International Journal of Veterinary Science and Medicine (2016) **4**, 46–53

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International Journal of Veterinary Science and Medicine

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Full Length Article

Molecular detection of Nigerian field isolates of *Mycoplasma mycoides* subsp. *mycoides* as causative agents of contagious bovine pleuropneumonia

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Received 13 May 2016; revised 18 October 2016; accepted 19 October 2016
Available online 10 December 2016

KEYWORDS

Mycoplasma;
CBPP;
PCR;
RFLP;
Cattle

Abstract Contagious bovine pleuropneumonia (CBPP) is a highly contagious respiratory disease affecting cattle and is widely distributed in the sub-Saharan Africa. The objective of this study was to detect *Mycoplasma mycoides* subspecies *mycoides* (*Mmm*) the causative agent of CBPP from 90 cattle at slaughter using polymerase chain reaction-Restriction fragment length polymorphism. In this study, 450 samples suggestive of CBPP in Maiduguri, Yola and Gombe township abattoirs were processed according to standard protocols. The isolation rate was found to be 3.33% and percentage of identification with PCR-RFLP yielded 1.56%. Subsequently, QIAxcel revealed molecular size of 574 bp for *Mycoplasma mycoides* subcluster. Further analysis of PCR amplicons with restriction digestion, confirmed the presence of *Mmm* 16 S rRNA of CAP 21 genomic region with molecular sizes of 180 bp and 380 bp. Thus, the 380 bp fragments delineated *Mmm* from *Mycoplasma mycoides* subsp. *capri*. Three isolates (BL5, BL6 and AL1) were from lungs and four from pleural fluids (APF2, APF8A, APF8B and APF9) were isolated and identified, while a vaccine strain T1/44 was re-detected along with the field isolates. No sample from Gombe had *Mmm*. In conclusion, the findings of this study have detected the presence of *Mmm* as causative agent of CBPP. Measures such as surveillance, quarantine and vaccination are hereby recommended for the control of CBPP in Nigeria.

1. Introduction

Mycoplasma mycoides subsp. *mycoides* (*Mmm*) is the causative agent of contagious bovine pleuropneumonia (CBPP), a severe, contagious respiratory disease affecting cattle and is

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Peer review under responsibility of Faculty of Veterinary Medicine, Cairo University.

http://dx.doi.org/10.1016/j.ijvsm.2016.10.007

widely distributed in Africa [1–3]. It is included among the Office International des Epizooties (OIE) diseases requiring official notification leading to banning of countries involved from participation in international cattle trade [4]. CBPP has continued to exist as an endemic disease in several countries in the sub-Saharan Africa [5].

CBPP could be manifested in hyperacute form, which may be rapidly fatal with no clinical signs observed, while the subacute form are frequent, characterized by mild or no clinical signs at all [6]. It is known that animals with these forms are able to transmit the infection to other cattle in the herd [7]. The acute form is characterized by increase in body temperature, moderate respiratory distress and painful and irregular cough, abduction of the forelimbs, extension of the neck, arched back and dilation of the nostrils [6,7]. In chronic form, the animal may remain in poor condition for a long period, depending on the size of the chronic lung lesion. Fever is intermittent and the animals with sequestra may be normal in appearance while harbouring bacteria [6,8]. Several countries have eradicated CBPP, but the disease still remains a serious problem in certain parts of Africa, which includes Nigeria where CBPP is endemic [9–14].

The causative agent of CBPP are presumptively identified as comet in broth and 'fried eggs' colonial appearance on the pleuropneumonia like-organism (PPLO) agar media [15,16]. Additionally, molecular typing could identify *Mycoplasma mycoides* subcluster and *Mmm* at the molecular sizes of 574 bp as well as 180 bp and 380 bp respectively [17–19].

Currently the control of CBPP in sub-Saharan Africa is largely dependent on a live vaccine, T144 [20,21]. The vaccine has low efficacy, limited duration of immunity, sometimes severe side effects and require cold chain to maintain its potency [21]. The low efficacy of the current live vaccine, together with the costs of vaccination campaign remains a serious challenge for the African countries in the control of CBPP [22–24]. After primary vaccination protection coverage may be as low as 30–60% [25].

The work hereunder report the typing of *Mmm* field samples taken from cattle at slaughter houses in Nigeria and possible step towards the identification of *Mycoplasma mycoides* subsp. *mycoides* with restriction fragment length polymorphism to establish the presence of the causative agent of CBPP.

2. Materials and methods

2.1. Study location

This study was carried out in Maiduguri, Yola and Gombe township abattoirs in Borno, Adamawa and Gombe States respectively in North-eastern zone of Nigeria (Fig. 1). Maiduguri is the Borno State capital. It lies between Latitude 11° 50′ 42″ N and Longitude 13° 9′ 36″ E. Yola is the State capital of Adamawa State. It lies between Latitude 9° 13′ 48″ N and Longitude 12° 27′ 36″ E. Gombe, the State capital of Gombe State is located on the latitude 10° 17′ N and Longitude 11° 10′ E in the centre of North-eastern Nigeria.

2.2. Collection, transportation, processing and storage of samples

A total of 450 samples of lungs, lymph nodes, ear, nasal, pleural swabs and pleural fluids were collected from 90 cattle with pathological lesions referring to CBPP at slaughter in the three different sampling abattoirs namely: Maiduguri, Yola and Gombe. In Maiduguri, Yola and Gombe abattoirs, samples were collected in the following order: Nasal swabs ($n = 30$), ear swabs ($n = 30$), lung tissues ($n = 30$), mediastinal lymph nodes ($n = 30$) and pleural swabs ($n = 30$). In Yola abattoir pleural fluids ($n = 10$) and pleural swabs ($n = 20$) were collected instead of 30 pleural swabs. Samples were transported in a refrigerated Coleman box to the National Veterinary Research Institute (NVRI) laboratory in Vom, Jos, where they were processed into 2 ml sterile Nalgene® cryo vials containing 1.5 ml of supplemented PPLO broth with horse serum, and then stored at −20 °C until required. These samples were later transported under cold chain by FEDEX® Courier to OIE Reference Laboratory for CBPP in Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise (IZSAM) 'G. Caporale', Campo Boario Teramo, Italy. On receiving the samples, they were stored at −20 °C until required. After the arrival at the OIE Reference laboratory, the samples were removed, verified and aseptically packaged and then registered under exotic diseases unit prior to further processing.

2.3. Isolation and growth condition of mycoplasmas

The strains used in this study were isolated from abattoir field samples. Mycoplasmas were grown at 37 °C under 5% CO_2 in PPLO broth and agar (Difco) supplemented as previously described [26,27]. The samples were processed in OIE Reference Laboratory for CBPP – Istituto 'G. Caporale', Teramo, Italy.

2.4. Molecular detection of Mycoplasma mycoides subcluster

2.4.1. Extraction of genomic DNA

Deoxyribonucleic acid (DNA) was extracted from 1000 μl of culture using commercially available Maxwell® 16 Tissue/cell DNA Purification Kits (Promega, USA) with Maxwell® 16 instrument (Promega, USA) according to the manufacturer's instructions. Briefly, the culture in the Eppendorf tubes was centrifuged at 1600*g*/10 min. The supernatant was discarded and the pellets diluted with 300 μl of lysis buffer in Eppendorf tubes and then pipetted into the first chamber of the DNA purification cartridge. The DNA purification kit was inserted into the Maxwell® 16 instrument and then run for 36 min.

2.4.2. Polymerase chain reaction oligos and PCR conditions

The protocol was adapted from the OIE Reference Laboratory for CBPP and a PCR-based test for the specific identification of *Mycoplasma mycoides* subspecies *mycoides* SC from [17]. Specific primers were used to amplify one band of 574 bp expected fragment for *Mycoplasma mycoides* subcluster (*Mmm* and *Mmc*). The forward and reverse primers were designed from CAP-21 genomic region.

MM450: 5′-GTATTTTCCTTTCTAATTTG-3′ MM451: 5′-AAATCAAATTAATAAGTTTG-3′ The 50 μl volume was required: 25 μl of Top Taq Master Mix containing; Taq Polymerase, dNTPs, $MgCl_2$ and Taq buffer, 18 μl nuclease free water, 1 μl forward primer, 1 μl reverse primer and 5 μl of genomic DNA.

The amplification reaction was carried out under the following conditions: initial denaturation temperature at 94 °C

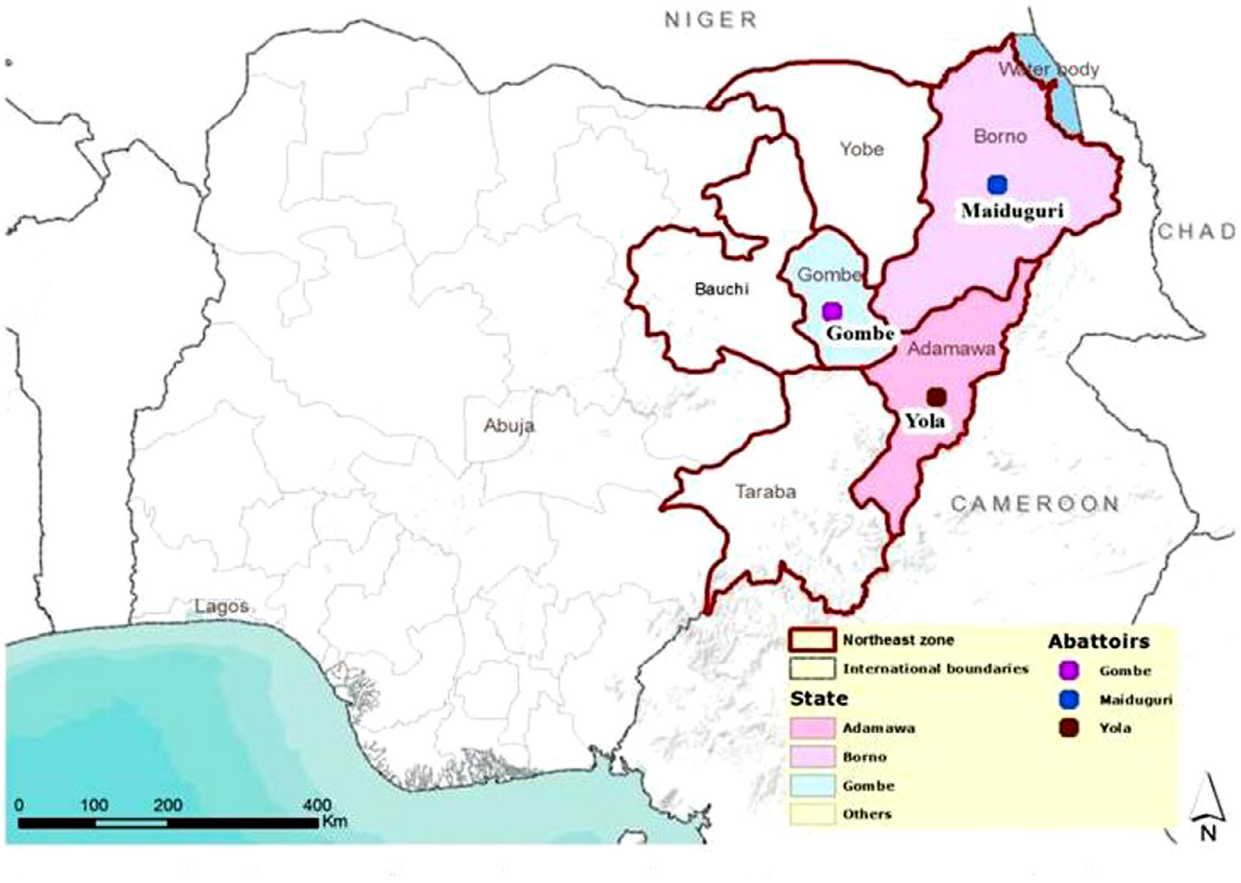

Fig. 1 Distribution of sampling location in North-Eastern Nigeria. Source: Designed by GIS Unit IZS, Teramo Italy.

Table 1 Profile of isolates of *Mycoplasma* and *Mycoplasma mycoides* subspecies *mycoides* confirmed with polymerase chain reaction-Restriction fragment length polymorphism.

Sample source	Sample type	No. of samples collected	No. of *Mycoplasma* grown on agar (%)	No. of positives strains with PCR-RFLP (%)
Maiduguri-Borno State	Lungs	30	2 (6.67)	2 (6.67)
	Lymph nodes	30	0 (0.00)	0 (0.00)
	Pleural swabs	30	1 (3.33)	0 (0.00)
	Ear swabs	30	2 (6.67)	0 (0.00)
	Nasal swabs	30	0 (0.00)	0 (0.00)
Yola-Adamawa State	Lungs	30	2 (6.67)	1 (3.33)
	Lymph nodes	30	3 (10.00)	0 (0.00)
	Pleural swabs	20	0 (0.00)	0 (0.00)
	Pleural fluids	10	4 (40.00)	4 (40.0)
	Ear swabs	30	0 (0.00)	0 (0.00)
	Nasal swabs	30	1 (3.33)	0 (0.00)
Gombe-Gombe State	Lungs	30	0 (0.00)	0 (0.00)
	Lymph nodes	30	0 (0.00)	0 (0.00)
	Pleural swabs	30	0 (0.00)	0 (0.00)
	Ear swabs	30	0 (0.00)	0 (0.00)
	Nasal swabs	30	0 (0.00)	0 (0.00)
	Total	450	15 (3.33)	7 (1.56)
NVRI-Vom, Jos Plateau State	T1/44 Vaccine vial	1 (100.00)	1 (100.00)	1 (100.00)

for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 30 s and a final extension step at 72 °C for 5 min and standby at 4 °C in GeneAmp® PCR System, 9700 (Applied Biosystems). *Mycoplasma mycoides* subsp. *mycoides* and *Mmc* were used as positive controls.

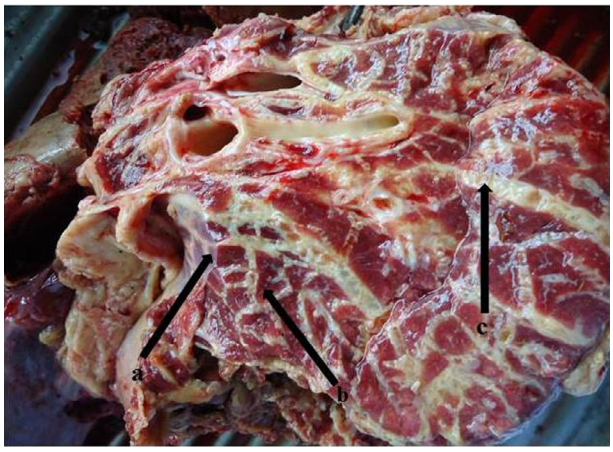

Fig. 2 A typical CBPP infected cattle lung cut open to reveal the marbled appearance from Maiduguri abattoir, North-Eastern Nigeria. Pneumonia and pleurisy (**a**), hepatization (**b**), thickened interlobular septae (**c**).

2.5. QIAxcel capillary electrophoresis

Polymerase chain reaction products were analyzed by capillary electrophoresis using QIAxcel-Pure Excellence (Qiagen). The technique was based on a DNA size marker or molecular marker set between 100 bp and 2500 bp, which was run with the 7 isolates of *Mycoplasma* (found to express fried eggs appearance on PPLO agar) and T1/44 in 96 wells plate. Pre-programmed marker table allowed the assigning of the electrophoresed DNA, the known size in bp to the detected peaks. Based on the bp/migration time ratio of the DNA size marker the software calculated the size of all unknown fragments.

2.6. PCR-RFLP for Mycoplasma mycoides subsp. mycoides

The typing method was adapted from the protocol used in OIE Reference Laboratory for CBPP and that of [18], but varied with respect to *Vsp1* (8–12 U/μL concentration) which replaced *Asn1* endonuclease in this study.

The master mix comprised of; 0.4 μl of restriction endonuclease (*Vsp*1), 3.6 μl of nuclease free water, 1 μl of specific buffer and 5 μl of the PCR product been a preparation for a single sample. The mixture was centrifuged at 16,875*g*/45 s and then incubated at 37 °C for 2 h. Polymerase chain reaction products were digested with restriction enzyme *Vsp*1. The products were separated by electrophoresis (agarose × 3% in TBE buffer) at 100 volts for a period of 20 min. At the end of the run, the resulting DNA fragments were analyzed by visualization under UV light to determine the expected base pairs of the fragments.

3. Results

3.1. Profile of samples for Mycoplasma and Mycoplasma mycoides subsp. mycoides isolates identified on PPLO agar media

Out of the total of 450 samples inoculated onto PPLO agar media, 2 (6.67%) lungs samples, 1 (3.33%) pleural fluids and 2 (6.67%) ear swab samples from Maiduguri, gave typical fried eggs colonies. Two (6.67%) lung samples, 3 (10.00%) lymph nodes, 4 (40.00%) pleural fluids and 1 (3.33%) nasal swab from Yola, yielded characteristic growth of *Mycoplasma*. The isolation rate on PPLO agar media was found to be 15 (3.33%). No sample gave typical colonies from Gombe (Table 1).

3.1.1. Lung sample showing CBPP lesion

The marbling appearance of the lung characterized by pneumonia and pleurisy, thickened inter-lobular septae and hepatization suggestive of CBPP is shown in (Fig. 2).

3.1.2. Colony morphology for Mycoplasma

The colonies of *Mycoplasma* showing typical fried eggs morphology were observed. The fried egg colony is labelled **a**, while **b** indicated the dense centre. The classical colonies were recorded at ten days of incubation (Fig. 3).

3.2. QIAxcel capillary electrophoresis for Mycoplasma mycoides subcluster

The QIAxcel capillary electrophoresis of PCR amplicons for *Mycoplasma mycoides* subcluster is shown in (Fig. 4). Lanes O1A to O7B are the samples in duplicate (50 μg and 30 μg concentration), lanes O8A to O8B is T1/44, followed by positive controls for *Mmm* and *Mmc* on lanes O9A and O9B and a negative control on lane O10A. The standard molecular marker

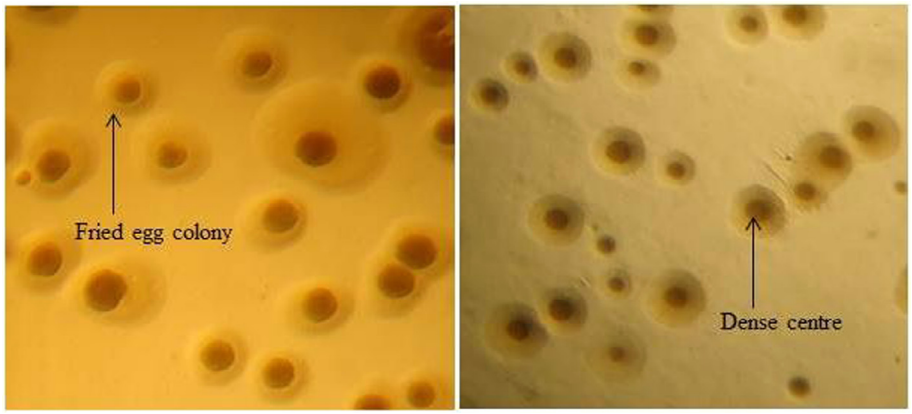

Fig. 3 *Mycoplasma* isolated from Yola at ten days of incubation (25X) at IZS, Teramo, Italy.

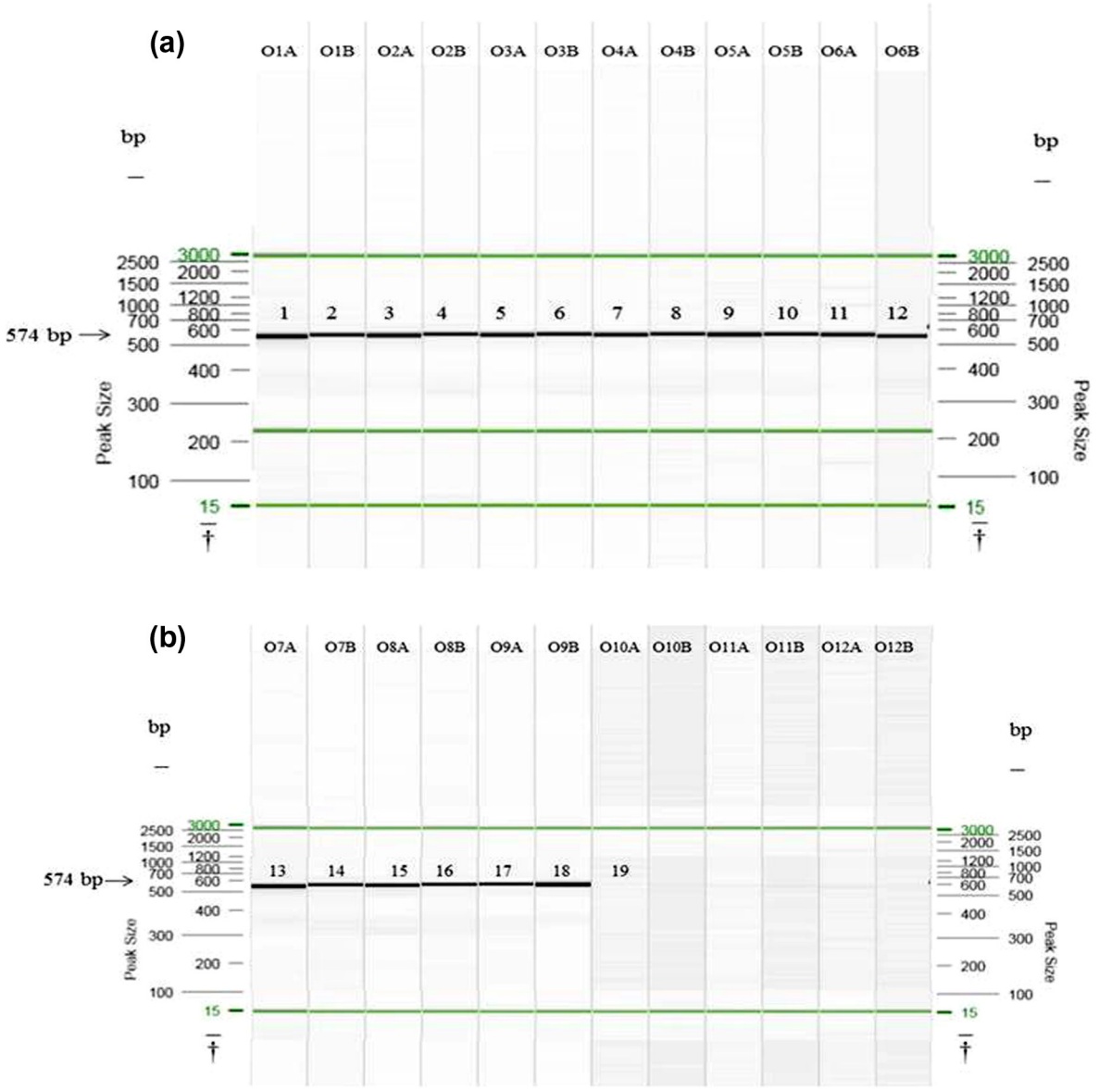

Fig. 4 (a) Capillary electrophoresis of PCR amplicons for *Mycoplasma mycoides* subcluster using Q1Axcel. Lances O1A to O6B: Samples in duplicate (OIA and OIB as sample number one in concentration of 50 µg and 30 µg and so on) † = Alignment marker, bp = Base pair. (b) Capillary electrophoresis of PCR amplicons for Mycoplasma mycoides subcluster using Q1Axcel. Lances Q1Axcel Lances O8A to O8B: T1/44,Lances: 09A and O9B: Positive controls for *Mmm* and *Mmc*, Lane O10A: Negative control, † = Alignment marker, bp = Base pair.

ranged from 100 bp to 2500 bp and the alignment marker is located between 15 bp and 3000 bp. The samples of *Mycoplasma mycoides* subcluster presented fragment sizes of 574 bp.

3.3. Percentage PCR-RFLP for Mycoplasma mycoides subspecies mycoides

Polymerase chain reaction-RFLP gave 2 (100.00%) from Maiduguri lungs, 1 (50.00%) and 4 (100.00%) from lung and pleural fluids in Yola. The percentage of identification by PCR-RFLP was 7 (1.56%). Seven out of the fifteen *Mycoplasma* isolates identified in cattle at slaughter; three samples from lungs and four from pleural fluids were confirmed as *Mmm* with PCR-RFLP. T1/44 was re-confirmed as *Mmm* (Table 1).

3.4. PCR-RFLP amplicons on 3% gel electrophoresis identified as Mycoplasma mycoides subsp. mycoides

Seven *Mycoplasma* isolates and T1/44 (in duplicate) were identified as specific fragments. The PCR-RFLP amplicons on 3% gel electrophoresis for the identification of *Mycoplasma mycoides* subspecies *mycoides* has been presented. Lanes 1 and 20 are the molecular markers, lanes 2 to 3 are

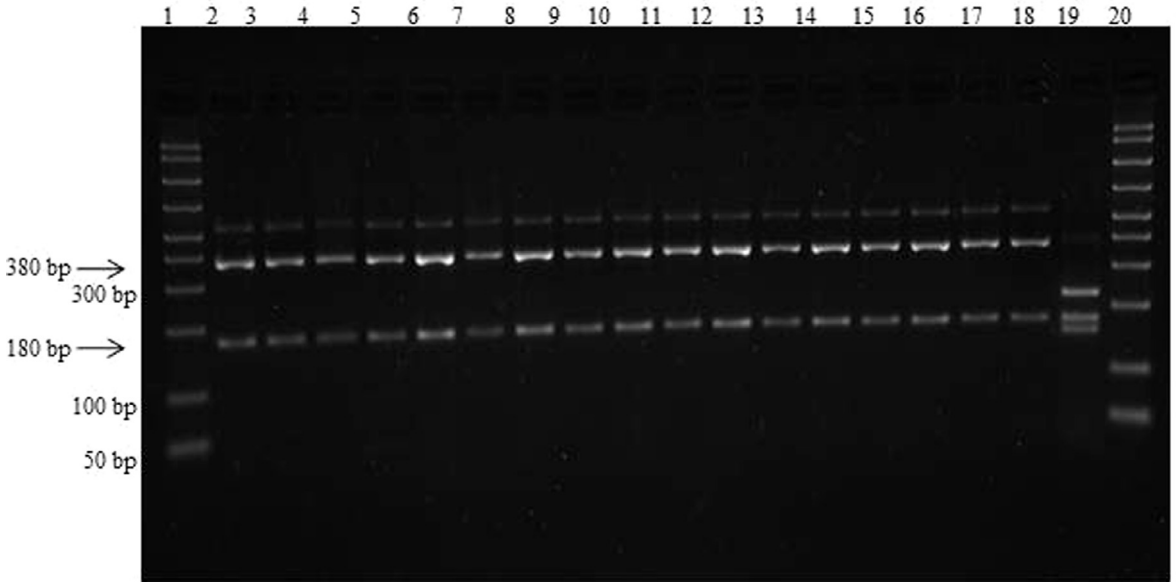

Fig. 5 PCR-RFLP amplicons on 3% agarose gel for the identification of *Mycoplasma mycoides* subspecies *mycoides* Lanes 1 and 20: Molecular marklers, lane 2 to 17: Samples in duplicate (T1/44 inclusive), Lane 18 and 19: *Mmm* and *Mmc* positive controls.

samples from Maiduguri (in duplicate), lanes 4 to 16 are samples from Yola, lane 17 is T1/44, and lanes 18 and 19 are *Mmm* and *Mmc* positive controls. Two different molecular sizes of 180 bp and 380 bp specific for *Mmm* was revealed by PCR-RFLP amplicons on 3% agarose gel. No band yielded 150 bp, 180 bp and 230 bp except the positive control for *Mmc*. The 380 bp fragment delineated *Mmm* from *Mmc* (Fig. 5).

4. Discussion

This study has demonstrated that cattle in Maiduguri and Yola harbour *Mmm* the causative agent of a highly contagious trans-boundary disease-CBPP. The isolation and molecular technique, PCR-RFLP provided means for specific identification of *Mmm* from slaughtered cattle in Northeastern zone of Nigeria. Although CBPP like lesions were sampled from all the three abattoirs, no *Mmm* was identified in samples obtained in Gombe abattoir. The identification of the causative agents of CBPP is in agreement with earlier report [11], who stated that CBPP is endemic in Borno (remained as Borno) and Gongola (now Adamawa). Additionally, an abattoir based study equally reported a lesion based prevalence of 0.29% in five States of Northern Nigeria [13]. The reason for the absence of *Mmm* isolates from Gombe abattoir, may not be unconnected with the fact that the disease might be absent in those animals at the time of sampling. The presence of antibiotics might have contributed to failure to recover the mycoplasmas and the CBPP-like lesions might have been mistaken for other causes of pleuropneumonia, such as *Pasteurella* species and other pathogens as earlier reported [27].

The small colonies with the classical 'fried-eggs' appearances of *Mycoplasma* on the agar media were in consonance with the report of [15]. Two of the strains (APF8A and APF8B) derived from APF8, which presented two different colonies on the agar media were presumptively identified as *Mycoplasma* in this study. The APF8A displayed typical fried egg colony, while APF8B failed to give the typical colony of *Mycoplasma*. The reason for this occurrence remained to be determined.

The isolation rate of 3.33% found in this study was lower than 6.9% reported in pneumonic cattle from Bosnia and Herzegovina [28]. The identification rate of *Mmm* in this study was found to be 1.56%, higher than 0.00% reported by [27] who could not isolate *Mmm* from cattle at slaughter in an abattoir study conducted in Turkey.

The *Mycoplasma* isolates found in this study were isolated from consolidated lungs, pleural fluids that were suggestive of CBPP. The 7 isolates confirmed as *Mmm* were from lungs (BL5, BL6 and AL1) and pleural fluids (APF2, APF8A, APF8B, APF9). This could be explained by the fact that the main predilection site for *Mmm* is the lung where *Mmm* causes pathological lesions accompanied with the production of pleural fluids- thus, creating a more conducive environment for the survival of *Mmm*.

The PCR amplification specific for *Mycoplasma mycoides* subcluster was found to be positive for all the 7 isolates and T1/44 which yielded molecular size of 574 bp specific for *Mmm* or *Mmc*. The identification of *Mycoplasma mycoides* subcluster at molecular size of 574 bp is in agreement with the findings of [18,19]. The molecular size of 574 bp is unable to distinguish between the two members of the *Mycoides* subcluster; *Mmm* which causes CBPP in cattle and *Mmc* which affects mostly small ruminants where it induce lesions such as mastitis, arthritis, kerato conjunctivitis, pneumonia and septicaemia [29].

The PCR-RFLP identified the 16S rRNA for CAP-21 genomic regions of 7 isolates of *Mmm*- the causative agent of CBPP, and re-confirmed T1/44 at the molecular sizes of 180 bp and 380 bp. Furthermore, the 380 bp delineated *Mmm* from *Mmc*. This finding is in consonance with the work of [18,19]. These further confirmed that strains of *Mmm* are

circulating in cattle in Maiduguri and Yola, North-eastern zone of Nigeria. The PCR-RFLP based on restriction endonuclease activity, could discriminate between isolates of *Mmm* and *Mmc* isolates. This method could be a useful tool to distinguish between the *Mycoplasma* isolates and for strain typing to enable detection of *Mmm* [17,18].

5. Conclusion

The findings of this study have detected the presence of *Mmm* as causative agent of CBPP. The PCR-RFLP is an important technique for the detection of *Mmm*. The lung lesions and pleural fluids are the most probable samples from which *Mmm* can be detect in cattle. We therefore recommend measures such as targeted abattoir surveillance, quarantine and vaccination for the control of CBPP in Nigeria.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the International Centre for Genetic Engineering and Biotechnology (ICGEB), Padriciano-Trieste, Italy (Grant No., S/NIG14-01).

Acknowledgements

The authors are most grateful to the management of Istituto Zooprofilattico Sperimentale dell Abruzzo e Molise 'G Caporale', OIE Reference Laboratory for CBPP, Campo Boario Teramo, Italy for granting us free bench space and for providing the technical support for this study.

References

[1] Westberg J, Persson A, Holmberg A, Goesmann A, Lundeberg J, Johansson KE, et al. The genome sequence of *Mycoplasma mycoides* subsp. *mycoides* SC type strain PG1T, the causative agent of contagious bovine pleuropneumonia (CBPP). Gen Res 2004;14:221–7.

[2] Citti C, Blanchard A. Mycoplasmas and their host: emerging and re-emerging minimal pathogens. Trends Microbiol 2013;21:196–203.

[3] Pilo P, Frey J, Vilei EM. Molecular mechanisms of pathogenicity of *Mycoplasma mycoides* subsp. *mycoides* SC. Vet J 2007;174:513–21.

[4] Regalla J, Caporale V, Giovannini A, Santini F, Martel JL, Goncalves AP. Manifestation and epidemiology of contagious bovine pleuropneumonia in Europe. Rev Sci Tech 1996;15:1309–29.

[5] Jores J, Mariner JC, Naessens J. Development of an improved vaccine for contagious bovine pleuropneumonia: an African perspective on challenges and proposed actions. Vet Res 2013;44:122.

[6] Egwu GO, Nicholas RAJ, Ameh JA, Bashiruddin JB. Contagious bovine pleuropneumonia: an update. Vet Bull 1996;66:875–88.

[7] Thiaucourt F, Van der Lugt, JJ, Provost A. Contagious bovine pleuropneumonia. In: Coetzer JAW, Tustin RC. editors. Infectious Diseases of Livestock. Oxford University Press; 2004; p. 2045–59.

[8] Nicholas RAJ, Bashiruddin JB. *Mycoplasma mycoides* subspecies *mycoides* (small colony variant): the agent of contagious bovine pleuropneumonia and member of the "*Mycoplasma mycoides* cluster". J Comp Path 1995;113:1–27.

[9] Ter laak EA. Contagious bovine pleuropneumonia. A review. Vet Quart 1992;15:104–10.

[10] Xin J, Li Y, Nicholas RAJ, Chen C, Liu Y, Zhang M, et al. A history of the prevalence and control of contagious bovine pleuropneumonia in China. Vet J 2012;191:166–70.

[11] Nwanta JN, Umoh JU. Epidemiology of Contagious bovine pleuropneumonia (CBPP) in Northern States of Nigeria. An Update. Rev Elev-et-de-Med Vet-des-Pay-Trop 1992;45:17–20.

[12] Tambi NE, Maina WO, Ndi C. An estimation of the economic impact of contagious bovine pleuropneumonia in Africa. Rev Sci Tech 2006;25, 999-11.

[13] Aliyu MM, Obi TU, Egwu GO. Prevalence of contagious bovine pleuropneumonia in Northern Nigeria. Prev Vet Med 2000;47:263–9.

[14] Musa JA, Egwu GO, Ameh JA. Prevalence of complement fixing antibodies to contagious bovine pleuropneumonia in slaughtered cattle in Maiduguri abattoir, Nigeria. Sah J Vet Sci 2011;10:51–6.

[15] Sori T, Zeleke A, Gelaye E, Regassa F. Isolation and identification of *Mycoplasma mycoides* subspecies *mycoides* Small Colony bovine biotype in Eastern Ethiopia. Int J App Res Vet Med 2005;3:30–4.

[16] Tardy F, Gaurivaud P, Tricot A, Maigre L, Poumarat F. Epidemiological surveillance of mycoplasmas belonging to the *'Mycoplasma mycoides'* cluster: is DGGE fingerprinting of 16S rRNA genes suitable? Lett Appl Microbiol 2009;48:210–7.

[17] Bashiruddin JB, Taylor TK, Gould AR. A PCR-based test for the specific identification of *Mycoplasma mycoides* subspecies *mycoides* SC. J Vet Diagn Invest 1994;6:428–34.

[18] Bashiruddin JB, de Santis P, Persson A, Ball H, Regalla J. Detection of *Mycoplasma mycoides* subspecies *mycoides* SC in bovine lung and lymph node tissues by culture, sandwich ELISA and polymerase chain reaction systems. Res Vet Sci 2005;78, 199-05.

[19] Muuka GM, Banda F, Bounavoglia D, Pini A, Scacchia M. Clinical cases of contagious bovine pleuropneumonia (CBPP) among calves in vaccinated cattle herds in Zambia: a case study. J Vet Sci Med Diag 2013;2:4. http://dx.doi.org/10.4172/2325-9590.1000124.

[20] Huebschle O, Aschenborn O, Godinho K, Nicholas R. Control of CBPP – a role for antibiotics? Vet Rec 2006;159:64.

[21] Schieck E, Liljander A, Hamsten C, Gicheru N, Scacchia M, Sacchini F, et al. High antibody titres against predicted *mycoplasma* surface proteins do not prevent sequestration in infected lung tissue in the course of experimental contagious bovine pleuropneumonia. Vet Microbiol 2014;172:285–93.

[22] Mbulu RS, Tjipura-Zaire G, Lelli R, Frey J, Pilo P, Vilei EM, et al. Contagious bovine pleuropneumonia (CBPP) caused by vaccine strain T1/44 of *Mycoplasma mycoides* subsp. *mycoides* SC. Vet Microbiol 2004;98:229–34.

[23] Nkando I, Ndinda J, Kuria J, Naessens J, Mbithi F, Schiner C, et al. Efficacy of two vaccine formulations against contagious bovine pleuropneumonia (CBPP) in Kenyan indigenous cattle. Res Vet Sci 2012;93:568–73.

[24] Kairu-Wanyoike SW, Kiara H, Heffernan C, Kaitibie S, Gitau GK, McKeever D, et al. Control of contagious bovine pleuropneumonia: knowledge, attitudes, perceptions and practices in Narok district of Kenya. Prev Vet Med 2014;115:143–56.

[25] Wesonga HO, Thiaucourt F. Experimental studies on efficacy of T1sr and T1/44 vaccine strains of *Mycoplasma mycoides* subsp.

mycoides (small colony). Rev d'élev de med vét pays trop 2000;53:313–8.

[26] Nicholas R, Ayling R, McAuliffe L. Isolation and growth of Mycoplasmas from ruminants. In: Mycoplasma Diseases of Ruminants. International C. Wallingford, UK; 2008; p. 3–14.

[27] Cetinkaya B, Ongor H, Karahan M, Kalender H, Lorenzon S, Thiaucourt F. Abattoir-based survey of contagious bovine pleuropneumonia in cattle in Turkey. Vet Rec 2003;152:254–8.

[28] Zinka M, Maid R. Mycoplasmas isolated from the respiratory tract of cattle in Bosnia and Herzegovina. An Vet Murcia 2012;28:79–83.

[29] Thiaucourt F, Manso-Silvan L, Salah W, Barbe V, Vacherie B, Jacob D, et al. *Mycoplasma mycoides*, from "mycoides Small Colony" to "capri". A microevolutionary perspective. BMC Genomics 2011;12:114.