

Pathological and Molecular Investigation of Some Bacterial in the Lungs and Livers of Red Sokoto Goats Slaughtered at Ilorin, Kwara State, Nigeria

Mohammed Adam¹ , Ahmed Olayiwola Akeem² , Sanni Abdul Barka³, Shoyinka Shodeinde Vincent Olu⁴, Ajadi Abdullateef Abiodun¹ , Ola-Fadunsin Shola David^{5*} 

¹Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Nigeria

²Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Nigeria

³Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria

⁴Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

⁵Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Nigeria

*E-mail: olafadunsin.sd@unilorin.edu.ng

ABSTRACT

This study was conducted to determine the pathological findings and molecular investigation of the bacteria flora associated with the lesions in the lungs and livers of Red Sokoto breed goats slaughtered for public consumption at the Ilorin abattoir. A total of 450 samples (240 lungs and 210 livers) were collected and examined from goats slaughtered at the Ipata slaughterhouse in Ilorin, Kwara State. Of this number, 58 (24.17%; 95% CI = 19.19–29.96) lungs and 17 (8.10%; 95% CI = 5.17–12.58) livers revealed gross and microscopic pathological lesions as a result of bacterial pathogens. Based on gross examination, lung lesions were categorized into bronchopneumonia (27.59%), congestion (22.41%), interstitial pneumonia (17.24%), hemorrhages (13.77%), emphysematous (10.34%), and hyperemia (8.62%). In the liver, hepatic congestion 8 (47.06%) was the most observed gross lesion. Others included hepatic enlargement (29.41), hepatic fibrosis (17.65%), and hepatic abscess (5.88%). The molecular investigation revealed lung pathological lesions were as a result of *Klebsiella* species (51.72%) and *Bacillus* species (48.28%), while *Escherichia* species (52.94%) and *Salmonella* species (47.06%) were detected in the liver. The molecular analysis showed that the bacteria organisms detected in this study did not fully cluster with similar species from other parts of the world. The infection caused by these bacteria showed

histopathological lesions in the lungs and livers. There is need for more studies to further characterize these bacteria species detected from Red Sokoto breed of goats.

Keyword: Bronchopneumonia, Emphysematous, *Escherichia* species, Hepatic congestion, *Klebsiella* species

INTRODUCTION

Ruminants are characterized by their "four" stomachs and "cud-chewing" behavior. The cud is a behaviorally altered food bolus. There are about 150 different ruminant species, including but not limited to cattle, sheep, goats, deer, buffaloes, bison, giraffes, moose, and elk. Ruminant species can be further classified as grazers, browsers, or intermediates (Iain, 2003). Small ruminants (sheep and goats) are a major component of the ruminant industry in Nigeria, with an estimated population of sheep and goats at 22.1 million and 34.5 million, respectively (Ola-Fadunsin and Ibitoye, 2017). Goats play a major role in the security and social well-being of rural populations living under conditions of extreme poverty (Ngambi et al., 2013). Goats are an important source of protein for humans in terms of meat and milk in both developed and developing economies (Wesongah et al., 2003; Ola-Fadunsin and Ibitoye, 2017). In Nigeria, goats are useful for the provision of meat, milk, household income, manure, skin, and sociocultural purposes (Okorafor et al., 2015; Ola-Fadunsin and Ibitoye, 2017).

The universal solution to malnutrition in rural and urban areas is the diversification of animal production, especially of food animals (Ugochukwu et al., 2017). Among the food animals that require less income to

start is goat production. The goat is an animal with a high source of quality meat, milk, and skin (Emikpe and Akpavie, 2012). In Nigeria, caprine diseases cause serious limitations that can deter the success of goat production (Emikpe and Akpavie, 2012; Ola-Fadunsin and Ibitoye, 2017). Respiratory conditions such as pneumonia are one of the most frequent infections in goats and are often diagnosed in veterinary clinics and abattoirs (Elsheikh and Hassan, 2012). The physical examination and observations such as emaciation, poor meat quality, and condemnation during abattoir meat inspection as a result of pneumonic lungs in goats have been connected to great economic losses to farmers (Al-Qudah et al., 2008; Ntiamoah et al., 2021).

Among the inflammatory and non-inflammatory disease conditions of the lungs are pneumonia, which could be acute or chronic (Alam et al., 2001; Ferdausi et al., 2008). In farm animals, the lungs are vulnerable to a wide range of infectious and non-infectious agents that cause a variety of pathological conditions. The liver plays numerous roles such as blood liver detoxification and purification, metabolism and storage of carbohydrates, proteins, fats, and vitamins, secretion of bile, and synthesis of plasma proteins (Sastri and Ramarao, 2001), thus any trouble in this organ will show on the health status of the animal, leading to enormous

economic losses in animals (Blood et al., 1989). Due to the highly specialized functions of the hepatic parenchymal cells and dual blood circulation, the liver is one of the first organs to be exposed to inimical agents," i.e., infectious agents, parasites (such as *Fasciola* species and *Oesophagostomum* species), and toxins (Kelly, 1985; Ola-Fadunsin et al., 2020). These two organs are vulnerable to many infectious and non-infectious agents that cause various pathological conditions in farm animals (Rashid et al., 2013). The prevalence of small ruminant diseases imposes a serious threat to livestock production (Ntiamoah et al., 2021).

It is well established that molecular detection techniques like the polymerase chain reaction (PCR) and subsequent DNA sequencing of target genes are highly effective means of detecting and genotyping organisms, including bacteria (Dantrakool et al., 2004; Ola-Fadunsin, 2017).

The survey of pathological lesions from slaughterhouses can provide information on a number of common diseases of small ruminants that are economically and zoonotically important (Ntiamoah et al., 2021). Pathological studies have been carried out in slaughterhouses to investigate small ruminant diseases (Kudi et al., 1987; Mugale and Balachandran, 2019). Given the importance of the information obtained from pathological lesions in abattoirs in the epidemiology of small ruminant diseases, there is a need for regular abattoir studies on the diseases of small ruminants in order to safeguard against zoonoses and to prevent economic losses in the small

ruminant industry (Al-Qudah et al., 2008).

This present study was carried out to investigate the pathological changes and associated bacterial to pneumo-hepatic disorders in Red Sokoto goats slaughtered at the Ipata slaughterhouse using gross, histopathology, and molecular techniques.

MATERIALS AND METHODS

Study Population

The study animals were Red Sokoto breed goats brought for slaughter in the Ipata goat abattoir in Ilorin, Kwara State. All the animals slaughtered were male and adults, and they were from the northern part of Nigeria.

Study location

Ilorin is located between latitudes 8°25'N to 8°32'N and longitudes 4°30'E to 4°41'E. The area is closer to the confluence of the Niger and Benue rivers, which demarcate the northern and southern regions of Nigeria (Olorunfemi and Oditia, 1998). Ilorin city covers approximately 50.2 square kilometers of land and is situated approximately 420 square kilometers from the Federal Capital Territory. It is strategically located as the gateway between the southern and northern parts of the country, making it easily accessible to all parts of the country by air, road, and rail transportation (Aiyedun and Olugasa, 2012; Ola-Fadunsin et al., 2019).

Study design

A cross-sectional survey study using a systemic random sampling technique was used to select the Red Sokoto breed of goats that were slaughtered at the Ipata abattoir from December 2019 to April 2020, to determine the pathological lesion caused by bacteria that led to the condemnation of the lungs and livers.

Gross examination

A total of 240 lungs and 210 livers, totaling 450 samples, were examined for gross abnormalities. The collected samples were thoroughly inspected using visualization, palpation, and multiple systemic incisions when and wherever required. The texture, consistency, color, adhesion, pattern, distribution, and number of lesions were recorded. Gross tissue changes were observed, meticulously recorded, and photographed with a Pentax 18-55mm digital camera manufactured in the Philippines by Hoya Corporation. Representative tissue samples containing lesions were stored in 10% neutral buffered formalin for histopathological studies for at least 24 hours.

Histopathological examination

After 24 hours, the fixed tissue sections were cut into pieces (3 mm thick) and dehydrated using ascending grades (70%, 80%, 90%, and 100%) of alcohol for 15 minutes, followed by clearing in absolute xylene and embedded in paraffin wax. Sections of 4-5 microns in thickness were cut and stained using the Harri's hematoxylin

and eosin method. Finally, the stained slides were examined at X4, X10, and X100 magnifications for the presence of characteristic and/or suggestive lesions using an ordinary light microscope. The different forms of lesions were then classified according to the involvement of anatomical sites and the nature of the inflammatory exudate and reaction present.

Bacteriological examination and identification of isolates

Swabs of lungs and liver samples were collected in 10 ml of buffered peptone water and incubated at 37 °C for 24 hours. Thereafter, growth was subcultured on 5 % sheep blood agar (BA) (Oxoid, Hampshire, UK) and MacConkey agar (MA) (Oxoid, Hampshire, UK) and incubated at 37 °C for another 24 hours. Growths on BA and MA were studied morphologically, and then the different colonies were subjected to biochemical tests including Gram staining, catalase, urease, citrate utilization, and triple sugar iron as previously described (Alam et al., 2001; Ferdausi et al., 2008; Tonu et al., 2011).

Molecular characterization of bacterial isolates

Genomic deoxyribonucleic acid extraction

The deoxyribonucleic acid (DNA) of the bacterial isolates was extracted from the lungs and livers positive for bacteria and purified using a Zymo Spin DNA Extraction kit according to the instructions of the manufacturer. The eluded DNA was stored at -20 °C until further molecular studies were carried out.

Polymerase chain reaction (PCR) amplification of 16S rRNA of bacterial isolates

Fragments of the 16S rRNA genes of each bacterial isolate were separately amplified using the pair of eubacteria universal primers 27F (AGAGTTTGATCMTGGCTCAG) and 1525R(AAGGAGGTGWTCARCCGA). The PCR cocktail mix consisted of 2.5 μ L of 10x PCR buffer, 1 μ L of 25mM MgCl₂, 1 μ L each of forward and reverse primers, 1 μ L of DMSO, 2 μ L of 2.5mM DNTPs, 0.1 μ L of 5u/ μ L Taq polymerase, and 3 μ L of the extracted DNA. The total reaction volume was made up to 25 μ L using 13.4 μ L Nuclease free water (Fermentas, St. Leon-Rot, Germany). The temperature program and the cycle of reactions were an initial denaturation step at 94 °C for 5 mins, followed by 36 cycles of denaturing at 94 °C for 30 sec, primer annealing at 56 °C for 30 secs, and primer elongation at 72 °C for 45 sec. This was followed by a final elongation step at 72 °C for 7 mins. About 5 μ L of the PCR products with 1 μ L loading dye was loaded in a 1.5% gel, 1kb bp DNA ladder was also loaded. The loaded gel was ran using the electrophoresis machine for 50 mins, at 45 volts. The ran gel was then stained with ethidium bromide for gel documentation. Amplified fragments were visualized using a gel doc. The size of the amplicon was about 1500 base pairs. Sequencing of the positive PCR products were done at the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Data and phylogenetic analyses

The Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS Inc., Chicago, Illinois) was used for the statistical analysis. The prevalence and corresponding 95% confidence interval (CI) were used to estimate the quantity of pathological lesions in the sampled lungs and livers of goats.

Sequences obtained from this study were searched for homologous sequences in GenBank® using BLASTn (www.ncbi.nlm.nih.gov/BLAST). Sequences were aligned using the Clustal W program. Based on these alignments, nucleotide alignments were performed and phylogenetic analyses were conducted using MEGA version 6.0 (Kumar et al., 2014). The resulting phylogenetic tree was an unrooted neighbor-joining tree constructed using Kimura 2 parameters and the Gama distribution (K 2-G). A bootstrap replicate of 1,000 was used to test the robustness of the tree. Seven hundred and eight (708) nucleotide regions were used for the construction of the phylogenetic tree. The sequences obtained from this study have been deposited in GenBank® under the accession number cited in Figure 1.

RESULTS

Of the 240 lungs and 210 livers sampled, 58 lungs and 17 livers had pathological lesions (Figure 2). The prevalence of pathological lesions was 24.17% (95% CI = 19.19-29.96) for lungs (Table 1), while that of livers was 8.10% (95% CI = 5.17-12.58) (Table 2). Six lesion types, which included bronchopneumonia, interstitial pneumonia, emphysema, hemorrhage, congestion, and hypereamia, were recorded as lung lesions.

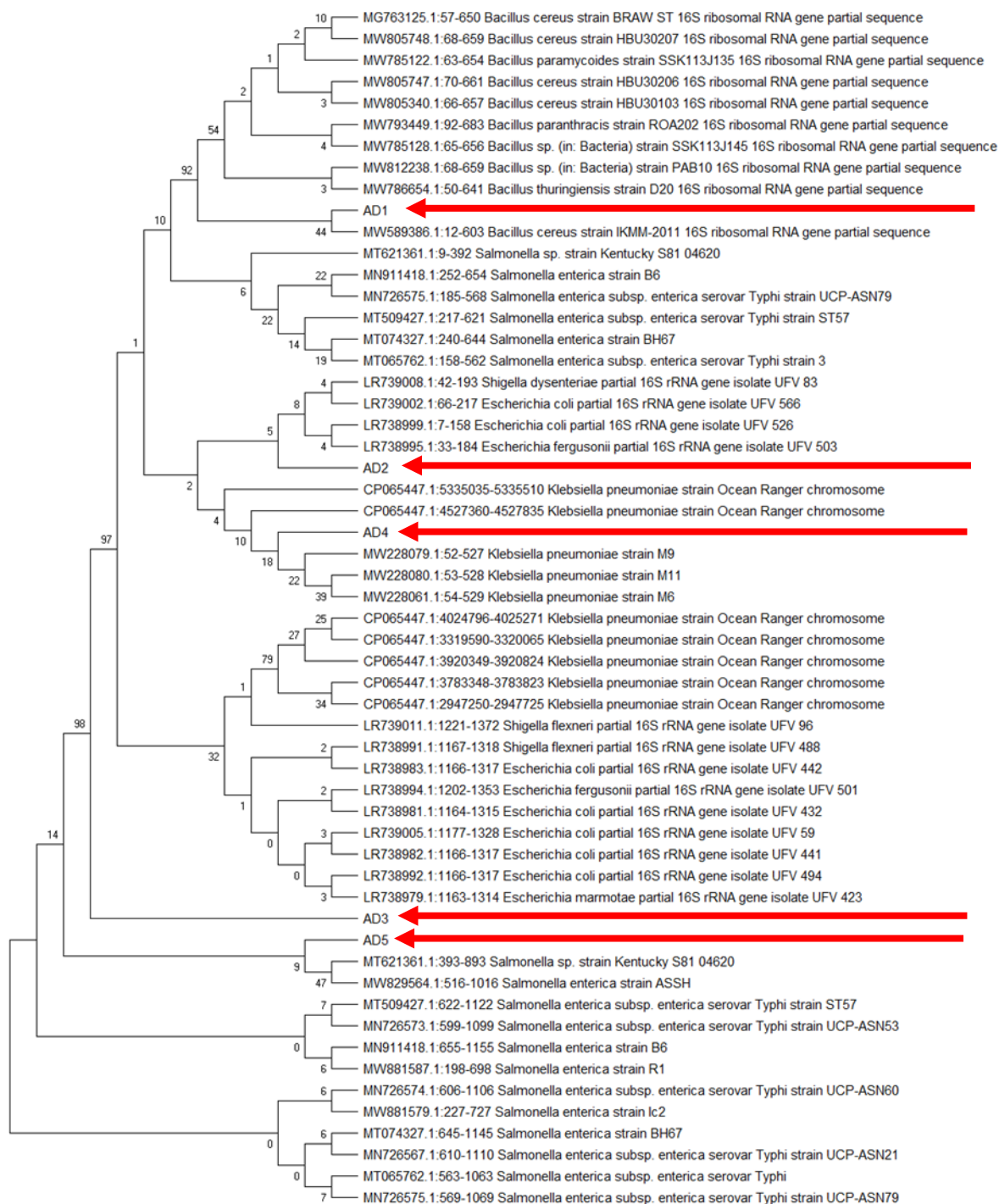


Figure 1. Phylogenetic tree of nucleotide sequence of 4 bacteria isolates from gross lesions of lungs, (AD1 and AD4); and livers (AD2, AD3, AD5). GenBank accession numbers were MW589386.1 (AD1), LR738995.1 (AD2), MT621361.1 (AD3), MW228079.1 (AD4), and MT621361.1 (AD5).

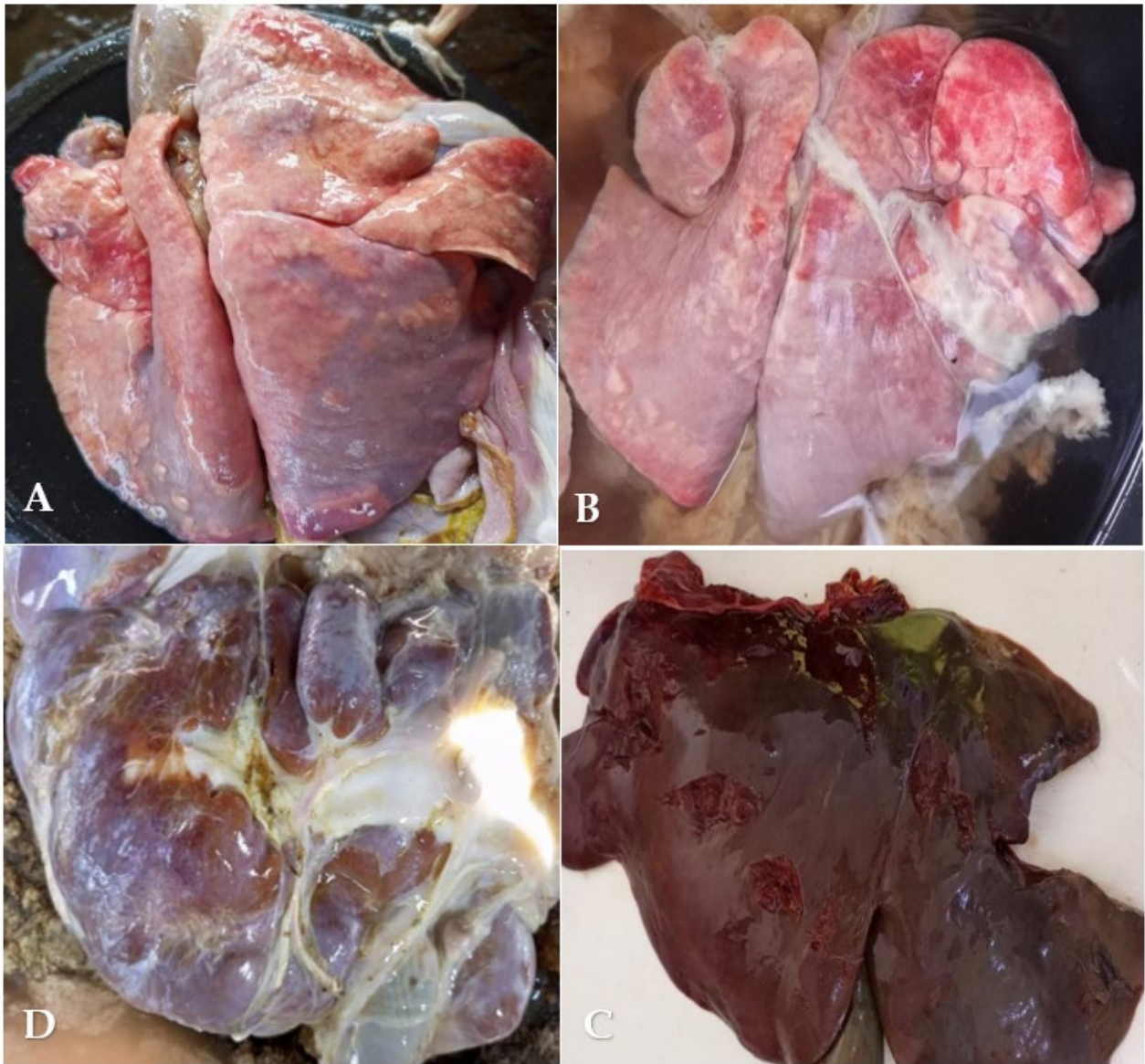


Figure 2. (A): show areas of pulmonary congestion. (B): show areas of hemorrhages. (C): shows severe hepatic fibrosis. (D): shows hepatic congestion.

Among the pathological lesions of the lungs, bronchopneumonia and congestion of the lungs were the most prevalent, representing 27.59% (95% CI = 17.75–40.20) and 22.41% (95% CI =

13.59–34.66), respectively. Hypereamia (10.34%) and hemorrhage (8.62%) of the lungs were the least prevalent pathological lesions recorded in the lungs (Table 1).

Table 1. Prevalence of different lesions from pathologic lungs sampled from Red Sokoto goats slaughtered in Ipata abattoir, Kwara State, Nigeria.

Lung gross lesions	N	Prevalence (%)	95% CI
Bronchopneumonia	16	27.59	17.75 – 40.20
Interstitial Pneumonia	10	17.24	9.65 – 28.91
Emphysema	6	10.34	4.83 – 20.79
Hemorrhage	8	13.79	7.16 – 24.93
Congestion	13	22.41	13.59 – 34.66
Hypereamia	5	8.62	3.74 – 18.64
Total	58	24.17	19.19 – 29.96

N= Number of pathologic lungs

CI= Confidence interval

Table 2. Prevalence of different lesions from pathologic livers sampled from Red Sokoto goats slaughtered in Ipata abattoir, Kwara State, Nigeria.

Liver gross lesions	n	Prevalence (%)	95% CI
Hepatic fibrosis	3	17.65	6.19 – 41.03
Hepatic enlargement	5	29.41	13.28 – 53.13
Hepatic congestion	8	47.06	26.17 – 69.04
Hepatic abscess	1	5.88	1.05 – 26.98
Total	17	8.10	5.17 – 12.58

n= Number of pathologic livers

CI= Confidence interval

In the livers, hepatic fibrosis, hepatic enlargement, hepatic congestion, and hepatic abscess were the lesions recorded. Of these lesions, hepatic congestion (47.06%) was the most prevalent, while hepatic abscess (5.88%) was the least prevalent. The prevalence of other lesions was 17.65% (3/17) for hepatic fibrosis and 29.41% (5/17) for hepatic enlargement (95% CI: 13.28–53.13) (Table 2).

Pasteurella species, *Bacillus* species, and *Klebsiella* species were the bacteria detected in the pathological lung samples of Red Sokoto goats

following the bacteriological examination, while *Escherichia* species and *Salmonella* species were detected in the pathological liver samples. The molecular studies confirmed the presence of *Klebsiella* species (51.72%; 95% CI = 39.16–64.07), and *Bacillus* species (48.28%) in the pathological lungs of the sampled Red Sokoto breed of goats. *Escherichia* species and *Salmonella* species were the bacterial species molecularly detected in the pathological livers of the Red Sokoto goat breed, with the former being the more prevalent species (52.94%) (Table 3).

Table 3. Molecular prevalence of bacteria species detected from pathologic lungs and livers sampled from Red Sokoto goats slaughtered in Ipata abattoir, Kwara State, Nigeria.

Bacterial isolated	N	Prevalence	95% CI
	Lungs		
<i>Klebsiella</i> species	30	51.72	39.16 – 64.07
<i>Bacillus</i> species	28	48.28	35.93 – 60.84
	Livers		
<i>Escherichia</i> species	9	52.94	30.96 – 73.83
<i>Salmonella</i> species	8	47.06	26.17 – 69.04

n= Number of pathologic organs

CI= Confidence interval

The molecular analysis showed that the bacterial organisms detected in this study did not fully cluster with similar species from other parts of the world (Figure 1).

Figure 3A showed a photomicrograph of bronchopneumonia as an extension of bacterial infection from the bronchioles into the surrounding lung parenchyma. There was also infiltration of inflammatory cells such as mononuclear cells, lymphocytes, and macrophages from the pulmonary vessel into the surrounding lung parenchyma (Figure 3B). As also shown in Figure 3C, the alveolar spaces were filled with oedema fluid, which appeared pinkish in color as labeled (p). Again, there was an enlargement of alveolar spaces, which coalesced to form a bulla (b). However, Figure 4E showed a photomicrograph of the liver at lower magnification with some areas

of necrosis and degeneration. As again shown in Figure 4F, the hepatic blood vessel was engorged with red blood cells, otherwise known as congestion. There was also infiltration of inflammatory cells in areas of fibrous connective tissue, as shown in Figure 4H.

DISCUSSION

The knowledge of the gross and histopathologic changes, as well as the pathogenesis of pathogens that affect the lungs and livers, will help veterinarians in the diagnosis and treatment of animal diseases for better profitability for farmers (Rashid et al., 2013; Ola-Fadunsin et al., 2020; Adam et al., 2022).

The 24.17% prevalence of pathologic lungs recorded in this study is lower than the 39.77% prevalence of pathologic lungs documented among slaughtered goats in a study carried out in Nigeria (Ugochukwu et al., 2017). Although the reported prevalence is

between 6.66% and 40.00%, as reported by Alam et al. (2001) and Rashid et al. (2013), For the livers, the 8.10% prevalence recorded in this study is close to the 9.47% prevalence of pathologic livers reported among

slaughtered goats in Ghana (Ntiamoah et al., 2021). In contrast, a 34.00% prevalence of pathological livers among slaughtered goats was reported in Ethiopia (Bekele and Szonyi, 2014).

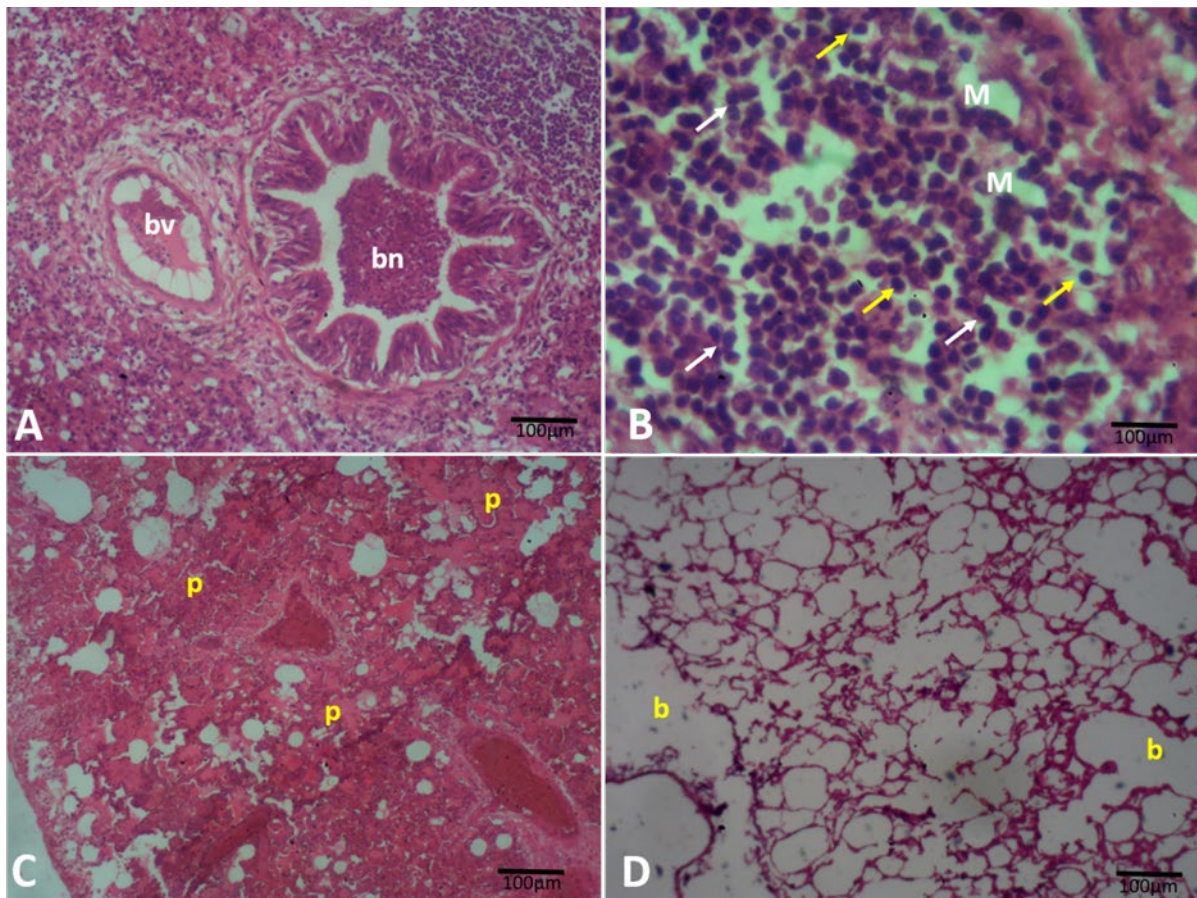


Figure 3. (A): Lung section of Red Sokoto goat naturally infected with *Klebsiella* species or *Bacillus* species showing bronchopneumonia (bn) and the blood vessel (bv) (H & E X100). (B): Showing inflammatory cells such as mononuclear cells (yellow arrow) lymphocytes (white arrow), and macrophages (M) (H & E X400). (C): Showing the pulmonary edema with fibrins (p) (H & E X100). (D): Showing coalesce of alveolar space (b) (H and E, X100).

The variation in prevalence can be attributed to a variety of factors, including the virulence of the causative agent and management factors.

Pathological lesions of bronchopneumonia, interstitial pneumonia, emphysema, hemorrhage, congestion, and hypereamia reported in

the lungs of goats in this study are similar to the lesions documented in the lungs of slaughtered goats in other parts of Nigeria (Alawa et al., 2011; Ugochukwu et al., 2017) and other parts of the world (Al-Qudah et al., 2008; Rashid et al., 2013; Sukanta et al. In line with our finding, Rashid et al. (2013), Ugochukwu et al. (2017), and Mugale and Balachandran (2019) reported that bronchopneumonia was the most prevalent lung pathologic lesion among goats in their studies. Bronchopneumonia is an inflammation of the bronchi and bronchioles, as well as the tubes that lead into the lungs. This condition is typically seen in bacterial infections, with viruses and fungi also causing it. Bronchopneumonia is the most common subtype of pneumonia, which is an infection that can affect any part of the lung tissue. There are different forms of the condition, which include fibrinous, purulent, and suppurative bronchopneumonia (Ugochukwu et al., 2017; Mahile et al., 2022; Moroz et al., 2022).

The detection of *Pasteurella* species, *Bacillus* species, and *Klebsiella* species in the lungs of goats at slaughter is a common phenomenon, as these bacteria have been isolated from the lungs of slaughtered goats in Nigeria (Ugochukwu et al., 2017) and outside of Nigeria (Rashid et al., 2013; Sukanta et al., 2018). Also, the isolation of *Escherichia* species and *Salmonella* species in this study concurs with the findings of Dutta et al. (2018), where these bacteria were isolated from the livers of slaughtered goats in Assam, India.

All these pathological lesions related to circulatory disturbances were associated with various pneumonia types. Based on these lesions, it could be believed that the route of infection might be the hematogenous route and associated with bacterial infections. Once the left ventricle or atrium ceases to empty as expected, there will be increased pressure in the affected chamber. This will cause the flow of blood to return to the pulmonary venous system and pulmonary capillaries. These capillaries then become dilated and congested with erythrocytes, leading to increased hydrostatic pressure and the transudation of plasma fluid into the alveolar spaces, characterized as pulmonary oedema. The result also showed emphysema, which is the rupture of the inter-alveolar wall. Emphysema grows gradually with gradual damage to the lung parenchyma, especially the alveoli, which eventually leads to rupture of the air sac. Gradually, this damage causes the air sacs to rupture and later coalesce to form a bigger one. This condition may be due to prolonged exposure to irritation that can injure the lung tissues and airways. The inequality between the pulmonary enzymes (protease and antiprotease) is suggested as an important pathomechanism leading to the formation of emphysema. An increase in the number of alveolar macrophages and neutrophils also aids this process (Sharafkhaneh et al., 2008). Again, in this study, the liver had focal areas of abscess in one part of the lobe. According to the report of Al-Qudah et al. (2008), liver abscess in ruminants is linked to grain overload, a known plight during the dry season, and, in

desert areas, to a lack of pasture. Grain overload causes a significant decrease in rumen pH, resulting in rumen acidosis, atony, and erosion of the rumen mucosa. The damaged rumen mucosa gives an opportunity for bacterial migration to the portal vein that eventually causes abscess formation in the liver (Radostitis et al., 2000).

CONCLUSIONS

This study showed that bronchopneumonia, interstitial pneumonia, emphysema, hemorrhage, congestion, and hypereamia were the pathological lesions of the lungs, while hepatic fibrosis, hepatic enlargement, hepatic congestion, and hepatic abscess were the pathological lesions of the livers of the Red Sokoto breed of goats. *Pasteurella* species, *Bacillus* species, and *Klebsiella* species were incriminated for causing the pathological lesions in the lungs, with *Escherichia* species and *Salmonella* species responsible for the pathological lesions in the livers. There is a need for more studies to further characterize these bacterial species detected in the Red Sokoto breed of goats.

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