ISOLATION AND PHENOTYPIC CHARACTERIZATION OF *MYCOPLASMA MYCOIDES* SUBSPECIES *MYCOIDES* FROM PNEUMONIC CATTLE SLAUGHTERED AT ZARIA ABATTOIR, KADUNA STATE

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ABSTRACT
This study was conducted to isolate and phenotypically characterize *Mycoplasma* species from pneumonic cattle slaughtered at Zaria Abattoir, Kaduna State using culture and isolation, and biochemical tests for identification. A total of 47 samples comprising of lung tissues, pleural fluid and nasal swabs were collected from 3 different breeds of cattle, namely White Fulani, Red Bororo and Sokoto Gudali with each having 27, 8 and 12 samples respectively. Isolation rate of 11.1% was recorded in White Fulani while no isolate was found from the other breeds, giving an overall isolation rate of 6.4%. The positive isolates identified based on their "fried egg" appearance under stereomicroscope were specifically isolated from pleural fluid 2(7.4%) and nasal swab 1(3.7%). Phenotypically, all the isolates hydrolyzed glucose, reduced tetrazolium, and did not catabolize arginine, hence, identified as *Mycoplasma mycoides* subspecies *mycoides* (*Mmm*). In CONCLUSION, this study established the presence of *Mmm* in cattle presented for slaughter at Zaria Abattoir. Therefore, intensive disease surveillance through isolation, serology and molecular detection techniques should be instituted to ensure accurate and timely diagnosis of this devastating disease.

Keywords: *Mycoplasma mycoides* subspecies *mycoides*, Pneumonic, Cattle, Zaria abattoir.

INTRODUCTION
Mycoplasma species of cattle are one of the most economically important contagious group of pathogens associated with respiratory distress, fatigue, consolidation and marbling of affected lungs with devastating consequences (OIE 2013; Suleiman *et al.,* 2015). Taxonomically, mycoplasma belongs to the genus *Mycoplasma* in the class *Mollicutes* and family *Mycoplasmataceae* (Pilo *et al.,* 2007; Li *et al.,* 2009). Mycoplasmas are facultative anaerobic microorganisms with prolong incubation period, they have the smallest free-living prokaryotic cells capable of auto-replication and are highly fastidious to grow on ordinary media (Nicholas and Baker, 1998). Similarly, mycoplasmas lack cell wall, therefore treatment with conventional drugs is often unsuccessful, thus, treatment is only indicated in endemic areas even though the OIE standard is test and slaughter (OIE, 2014). The first incidence of Mycoplasma infection from cattle in Nigeria was recorded in 1924 as Contagious Bovine Pleuropneumonia (CBPP), and since then, it has remained endemic in the country (Suleiman *et al.,* 2015; Tambuwal and Egwu, 2017; Olorunshola *et al.,* 2020). The disease has been reported in some Nigerian major cities including Borno state (Adamu and Aliyu, 2006), Kaduna state (Billy *et al.,* 2017), Plateau state (Ankeli *et al.,* 2017) and Nasarawa state (Ikpa *et al.,* 2020). Transmission occurs through direct and close repeated contacts between diseased and susceptible animals in herd at water points or common grazing area (Okaiyeto *et al.,* 2013; Suleiman *et al.,* 2015). Currently, *Mycoplasma mycoides* subspecies *mycoides* (*Mmm*) and *Mycoplasma bovis* (*M. bovis*) are the two most important *Mycoplasma* species known to cause significant diseases in cattle (Admassu *et al.,* 2015; Tambuwal and Egwu, 2017). Considering the devastating economic implications of Mycoplasma infections in Nigeria, and with the escalating trend of highly contagious diseases, it is necessary that more attention

be given to this important disease. Thus, this study was designed to assess the occurrence of *Mycoplasma* species among pneumonic cattle presented for slaughter at Zaria Abattoir, Kaduna State, Nigeria.

MATERIALS AND METHODS

Study area

A purposive sampling method was adopted and all the samples used in this study were collected from Zaria Abattoir located in Zangon shanu, Zaria, Kaduna State, Nigeria. Collected samples were processed at Mycoplasma laboratory, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University (ABU), Zaria.

Mycoplasma media

The Mycoplasma Agar (Oxoid CM0401), and Broth (Oxoid CM0403) media used in this study were prepared in accordance with manufacturer's instruction. They were adequately enriched with 20% decomplemented horse serum, and 10% (v/v) yeast extract as described by Nicholas and Baker, (1998).

Study design

A total of 47 samples comprising of lung tissues, pleural fluid and nasal swabs were collected from White Fulani, Red Bororo and Sokoto Gudali breeds of cattle as outlined below (Table 1). Samples were collected and transported in broth media, then cultured and subcultured onto Agar media following incubation in Microaerophilic jar at 37°C. The cultured plates were examined under stereomicroscope (at 60× magnification) daily starting from day 3 up to 14 days post incubation, after which the plates with no observable growth were regarded as negative.

Table 1: Breed Distributions of Samples Collected from Pneumonic Cattle Slaughtered at Zaria Abattoir, Kaduna state**,** Nigeria

S/N	Breed	No. of samples	Sample type		
			Lung tissue	Pleural fluid	Nasal swab
1.	White Fulani	27	7	5	15
2.	Red Bororo	8	2	2	4
3.	Sokoto Gudali	12	4	3	5
Total		47	13	10	24

Results and Discussion

The colonies of the positive samples have the characteristic "fried egg" appearance evidently with center embedded beneath the surface and peripheral growth at the surfaces (Plate 1).

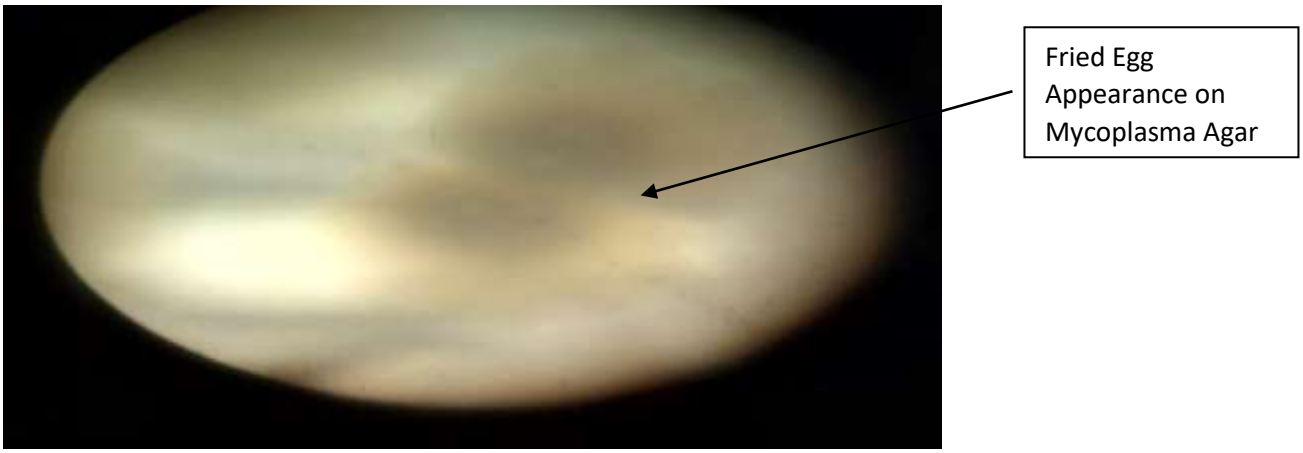

Plate 1: Isolated *Mycoplasma mycoides* subspecies *mycoides* from Pneumonic Cattle in Zaria Abattoir
All the isolated organisms hydrolyzed glucose, reduced tetrazolium, and do not catabolize arginine, hence, identified as *Mycoplasma mycoides* subspecies *mycoides* (Mmm) (Table 2).

Table 2: Biochemical tests for the identification of *Mycoplasma* species from Pneumonic Cattle slaughtered at Zaria Abattoir, Kaduna State

Isolate	GF	TR	AU	Remarks
Isolate 1(NS)	+	+	-	*Mmm*
Isolate 2 (PF)	+	+	-	*Mmm*
Isolate 3 (PF)	+	+	-	*Mmm*

Key: NS = nasal swab, PF = pleural fluid, GF = glucose fermentation, TR = tetrazolium reduction, AU = arginine utilization, + = positive and - = negative

This study has established the occurrence of *Mmm* in cattle presented for slaughter at Zaria Abattoir, and the 6.4% overall isolation rate reported presents significant clinical finding since isolation and identification of mycoplasma organisms is required for CBPP diagnosis (Egwu *et al.*, 2012; OIE 2014). Similarly, the study has shown that White Fulani breed of Cattle with 11.1% isolation rate, are more predisposed to CBPP when compared to the 0% rate recorded in both Red Bororo and Sokoto Gudali breeds. This could be as a result of being the most dominant breed in the study area, and account for 57.4% of the animals sampled. Nomadism could also be another reason because CBPP is highly contagious disease exacerbated by unrestricted movement which is peculiar to White Fulani herders.

The overall isolation rate reported in the present study correlate well with the 6.9% isolation rate reported by Tambuwal and Egwu (2017), but slightly differs to the 4.8%, 3.3% and 4.0% independently reported by Ankeli *et al.*, (2016), Musa *et al.*, (2016) and Ikpa *et al.*, (2020), respectively all in the northern Nigeria. The reasonable isolation rate in this study could be as a result of the diminishing control measures due to inadequate ante-mortem inspection at abattoir level, low annual CBPP vaccination coverage and as well as steady unrestricted movement of cattle to and from the state.

CONCLUSION AND RECOMMENDATIONS

This research established the presence of *Mmm* in cattle presented for slaughter at Zaria Abattoir. Therefore, intensive disease surveillance through isolation, serology and molecular detection techniques should be instituted to ensure accurate and timely diagnosis of this devastating disease. Likewise, there is a need for the government to restore annual CBPP vaccination and awareness campaign programs.

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