Evaluation of Bacterial Pneumonia and Pathological Changes in Lungs of Cattle Slaughtered in Maiduguri Central Abattoir, Borno state Nigeria

[1]Abdullahi, A. M., [2]Iliyasu, D., [3]Galadima, H.B., [4]Mohzo, D. L., [5]Abba, M. I

[1]Veterinary Teaching Hospital, [2]Department of Theriogenology, [3]Department of Veterinary Medicine, [4]Department of Veterinary Pathology, University of Maiduguri, Borno State, [5]Ministry of Agriculture, Yobe State, Nigeria

*Correspondence: drdisambogmail.com; +2348063294756

ABSTRACT

The study was designed to determine level of lungs inflammation and associated bacterial flora that influenced gross and histopathological lesions related to bovine pneumonic lungs amongst cattle slaughter in Maiduguri central abattoir. The study was conducted in Maiduguri, North-Eastern Nigeria. A total of 100 pneumonic lungs sampled from cattle slaughtered at Maiduguri central abattoir, 63 (63 %) were positive isolate and 37 were negative isolate (37 %). The results indicate isolates recovered were gram-positive and gram-negative bacteria with five bacterial genera isolated consist of 3 Gram-positive and 2 Gram-negative bacteria. The isolates were subjected to sensitivity test of various antibiotics like Ciprofloxacin, Pefloxacin, and Oflaxacinm and they were sensitive, while Chloramphenicol, Ciprofloxacin, Gentamicin, Streptomycin and Oflaxacin were Intermediate and Amoxicillin, Erythromycin, Cotrimoxazole, Gentamycin, and Ceftriaxone were resistance to the antimicrobial sensitivity tests. Pneumonic bacteria isolates based on sex and age of the cattle slaughtered at Maiduguri abattoir were recorded. Bull has the highest 58 (92.0%) compare to the cow 5 (8.0%). While age-wise, Isolates were recorded with highest 97.0 % among adult cattle when compare to the young cattle (3.0%). Three types of pneumonia were identified based on the Pathological examinations of 25 pneumonic lungs randomly selected from the 100 lungs samples which were recorded.

Keywords: Bacterial Pneumonia, Pathological lesions, Lungs, Cattle Slaughtered, Maiduguri Central Abattoir

INTRODUCTION

Cattle are large ruminant domesticated cloven-hooved herbivores farm animals that are raised for their meat, milk, hides or fur and transportation purposes. They belong to the subfamily Bovidae, and widespread species of the genus Bovine (Ola *et al.*, 2020). Bovine respiratory disease (BRD) is one of the most important diseases of cattle and is the second to gastrointestinal disease which leads to significance losses in dairy cows (Yesuf *et al.*, 2012). Bovine respiratory disease is associated with infectious and non-infectious courses, the infectious course is usually related to acute and severe bacterial pneumonia that is characterised with death as reported by Yesuf, (2012). Transient illness like chronic pneumonia usually becomes complicated due to poor feeding and management practice (Mandeel and Dabhawi, 2020). The situation might also lead to anorexia, anemia and decreased body weight due to immune suppression and deterioration of the health of the infected animals (Fernández *et al.*, 2020).

The most common bacteria frequently associated with pneumonia in BRD are *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), *Pasteurella multocida,* and *Histophilus somni* (formerly *Haemophilus somnus*, *Mannheimia haemolytica*) as reported by Mekibib, *et al*. (2019).

The most frequently isolated bacterium from cases of acute fibrinous pleura-pneumonia in transported beef is *Pasteurella multocida,* as reported by Mekibib, *et al*. (2019). However, *Pasteurella multocida,* is also associated with fibrin purulent bronchopneumonia in dairy calves (Radaelli, 2008). All the three organisms can be associated with bacterial pneumonia in both beef and dairy cattle.

An abattoir is a special facility designed and licensed for receiving, holding, slaughtering, and inspecting meat animals and meat products before release to the public as wholesome products. The establishment and management of abattoirs and its waste in Nigeria have always been regarded as social services by all three tiers of the government (Ibrahim *et al*., 2021). Bacterial culture and pathologic lesions of Caprine

pneumonic lungs in Maiduguri abattoir were conducted with *Manheinmia haemolytica* being the most common bacterial isolated (Tijjani *et al.,* 2012). There is paucity of information on bacterial components of pneumonic lungs in cattle in Maiduguri abattoir hence the need for the current research.

MATERIALS AND METHODS

STUDY AREA
The samples were collected from Central Abattoir Maiduguri, and processed in the Department of Veterinary Medicine, Teaching Laboratory Unit, Faculty of Veterinary Medicine University of Maiduguri. Borno State Situated in North Eastern part of Nigeria. It is Cosmopolitan in nature, located at an elevation of 354 meters above sea level located between latitudes 11^0 and 14^0N and longitudes 10^0 and 14^0E, within the Sahel zone and has a total land mass of 50,778 square kilometers (BMLS, 2007). It has a population density of 1,738 people per square kilometers, and a total population of 521,492 (NPC, 2006). The temperature ranges from 35-40 ^{0}C for most parts of the year with two distinct seasons, a rainy season with mean annual rainfall of 647mm from July to October and a prolonged dry season for the rest of the year (LCRI, 2007). The state due to its geographical features favors the production of honey even though not at colossal economy, and similarly, the state derives great economic activity from its rich livestock and fishery production (NPC, 2006).

STUDY DESIGN
The study designed was cross-sectional investigation linking cattle of numerous ages in the targeted area. Cattle with gross pneumonic lung lesions were sampled from the abattoir and samples sent to the Veterinary Microbiology unit for investigation.

SAMPLE SIZE DETERMINATION
The determination of sample size for the study was determined based on the formula provided by Thrusfield (1995)

$$n = b \ \frac{z^2 pq}{d^2}$$

Where:
n= sample size
z=desired confidence 1.96
p=prevalence= 3.6 % = 61% Edwards (1996).
q= 1-p
d=allowable error 5 %
n= 100
Design effect= 3.65
Thus total sample sizes of 100 pneumonic lungs were determined considering the 5% desired absolute precision (Thrusfield, 2005)

SAMPLES COLLECTION

A Total of 100 pneumonic lungs were sampled from slaughtered cattle at the Maiduguri central abattoir. All samples were collected aseptically, placed into sterile containers (polythene bags), were labeled and placed according to sex, and age. The samples were then transported to the Department of Veterinary Medicine, Teaching Laboratory Unit at the Faculty of Veterinary Medicine, University of Maiduguri, Borno State Nigeria.

GROSS AND HISTOPATHOLOGY
Lungs collected were examined for gross pathological lesions due to pneumonia based on the location, colour, size, palpation and examination of the cut surface of the lesions and major airways of the lugs as illustrated by Murray *et al.* (2017). Samples for histopathology were fixed in 10% formalin. Blocks of the fixed tissues were embedded in paraffin wax and 5 μm thick sections was cut from them and stained with hematoxylin and eosin (H&E) for histopathological examination as described by Chan (2014**).**

LABORATORY ASSESSMENT FOR BACTERIA
Samples for bacteriologic examination were collected on the surface of the lung seared with a hot spatula. The lungs were incised using sterilized scalpel and the exudates were collected using sterile swab sticks and inoculated onto the blood and MacConkey agar and incubated aerobically and an-aerobically at 37°C for 24 hours to acquire the distinct bacteria colonies. After the incubation period, the plates were brought out and the colonies were observed and recorded based on the size, shape, colour and nature of the colonies. Thereafter, the colonies were stained using Gram's staining techniques and examined for cellular morphology under light microscope at objective × 100. Single colony type from both the blood and MacConkey agar, were sub cultured onto nutrient agar and nutrient broth to obtain pure culture according to the method described by Murray *et al.* (2017) and Tijjani *et al.* (2012). The pure cultures were then transferred on to nutrient agar slants for biochemical test using standard procedures as illustrated by Happy *et al.* (2018).

PRESERVATION OF CULTURE
Preservation was made by sub-culturing the purified isolates on nutrient agar slant, which were then stored in a refrigerator at 4 ^{0}C. Conventional bacteriological test were used to determine the cultural and biochemical characteristics of the isolates.

DATA ANALYSIS
Data obtained from this research was analyzed using descriptive statistics of percentage and frequency to determine the pulmonary lesions, bacterial culture, and sensitivity testing in slaughtered cattle in Maiduguri central abattoir.

RESULTS

The gross appearance of the lungs revealed various lesions on the lung which indicated presence of pneumonia as presented in (Plate 1).

The histopathology findings revealed degree of cellular lesions of the organs as presented in (plate II) and (plate III) below.

Of the total 100 pneumonic lungs sampled from cattle slaughtered in Maiduguri central abattoir, 63 (63 %) were positive for microbial isolates and 37 (37 %) were negative for microbial isolates as presented in (Figure I).

The isolates recovered were both gram-positive gram-negative bacteria. Five bacterial genera were isolated with 3 Gram-positive and 2 Gram-negative bacteria as presented in (Figure II). However, the most frequent isolated bacteria was *Escherichia coli* (78.19 %), followed by *Staphylococcus spp* (69.84 %), *Streptococcus* spp (28.57 %), *Bacillus spp.* (17.46 %) and *Proteus spp.* (7.94 %) as seen in (Figure II)

The isolates were subjected to sensitivity test to various antibiotics. Ciprofloxacin, Pefloxacin, and Oflaxacinm were sensitive, Chloramphenicol, Ciprofloxacin, Gentamicin, Streptomycin and Oflaxacin were Intermediate and

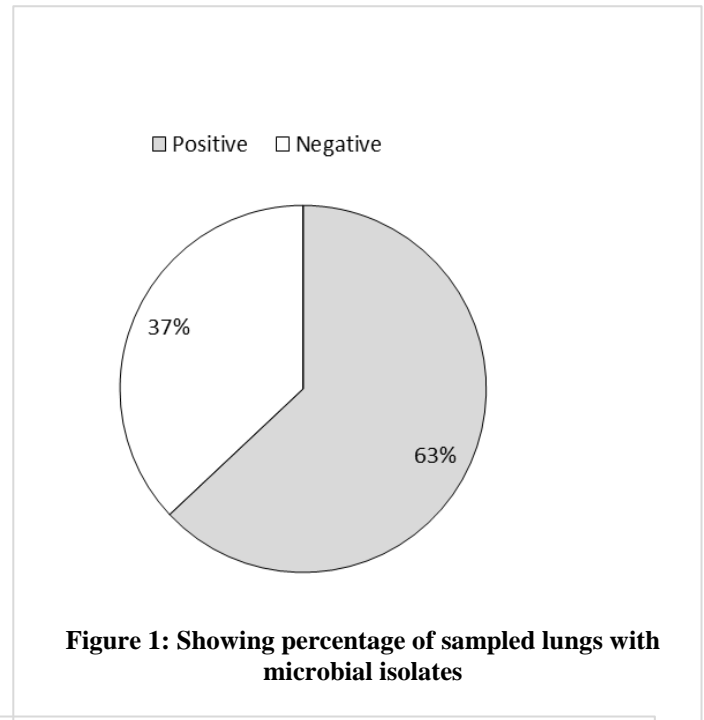and Ceftriaxone were resistant to the antimicrobial sensitivity tests as presented in (Table 1).

Figure 1: Showing percentage of sampled lungs with microbial isolates
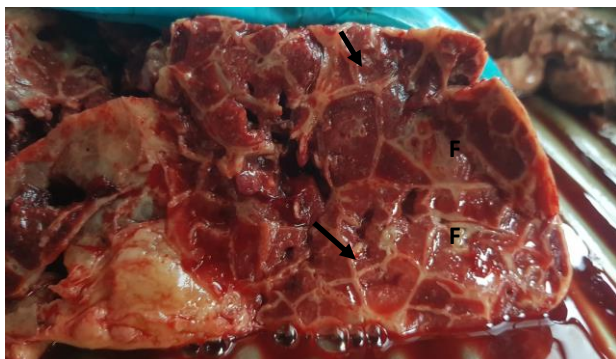

Plate 1. Gross photograph portion of the lung showing consolidated lobe with marble appearance and widened interlobular septa (arrows) with fibrin deposits (F)
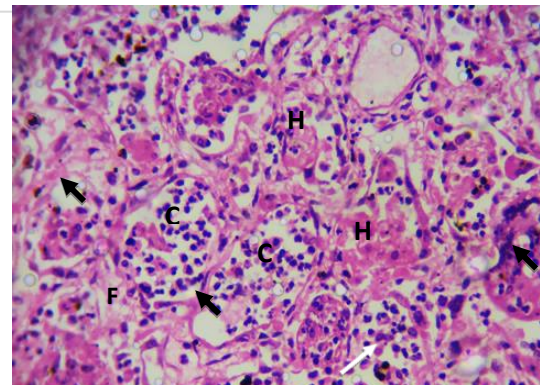

Plate II: Photomicrograph showing severe inflammatory cellular infiltration predominantly mononuclear leucocytes in the alveolar space(C), thickened alveolar walls (arrows), fibrin deposits (F) and hyaline degeneration (H) H & E×40
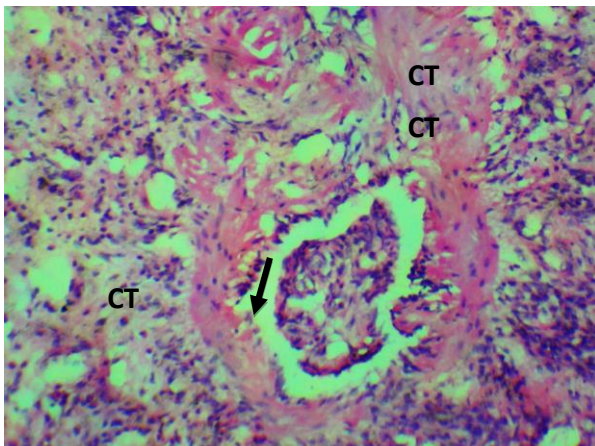
Plate III. Gross photograph portion of the lung showing consolidated lobe with marble appearance and widened interlobular septa (arrows) with fibrin deposits (F)

Amoxicillin, Erythromycin, Cotrimoxazole, Gentamycin,

Table 1: Antibiogram Profile of Bacterial Isolates Using Gram-Positive Antibacterial Drugs

Genera	Amox	Erith	Cipro	Cotrim	Pefro	Genta	Ceftri	Chl	Strep	Oflax
Staphylococcus spp	R	R	S	R	S	R	R	I	R	S
Bacillus spp	R	R	S	R	S	R	R	R	R	S
Streptococcus spp	R	R	I	R	R	I	R	R	I	I

Keys: - Amox- Amoxicillin, Eryth- Erythromycin, Cipro- Ciprofloxacin, Cotrim- Cotrimoxazole, Pefro- Pefloxacin, Genta-Gentamicin, Cefri-Cefriaxone, Chl- Chloramphenicol, Strep- Streptomycin, Oflaxacin, S-Sensitive, I-Intermediate, R-Resistant.

Table II. Antibiogram Profile of Bacterial Isolates Using Gram-Negative Antibacterial Drugs.

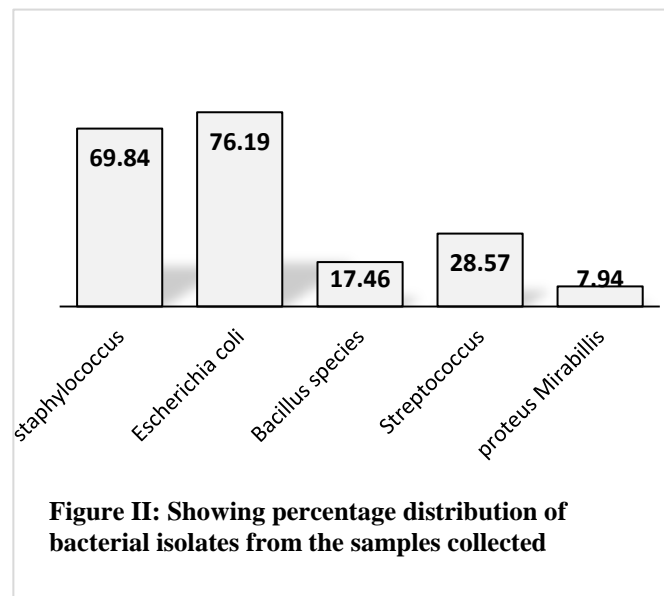| Genera	Aug	Cef	Nitro	Cotrim	Ofla	Amox	Cipro	Tetra	Peflo
Escherichia coli	R	R	R	R	I	R	I	R	R
Proteus spp	R	R	R	R	I	R	I	R	R

Keys:- Aug-Augmentin, Cef-Ceftriaxone, Nitro-Nitrofuran, Cotrim- Cotrimoxazole, Ofla- Oflaxacin, Amox- Amoxicillin, Cipro- Ciprofloxacin, Tetra-Tetracycline, Peflo- Pefloxacin, I-Intermediate, R-Resistant

Table III: Types and Frequency of Gram-Positive and Gram Negative Bacteria Isolated from Pneumonic Lungs of Slaughtered Cattles.

Types of bacteria	Number of isolates	Frequency (%)
Gram-positive		
Streptococcus spp	15	12.6%
Staphylococcus spp	38	32.0%
Bacillus spp	12	10.0%
Total	65	54%
Gram-negative Genera		
Escherichia coli	48	40.0%
Proteus spp	6	5.0%
Total	54	45.0%

Table IV Pneumonic Bacteria Isolate base on Sex and Age of the cattle slaughtered in the Maiduguri abattoir

Sex	Number Examined	Isolate Prevalence	Percentage
Bull	76	58	92.8%
Cow	24	5	8.0%
Total	100	63	100%
Age			
Adult Cattle	87	61	97.0%
Young Cattle	13	2	3.0%
Total	100	63	100%

Figure II: Showing percentage distribution of bacterial isolates from the samples collected

DISCUSSION

This study was designed to isolate and identify bacteria associated with pneumonic lungs of cattle slaughtered in Maiduguri central abattoir, Borno state. Gross lesions of the pneumonic lungs indicated bronchopneumonia mixed with interstitial pneumonia characterized by patchy to diffuse reddish brown to grayish red areas of consolidation and hepatization and this agreed with the findings reported by Mugale and Balachandran, (2019) in small ruminants. The cut surface of the lungs showed straw yellow colored fluid oozing out of the bronchi which is in line with the research findings reported by Dorso, *et al*. (2021)) in cattle and (Dar *et al.,* 2014) in sheep.

The Histopathologic lesion characterized with leukocytic infiltration of the alveoli, edema, coagulative necrosis, degeneration of bronchiolar epithelium, leukocytic infiltration into the interstitium and fibrin deposits in the interstitium and peribronchiolar area an indicative of bronchopneumonia and interstitial pneumonia. Presence of these lesions agrees with the conclusions reported by Dorso *et al*. (2021) on cattle and sheep respectively.

Out of the total pneumonic lungs sampled, 63 % had bacterial growth on blood and MacConkey agar and 37 % did not show any bacterial growth despite clear pneumonic discoloration and the gross lesions available on the lungs sampled. The failure of bacterial isolation from the lung sampled might be attributed to the short incubation period of the inoculated plates and the use of limited types of media.

However, aerobic incubation might also be a factor and this in agreement with the findings reported by Adam *et al.* (2023) in goats. Furthermore, no attempt was made to isolate other causes of pneumonic and fastidious bacteria like *Mycoplasma*, viruses, or fungi from the samples because it was out of the scope of the current research. The current findings differed with the report of Abo-Elnaga and Osman, (2012) who reported isolates of both Gram-positive and Gram-negative bacteria from pneumonic lungs in camels, this might be linked to the uncommon detection or mixed pulmonary infections associated with bovine respiratory air pathways diseases which also act as reservoirs for potential pathogenic microorganisms responsible for respiratory diseases in cattle, which may also develop and cause pneumonia especial on animals that are subjected to stress and other factors associated with declining hygienic measures and adverse climatic changes which might also result to poor health conditions (Ebi *et al.*, 2020).

Isolation of bacteria from pneumonic lungs demonstrates the complexity of the disease as reported by Roberson *et al.*, (1994) and Sedeek and Thabet (2001), but isolation of *Staphylococcus aureus* and *E. coli* from infected lungs, is usually assumed that *Staphylococcus aureus* are predisposed organisms to infection by coliform organisms or other pathogens as reiterated by Jaja *et al.* (2016).This finding implies that both bulls and cows are equally susceptible and may also be exposed to infection but relatively at higher frequency in bulls when compared with the cows. This might be attributed to the low immunity associated with cows especially during gestation and lactation period. Similar findings were also reported in cattle by Samadipoor, (2022).

The findings of the current study on age showed that young cattle had lower infection when compared to the adult cattle. This variability may be associated with acquired maternal immunity that can protect young cattle against diseases when compared the status of the adult cattle in struggling to survive (Ali and Mohammed, 2023). The current study showed that *Staphylococcus species* and other Gram-positive bacteria were the highest isolates than the Gram negative bacteria of *Escherichia coli* and other *species*. This finding also agreed with the findings reported by Francis and Ameh, (2015) who reported presence of *Staphylococcus species* in cattle in Maiduguri. Similarly, Lindström *et al.*, (2018) and Ali and Mohammed, (2023) who reaffirmed and considered these as the most common pneumonic bacteria isolated from lung of cattle presented with a clinical signs of respiratory diseases.

The antibacterial sensitivity tests of the isolated bacteria obtained in the study showed variable response to the bacteria isolated. Some showed resistance to most of the antibiotics commonly used for the treatment of bacterial disease associated with Gram-positive and Gram-negative bacteria origin. This finding is divergent to the report of Francis and Ameh, (2015) who reported sensitivity to most of the antibiotics tested as evidence of a large zone of inhibition (22-38mm). This may be attributed to the indiscriminate use of drugs and abuse of the drugs by the farmers on their cattle coupled with lack of adequate knowledge and awareness on the use of antibiotics on animals (Jaja *et al.*, 2016).

CONCLUSION

In conclusion, the current study illustrated that numerous bacterial pathogens were isolated from the pneumonic lungs of cattle sampled at Maiduguri central abattoir and the prevalence of various organisms associated with clinical respiratory diseases were determined. Some of the isolates were resistant to most of the available antibiotics used in the current study. However, some few antibiotics drugs used in the current study were effective against the bacterial isolates found in the respiratory system of the cattle.

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