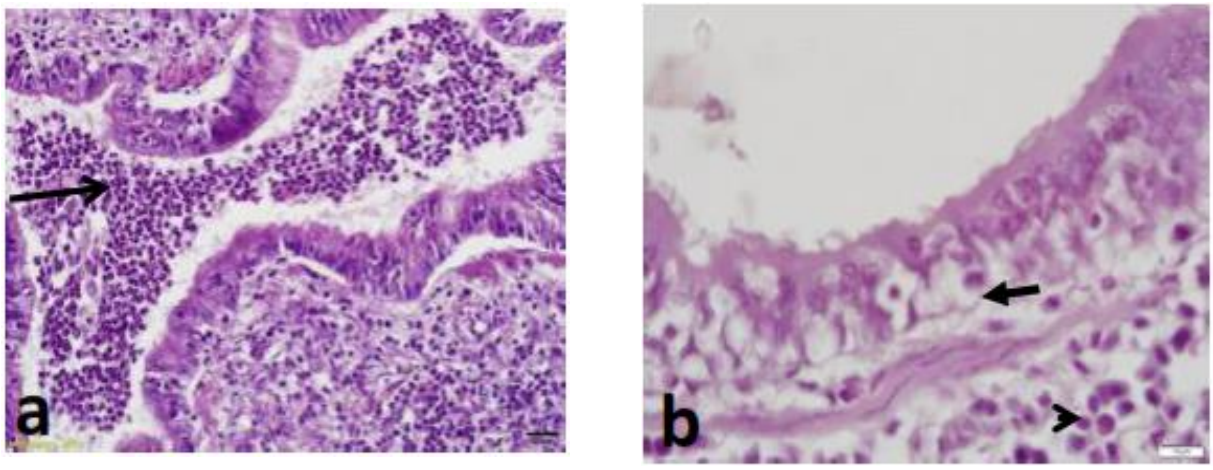

Plate V: Lung sections showing (a) acute suppurative bronchiolitis with intra-luminal cellular exudate consisting predominantly of neutrophils (arrow) (b) chronic bronchiolitis with lymphoplasmacytic infiltration (arrow) and widespread epithelial cell necrosis (arrowhead). H&E stain, Bar = 10µm

Table 1: Frequency of bacterial pathogens isolated from lungs that had CVPC (n = 104) and APNL (n = 60)

Pathogen	CVPC		APNL		
	n	%	n	%	p- value
β- haemolytic *Streptococcus spp.*	33	31.7	3	5.0	< 0.05
Pasteurella multocida	51	49.0	7	11.7	< 0.05
Haemophilus species	11	10.6	3	5.0	< 0.05
Staphylococcus species	05	4.8	4	6.7	< 0.05
Escherichia coli	02	1.9	20	33.3	NS
Proteus species	02	1.9	23	38.3	NS

NS = Not significant, CVPC = Cranio-ventral pulmonary consolidation, APNL = Apparently normal lung

Mycoplasma hyopneumoniae antigens were strongly immunolabelled and detected as a granular brown reaction on the bronchial and bronchiolar epithelial cells of all positive lung tissues that showed bronchopneumonia (42.2%) (Plate VIa). There was also less intense immunosignalling of the mononuclear cells in the BALT in one of the samples (Plate VIb).

Discussion

Porcine Respiratory Disease Complex (PDRC) is due to a number of pathogenic microbes including *M. hyopneumoniae*, *Pasteurella multocida* and

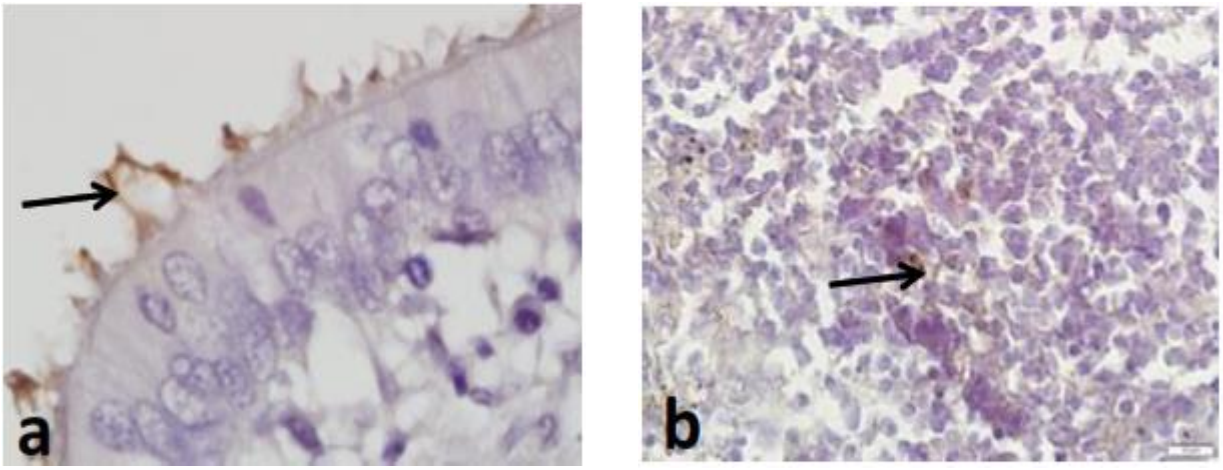

Plate VI: Photomicrograph of lung sections showing (a) Strong immuolabelled MHYO antigens on the surface of the bronchiolar epithelial cells (arrow) (b) MHYO-infected cells bearing mild immunolabelled antigens in the BALT (b) (arrow). IHC, Gill haematoxylin counterstain, Bar = 10µm

Actinobacillus pleuropneumoniae (Choi *et al.,* 2003; Hansen *et al.,* 2010; Fablet *et al.,* 2012). Respiratory parasites and migratory stages of helminths have also been reported to have an impact on the PRDC (Wallgren & Pettersson, 2022). In this study, the mechanism leading to MYHO-lungworm interaction leading to co-infection is not known. A few studies have investigated the interaction of pathogens in cases of co-infections and their molecular consequences in the porcine respiratory tract of infected pigs. However, no clear elucidation of the mechanism shaping the complex interaction between the pathogens has been reported (Saade *et al.,* 2020). The finding of this study is similar and agrees with other studies that reported high prevalence of pneumonia in pigs (Oba *et al.,* 2021; Sibila *et al.,* 2010; Wallgren & Pettersson, 2022).

A study carried out by Oba *et al.* (2021) established a co-infection of *M. hyopneumoniae* and *Metastrongylus* spp. in matured swine of Northern Uganda, and increased risk in pigs with multiple pathogens and *Metastrongylus* spp. infection, this suggests possible interactions in the infected pigs.

It is therefore plausible to suggest that MHYO and other opportunistic bacterial infections may overwhelm the host immune response, which could possibly trigger a severe reaction resulting into granulomatous bronchopneumonia. Immune compromise as a result of lymphoid hyperplasia of the BALT had been previously reported in pigs with MHYO infection (Cheebrough 2006; Bancroft & Gamble, 2014; Olaniyi *et al.,* 2020b).

In the present study, bacterial culture from the lung yielded *Pasteurella multocida* and β-haemolytic *Streptococcus* spp. Severe suppurative bronchiolitis , necrosis and desquamation of the airway epithelium recorded in this study had been reported in cases of infections with *Pasteurella multocida* and *Streptococcus suis* by Reams *et al.* (1994); and swine influenza A virus (H1N1) infection (Valheim *et al.,* 2011; Janke, 2014; Lopez, 2016; Olaniyi *et al.,* 2020a). In this study, the demonstration of MHYO antigens on the airway epithelial cells further confirms the role of MHYO. This has also been demonstrated by by Sarradell *et al.* (2003), Lorenzo *et al.* (2006), Redondo *et al.* (2009) and Olaniyi *et al.* (2020a).

Parasitic pulmonary helminths have been reported in wild boars (Ewing *et al.,* 1982; de-la-Muela *et al.,* 2001) and in slaughtered pigs (Marruchella *et al*., 2012; Taylor *et al.,* 2016) but are quite scarce now (Wallgren & Pettersson, 2022). In Nigeria, a high prevalence of 61.38% was reported in indigenous pigs (Nssien & Adesehinwa, 1999). The appropriate housing design with concrete flooring, reduce access to earthworm, has tremendously reduced the prevalence of metastrongylosis in many countries (Marruchella, 2012) including Nigeria. Prevalence of 2.9% recorded in this study supports this assertion. None the less, verminous pneumonia due to *M. apri* could still be a major challenge to feral and outdoor-reared pigs (Marruchella *et al*., 2012). Lungworm infection in pigs is associated with few helminth parasites (Ewing *et al.,* 1982; Leignel *et al.,* 1997; Poglayen *et al.,* 2016). The findings of the present study could not conclude on the number of parasite species involved, therefore, further molecular studies are thus warranted. Lack of pasture rotation as well as allowing pigs to have access to moist soil which is

the ideal habitat for earthworms have been reported to contribute to the incidence (Stockdale 1976). Therefore, these factors are to be taken into consideration in pig management if free-range is to be adopted. In addition, regular deworming has also been advocated using Ivermectin 1% injectable (Ivomec®). The efficacy of this drug in pigs had long been documented (Leignel *et al.*, 1997). On the other hand, hygience, vaccination, chemotherapy and good husbandry are effective for control of *M. hyopneumoniae* (Pieters & Maes 2019). Unfortunately, no vaccine is available to control MHYO in Nigeria at present. It is suggested that further molecular studies be carried out on *M. hyopneumoniae* strain circulating in Nigeria pigs with a view to developing appropriate vaccine to control the infection.

In conclusion, the present study recorded more pneumonic changes in pigs, and also demonstrated lesions due to *Metasrongylus* spp. associated with *M. hyopneumoniae,* and *P. multocida* in slaughtered pigs in Nigeria. The detection of respiratory pathogens such as *M. hyopneumoniae, Metasrongylus* spp. and *P. multocida* suggests that they are potential contributors to lung pathology observed in this study. The high prevalence of pneumonic lesions recorded underscores the need to place more emphasis on virus-bacterium-parasite synergism rather than the widely reported virus-bacterium and virus-virus synergism in pigs respiratory diseases. In Nigeria, metastrongylosis may be more common than reported herein. The 2.9% reported in this study is significant because a larger percentage of these slaughtered pigs were from commercial piggeries. It is suggested that further molecular studies be carried out on *M. hyopneumoniae* strains circulating in Nigerian pigs to develop an appropriate vaccine to control the infection.

Acknowledgement

The authors are grateful to the staff in the Department of Veterinary Pathology, College of Veterinary Medicine, Federal Universiy of Agriculture, Abeokuta for their technical support.

Funding

No funding was received.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Adetunji MO & Adeyemo KE (2012). Economic efficiency of pig production in Oyo State, Nigeria: A stochastic production frontier approach. *American Journal of Experimental Agriculture*, **2**(3): 382-394.

Alawneh JI, Parke CR, Lapuz EJ, David JE, Basinang VG & Baluyut AS (2018). Prevalence and risk factors associated with gross pulmonary lesions in slaughtered pigs in smallholder and commercial farms in two provinces in the Philippines. *Frontiers in* Veterinary Science, doi.10.3389/fvets.2018.00007.

Amass SF, Clark LK, van Alstine WG, Bowersock TL, Murphy DA, Knox KE & Albregts, SR (1994). Interaction of *Mycoplasma hyopneumoniae* and *Pasteurella multocida* infections in swine. *Journal of the American Veterinary. Medical Association,* **204**(1): 102-107.

Asenso N, Emikpe B, Folitse R, Opoku-Agyemang T & Burimuah V (2015). The incidence and pattern of pneumonia in pigs slaughtered at the Kumasi abattoir, Ghana. *Bulletin of Animal Health and Production in Africa*, **63**(1): 101–107.

Bancroft JD & Gamble M (2014). Theory and Practice of Histological Techniques, Sixth edition. Elsevier Publication. Pp 10–15.

Barrow GS & Feltham RKA (1993). *Cowan and Steel Manual for Identification of Medical Bacteria*. Thrid edtion. Cambridge University Press, Cambridge. Pp 50 - 164.

Bowman DD (2014). *Georgi's Parasitology for Veterinarians*. Tenth edition. Elsevier Publ. pp 198—199.

Cheebrough M (2006). District Laboratory Practice in Tropical Countries. Cambridge University Press. Pp 62.

Choi YK, Goyal SM & Joo HS (2003). Retrospective analysis of etiologic agents associated with respiratory diseases in pigs. *Canadian Veterinary Journal*, **44**(9): 735–737.

de-la-Muela N, Hernández-de-Luján S & Ferre I (2001). Helminths of wild boar in Spain. *Journal of Wildlife Diseases,* **37**(4): 840–843.

Ewing MS, Ewing SA, Keener MS & Mulholland RJ (1982). Mutualism among parasitic nematodes: A population model. *Ecological Modelling,* **5**(3): 15353–15366.

Fablet C, Marois-Crehan C, Simon G, Grasland B, Jestin A, Kobisch M, Madec F & Rose N (2012). Infectious agents associated with

respiratory diseases in 125 farrow-to-finish pig herds: a cross-sectional study. *Veterinary Microbiology*, **157**(1-2):152–163.

Ferraz MES, Almeida HMS, Storino GY, Sonálio K, Souza MR, Moura CAA, Costa WMT, Lunardi L, Linhares DCL & de Oliveira LG (2020). Lung consolidation caused by *Mycoplasma hyopneumoniae* has a negative effect on productive performance and economic revenue in finishing pigs. *Preventive Veterinary Medicine*, doi.10.1016/j.prevetmed.2020.105091.

Fraile L, Alegre A, Lopez-Jimenez R, Nofrarias M, Segales J & Opriessnig T (2010). Risk factors associated with pleuritis and cranio-ventral pulmonary consolidation in slaughter-aged pigs. *Veterinary Journal*, **184**(3): 326–333.

Galdeano JVB, Baraldi TG, Ferraz MES, De Souza Almeida HM, Mechler- Dreibi ML, Costa WMT, Montassier HJ, Mathias LA & de Oliveira LG (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. *Porcine Health Management*, doi.10.1186/s40813-019-0130-0.

Garcia-Morante B, Segalés J, Fraile L, Pérez de Rozas A, Maiti H, Coll T & Sibila M (2016). Assessment of *Mycoplasma hyopneumoniae*-induced pneumonia using different lung lesion scoring systems: A comparative review. *Journal of Comparative Pathology*, **154**(2-3): 125–34.

Hansen MS, Pors SE, Jensen HE, Bille-Hansen V, Bisguard 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.

Janke BH (2014). Influenza A virus infection in Swine: Pathogenesis and diagnosis. *Veterinary Pathology,* **51** (2):410-426.

Leignel V, Humbert JF & Elard L (1997). Study by ribosomal DNA ITS 2 sequencing and RAP analysis on the systematics of four *Metastrongylus species* (*Nematoda Metastrongyloidea*). *Journal of Parasitology,* **83**(4): 606–611.

Lopez A (2016). Respiratory System, Mediastinum, and Pleurae. In: *Pathologic Basis of Veterinary Diseases*. Chapter 9 (fifth edition). MD MaGavin, JF Zachary, editors). Mosby Elsevier Publisher. Pp 514–519.

Lorenzo H, Quesada O, Assuncao P, Castro A & Rodriguez F (2006). Cytokine expression in porcine lungs experimentally infected with *Mycoplasma hyopneumoniae*. *Veterinary Immunology and Immunopathology,* **109** (3-4): 199–207.

Maes D, Sibila M, Kuhnert P, Segales J, Haesebrouck F & Pieters M (2018). Update on *Mycoplasma hyopneumoniae* infections in pigs: knowledge gaps for improved disease control. *Transboundary Emerging Diseases,* **65**(1): 110–124.

Marruchella G, Paoletti R, Speranza R & Di Guardo G (2012). Fatal bronchopneumonia in a *Metastroglylus elongatus* and Porcine Circovirus type 2 co-infected pigs. *Research in Veterinary Sciences,* **93**(1): 310-312.

Nssien MAS & Adesehinwa AOK (1999). Seasonal variation and prevalence of lungworm infection in three different breeds of pigs slaughtered at Bodija Municipal Government Abattoir, Ibadan, Nigeria. *Nigerian Journal of Animal Science*, **2**(1): 209-214.

Nwanta JN, Shoyinka SVO, Chah KF, Onunkwo JI, Onyenwe IW, Eze JI, Iheagwam CN, Ngoja EO, Ogbu KI, Mbegbu EC, Nnadizie PN, Ibe EC & Oladimeji KT (2011). Production characteristics, disease prevalence and herd-health of pigs in Southeast Nigeria. *Journal of Swine Health and Production,* **19**(6): 331-339.

Oba P, Dione MM, Wieland B, Mwiine FN & Erume J (2021). Correlations between lung pneumonic lesions and serologic status for key respiratory pathogens in slaughtered pigs in Northern Uganda. *Porcine Health Management,* doi.10.1186/s40813-021-00233-y.

Olaniyi MO, Adebiyi AA, Ajayi OL, Alaka OO & Akpavie SO (2020a). Localization and immunohistochemical detection of swine influenza A virus subtype H1N1 Antigen in formalin-fixed, paraffin-embedded lung tissues from naturally infected Pigs. *Ben Suef University Journal of Applied Science*, doi.10.1186/s43088-020-0039-3.

Olaniyi MO, Ajayi OL, Alaka OO, Mustapha OA, Brown CC, Shields JP, Ard MB & Nagy T (2020b). Immunohistochemical and ultrastructural studies of *Mycoplasma hyopneumoniae*

strain in naturally infected pigs in Nigeria. *Folia Veterinaria,* **64**(1):1-10.

Olaniyi MO, Akinniyi OO, Alaka OO, Ajayi OL, Jubril JA, Adebiyi AA, Jarikre TA & Emikpe BO (2020c). Retrospective study of swine respiratory diseases in Ogun and Oyo States, Nigeria: Immunohistochemical detection of *Mycoplasma hyopneumoniae* in naturally infected pigs. *Sokoto Journal of Veterinary Sciences,* **18**(2): 72-82.

Opriessnig T, Giménez-Lirola LG & Halbur PG (2011). Polymicrobial respiratory disease in pigs. *Animal Health Research Reviews*, **12**(2): 133-148.

Pallarés F, Añón J, Rodríguez-Gómez I, Gómez-Laguna J, Fabré R, Sánchez- Carvajal J, Ruedas-Torres I & Carrasco L (2021). Prevalence of mycoplasma-like lung lesions in pigs from commercial farms from Spain and Portugal. *Porcine Health Management,* doi.10.1186/s40813-021-00204-3.

Pieters MG & Maes D (2019). Mycoplasmosis In: *Diseases of Swine*, eleventh edition. (*JJ Zimmerman, LA Karriker, A, Ramirez, KJ Schwartz,GW Stevenson, J Zhang, editors*). Wiley-Blackwell Incoporated. Pp 102-113.

Poglayen G, Marchesi B, Dall'Oglio G, Barlozzari G, Gallup R & Morandi B (2016). Lung parasites of the genus *Metastrongulus* Molin, 1861 (Nematoda: *Metastrongiliade*) in wild boar (*Sus scrofa* L. 1758) in central Italy: an ecoepidemiological study. *Veterinary Parasitology,* doi.10.1016/j.vetpar.2015.12.007.

Raymond BBA, Turnbull L, Jenkins C, Madhkoor RI, Schleicher I, Uphoff CC, Whitchurch CB, Rohde M & Djordjevic SP (2018). *Mycoplasma hyopneumoniae* resides intracellularly within porcine epithelial cells. *Nature Scientific Reports*, doi.10.1016/j.vetpar.2015.12.007.

Reams RY, Glickman, LT, Harrington DD & Bowersock TY (1994). *Streptococcal suis* infection in swine: A retrospective study of 256 cases. Part II. Clinical signs, gross and microscopic lesions and coexisting microorganisms. *Journal of Veterinary Diagnostic Investigation*, **6**(3): 326-334.

Redondo F, Masot AJ, Fernandez A & Gazquez A (2009). Histopathological and Immunohistochemical findings in the lung of pigs infected experimentally with *Mycoplasma hyopneumoniae*. *Journal of Comparative Pathology,* **140**(4): 260–270.

Saade G, Deblan C, Bougon J, Marois-Crehan C, Fablet C, Auray G, Belloc C, Leblanc-Maridor M, Gagnon CA, Zhu J, Gottschalk M, Summerfield A, Simon G, Bertho N & Meurens F (2020). Coinfections and their molecular consequences in the porcine respiratory tract. *Veterinary Research,* doi. 10.1186/s13567-020-00807-8.

Sarradell J, Andrada M, Ramirez AS, Fernandez A, Gomez-Villamandos JC & Jover A (2003). A morphologic and Immunohistochemical study of the bronchus associated lymphoid tissue of pigs naturally infected with *Mycoplasma hyopneumoniae*. *Veterinary Pathology* **40**(4): 395–404.

Shima FK & Garba HS (2014). Prevalence of characteristic macroscopic lung pathologies in pigs at slaughter in Makurdi, Benue State, Nigeria. *Bulletin of Animal Health and Production in Africa*, **62**(4): 377–385.

Sibila M, Mentaberre G, Mariana Boadella E, Huerta E, Casas-Díaz J, Vicente C, Gortázar I, Marco S, Lavín, S & Segalés J (2010). Serological, pathological and polymerase chain reaction studies on *Mycoplasma hyopneumoniae* infection in the wild boar. *Veterinary Microbiology,* **144**(1-2): 214-218.

Stockdale PHG (1976). Pulmonary pathology associated with metastrogyloid infections. *British Veterinary Journal,* **132**: 595-608.

Surendran Nair M, Yao D, Chen C & Pieters M (2019). Serum metabolite markers of early *Mycoplasma hyopneumoniae* infection in pigs. *Veterinary Research,* **50**: 80. doi.10.1186/s13567-019-0715-2.

Taylor MA, Copop R & Wall RL (2016). *Veterinary Parasitology*. Forth edition. Wiley and Blackwell Publish. West Sussex, UK. Pp 231-252.

Valheim M, Gamlem H, Gjerset B, Germundsson A & Lium B (2011). Pathological findings and distribution of pandemic influenza A (H1N1) (2009). virus in lungs from naturally infected fattening pigs in Norway. *Influenza Research and Treatment*, doi.10.1155/2011/565787.

Wallgren P & Pettersson E (2022). Lungworms (*Metastrongylus* spp.) demonstrated in domestic pigs with respiratory disease: was there a clinical relevance? *Porcine Health Management*, doi.10.1186/s40813-022-00258-x.