
PATHOMORPHOLOGY AND AEROBIC BACTERIA ASSOCIATED WITH PNEUMONIA IN SMALL RUMINANTS SLAUGHTERED AT THE NSUKKA ABATTOIR

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ABSTRACT

*Pneumonia occurs in all ages of sheep and goats, in all breeds, in every country of the world causing heavy economic losses. The aim of this study was to determine the prevalence of pneumonia and aerobic bacteria flora associated with it in small ruminants slaughtered at the Nsukka abattoir. Pneumonic lung of small ruminants were examined for gross lesions. Lung samples were collected and processed using standard protocols for histopathological and bacteriological examinations. Lung samples from 342 goats and 40 sheep were examined. A total of 116(30.36 %) lungs had various types of pneumonia. Two major types of pneumonia were observed during histopathological examination; bronchopneumonia 64(55.17 %) and interstitial pneumonia 52(44.82 %). Out of the 116 pneumonic lungs collected over a six months period, 98 were caprine lungs and 18 were ovine lungs. Aerobic bacteria isolated from the pneumonic lungs were *Escherichia coli*, *Klebsiella pneumoniae*, *Mannheimia haemolytica*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pasteurella multocida* respectively. There was no significant seasonal, species and breed associations ($p>0.05$) between pneumonic lesions observed and the associated aerobic bacteria.*

Keywords: Small ruminants, Pneumonia, Interstitial pneumonia, Bronchopneumonia, Aerobic bacteria flora

INTRODUCTION

Small ruminants play an important role in the nutrition and income of people around the world. They serve as source of meat, milk, skin and wool (Habashy *et al.*, 2009). Small ruminants contribute significantly to the

economy of farmers in the Mediterranean as well as African and Southeast Asian countries (Chakraborty *et al.*, 2014). The mass production of small ruminant in the country is constrained by disease, inadequate nutrition, poor genetic resources of the local stock, marketing, social factors, structural constraints

and a shortage of high level trained manpower (Yesuf *et al.*, 2012). Mohamed and Abdelsalam (2008) reported that respiratory tract infections were of common occurrence in various species of domestic and farm animals. Pathogenesis is multifactorial, and the diseases appear due to the interaction of infectious micro-organisms (bacteria, mycoplasma, viruses and fungi), host defense, environmental factors and stress. Bacterial infection of the respiratory tract may be primary, occurring in healthy individuals or secondary to a large number of conditions which causes immunosuppression (Yesuf *et al.*, 2012). Secondary bacterial infection occurs especially when the resistance of the respiratory mucosa is lowered and bacteria growing in the upper respiratory tract extend downwards (Yesuf *et al.*, 2012). Pneumonia is a major respiratory disease of domestic animals worldwide, especially in countries where livestock management and husbandry are yet to be developed. The disease incidence is usually very high in these areas and this causes serious financial losses to the livestock industry (Raji *et al.*, 2000).

Pneumonia in goats is one of the most important infections that are frequently diagnosed in veterinary clinics and abattoirs (Elsheikh and Hassan, 2012). Sayed and Zaitoun (2009) pointed out that pneumonia is the most frequently occurring respiratory infections in domestic animals, their aetiologic agents being bacteria, viruses, parasites or concert effect of all of them, often predisposed to by several factors. Pneumonias can be acute, chronic or progressive (Bell, 2008). In summary, in Nigeria, some livestock owners dispose of sick, debilitated and infertile animals in an effort to minimize losses, thereby leading to an increase in the risk of slaughtering and consumption of sick animals (Bala *et al.*, 2011). There has been little work done on this topic with regards to extensive aerobic bacteria isolation and histopathological examination in Nsukka agroecological zone of Nigeria. The objectives of this study were to determine the prevalence of pneumonia in small ruminants slaughtered at the Nsukka abattoir, determine the effects of season, species and breed on the prevalence of pneumonia in the small ruminants and identify

the aerobic bacteria associated with the pneumonia cases.

MATERIALS AND METHODS

Study Area: This study was conducted in Nsukka, a town in Nsukka Local Government Area of Enugu State, Nigeria. As of 2007, Nsukka Cultural Zone had an estimated population of 1,377,001. Nsukka is situated at latitude 6°51'24"N and longitude 7° 23'45"E. The town has one abattoir located in the Ikpa Commodity Market, about 55 m from the University of Nigeria, Nsukka campus.

Study Design and Sample Collection: A cross sectional survey was conducted over a 6-months period in the year 2014; 3 months in the dry season (December to February) and 3 months in the wet season (April to June). Immediately after slaughter, ovine and caprine lungs were examined and sections from lungs with gross pneumonic lesions were collected in duplicates, placed in sterile sample bottles and appropriately labelled. One portion was used for histopathological study, while the other portion was used for bacteriological study. Ethical approval was not necessary as samples were collected from dead animals.

Gross and Histopathological Examination: The lungs of each slaughtered small ruminant were examined and the nature and distribution of the pneumonic lesions of the small ruminants slaughtered at the Nsukka abattoir were documented as described by Thompson (1981).

Each pneumonic lung specimen was fixed in 10 % buffered formalin. After fixation, the tissue was dehydrated in graded alcohol (70 – 100 %), clearing in xylene, paraffin wax embedded, sectioned (15 µm) using microtome, mounted on slides and differentially stained with Haematoxylin and Eosin (Drury and Wallington, 1980). Slides were examined with Olympus light microscope at x1000 magnification. Photomicrographs of representative pneumonic lesions were taken using Motic camera.

Isolation and Identification of Aerobic Bacteria: Pneumonic samples were aseptically

collected and streaked on blood and MacConkey agar plates. Inoculated plates were incubated at 37°C for 24 hours. The representative morphological colony types were picked and purified on blood, nutrient and MacConkey agar. Gram positive *Cocci* were subjected to Mannitol salt agar, catalase, CAMP and coagulase tests following standard protocols (Chessbrough, 2000). Gram negative rods and *Coccobacilli* were cultured on Eosin methylene blue agar. Giemsa staining, indole, methyl red, Voges-proskauer, citrate utilization, nitrate reduction and urease tests as well as reaction on triple sugar iron agar were accompanied using standard procedures (Chessbrough, 2000). The identity of the aerobic bacteria isolates were determined based on their colonial, microscopic and biochemical characteristics using standard procedures (Chessbrough, 2000).

Data Analysis: Descriptive statistics involving frequencies and percentages of occurrence of pneumonia in small ruminants and aerobic bacterial isolates were used. Chi-square statistics was used to determine the level of association between the season, species, breed, sex and the occurrence of pneumonia. Significance was accepted at $p < 0.05$.

RESULTS

Three hundred and eighty-two (382) lungs were examined, 116 ovine and caprine lungs were positive for different types of pneumonia namely bronchopneumonia and interstitial pneumonia. Bronchopneumonia (41.29 %) was more prevalent than interstitial pneumonia (33.54 %) (Figure 1). The distribution of pneumonia among breeds of small ruminants slaughtered at Nsukka abattoir indicated that the Kano brown breed of goat recorded the highest occurrence of pneumonia (Table 1).

The frequency distribution of the histopathological findings and associated aerobic bacteria isolates in goats and sheep slaughtered at the Nsukka abattoir, during the dry and wet seasons indicated that bronchopneumonia and interstitial pneumonia were common in the ruminants regardless of the season (Table 2).

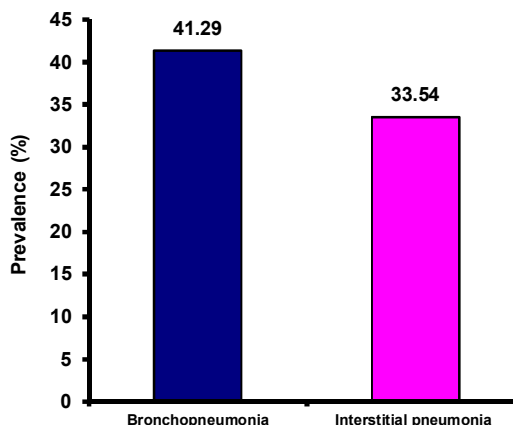


Figure 1: Prevalence of the different observed pneumonia in small ruminants slaughtered at the Nsukka abattoir

The aerobic bacteria associated with bronchopneumonia in the ruminants during the dry season were *Mannheimia haemolytica*, *Escherichia coli*, *Pasteurella multocida*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, while the aerobic bacteria associated with bronchopneumonia in the ruminants during the wet season were *E. coli*, *M. haemolytica*, *Streptococcus pyogenes* and *K. pneumoniae*. Furthermore, the aerobic bacteria associated with interstitial pneumonia in the ruminants during the dry season were *S. aureus*, *S. pyogenes*, *E. coli* and *K. pneumoniae*, while the aerobic bacteria associated with interstitial pneumonia in the ruminants during the wet season were *S. aureus*, *S. pyogenes*, *K. pneumoniae* and *E. coli* (Table 2).

The percentage of aerobic bacteria isolated from the caprine and ovine lungs observed with pneumonia were 37(32.47 %), 16(13.39 %), 12(9.97 %), 19(16.23 %), 23(19.94 %), 4(3.13 %), 4(3.41 %) and 2(1.42 %) positive for *E. coli*, *K. pneumoniae*, *M. haemolytica*, *S. pyogenes*, *S. aureus* and *P. multocida* respectively (Figure 2).

The gross lesions observed were majorly, suppurative pneumonia, exudative pneumonia, congestion (Figure 3). Enlarged dark red wet lung (arrow) in a Kano brown goat. Note the inset - blood expressed from alveoli of the cut surface of the lungs in Figure 3 and the consolidation in Figure 4 and hyperaemia in Figure 5.

Table 1: Breed distribution of small ruminants with pneumonia slaughtered at Nsukka abattoir

Species Breeds	Goats		Sheep		Total
	Kano Brown	WAD Goat	Yankasa	WAD Sheep	
Seasons					
Dry season	31	11	12	0	54
Wet season	42	14	5	1	62
Total	73	25	17	1	116

Table 2: Frequency distribution of the histopathological findings and associated aerobic bacteria isolates in small ruminants slaughtered at the Nsukka abattoir, during the dry and wet seasons

Histopathological Findings	Number of Goats	Number of Sheep	Most frequent aerobic bacteria isolates
Bronchopneumonia	24	5	<i>Mannheimia haemolytica</i> , <i>E. coli</i> , <i>Pasteurella multocida</i> , <i>Klebsiella pneumoniae</i> and <i>Staphylococcus aureus</i>
Interstitial pneumonia	18	7	<i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i> , <i>E. coli</i> and <i>Klebsiella pneumoniae</i>
Wet season			
Bronchopneumonia	30	5	<i>E. coli</i> , <i>Mannheimia haemolytica</i> , <i>Streptococcus pyogenes</i> and <i>Klebsiella pneumoniae</i>
Interstitial pneumonia	26	1	<i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i> , <i>Klebsiella pneumoniae</i> and <i>E. coli</i>

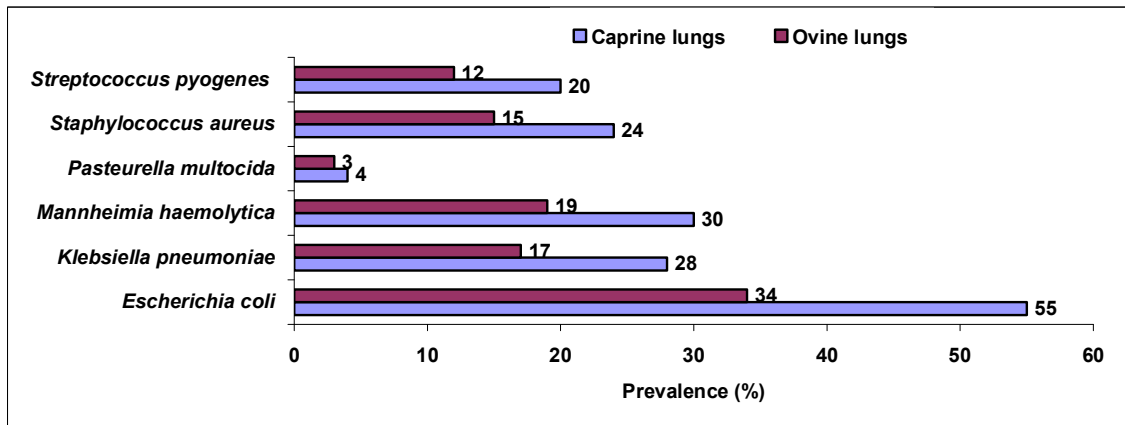


Figure 2: Percentage of aerobic bacteria isolated from the caprine and ovine pneumonic lungs of small ruminants slaughtered at the Nsukka abattoir

Histopathological examination revealed the two types of bronchopneumonia, fibrinous pneumonia in 17 out of 155 (10.96 %) ruminants and catarrhal bronchopneumonia in 47 out of 155 (30.32 %) ruminants, and interstitial pneumonia occurred in 52 out of 155 (33.54 %) ruminant lungs examined. The lungs

diagnosed with bronchopneumonia were characterised by neutrophilic exudates were present in the alveolar spaces and lumens of the bronchioles and bronchi, and in some occasions a mixture of various amounts of cell debris, neutrophils and macrophages were observed in these areas and there are also distended

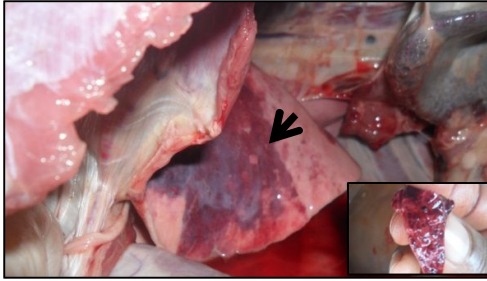


Figure 3: Enlarged dark red wet lung (arrow) in a Kano brown goat. Inset showing blood expressed from alveoli of the cut surface of the lungs



Figure 4: Consolidation (arrowed) of the caudal lung lobe in a West African dwarf goat

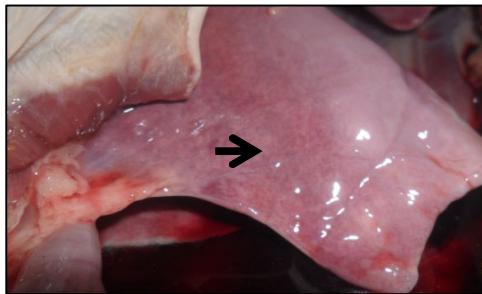


Figure 5. Hyperemia (arrowed) of the caudal lung lobe of a Yankasa sheep

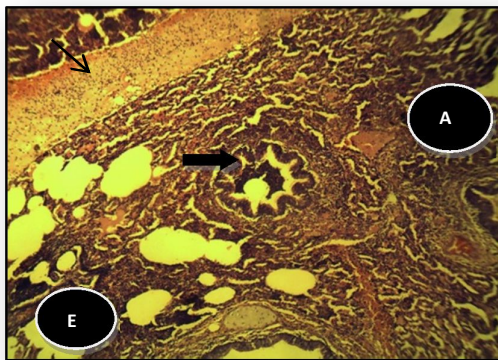


Figure 6: Bronchopneumonia case characterised by neutrophilic exudates were present in the alveolar spaces and lumens of the bronchioles and bronchi (thick arrow), there are also distended interlobular space (thin arrow), infiltrated with inflammatory cells, distended alveoli (E) and collapsed alveoli (A)

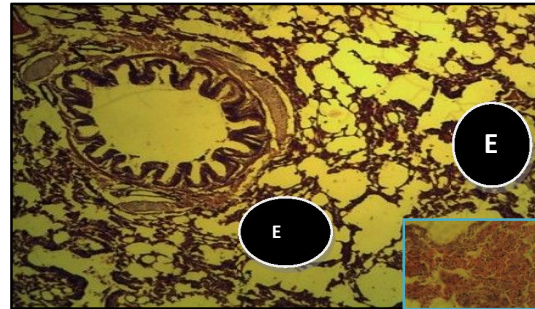


Figure 7: Interstitial pneumonia case characterised by interalveolar space infiltrated with predominantly polymorphonuclear cells. Inset showing lymphocytes, macrophages and a few neutrophils

interlobular space, infiltrated with inflammatory cells, distended alveoli (E) and collapsed alveoli (A) (Figure 6), while the lungs with interstitial pneumonia were characterised by interalveolar space infiltrated with predominantly polymorphonuclear cells namely lymphocytes, macrophages and a few neutrophils (Figure 7).

DISCUSSION

The major types of pneumonia namely bronchopneumonia and interstitial pneumonia were observed during the histopathological examination. The types of pneumonia observed were the most common types of pneumonia encountered in most studies. Tijjani *et al.* (2012) reported bacterial flora and pathologic lesions of caprine pneumonic lungs in Maiduguri, Nigeria, Yesuf *et al.* (2012) had equally reported histopathological changes and bacterial flora associated with pneumonic lungs of small ruminants slaughtered at Gondar, Ethiopia, and Ashraf *et al.* (1986) had reported incidence and pathology of pneumonias in sheep and goats slaughtered at Faisalabad, Pakistan. Sheep and goats slaughtered in this study were positive for *E. coli*, *K. pneumoniae*, *M. haemolytica*, *S. pyogenes*, *S. aureus* and *P. multocida*. The aerobic bacteria isolated from the pneumonic lungs agreed with the ones isolated by Raji *et al.* (2000) from ovine and caprine in Zaria, Nigeria and Asaduzzaman *et al.* (2013) from black Bengal goats in Bangladesh.

The observed variation in the prevalence of different types of pneumonia among different species of small ruminant in this study agreed with Obasi *et al.* (2001). This may be attributed to the fact that the ratio of the alveolar surface to metabolic weight is very low in sheep when compared to other ruminant species (Aden *et al.*, 2012).

Above 55 % of the pneumonic lung sampled were positive for *E. coli*, this was higher than reports by previous researchers (Sayed and Zaitoun, 2009; Enany *et al.*, 2012) but lower than the report of Tijjani *et al.* (2012). *E. coli* was the most prevalent aerobic bacteria, this agreed with the findings of Ouchriah *et al.* (2015) who also isolated more *E. coli* than any other aerobic bacteria from newborn calves slaughtered in Batna, Algeria but disagreed with the findings of Enany *et al.* (2012) for buffalo calves slaughtered in Ismailia Governorate, Egypt.

Furthermore, 27.58 % of the pneumonic lung samples were positive for *K. pneumoniae*, this was higher than the results reported by previous researchers Enany *et al.* (2012), Sayed and Zaitoun (2009), but lower than the results reported by Tijjani *et al.* (2012).

Thirty-five of the pneumonic lungs sampled were positive for *M. haemolytica* was higher than the results reported by Enany *et al.* (2012) but lower than the results reported by Tijjani *et al.* (2012). 24.13 % of the pneumonic lungs sampled were positive for *S. pyogenes*, this was higher than the results reported by Sayed and Zaitoun (2009), Enany *et al.* (2012) and Tijjani *et al.* (2012) but lower than the results reported by Enany *et al.* (2012).

Twenty-three representing 19.82 % of the pneumonic lung samples were positive for *S. aureus*, this was higher than the results reported by previous researchers Enany *et al.* (2012), Tijjani *et al.* (2012) but lower than the results reported by Sayed and Zaitoun (2009).

Five representing 4.31 % of the pneumonic lung samples were positive for *P. multocida*; this was higher than the results reported by Ugochukwu (2008) but lower than the results reported by Sayed and Zaitoun (2009) and Enany *et al.* (2012). In agreement with previous reports of Yimer and Asseged

(2007) and Azizi *et al.* (2013) in Ethiopia and Iran respectively, in terms of infection intensity and pathogenicity, it seems that *M. haemolytica* assumes greater prominence than *Pasteurella* in Nsukka environment. Although Ugochukwu (2008) reported relatively low prevalence of *P. multocida* (1.15%) in Nsukka as against 4.31% in this study, the possible role of *P. multocida* in the aetiology and pathogenesis of caprine and ovine pneumonia should not be under estimated.

No bacteria could be isolated from 8(5.80 %) sampled lungs supported the findings of Yimer and Asseged (2007) in Dessie, Ethiopia, Elsheikh and Hassan (2012) in Sudan and Azizi *et al.* (2013) in South western Iran.

The variation in the prevalence of different types of pneumonia in small ruminants in different seasons may be attributed to the variation in nutritional status, breed and the effect of stressors involved during transportation, overcrowding and seasonal variation in climatic factors such as temperature and humidity (Obasi *et al.*, 2012). For the caprine species, more of the Kano brown than the WAD and Sahel goats examined had pneumonia, this was also similar to what was observed in the sheep, where more of the Yankasa breed had pneumonia. This difference was mainly due to the fact that the butchers slaughtered more of these breeds and could also occur due to genetic variation of the breed which influences their immune response and host receptor interaction with colonizing bacteria of the selected species (Yesuf *et al.*, 2012). The results confirmed the findings by Raji *et al.* (2000) that rainstorms, heavy rainfall and dry harmattan are important stress factors in the pathogenesis of pneumonia in sheep and goats.

Conclusion: In conclusion, based on the results obtained in this study, the prevalence of the diagnosed pneumonia were higher in the wet season than in the dry season, higher in sheep than the goats examined and higher in females than in males examined. It was also concluded that several bacterial species inhabit the respiratory passageways of apparently normal goats and sheep but stressful conditions

like transport stress, overcrowding, poor ventilation in the houses where these animals are housed prior to slaughtering and poor nutrition could turn these supposedly harmless commensals into pathogenic bacteria. In cases of bronchopneumonia, *E. coli*, *M. haemolytica* and *Pasteurella* species were the most frequently isolated aerobic bacterial organisms for fibrinous types of bronchopneumonia; *Staphylococcus* spp., *Streptococcus* spp. and *E. coli* were the most frequently isolated from catarrhal bronchopneumonia and in this study, bacteriological examination results showed that for interstitial pneumonia cases, *S. aureus*, *S. pyogenes* and *E. coli* were the major aerobic bacteria isolated. The unhygienic condition of the slaughter slab at the Nsukka abattoir was a great concern. A concert effort should be made by Veterinarians, Meat inspectors and Public health specialists to ensure cleanliness of the abattoir and provision of a source of clean and potable pipe-borne water at the Nsukka abattoir. Occurrence of supposedly harmless bacteria flora as pathogenic bacteria has been attributed to transport stress, therefore proper resting of small ruminants prior to slaughter at the lairage and minimal stress during transportation is with great emphasis, recommended to minimize the incidence of caprine and ovine pneumonia. It is also recommended that a detailed research work be carried out to look into the economic and public health implications of these aerobic commensal organisms.

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