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PATHOMORPHOLOGICAL CHANGES AND BACTERIAL PATHOGENS ASSOCIATED WITH SWINE PNEUMONIAS IN SOUTHWEST NIGERIA

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ABSTRACT

Pneumonia is a major economic threat to swine industry worldwide, however, there is still dearth of information on the pathology and associated pathogens in Nigeria, and these were therefore investigated. Lungs from 408 slaughtered pigs were randomly collected from abattoirs in Abeokuta, Ibadan and Lagos. The lung samples were cultured for bacterial pathogens using standard techniques, while formalin-fixed tissues were processed for histopathological examination. Grossly, the most consistent lesion was bronchopneumonia (35.3%). The main histopathological findings were lymphoid hyperplasia of bronchus-associated lymphoid tissue (BALT) (88.2%), suppurative bronchopneumonia (63.6%), suppurative bronchitis and bronchiolitis with concurrent epithelial hyperplasia (57.1%), as well as thickened alveolar septa due to cellular infiltration consisting predominantly of neutrophils (54.1%). Ten different species of bacteria were isolated from the lung samples in which two or more pathogens were isolated from each sample (82.7%). *Pasteurella multocida* was the most frequently isolated bacterium (54.8%). Among the bacteria isolated, there were significant ($P < 0.05$) differences in the frequencies of isolation of β-*haemolytic Streptococci*, *P. multocida*, *Haemophilus species* and *Escherichia coli* between the pneumonic lungs and apparently normal lungs. The results of bacterial culture, gross and histopathological changes recorded in this study are consistent with bacterial pneumonia possibly caused by most of the bacteria identified in the present study.

Keywords: Pig, pathomorphology, bacterial pathogens, bronchopneumonia, slaughterhouse.

INTRODUCTION

Pneumonia is one of the most important health concerns to swine production worldwide (Harms et al., 2002; Brockmeier et al., 2003; Emikpe et al., 2015) due to its devastating effects on intensive pig farming (Thacker, 2001; Hansen et al., 2010; Emikpe et al., 2018). Published data have confirmed that pneumonias in pigs are very commonly observed at slaughter and bronchopneumonia is one of the most frequent gross lesions encountered (Pijoan, 2006; Martinez et al., 2009; Emikpe et al., 2015; Garcia-Morante et al., 2016; Olaniyi, 2017; Emikpe et al., 2018).

A previous study on swine pneumonia by Antia et al. (1981) and Emikpe et al. (2015) in Ibadan, Nigeria reported about 60% mortality directly attributable to pneumonia.

The prevalence of pneumonia at slaughter previously reported varied from country to country and ranged between 15.4% and 69.3%. A prevalence of 61.3% was recorded in Denmark (Hansen et al., 2010), 55.7% in Spain (Fraile et al., 2010), and in France it ranged between 55.0% and 69.3% (Fablet et al., 2012). In Nigeria, a prevalence range of 35.3% to 44.75% had been previously reported for bronchopneumonia (Antia et al., 1981; Emikpe et al., 2015; Olaniyi, 2017).

Bacterial pathogens including *Mycoplasma hyopneumoniae, β-haemolytic Streptococcus species*, *P. multocida* and *Haemophilus species* have long been reported to be associated with swine respiratory diseases (Choi et al., 2003; Sibila et al., 2009; Fraile et al., 2010; Fablet et al., 2012; Thacker and Minion, 2012; Garcia-Morante et al., 2016, Raymond et al, 2018) and are considered to play a major role in porcine respiratory disease complex (PRDC) (Harms et al., 2002; Brockmeier et al., 2003; Drolet et al., 2003; Opriessnig et al., 2004; Hansen et al., 2010; Morandi et al., 2010; Opriessnig et al., 2011; Thacker and Minion, 2012). However, their roles in swine pneumonia in Nigeria is relatively unknown.

While studies in grower-finishing pigs from different parts of the world showed varying prevalence of bronchopneumonia at slaughter, (Davies et al., 1992; Christensen and Enoe, 1999; Grest et al., 1997; Hansen et al., 2010), only a few records exist in Nigeria. Pathology of swine pneumonias and associated pathogens have been studied extensively in developed countries of the world (Sarradell et al., 2003; Cho et al., 2006; Lorenzo et al., 2006) probably due to the significant impact on commercial pig production and productivity in terms of economic losses (Stark, 2000; Raymond et. al., 2018). Recent studies in Nigeria (Shima and Garba, 2014; Emikpe et al., 2015, 2018) had described the pulmonary pathology of swine pneumonia and the negative effects on pig production in different parts of Nigeria. However, no in-depth study on the pathogens associated with swine pneumonia particularly bacterial pathogens has been performed for decades in Nigeria particularly in the Southwest where pigs are raised on commercial scale.

This study, therefore, investigated the pathomorphological changes and bacterial pathogens associated with pneumonias in slaughter-age pigs in three Southwestern States of Nigeria. The knowledge of the pathology and the pathogens involved in swine pneumonias is critical to effective prevention and control strategies.

MATERIALS AND METHODS

Sample Collection

Lung samples were collected from abattoirs located at Abeokuta, Ibadan and Lagos; all in southwest of Nigeria and from cases submitted for postmortem examination in the Department of Veterinary Pathology, Federal University of Agriculture, Abeokuta and examined grossly for the presence of pneumonic lesions and for bacteria isolation. A total of 408 lung samples consisting of 244 pneumonic lungs (case group) and 160 apparently normal lungs (control group) were randomly collected aseptically from slaughtered pigs between February 2014 and September 2015. The number of animals, age, sex and breed and site of the lesion(s) were recorded to determine the prevalence of

pneumonia in pigs. Samples were labelled and stored in a cool box prior to submission to the laboratory for bacteriology. Part of the samples were immediately fixed in 10% neutral buffered formalin, left to fix for at least 48 hours and processed for histopathological examination.

Gross pathology

Lungs were evaluated grossly and morphological patterns recorded (Caswell and Williams, 2016). Gross lesions were observed in the three cranioventral lobes (i.e. the apical, cardiac and intermediate lobes) of the pneumonic lungs. A preliminary diagnosis of bronchopneumonia was made based on these gross lesions. The bronchopneumonia was considered lobular as evidenced by sharp delineation between the lesion and apparently normal tissues (Figure 1). The lungs were considered pneumonic when they appeared mottled, tan to gray in colour. Pneumonia was considered acute when characterized by oedema, hyperaemia, frothy exudate in the airways; and the lungs were non-collapse and rubbery. Chronic lesions were considered when characterized by fibrosis and firm consistency.

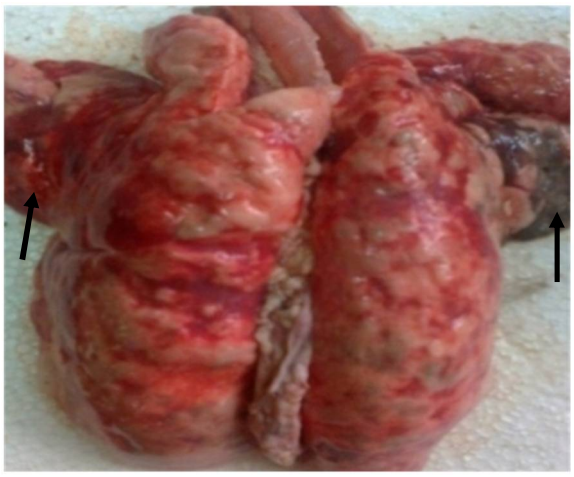

Figure 1: Photograph of the lung showing gross appearance of lungs with acute lobular pneumonia (arrowed).

Histopathological Technique

Two hundred and four (204) formalin-fixed lung tissues (60 apparently normal lungs and 144 pneumonic lungs) were dehydrated in ascending grade of alcohol, cleared in xylene, embedded in paraffin, sectioned at 3 -5µm and stained with hematoxylin and eosin (H&E) according to the method described by Bancroft and Gamble (2014). Selected tissue sections were also stained with Masson's trichrome and phosphotungistic acid haematoxylin to detect collagen and fibrin respectively. Sections were examined carefully by evaluation of the following structures in the section: pleura, bronchi, bronchioles, and bronchus-associated lymphoid tissue (BALT); alveolar ducts and alveoli including alveolar septa; peribronchial and peribronchiolar and interlobular connective tissue. BALT hyperplasia was graded according to Hansen et al. (2010) as absent (0), mild (+), moderate (++),

marked (+++) or extensive (++++).

Isolation and identification of bacterial pathogens

One hundred and forty four (144) lung samples (84 pneumonic lungs and 60 apparently normal lungs) were examined for bacterial pathogens. Standard bacteriological methods as described by Cheesbrough (2006) were employed for the isolation of bacteria. Each lung sample was transferred into a sterile bottle containing 9 ml of buffered peptone water (BPW) for pre-enrichment. It was mixed very well by agitation and incubated at 37oC overnight for 18 hour. A loopful of the overnight broth culture was then inoculated directly on MacConkey Agar, Chocolate Agar and Blood Agar (Oxoid® Basingstoke, England). MacConkey Agar was used to isolate lactose fermenting and non-lactose fermenting gram negative bacteria, Blood Agar were used to isolate fastidious organisms while Chocolate Agar was used to isolate Haemophilus spp. All the plates were incubated at 37oC for 24 hours but for Haemophilus spp. 10% CO2 was provided to enhance its growth. Identification of bacteria was based on cultural; Gram's staining test and biochemical characteristics such as triple sugar iron, urease test, catalase, hydrogen sulphide, indole, motility, coagulase, citrate and oxidase tests. Only pure isolates were used in the identification process.

Statistical Analysis

Data obtained from this study were analysed using descriptive statistics and presented in percentages (%). Inferential statistics was performed by Chi square test and Fisher's exact test to test the association between the variables (age, breed, sex and season) and pulmonary lesions. All analyses were carried out with SPSS statistical package version 16 (SAS Institute, Inc., US) at $\alpha 0.05$.

RESULTS

Gross pathology

Grossly, 35.3% (144/408) lungs showed varying degrees of pneumonia. The most prominent gross morphological pathology recorded was bilateral, lobular cranio-ventral pulmonary consolidation affecting the apical, middle and accessory lobes (Fig.1). Multifocal fibrotic pleurisy of the diaphragmatic lobes, without any relationship to pneumonic lesions was observed in 29.9% (43/144) of the samples collected. Only 5% (12/260) of the apparently normal lungs showed gross lesions in the form of mild congestion. Grower pigs had the highest frequency of bronchopneumonia than other age groups (59.1%), while the weaner pigs had the least frequency (Table 1). Generally, there was statistically significant (P = 0.027) association between pulmonary lesions and the age group of the pigs (Table 1).

Table 1: Frequency distribution of lungs with bronchopneumonia (BP) and apparently normal lung (APNL) in different age group of pigs

Age group	BP (%) (N =144)	APNL (%) (N = 264)	Total (%) (N = 408)	p-value
Weaner (1-2months)	12 (8.3)	35 (13.3)	47 (11.5)	
Grower (2-6 months)	85 (59.1)	127 (48.1)	212 (52)	0.027
Adult (> 6 months)	47 (32.6)	102 (38.6)	149 (36.5)	
Total	144 (35.3)	264 (64.7)	408 (100)	

N= number of samples collected

There was no significant (P > 0.05) different in frequency distribution of bronchopneumonia among breed of pigs in this study, even though highest frequency was recorded for the Large White breed. Frequency distribution of lungs with bronchopneumonia in different breeds of pig is shown in Table 2.

Table 2: Frequency Distribution of Lungs with Bronchopneumonia (BP) and Apparently Normal Lung (APNL) in Different Breeds of Pigs

Breed	BP (%) (N =144)	APNL (%) (N =264)	Total (%) (N =408)	p-value
Large white	68 (47.2)	118 (63.4)	186 (45.6)	
Landrace	16 (11.1)	34 (68.0)	50 (12.3)	
Duroc	16 (11.1)	28 (63 .6)	44 (10.8)	0.124
Local breed	19 (13.2)	36 (65.5)	55 (13.5)	
Cross breed	25 (17.4)	48 (65.8)	73 (18.8)	
Total	144 (35.2)	264 (64.8)	408 (100)	

N= number of samples collected

Histopathology

Based on the histopathological examination, lesions were divided into acute (8/144, 5.6%), sub-acute (22/144, 15.3%) or chronic (114/144, 79.2%) cases of bronchopneumonia. All acute cases (n = 8) were mainly suppurative bronchopneumonia (BP). The sub-acute cases (n = 22) were subdivided into suppurative (14/22, 63.6%), mixed (6/22, 27.3%) and non-suppurative (2/22, 9.1%) bronchopneumonia. The chronic cases (n = 114) were subdivided into mixed (48/114, 42.1%) and non-suppurative (66/114, 57.9%) bronchopneumonia. The main histopathological findings of this study are summarized in Table 3.

Microscopic lesions were found in all the lungs with gross pneumonic lesions. In many of the lung sections (62.5%), there were bronchitis and bronchiolitis with presence of cellular exudate in the bronchiolar lumen (Figure 2a). Bronchitis and bronchiolitis were mainly suppurative with concurrent epithelial hyperplasia (57.1%) (Figure 2b). There were varying degrees of BALT hyperplasia (Figure 3) which was more pronounced in the chronic stage where partial or complete compression and obliteration of the bronchial or bronchiolar lumen was observed (Figure 3D). BALT hyperplasia was recorded in 88.2% of the cases.

Varying degrees of thickening of the alveolar septa, mainly by cellular infiltration consisting of predominantly of neutrophils, lymphocytes and macrophages were recorded in 54.1%, and in a few chronic cases (34/114, 29.29.2%) by fibrous connective tissue (Figure 4).

Pulmonary congestion (Figure 5A) and interstitial oedema were common findings in both acute and sub-acute stages with the degree being more pronounced in acute stage of the infection. In some cases of bronchiolitis, degeneration and/or necrosis of the epithelial cells (Figure 5B) were observed especially in sub-acute and chronic cases of the infection.

Other histological changes observed were hypertrophy of the muscle around the bronchioles which was recorded in 12/114 (10.4%) of the chronic cases and hyperplasia of bronchial submucosal glands found mostly in the acute phase of the infection as well as thickening of the pleural (Table 3).

Table 3: Summary of Histopathological Changes in the Lungs with Bronchopneumonia (N = 144) and Apparently Normal Lung (APNL) (N = 60)

Histopathological changes	APNL (n = 60)		Bronchopneumonia+ Acute (n=8)		Sub-acute (n=22)		Chronic (n =114)	
	n	%	n	%	n	%	n	%
BALT hyperplasia *								
0	51	85.0	5	62.5	2	9.1	12	10.5
+	7	11.7	6	75.0	6	27.3	27	27.7
++	2	3.3	0	-	8	36.4	51	44.7
+++	0	-	0	-	4	18.2	23	12.3
++++	0	-	0	-	2	9.1	37	32.8
Bronchitis	1	1.7	2	25.0	6	27.3	15	13.2
Bronchiolitis	2	3.3	4	50.0	9	40.9	37	32.5
Thickening of alveolar septa	13	21.7	1	12.5	15	68.2	42	36.9
Alveolar/interstitial oedema	4	6.7	5	62.5	17	77.3	2	1.3
Epithelial necrosis	0	-	0	-	3	13.6	30	26.3
Pulmonary congestion	4	6.7	5	62.5	18	81.9	0	-
Thickening of pleura	2	3.3	0	-	0	-	10	8.8
Epithelial hyperplasia	0	-	6	75.5	15	68.2	60	52.6
Interstitial fibroplasia	0	-	0	-	0	-	34	29.9
Interstitial cellular infiltration	0	-	6	75.5	16	72.3	31	27.2
Sub-mucosal gland hyperplasia	10	16.7	5	62.5	6	27.3	0	-
Smooth muscle hyperplasia	0	-	0	-	5	22.8	12	10.4

+Grouping of lesions as acute, sub-acute or chronic is based on histopathological evaluation.

*BALT hyperplasia was scored as absent (0), mild (+), moderate (++), marked (+++), or massive (++++)

N = Number of samples examined

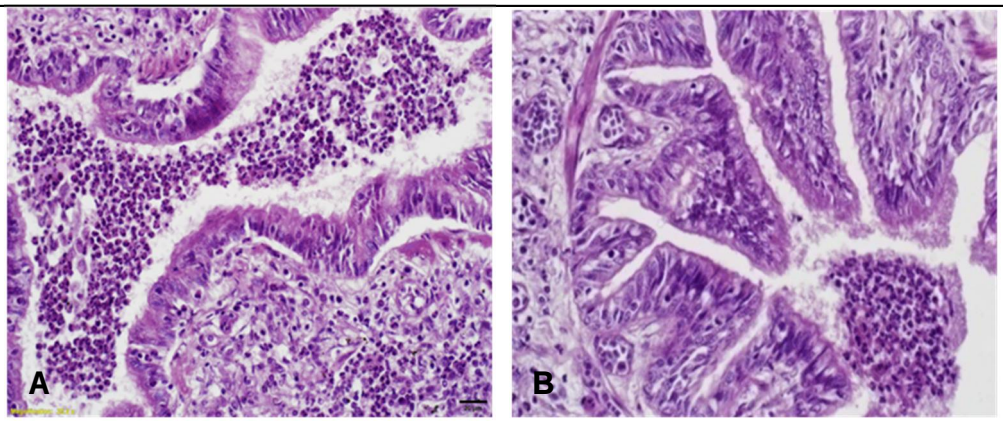

Figure 2: Photomicrograph of lung sections showing suppurative bronchiolitis with concurrent epithelial hyperplasia and intra-luminal cellular exudate consisting predominantly of neutrophils (H &E stain, Bar= 20µm)

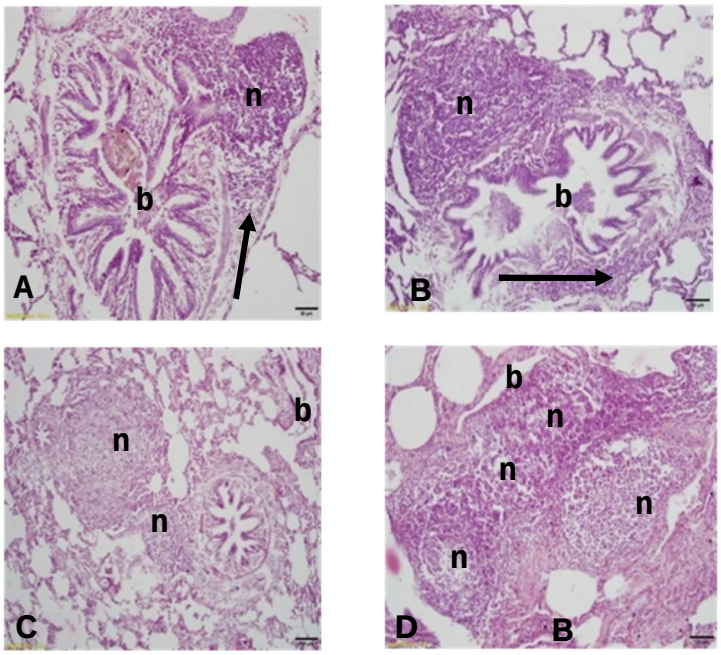

Figure 3: Photomicrograph of lung sections showing (A) mild BALT hyperplasia (+) with diffuse infiltration of inflammatory cells (arrowed) into the peribronchiolar tissues including the lamina propria of an apparently normal bronchiole (b). (B) Moderate BALT hyperplasia (++) with greater diffuse infiltration of lymphocytes (arrowed) with formation of a lymphoid nodule (n) and a slightly compressed bronchiolar lumen (b). (C) Severe BALT hyperplasia (+++) with presence of a few lymphoid nodules (n), a compressed bronchiole (b) can be seen. (D) Extensive BALT hyperplasia (++++) with presence of numerous lymphoid nodules (n) affecting a large portion of the lung parenchyma and a few compressed bronchioles (b). (H&E stain, Bar = 100µm).

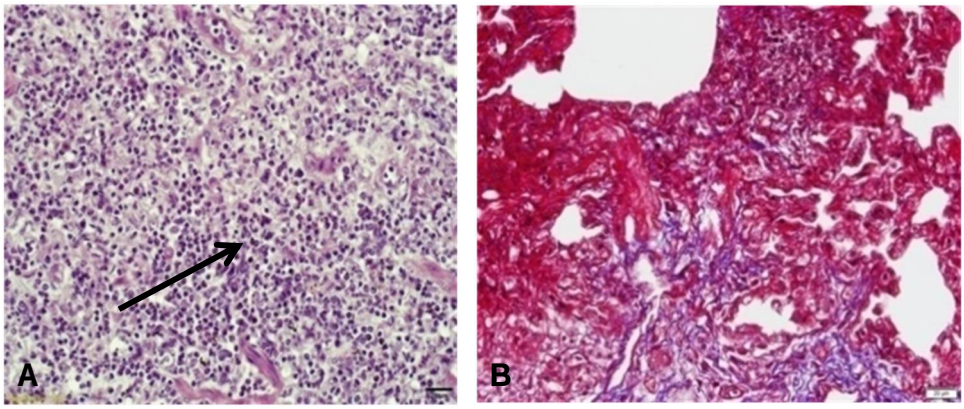

Figure 4: Photomicrograph of lung sections showing (a) thickened alveolar septa due to cellular infiltration dominated by neutrophils (arrowed). (H&E stain, Bar = 100µm). (b) interstitial fibroplasia by fibrous (blue) connective tissue. (Masson's trichrome stain, Bar = 20µm)

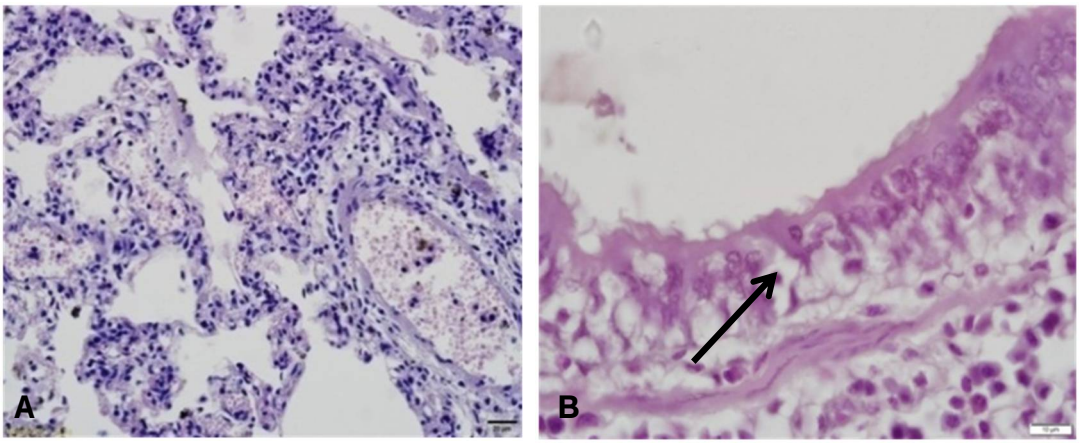

Figure 5: Photomicrograph of lung sections showing (a) acute pulmonary congestion. (b) chronic bronchiolitis with concurrent widespread necrosis of bronchiolar epithelial cells (arrowed). (H&E stain, Bar = 20 µm).

Bacteriology

Ten different species of bacterial pathogens were isolated in this study. At least one species of bacterium was isolated from each lung sample. Two or more species of bacteria were isolated from 82.7% of the pneumonic lung samples. The frequency of isolation of the bacterial pathogens is shown in Table 3. In general, the frequencies of isolation of the bacterial isolated were significantly higher ($P < 0.05$) in the lung samples with pneumonic lesions compared with samples from apparently normal lungs.

Pasteurella multocida was most frequently isolated bacterium from the pneumonic lungs while *Escherichia* coli was the most frequently isolated bacterial species from the apparently normal lungs (Table 4).

TABLE 4: FREQUENCY OF BACTERIAL PATHOGENS ISOLATED FROM LUNG SAMPLES THAT HAD BRONCHOPNEUMONIA AND APPARENTLY NORMAL LUNG (APNL) SAMPLES

Pathogens	Bronchopneumonia (N = 84)	%	APNL (N = 60)	%	p- value
β- *haemolytic streptococcus spp*	38	45.2	3	5.0	< 0.05
Pasteurella multocida	46	54.8	6	10.0	< 0.05
Haemophilus species	22	26.2	4	6.7	< 0.05
Mannheimia haemolytica	4	4.8	1	1.7	NS
Staphylococcus species	18	21.4	3	5.0	< 0.05
Klebsiella species	9	10.7	3	5.0	NS
Escherichia coli	28	33.3	10	16.7	<0.05
Pseudomonas aeruginosa	11	13.1	5	8.3	NS
Bacillus species	7	8.3	4	6.7	NS
Proteus species	4	4.8	3	5.0	NS

NS = Not significant, N = number of samples

There were significant (P < 0.05) differences between pneumonic and apparently healthy lungs in the frequency of isolation of β-*haemolytic streptococcui*, *Pasteurella multocida*, *Haemophilus* species and *Escherichia* coli.

Pasteurella multocida was frequently isolated either as single or in combination with other bacteria from the pneumonic lung samples and had the most significant association, especially with *Haemophilus* species and β-*haemolytic* streptococci. Three significant associations (P < 0.05) were observed for P. *multocida*, and two for β-*haemolytic* streptococci. Significant (P < 0.05) associations were observed between four pairs of bacteria species, the most frequent combinations are presented in Table 5.

TABLE 5: SIGNIFICANT ASSOCIATION BETWEEN PAIRS OF BACTERIAL PATHOGENS IN 84 LUNG SAMPLES WITH BRONCHOPNEUMONIA

Association between pathogens	P- value
β- *haemolytic streptococcus spp* and *Escherichia coli*	NS
β- *haemolytic streptococci* and *Haemophilus species*	< 0.05
Pasteurella multocida and *Haemophilus species*	< 0.05
Pasteurella multocida and β- *haemolytic streptococci*	< 0.05
Pasteurella multocida and *Proteus species*	NS
Haemophilus species and *Escherichia coli*	NS
Pasteurella multocida and *Staphylococcus species*	< 0.05
β- *haemolytic streptococci* and *Staphylococcus species*	NS

NS = Not significant

There were significant associations between the following pairs of bacteria: β-haemolytic streptococci and Haemophilus species, Pasteurella multocida and Haemophilus species, Pasteurella multocida and β-haemolytic streptococci, and Pasteurella multocida and Staphylococcus species ($P < 0.05$). No significant ($P > 0.05$) differences were observed for other species of the bacterial pathogens isolated (Table 5).

DISCUSSION

Pneumonia is a worldwide problem in the pig industry and one of the most important disease factors limiting pig production (Brockmeier et al., 2003; Emikp et al., 2015, Raymond et al., 2018). It can be classified as broncho-, interstitial, broncho-interstitial and embolic pneumonia based on the morphological pattern (Caswell and Williams, 2007). This may be the first study investigating pathomorphology and bacterial pathogens associated with swine pneumonias in slaughter-age pigs on a large scale.

In the present study, a prevalence of 35.3% was recorded for swine pneumonia which was mostly seen in grower pigs. A previous study of swine pneumonia in intensively managed pig farms in Ibadan, Nigeria reported a prevalence of 44.75% for bronchopneumonia in pigs (Antia et al., 1981; Emikpe et al., 2015, 2018) which was mostly seen in weaner pigs. The prevalence of swine pneumonia at slaughter previously reported from various countries also varied and ranged between 15.4% and 79% (Christensen and Enoe, 1999; Fraile et al., 2010; Fablet et al., 2012). Factors that may account for this great variation may include different sampling methods, season of year when investigation was conducted, age at slaughter, environment and management conditions (Cho et al., 2006). In this study, age of pig at slaughter appears to be an important associated predisposing or risk factor in the development of pneumonia in pigs as the condition was observed in grower pigs more than any other age groups. This has been reported to be associated with waning of passively acquired antibodies as weaner pigs still carry sufficient antibodies against respiratory infections (Cho et al., 2006; Pomorska-Mol et al., 2011). However, this does not agree with the findings of Antia et al. (1981) who reported highest susceptibility to pneumonia in weaner pigs. The variation in prevalence may be due to differences in sampling methods used; modern management strategies and improved biosecurity measures being presently put in place in most of the farms in Nigeria. The present study also showed that Large White had the highest percentage occurrence of pneumonia, this could probably be due to the low pulmonary to body ratio in this breed of pig (Konig and Liebich, 2009). Evidence of increased susceptibility of certain breeds of pigs to respiratory diseases induced by porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae had been previously reported (Opriessnig et al., 2004; Vincent et al., 2006; Opriessnig et al., 2009).

The histopathological findings in the present study were consistent with bacterial pneumonia caused by most of the bacterial species isolated and resemble lesions associated with pneumonia caused by combination of bacterial pathogens including M. hyopneumoniae, β-haemolytic Streptococcus species, Pasteurella multocida, Haemophilus species as described by various workers (Kwon et al., 2002, Sarradell et al., 2003, Lorenzo et al., 2006; Redondo et al., 2009; Hansen et al., 2010; Merialdi et al., 2012). The partial or complete compression and obliteration of

bronchiolar lumen associated with these lesions resulting in collapse of the surrounding alveoli, which was recorded in the present study had been previously described in *M. hyopneumoniae*-infected pigs (Redondo *et al.,* 2009; Hansen *et al.,* 2010, Olaniyi, 2017, Raymond *et al.*, 2018). The obliteration of the bronchiolar lumen had been attributed to accumulation of mucous and inflammatory exudates in the bronchial lumen as a result of decreased ciliary activities, increased activities of mucous secreting cells and altered glycoprotein (Park *et al.,* 2016), release of pro-inflammatory chemical mediators by alveolar macrophages (van Reeth and Nauwynck, 2000) and the presence of hyperplastic lymphoid aggregates (Maes *et al.,* 1996; Sarradell *et al.,* 2003, Redondo *et al.,* 2009, Hansen *et al.,* 2010). This hyperplasia has been associated with constant antigenic stimulation which associated with chronic recurrent infections. The damage caused by *M. hyopneumoniae* to the airway epithelium had been reported to predispose pigs to secondary infection by organisms such as *P. multocida* (Thanawongnuwech and Thacker, 2003; Thanawongnuwech *et al.,* 2004) and porcine circovirus type 2 (Opriessnig *et al.,* 2004; Ellis, 2014).

Other histopathological findings were widespread degeneration and necrosis of the bronchial and bronchiolar epithelium lining of the affected lungs, bronchiolar lumen and alveoli were filled with exudate containing desquamated cells, neutrophils and mononuclear cells and these lesions have been reported in swine influenza virus (SIV)-infected pigs (Thacker et al., 2001; Jung et al., 2002; Janke, 2014). SIV infection is a commonly encountered respiratory disease of pigs throughout swine raising countries (Thacker et al., 2001; Jung et al., 2005; van Reeth et al., 2012; Janke, 2014). In Nigeria, serological evidence of this virus in commercial piggeries had earlier been reported (Olaleye et al., 1990; Meseko et al., 2014). This study showed that 24.3% of lung samples had lesions described above and so may suggest the presence of sub-clinical SIV infection in pigs in the study area. Therefore, further study on molecular epidemiology of swine influenza virus is thus warranted.

In this study, 10 species of bacteria were isolated. Two or more species were isolated from 82.7% of the pneumonic lungs. The species of bacteria isolated in this study were similar to the ones previously reported by Antia et al. (1981), Choi et al. (2003), Ross, (2006), Palzer et al. (2008) and Fablet et al. (2012). However, each of these pathogens was isolated more frequently or less frequently than in the earlier studies. In this study, *P. multocida* was isolated from a higher proportion of lung samples that had pneumonia (54.8%) than from apparently normal lung. This was closely followed by *β-haemolytic* streptococcus species which were detected in 45.2% of the samples and was similar to the findings of Hansen et al. (2010) and Fablet et al. (2012).

In the present study, *E. coli* and *Proteus* species were isolated from lung samples that had pneumonia and apparently normal lungs. These two species have been shown to be pathogens that are not normal inhabitant of the lung (Palzer et al., 2008), although are normally common contaminants during sample collection.

A few of the association between bacterial pathogens recorded in this study had been established either experimentally or naturally (Opriessnig et al., 2004, Opriessnig et al., 2011). The positive association ($P < 0.05$) between P. multocida and Staphylococcus

species recorded in this study has not been reported in previous studies. However, the pathogenic effects of the individual organisms had been previously described; both agents are found to be pathogenic to the lung. (Pijoan, 2006; Ross, 2006; Brockmeier and Register, 2007; Wilson and Ho, 2010; Opriessnig et al., 2011). Pasteurella multocida had been shown to be an important bacterium associated with suppurative bronchopneumonia in swine (Pijoan, 2006; Ross, 2006; Olaniyi, 2017) and is generally considered to occur secondary to infections with viruses (Ross, 2006;) or M. hyopneumoniae (Amass et al., 1994; Hansen et al., 2010). The possible role of Streptococcus spp in the pathogenesis of bronchopneumonia had been suggested (Higgins and Gottschalk, 2006). Proliferation of α-haemolytic streptococci had been reported to affect epithelial surface, making it more receptive to colonization by other facultative pathogens such as P. multocida (Higgins and Gottschalk, 2006; Palzer et al., 2008). The organism is generally considered to be a secondary invader that causes suppurative bronchopneumonia (Higgins and Gottschalk, 2006) usually in conjunction with Mycoplasma spp or Pasteurella spp (Thacker, 2001; Hansen et al., 2010). However, it can also act as a primary pathogen of pneumonia in pigs (Higgins and Gottschalk, 2006).

CONCLUSION

In conclusion, this study showed that pneumonias occurred more frequently among grower pigs in Nigeria. Pathomorphological findings were consistent with bacterial pneumonia caused by most of the species identified in the present study. The present study supports previously reported pathogen profile and multifactorial aetiology of pneumonias in pigs. The result of this study also showed that over 80% of the cases of pneumonia in pigs were caused by co-infection; therefore, any management procedure that will break the opportunity for combined infections would be beneficial and should be enforced in the farm. The implications of these findings is that measures including appropriate managemental practices, vaccination and adequate biosecurity to control these pathogens be put in place and promptly implemented, and these would significantly reduce respiratory diseases particularly *Mycoplasma hyopneumoniae* infection in pigs. This study underscores the need for molecular epidemiological investigation on the viral agents of swine pneumonia in the study area.

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REFERENCES

Amass, S.F., Clark, L.K., van Alstine, W.G., Bowersock, T.L., Murphy, D.A., Knox, K.E., Albregts, S.R. 1994. Interaction of *Mycoplasma hyopneumoniae* and *Pasteurella multocida* infections in swine. *Journal of the American Veterinary Medical Association* 204: 102-107.

Antia, R.E., Akpavie, S.O., Ikede, B.O. 1981. Observations on the pathology of pig pneumonias in Ibadan, Nigeria. *Bull. Anim. Hlth. Prod.* (29): 309-316.

Bancroft, J.D., Gamble, M. 2014. Theory and Practice of Histological Techniques. Sixth Edition: Elsevier Publ., Pp. 10–15.

Brockmeier, S., Halbur, P., Thacker, E. 2003. Porcine Respiratory Disease Complex. In Polymicrobial Diseases, Brogden, K.A. and Guthmiller, J.M. (Eds). Pp. 231-258. ASM Press, Washington DC.

Brockmeier, S.L., Register, K.B. 2007. Expression of the dermonecrotic toxin by *Bordetella bronchiseptica* is not necessary for predisposing to infection with toxigenic *Pasteurella multocida*. *Veterinary Microbiology* 125: 284–289.

Caswell, J.L., Williams, K.J. 2016. Respiratory system. In: *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*, 5th Edition, Maxie M.G. (Eds). Pp. 534-537. Saunders Elsevier, Philadelphia.

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

Cho, J.G., Dee, S.A., Deen, J., Guedes, A., Trincado, C., Fano, E., Jiang, Y., Faaberg, K., Murtaugh, M.P., Guedes, A., Collins, J.E., Joo, H.S. 2006. The impact of animal age, bacterial coinfection, and isolate pathogenicity on the shedding of porcine reproductive and respiratory syndrome virus in aerosols from experimentally infected pigs. *Canadian Journal of Veterinary Research* 70: 297–301.

Choi, Y.K., Goyal, S.M., Joo, H.S. 2003. Retrospective analysis of etiologic agents associated with respiratory diseases in pigs. *Canadian Veterinary Journal* 44: 735–737.

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: 1006-1015.

Christensen, G., Mousing, J. 1992. Respiratory System. In *Diseases of Swine*. 7th Edition, Leman, A.D.; Straw, B. E., Mengeling, W.L, D'allaire S. and Taylor, D.J. (Eds). Pp. 128-162. Ames, Iowa. Iowa State University Press.

Ciprian, A., Pijoan, C., Cruz, T., Camacho, J., To´rtora, J., Colmenares, G., Lo´pez-Revilla, R., De la Garcia, M. 1988. *Mycoplasma hyopneumoniae* increases the susceptibility of pigs to experimental *Pasteurella*. *Canadian Journal of Veterinary Research* 52: 434–438.

Davies, P., Moore, M.J., Pointon, A.M. 1992. The association between lesions of pleuritis and enzootic pneumonia in slaughtered pigs. In: Proceedings of the 12th International Pig Veterinary Society Congress, The Hague. 306pp.

Drolet, R., Larochelle, R., Morin, M., Delisle, B., Magar, R. 2003. Detection rates of porcine reproductive and respiratory syndrome virus, porcine circovirus type 2, and swine influenza virus in porcine proliferative and necrotizing pneumonia. *Veterinary Pathology* 40: 143-148.

Emikpe, B.O., Jarikre, T.A., Adediran, O.A., Olaniyi, M.O., Dikeogu, T.C. 2018. Haematology, bronchoalveolar cellular changes and pathology of swine pneumonia in Nigeria. *Sokoto Journal of Veterinary Sciences* 16(2): 1–9.

Emikpe, B.O., Adediran, O.A., Jarkre, T.A., Olawumi, O., Jubril, A.J. 2015. Evaluation of lung lesions and associated predisposing factors from slaughtered pigs in Nigeria. *Archieves of Basic and Applied Medicine* 3: 119–122.

Ellis, J. 2014. Porcine Circovirus: A Historical Perspective. Veterinary Pathology 51 (2) 315-327. DOI: 10.1177/0300985814521245

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* 157: 152-163.

Fraile, L., Alegre, A., Lopez-Jimenez, R., Nofrarias, M., Segale's, J., Opriessnig, T. 2010. Risk factors associated with pleuritis and cranio-ventral pulmonary consolidation in slaughter-aged pigs. *Veterinary Journal* 184: 326–333.

Garcia-Morante, B., Segale's, J., Fraile, L., Perez 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: 125-134.

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: 500-506.

Hansen, M.S., Pors, S.E., Jensen, H.E., Bille-Hansen, V., Bisguard, M., Flachs, E.M., Nielsen, O.L. 2010. An investigation of the Pathology and Pathogens associated with Porcine Respiratory Disease Complex in Denmark. *Journal Comparative Pathology* 143: 120-131.

Harms, P.A., Halbur, P.G., Sorden, S.D. 2002. Three cases of porcine respiratory disease complex associated with porcine circovirus type 2 infections. *Journal of Swine Health and Production* 10: 27-30.

Higgins, R., Gottschalk, M. 2006. Streptococcal Diseases. In: *Diseases of Swine*, 9th Edition., B.E. Straw, J.J. Zimmerman, S. D'Allaire and D.J. Taylor (Eds). Pp. 769-783. Blackwell Publishing, Oxford.

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

Jung, K, Ha, Y., Chae, C. 2005. Pathogenesis of swine influenza virus subtype H1N2 infection in pigs. *Journal of Comparative Pathology* 132(2-3): 179–184.

Jung, T., Choi, C., Chae, C. 2002. Localization of swine influenza virus in naturally infected pigs. *Veterinary Pathology* 39(1): 10-16.

Konig, H.E., Liebich, H. G. 2009. Respiratory system. In: Veterinary Anatomy of Domestic mammals 4th edition. Schatteaer Stuttgart Germany Publ. Pp. 385-390.

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

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: 199-207.

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, E.A., Corpa, J.M. 2009. The relationship between infectious and non-infectious herd factors with pneumonia at slaughter and productive parameters in fattening pigs. *Veterinary Journal* 179: 240– 246.

Merialdi, G., Dottori, M., Bonilauri, P., Luppi, A., Gozio, S., Pozzi, P., Spaggiari, B., Martelli, P. 2012. Survey of pleuritis and pulmonary lesions in pigs at abattoir with a focus on the extent of the condition and herd risk factors. *Veterinary Journal* 193: 234–239.

Meseko, C., Odaibo, G., Olaleye, O. 2014. Detection and Isolation of (2009) Pandemic influenza A/H1N1 virus in commercial piggery, Lagos Nigeria. *Veterinary Microbiology* 168: 197-201.

Morandi, F., Ostanello, F. Fusaro, L. Bacci, B., Nigrelli, A., Alborali, L., Dottori, M., Vezzoli, F., Barigazzi, G., Fiorentini, L., Sala, V., Leotti, G., Joisel, F., Sarli, G. 2010. Immunohistochemical detection of aetiological agents of proliferative and necrotizing pneumonia in Italian pigs. *Journal of Comparative Pathology* 142:74–78.

Olaleye, O.D., Omilabu, S.A., Baba, S.A., Fagbami, A.H. 1990. Haemaglutination-inhibition (HI) Antibodies against strains of influenza a virus in Horses and Pigs Sera in Nigeria. *J. Hyg. Epidemiol. Microbial and Immunology* 34(4): 365-370.

Olaniyi, M.O. 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., Giménez-Lirola, L.G., Halbur, P.G. 2011. Polymicrobial Respiratory Disease in Pigs. *Animal Health Research Reviews* 12: 133-148.

Opriessnig, T., Patterson, A.R., Madson, D.M., Pal, N., Rothschild, M., Kuhar, D., Lunney, J.K., Juhan, N.M., Meng, X.J., Halbur, P.G. 2009. Difference in severity of porcine circovirus type 2 (PCV2)-induced pathological lesions between Landrace and Pietrain pigs. *Journal of Animal Science* 87: 1582–1590.

Opriessnig, T., Thacker, E.L., Yu, S., Fenaux, M., Meng, X.J., Halbur, P.G. 2004. Experimental re-production of post-weaning multisystemic wasting syndrome in pigs by dual infection with *Mycoplasma hyopneumoniae* and porcine circovirus type 2. *Veterinary Pathology* 41: 624-640.

Palzer, A., Ritzmann, M., Wolf, G., Heinritizi K. 2008. Associations between pathogens in healthy pigs and pigs with pneumonia. *Veterinary Record* 162: 267-271.

Park, C., Jeong, J., Kang, I., Choi, K., Park, S., Chae, C. 2016. Increased fucosyl glycoconjugate by *Mycoplasma hyopneumoniae* enhances adherence of *Pasteurella multocida* type A in the ciliated epithelial cells of the respiratory tract. *BMC Vet. Res.* 12: 25-31.

Pijoan, C. 2006. Pneumonic Pasteurellosis. In: *Diseases of Swine*, 9th Edit., B.E. Straw, J.J. Zimmerman, S. D'Allaire and D.J. Taylor (Eds). Pp. 701-717. Blackwell Publishing, Oxford.

Pomorska-Mol, M., Markowska-Daniel, I., Rachubik, J., Pejsak, Z. 2011. Effect of maternal antibodies and pig age on the anti-

body response after vaccination against Glassers disease. *Veterinary Research Communications* 35: 337–343.

Raymond, B.B.A., Turnbull, L., Jenkins, C., Madhkoor, R.I., Schleicher, I., Uphoff. C.C, Whitchurch, C.B., Rohde, M., Djordjevic, S.P. 2018. *Mycoplasma hyopneumoniae* resides intracellularly within porcine epithelial cells. *Nature Sci. Rep.* 8, 17697. DOI:10.1038/s41598-018-36054-3.

Redondo, F., Masot, A.J., Fernandez, A., Gazquez, A. 2009. Histopathological and Immunohistochemical findings in the lung of pigs infected experimentally with *Mycoplasma hyopneumoniae*. *Journal of Comparative Pathology* 140: 260-270.

Ross, R.F. 2006. *Pasteurella* and its role in porcine pneumonia. *Animal Health Research Reviews* 7: 13-29.

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

Shima, F.K., Garba, H.S. 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.

Sibila, M., Pieters, M., Molitor, V., Maes, D., Haesebrouck, F., Segalés, J. 2009. Current perspectives on the diagnosis and epidemiology of *Mycoplasma hyopneumoniae* infection. *The Veterinary Journal* 181 (3): 221–231.

Stark, K.D.C. 2000. Epidemiological investigation of the influence of environmental risk factors on respiratory diseases in swine: A literature review. *Vet. Journal* 159: 37 –56.

Thacker, E.L. 2001. Porcine respiratory disease complex- What is it and why does it remain a problem. *Pig Journal* 48: 66-70.

Thacker, E.L., Thacker, B.J., Janke, B.H. 2001. Interaction between *Mycoplasma hyopneumoniae* and Swine Influenza virus. *Journal of Clinical Microbiology* 39: 2525-2530. DOI: 10.1128/JCM.39.7.2525–2530.2001

Thacker, E.L., Minion, F.C. 2012. Mycoplasmosis. In: *Diseases of Swine,* 10th Edit., Zimmerman, J.J., Ramirez, A., Schwartz, K.J., Stevenson, G.W. (Eds). Wiley-Blackwell, Ames. Pp. 779-798.

Thanawongnuwech, R., Thacker B., Halbur, P., Thacker, E. 2004. Increased Production of Proinflammatory Cytokines following infection with Porcine Reproductive and respiratory Syndrome virus and *Mycoplasma hyopneumoniae*. *Clinical and Diagnostic Laboratory Immunology* 11: 901-908.

Thanawonguuwech, R., Thacker, E.L. 2003. Interlukin-10, Interlukin-12 and Interferon gamma levels in the respiratory tract following *Mycoplasma hyopneumoniae* and PPRS infection in pigs. *Canadian Veterinary Journal* 44: 735-737.

Van Reeth, K., Brown, I.H., Olsen, C.W. 2012. Influenza Virus. In: Diseases of Swine, 10th Edit., Zimmerman, J.J., Ramirez, A., Schwartz, K.J., Stevenson, G.W. (Eds). Pp. 557-570. Wiley-Blackwell, Ames.

Vincent, A.L, Thacker, B.J., Halbur, P.G., Rothschild, M.F., Thacker, E.L. 2006. An

investigation of susceptibility to porcine reproductive and respiratory syndrome virus between two genetically diverse commercial lines of pigs. *Journal of Animal Science* 84: 49–57.

Wilson, B.A., Ho, M. 2010, Recent insights into *Pasteurella multocida* toxin and other G-protein-modulating bacterial toxins. *Future Microbiology* 5: 1185–1201.

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