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## Full Length Research Paper

# Isolates of *Mycoplasma mycoides* subspecies *mycoides* (SC) in small ruminants in Sahel zone of Nigeria and its implications on disease control

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A study on the isolation of *Mycoplasma mycoides* subspecies *mycoides* (SC) in small ruminants and its implication on disease control was carried out in the Sahel zone of Nigeria. This was achieved by the examination of pneumonic lesions in apparently normal and affected lungs of sheep and goats slaughtered at Maiduguri municipal abattoir. A total of 400 lungs (200 each from sheep and goats) were examined at post-mortem (PM) for pneumonic lesions. Of this number, 50 (25%) sheep had pneumonic lungs, while almost double the number 89 (44.5%) of goats showed pneumonic lungs. The prevalence of pneumonic lungs in the sheep was higher amongst the females (34.3%) than the males (23%), while in the goats, the prevalence was higher in the males (48%) compared to the females (38.7%) examined. Seven different *Mycoplasma* species were isolated from both unaffected and affected lungs of sheep and goats. 42 isolates were obtained from 150 unaffected sheep samples, whilst 36 isolates were obtained from 50 affected sheep. Of the 111 unaffected and 89 affected caprine lung samples, a total of 55 and 66 *Mycoplasma* isolates were recovered respectively. The commonly occurring *Mycoplasmas* in both unaffected and affected lungs of sheep and goats were *Mycoplasma ovipneumoniae* (30%), *Mycoplasma mycoides* subspecies *capri* (29.5%), *Mycoplasma mycoides* subspecies *mycoides* SC (13.5%) and *Mycoplasma capricolum* (11.5%) with *Mycobacterium bovis* (1.5%) being the least isolated. To the best of the authors' knowledge, this is the first report of the occurrence of *Mycoplasma mycoides* subspecies *mycoides* SC the causative agent of contagious bovine pleuropneumonia (CBPP) in cattle in small ruminants (sheep and goats) in Nigeria. Although the isolation rate of *M. bovis* was low in this study, its isolation in non-bovine ruminants is significant as it shows evidence of mycoplasma circulation between various animal species reared in close contact. These findings may pose serious impediments to the control of endemic CBPP in Nigeria.

**Key words:** Isolates, *Mycoplasma mycoides* subspecies *mycoides*, sheep, goats, Nigeria.

## INTRODUCTION

The Nigerian livestock resources are mostly found in the northern parts of the country (Ajayi et al., 1987; Bourn et

al., 1994). Borno state, located in the extreme northern Sudano-sahelian zone of Nigeria, accounts for about one quarter of the ruminant population (Ngere et al., 1984). These animals are reared under the extensive, intensive and semi-intensive systems (Williamson and Payne, 1978; Devendra and Mcleroy, 1982); however, the extensive system, which is predominantly practiced in Nigeria (Gefu, 1982) is becoming less common; perhaps due to the increasing pressure on land. The use of this

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**Abbreviations:** PM, Post-mortem; CBPP, contagious bovine pleuropneumonia.

management system has resulted to low productivity as a result of constant exposure of the animals to low levels of nutrition and disease (Egwu et al., 1995). Disease problems have constituted serious constraints to small ruminant production in Nigeria. Amongst these, bacterial and viral respiratory diseases, such as contagious caprine pleuropneumonia (CCPP) and pneumo-enteritis complex (Kata) are notably devastating to these species (Ameh et al., 1993; Egwu et al., 2000; Srivastava et al., 2010).

Since ruminant respiratory disease complex is of multi-factorial aetiology, the role and distribution of other specific pathogens involved in this disease have not been clearly elucidated in the epidemiology of respiratory infections in small ruminants. Amongst these respiratory pathogens of sheep and goats, mycoplasmas have been incriminated in the respiratory disease complex of sheep, contagious agalactia and CCPP which is an important World Organisation for Animal Health (OIE) recognised disease.

CCPP is caused by *Mycoplasma capricolum* sub-species *capripneumoniae* (*Mccp*), with severe losses in goat herds in developing countries. It has been indicated recently that the disease has spread to new territories in Africa, south-eastern Europe, Asia and the Middle East (Srivastava et al., 2010). *Mccp* was earlier thought to be primarily a goat pathogen, but has recently been isolated in captive wild non-bovine ruminant species in Qatar and the United Arab Emirates (Arif et al., 2007; Nicholas et al., 2008). In acute infections in goats, obvious signs of respiratory disease, including laboured and painful breathing, frequent coughing episodes and eventual death within few days of onset of disease are commonly seen. At postmortem examination, lesions confined to the thorax such as extensive fibrinous pleuropneumonia, massive lung hepatisation and pleurisy with large quantities of straw-coloured fluid in the pleural cavity are common findings in affected animals (Srivastava et al., 2010).

Another important mycoplasma disease which has devastating consequences on cattle population is contagious bovine pleuropneumonia (CBPP) (Egwu et al., 1996; Tambi et al., 2006; Tambuwal et al., 2011b; Thiaucourt et al., 2011). The aetiological agent of this disease, *Mycoplasma mycoides* ssp. *mycoides* small colony (*MmmSC*) which mainly affects cattle, has been isolated from buffaloes (*Bubalus bubalus*) in Italy (Santini et al., 1992), sheep and goats in Africa, and more recently in Portugal and India (Srivastava et al., 2000; Anon, 2008) and is widespread in other parts of the world (Egwu et al., 1996; Srivastava et al., 2000; Kusikula et al., 2001; Totte et al., 2008; Yaya et al., 2008; Manson-Silvan et al., 2009). Although CBPP has been eradicated from most continents, the disease still persists in Africa (Anon, 2008; Thiaucourt et al., 2011) and has remained endemic in Nigeria with massive spread across the country. This is encouraged by the mass trade cattle

movement, seasonal migration especially by the pastoralist farmers and transhumance activities (Aliyu et al., 2000; Ayuwape, 2004; Tambuwal 2011a). A recent report (Ikhatua, 2011) has indicated that outbreaks of the disease still occur with some published evidence of its occurrence in Nigeria (Aliyu et al., 2003; Ayuwape et al., 2004; Danbirni et al., 2010; Mailafiya et al., 2010; Tambuwal et al., 2011a, b). Despite the wide spread presence of CBPP in northern Nigeria, a current report (Tambuwal et al., 2011b) showed that annual vaccination coverage against the disease in some parts of the country is less than 40%. At this rate, the control of the disease in Nigeria may not be achieved easily. Also, in Nigeria, it is a common occurrence for small ruminants to be found co-herding with cattle. Taking into cognisance the cross-reactivity between *Mycoplasma* sp. and ruminant species, it was thought worthwhile to investigate the pneumonic lesions of sheep and goats and isolates of mycoplasmas with special reference to *MmmSC* so as to elucidate their epidemiological significance, distribution in small ruminant species and implications on disease control.

## MATERIALS AND METHODS

### Samples

A total of 400 lungs from slaughtered sheep and goats of various age groups and both sexes were examined at post mortem for pneumonic lesions at the Maiduguri municipal abattoir. Both halves of the lungs including the various lobes were examined for typical lesions. Those lungs with characteristics CCPP lesions (Srivastava et al., 2010) were classified as affected (or pneumonic) on the one hand and those without obvious lesions were classified as unaffected (or apparently normal) on the other hand.

### Collection of samples for culture

50 samples each from pneumonic and 150 apparently normal ovine lung samples were collected aseptically into sterile containers containing 2 ml mycoplasmal broth medium. Similarly, 89 affected and 111 unaffected lung samples from goats were also collected. They were then immediately transported to the laboratory for immediate culture while some samples were stored for a few days at -70°C before culture.

### Laboratory culture of suspect *Mycoplasma* samples

The *Mycoplasmas* to be isolated were cultured and sub-cultured on modified Eaton's medium as previously described in the Central Veterinary Laboratory's manual on mycoplasmaology (Boughton and Thorn, 1993).

Inoculated broth and agar plates were incubated at 37°C in 5% carbon dioxide for up to three weeks. Inoculated broth medium which showed a colour change from pink to yellow as denoted by a colour change by half of PH change of 0.5 units were sub-cultured into fresh broth medium and onto agar plates. Following further incubation, representative colonies were then sub-cultured by agar push technique.

Representative *Mycoplasma* colonies in single or mixed cultures

**Table 1.** Number of sheep with pneumonic and apparently normal lungs grouped according to age and sex from Maiduguri municipal abattoir, Borno State.

Age range (years)	Number of animals examined with pneumonic lesion					
	Male	PL (%)	Female	PL (%)	Total	PL (%)
1-2	14	4 (28.6)	8	3 (37.5)	22	7 (31.8)
3-4	135	31 (15.6)	18	7 (38.9)	153	38 (24.8)
5 above	16	3 (18.8)	9	2 (22.2)	25	5 (20.0)
Total	165	38 (23.0)	35	12 (34.3)	200	50 (25.0)

PL, Number of sheep with pneumonic lung lesions that exhibited areas of consolidation, pin point areas of necrotic foci, haemorrhages, enlargement and distension of interlobular septa.

**Table 2.** Number of goats with pneumonic and apparently normal lungs grouped according to age and sex from Maiduguri municipal abattoir, Borno State.

Age range (years)	Number of animals examined with pneumonic lesion					
	Male	PL (%)	Female	PL (%)	Total	PL (%)
1-2	18	6 (33.3)	7	3 (42.9)	25	9 (36.0)
3-4	75	37 (49.3)	48	13 (27.1)	23	50 (40.7)
5 above	32	17 (53.1)	20	13 (65.0)	52	30 (57.7)
Total	125	60 (48.0)	75	29 (38.7)	200	89 (44.5)

PL, Number of goats with pneumonic lung lesions that exhibited areas of consolidation, pin point areas of necrotic foci and haemorrhages, enlargement and distension of interlobular septa.

were identified by the growth inhibition test (GI) and indirect immunofluorescent test (IFA). Briefly, the GI test consisted of adding an over-night broth culture of the suspect *Mycoplasma* culture onto an agar plate, and allowing the drop to run down the plate as a lane when slightly tilted. Then, specific antisera impregnated to a disk were placed at the centre of the lane. The plate was incubated at 3°C and checked for inhibition of growth for the specific *Mycoplasma*(s). Growth inhibitory zones of  $\geq 2$  mm were considered positive.

The IFA technique consisted essentially of washing cut agar bearing *Mycoplasma* colonies following the addition of specific antisera and fluorescent labelled antiglobulin (conjugate). These washed colonies reacted with specific antisera and conjugate were visualized under the UV light for greenish yellow fluorescent as earlier described (Rosendel and Black, 1972).

### Statistics

The data was analysed using simple percentages while Chi-square was used to test the level of significance at a probability level of  $p = 0.05$ .

### RESULTS

The number of pneumonic and apparently normal lungs of 200 sheep aged between 1 to >5 years examined at post-mortem (PM) is shown in Table 1. Of this number, 165 (82.5%) and 35 (17.5%) were from males and females respectively. Of the number of males examined, 38/165 (23%) had pneumonic lesions, compared to 12/35 (34.3%) of the females. Similarly, of the 200 goats

examined at PM, 125 (62.5%) and 75 (37.5%) were also males and females respectively. Of the number of male goats examined, 60/125 (48%) had pneumonic lesions, compared to 29/75 (38.7%) of the females (Table 2). Overall, the result indicates that there were more male than female sheep and goats slaughtered at the Maiduguri municipal abattoir.

There were seven different *Mycoplasma* sp. isolated from both the apparently normal and pneumonic lungs of sheep and goats, with a total of 199 isolates recovered from all animals examined in this study. The distribution of these isolates among the different categories of animals examined is shown in Table 3. The isolation rates of these organisms is also shown in Figure 1, with *Mycoplasma ovipneumoniae* (*Mo*) being the most highly isolated (15%) followed by *M. mycoides* ssp. *Capri* (*Mmc*) with isolation rate of 14.5%, *MmmSC* with 6.8% and *M. capricolum* ssp. *capricolum* (*Mcc*) (5.8%) and *M. bovis* (1.5%) being the least isolated. There were no significant differences ( $P > 0.05$ ) in the number of *Mycoplasma* isolates from unequal number of samples obtained from either unaffected or affected lungs of sheep and goats.

### DISCUSSION

Respiratory disease problems have constituted serious handicaps to improved small ruminant production in the tropics and temperate regions of the world (Ameh et al., 2000; Egwu et al., 2000). Small ruminant respiratory

**Table 3.** *Mycoplasma* species isolated from apparently normal and pneumonic lungs of sheep and goats in Maiduguri municipal abattoir, Borno State.

<i>Mycoplasma</i> species	Sheep			Goat			Goat and sheep
	Unaffected n=150 (%)	Affected n= 50 (%)	Total n=200 (%)	Unaffected n= 111 (%)	Affected n= 89 (%)	Total n= 200 (%)	Total lungs n= 400 (%)
<i>M. mycoides</i> ssp. <i>mycoides</i> SC	6 (4)	10 (20)	16 (8)	8 (7.2)	3 (3.4)	11 (5.5)	27 (6.8)
<i>M. mycoides</i> ssp. <i>Capri</i>	10 (6.7)	7 (14)	17 (8.5)	16 (14.4)	25 (28)	41 (20.5)	58 (14.5)
<i>M. ovipneumoniae</i>	23 (15)	16 (32)	39 (19.5)	8 (7.2)	13 (14.6)	21 (10.5)	60 (15)
<i>M. capricolum</i> ssp. <i>Capricolum</i>	-	-	-	10 (9)	13 (14.6)	23 (11.5)	23 (5.8)
<i>M. putrefaciens</i>	3 (2)	1 (2)	4 (2)	2 (1.8)	3 (3.4)	5 (2.5)	9 (2.3)
<i>M. agalactiae</i>	-	-	-	8 (7.2)	9 (10)	17 (8.5)	17 (4.3)
<i>M. bovis</i>	-	2 (4)	2 (1)	3 (2.7)	-	3 (1.5)	5 (1.5)
Total	42 (28)	36 (72)	78 (39)	55 (49.5)	66 (74.2)	121 (60.5)	199 (49.8)

syndrome is usually of multifactorial aetiology (Egwu et al., 2000), there is therefore need to ascertain the aetiology and distribution of the specific pathogens involved in this respiratory disease complex.

*Mycoplasmas* have long been known to cause various diseases of sheep and goats (Jones, 1983; Egwu et al., 1989, 2000). Among the *Mycoplasmas* known to cause disease of sheep and goats, *MmmSC* and *M. capricolum* ssp. *capripneumoniae* (*Mccp*) are known to cause contagious bovine and caprine pneumonia respectively (Egwu et al., 1996).

During the course of investigation of the occurrence of *Mycoplasmas* in the lungs of slaughtered sheep and goats in the present study, more males aged between three and four years were frequently slaughtered compared to the females. This observation is similar to our previous report (Egwu et al., 1995), and it is likely due to the fact that fewer males are usually kept for breeding purposes, and can easily be sold off to meet family financial demands. In addition, males are usually in high demand during festivities and regularly so in hotels and restaurants (Egwu et al.,

1995).

In this present study, we observed the presence of pneumonic lesions in both sexes of sheep and goats examined at PM which shows that these species are prone to mycoplasmal pneumonia as buttressed by the increased number of pathogenic isolates in both apparently normal and affected lungs of sheep and goats. Similar observations were reported by Jones (1983).

Although, in this study, we were not able to examine the animals before slaughter, it was not possible to ascertain those animals that had clinically expressed the disease prior to their entrance into the abattoir. In the present study, *MmmSC*, an agent of CBPP, was isolated from apparently normal and affected lungs of sheep and goats.

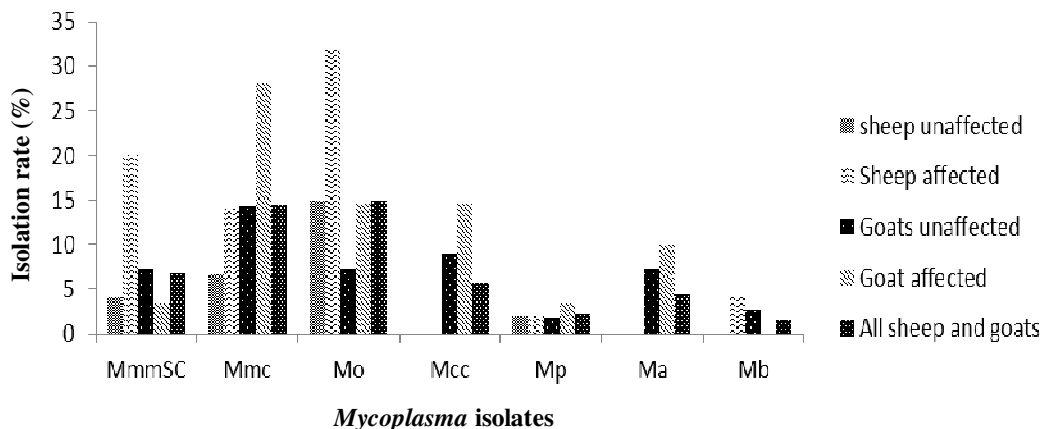
Although there are published evidence of the isolation of this agent in small ruminants from other parts of the world (Srivastava et al., 2000; Anon, 2008), this is the first time it is being reported in Nigeria, a place where CBPP is highly endemic (Egwu et al., 1996; Tambuwal et al., 2011a, 2011b).

Moreover, the occurrence of the mycoplasmas

in unaffected lungs could signify a carrier status in these species, which could constitute a serious epidemiological consequence in disease control (Egwu et al., 1996).

Contagious caprine pleuropneumonia is not caused by the same agent of CBPP, neither is it known to cause any desirable respiratory disease in sheep. Their occurrence in the lungs of sheep and goats may be presented with serious consequences and have far-reaching epidemiological implications in the control of CBPP in Nigeria. This is because they may continue to harbour the organism and hasten the spread of CBPP particularly at drinking and feeding sites as a result of co-herding of these species which is a common practice in this part of the world.

Furthermore, the occurrence of increased numbers of mycoplasmal species isolated from unaffected lungs of sheep and goats in the present study clearly parallels the findings of Jones (1983), who indicated that *Mycoplasma* infections are usually chronic and probably no inflammatory changes are usually obvious during early infections with this species. This study has also highlighted the occurrence of pathogenic



**Figure 1.** Isolation rate of *Mycoplasma* species cultured from pneumonic and apparently healthy lungs of sheep and goats slaughtered at the Maiduguri municipal abattoir, Nigeria. MmmSC, *M. mycoides* subsp. *mycoides* SC; Mmc, *M. mycoides* subsp. *Capri*; Mo, *M. ovipneumoniae*; Mcc, *M. capricolum* subsp. *capricolum*; Mp, *M. putrefaciens*; Ma, *M. Agalactiae*; Mb, *M. bovis*.

mycoplasmas in apparently normal and diseased lungs of sheep and goats. It demonstrates that *M. mycoides* ssp. *mycoides* SC can be harboured by small ruminant species such as sheep and goats, indicating the need for these species to be considered during epidemiological control of CBPP especially when they are herded in close contact with cattle. In this case, extreme caution needs to be taken as cross-infection of mycoplasmas can easily occur between these ruminant species which will likely pose serious consequences in the control of pathogenic mycoplasmas found in both large and small ruminants, particularly in areas and countries where cross infection of isolates occur.

There is therefore the need to monitor small ruminants serologically and by culture for bovine pathogenic *Mycoplasma* sp. and vice-versa during the control of economically endemic mycoplasmal diseases. The detection of these species by PCR has been shown to be highly more efficient than cultural techniques (Wade, 2010), so, where such facilities are available, they should be employed in order to get better results. In the present study, we were not able to isolate *Mccp* by cultural techniques even though typical lesions (Srivastava et al., 2010) of the disease (CCPP) were commonly observed in the affected lungs examined at PM. The non-application of PCR could be a major limitation in this study, nonetheless, the positive isolation of *MmmSC* and *Mcc* from the lungs of these species is significant.

Finally, as molecular techniques such as multilocus sequence analysis (MLSA), PCR and sequence analysis (Yaya et al., 2008) are becoming more available and affordable, and their applications in the investigation of African *Mycoplasma* isolates have begun to elucidate more information on the epidemiology of these agents (Kusikula et al., 2001; Yaya et al., 2008; Tambuwal, 2009; Wade et al., 2010). They should therefore be used to monitor new epidemics and enhance the surveillance

and control of the disease in endemic countries. Although current efforts are underway towards the search for *MmmSC* antigens that have potential for the development of subunit vaccine against CBPP (Totte et al., 2008), it certainly remains a fertile ground for future work. More work may also be needed experimentally to demonstrate the pathogenicity of cross infecting mycoplasmas in some definitive host species.

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