Scientific Research Publishing

Open Journal of Veterinary Medicine, 2020, 10, 15-26
https://www.scirp.org/journal/ojvm
ISSN Online: 2165-3364
ISSN Print: 2165-3356

Isolation and Molecular Characterization of *Mycoplasma mycoides* Subspecie *mycoides* in 3 Agro Ecological Zones of Nasarawa State, Nigeria

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How to cite this paper: Ikpa, L.T., Bwala, D.G., Ankeli, P.I., Kaikabo, A.A., Maichibi, M.S., Maurina, I.A., Abenga, J.N., Nwankpa, N.D. and Adah, M.I. (2020) Isolation and Molecular Characterization of *Mycoplasma mycoides* Subspecie *mycoides* in 3 Agro Ecological Zones of Nasarawa State, Nigeria. *Open Journal of Veterinary Medicine*, **10**, 15-26.
https://doi.org/10.4236/ojvm.2020.102002

Received: August 7, 2019
Accepted: February 25, 2020
Published: February 28, 2020

Abstract

Contagious bovine pleuropneumonia is a disease caused by *Mycoplasma mycoides* subspecie *mycoides*, a transboundary animal disease causing serious devastation to cattle producers in Africa. The study was designed to identify and characterize the pathogenic member of mycoplasma cluster the *Mycoplasma mycoides* subspecie *mycoides* (Mmm) isolated from cattle infected with the disease. Three hundred (300) samples of nasal swabs and pleural fluid from cattle showing signs of CBPP were analyzed using culture and biochemical identification techniques and polymerase chain reaction (PCR) using specific primers to determine the prevalence of contagious bovine pleuropneumonia in Nasarawa State, Nigeria. Isolation recorded a prevalence of 4% and PCR recorded a prevalence of 67.7%. Isolates subjected to PCR analysis produced an amplicon size of 548 bp and 1.1 k bp respectively for the *Mycoplasma mycoides* cluster and *Mycoplasma mycoides* subspecie *mycoides*. Sequencing of the 16 S rRNA gene blast search revealed 96% to 99% sequence homology of *Mycoplasma mycoides* subspecie *mycoides* compared with 14 available sequences in the gen bank at NCBI. Based on this investigation mass vaccination of cattle is recommended, isolation and PCR techniques could be used as diagnostic tools for CBPP disease in three agro ecological zones of Nasarawa state, Nigeria.

Keywords

Polymerase Chain Reaction, Amplicon Size, Specific Primer, Sequence Homology, *Mycoplasma mycoides*

1. Introduction

Contagious bovine pleuropneumonia is among the transboundary animal diseases classified under OIE list A. The disease is caused by *Mycoplasma mycoides* subspecie *mycoides*, the disease is manifested by respiratory disorders; dyspnea, polypnoea, pyrexia, anorexia, nasal discharges, extension of the neck and coughing when animal is forced to move [1] [2].

CBPP has low morbidity and mortality in Europe compared to Africa with high percentage of infected animals due to chronic carriers [3]. The disease is endemic in Nigeria causing serious devastation to the economy [4]-[12]. The nomadic culture of Nigerian herdsmen due to their transhumance activities has significantly contributed to the spread of disease in Nigeria [13].

There has been a substantial re-emergence of the disease despite vaccination campaigns using freezed dried broth cultures of live attenuated *Mycoplasma mycoides* subspecie *mycoides* strain T1/44 [14]. Cultural identification of *Mycoplasma* species can be difficult due to the fastidious nature of the pathogens. However, identification of the *Mycoplasma mycoides* species by molecular techniques is very efficient, specific and rapid. Therefore Polymerase Chain Reaction and sequencing is the most accurate tool for the identification and confirmation of different *mycoplasma* species [4] [15]. The technique is capable of confirming the exact specie of microorganism even in mixed infection and directly from clinical samples like nasal swab, lung tissue and pleural fluids [16].

The 16 S rRNA gene codes for variable region of *Mycoplasma mycoides* subspecie *mycoides* with both gene and species specific primers, which can be used in the identification of particular species of Mycoplasma cluster [17] [18] [19] [20].

This study is carried out to isolate, identify and characterize *Mycoplasma mycoides* subspecie *mycoides* in 3 zones of Nasarawa state, Nigeria (**Figure 1**). This will eventually lead to proper design of control measures of the disease in Nasarawa state.

2. Methodology

2.1. Sampling

A total of 300 samples consisting of 150 nasal swabs and 150 pleural fluids were collected from cattle manifesting signs of respiratory disorder suspected for CBPP in cattle markets and abattoir in Nasarawa state, Nigeria. The samples were taken by sterile swab sticks and transferred to 2 ml of Pleuro pneumonia

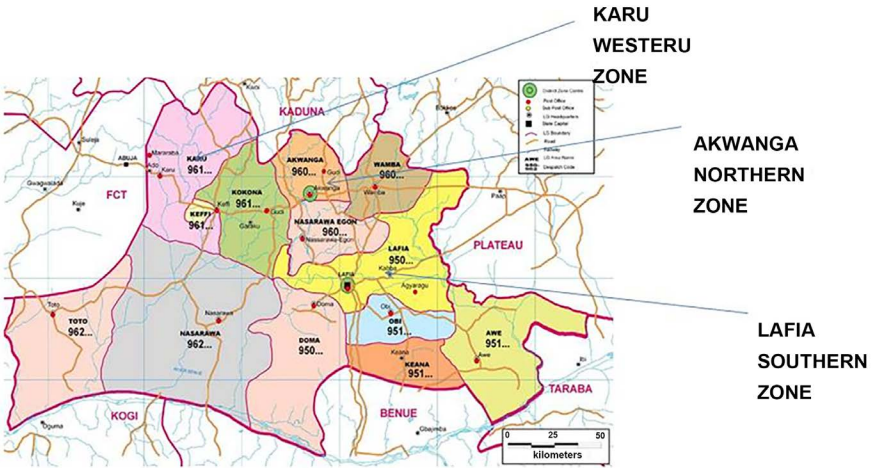

Figure 1. Map of Nasarawa State showing the agro ecological zones. (http://nigeriazipcodes.com/458/nasarawa.state-zip-code-map/).

like organism transport media. The collected samples in bijou bottles were kept under ice and transported to the *Mycoplasma* Laboratory of National Veterinary Research Institute Vom for analysis or preserved at −20˚C before analysis.

2.2. Isolation and Identification

The samples were taken aseptically by sterile swab stick and transferred to Pleuro pneumonia-like organism (PPLO) broth. All the collected samples were incubated in an anaerobic incubator (Memmert® Germany) with 5% CO_2 at 37˚C for 48 hours before sub culturing onto Pleuro pneumonia-like organism (PPLO) agar followed by incubation at 37˚C anaerobically for 3 to 10 days. The cultured plates were viewed using the stereomicroscope (h33 hund wetzlar®) for the appearance of nipple-like or fried egg typical *mycoplasma* colonies. To obtain pure culture the positive colonies were cultured three times according to standard protocol of [21].

2.3. Biochemical Assay

Biochemical assay of the 12 local isolates was carried out for the identification of *mycoplasma* species according to standard protocol of [22]. A volume of 200 ul from each isolate was diluted in 2.5 ml of *mycoplasma* broth and subjected to different biochemical tests like glucose fermentation, serum digestion, tetrazolium salt reduction, casein digestion, and arginine hydrolysis test for the identification of *mycoplasma* species.

2.4. Polymerase Chain Reaction

The biochemical identified samples of the species of *mycoplasma* were subjected to DNA extraction for confirmation through PCR. The polymerase chain reaction was performed for the detection of *Mycoplasma mycoides* subspecie *mycoides* by using two set of primers IS1296F (CTAAAGAGCTTGGAGTTCAGTG) and CA (F) (CGA AAG CGG CTT ACT

GGC TTG TT); the *Mycoplasma* mycoides subspecie mycoides and the *Mycoplasma mycoides* cluster primer. This was carried out according to the method described by [23]. All reactions were carried out in a final volume of 25 µl, and contained 6.5 µl molecular grade nuclease-free water, 12.5 µl 2X Dream Taq® DNA polymerase Master Mix. Specific detection of *MmmSC* isolates was carried out using 2 µl (0.4 mM) IS1296F (CTAAAGAGCTTGGAGTTCAGTG) and 2 µl (0.4 mM) R (all) (CCAGCT CAACCAGCT CCA G). To each 23 µl PCR master mix was added 2 µl of the DNA extract from the isolates and amplification reactions were carried out in thermocycler (AB Applied Biosystems Gene Amp® PCR system 9700, Singapore) with an initial denaturation/enzyme activation step of 95˚C for 5 minutes, followed by 32 cycles of denaturation at 95˚C for 30 seconds; annealing at 62˚C for 30 seconds; and extension at 72˚C for 1 minute 20 seconds. A final extension at 72˚C for 5 minutes was included. All the *Mycoplasma* isolates were screened by PCR. These primers targeted the 16 S rRNA gene of the *Mycoplasma* with an amplicon size of 1.1 kbp and 548 bp for *Mycoplasma mycoides* subspecie *mycoides* and *Mycoplasma* cluster respectively (**Figure 2**, **Figure 3**).

2.5. Homology and Phylogenetic Analysis

The gel product of specific amplicon size was taken and submitted for sequencing. The obtained sequence was subjected to NCBI BLAST to screen for homologous sequence for phylogenetics relationship of the local isolates of *Mycoplasma mycoides* subspecie *mycoides* with available sequences in the Gen Bank. Sequences of the isolates were downloaded from NCBI and then multiple aligned by using Bio Edit version 7.05.2 [24]. Furthermore, phylogenetic tree topology was constructed for the obtained sequences using software MEGA version 7.1 for evolutionary study and to build a correlation with other strains of different regions (**Figure 4**).

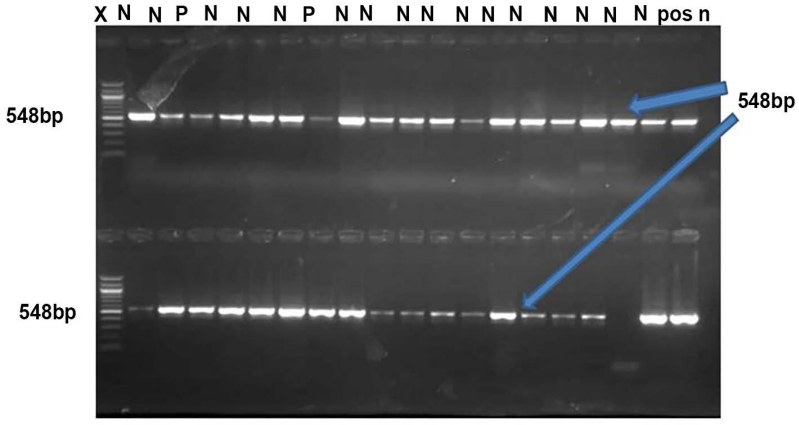

Figure 2. Gel electrophoresis of PCR products of seventeen field samples (Mmm cluster 548 bp) and control using primer CA (F)/CA (R). Lane X = Molecular weight markers (Bio-rad: 100 - 1000 bp).

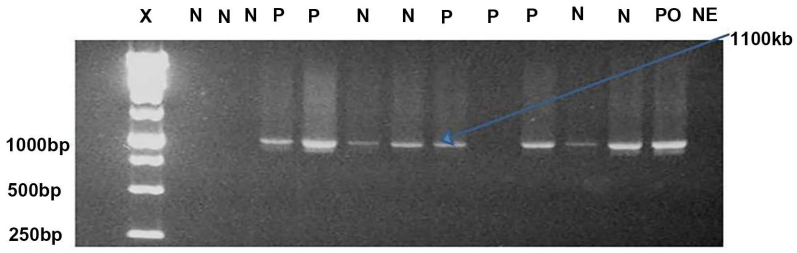

Figure 3. Gel electrophoresis of PCR products of twelve field samples (Mmm 1.1 bp) and control using primer IS1296. Lane X = Molecular weight markers (Bio-rad: 50 - 2000 bp).

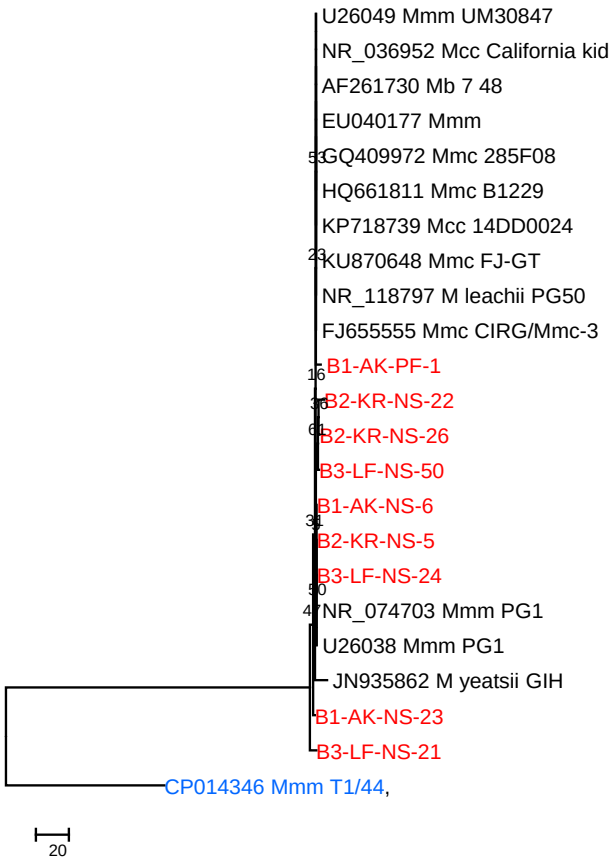

Figure 4. Phylogenetic relationships between 16S rRNA gene of Mycoplasma mycoides subspecies mycoides (Mmm) detected from nasal swabs and pleural fluid samples in Nasarawa State, Nigeria. Mmm sequenced in this study are B1-AK-PF1 (MG766272), B2-KR-NS5 (MG766273), B1-AK-NS6 (MG766270), B3-LF-NS21 (MG766276), B2-KR-NS22 (MG766274), B1-AK-NS23 (MG766271), B3-LF-NS24 (MG766277), B2-KR-NS26 (MG766275) and B3-LF-NS50 (MG766278). Phylogenetic analyses were completed with MEGA 6 software that used a neighbor-joining algorithm.

2.6. Statistical Analysis

Data obtained were subjected to statistical analysis using Chi-square of Open Epi (Version 3.02a) software to check level of significance between different samples.

3. Results

3.1. Isolation of Mycoplasma

Out of 300 samples, 12 (4%) were positive on culture for *Mycoplasma* species showing the classical comet with turbidity in Pleuro pneumonia like organism broth media. A typical nipple like and fried egg micro colonies appeared on day four and seven post-incubation on Pleuro pneumonia-like organism agar (Figure 5). More micro colonies were observed by microscopy (Stereomicroscope hund wetzlar®) nasal swab compared to pleural fluid. On statistical analysis of the data by Chi-square test (X^2) there was no significant difference ($P > 0.05$) found between the two samples obtained from cattle suspected of CBPP. The result of *Mycoplasma mycoides* subspecie *mycoides* are presented in Table 1.

The positive culture of *Mycoplasma* species was sub cultured on Pleuro pneumonia-like organism agar till the characteristic typical fried egg appearance growth were observed. On biochemical analysis 7 (58.3%) fermented glucose, reduced tetrazolium salt and hydrolyzed casein. They did not hydrolyze arginine, urea and negative for both phosphatase activity and film/spot formation. 3 (25%) hydrolyze arginine, positive for phosphatase activity, negative to tetrazolium salt, negative to casein, did not reduce glucose and negative for film/spot formation. 2 (16.6%) reduced tetrazolium salt, positive for phosphatase activity and produced film/spot (Table 2).

3.2. Molecular Characterization

Based on Polymerase Chain Reaction analysis, out of 300 samples, 203 (67.7%) were identified as *Mycoplasma mycoides* subspecie *mycoides* with amplicon size of 1.1 kbp (Figure 3). The nasal swab samples recorded 98 (32.7%) positives, while pleural fluid recorded 105 (35%) positives in the study (Table 3). On statistical analysis of data (X^2) there was significant difference ($P < 0.05$) obtained between the samples collected. The *Mycoplasma mycoides* cluster, 3 (25%) were confirmed to be positive with an amplicon size of 548 bp (Figure 2). *Mycoplasma mycoides* subspecie *mycoides* was identified for the first time in three agro ecological zones of Nasarawa state, Nigeria. Nine Polymerase Chain

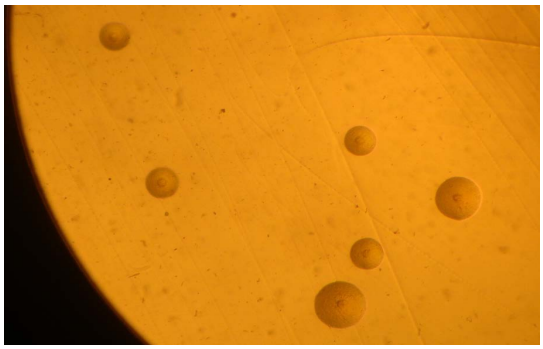

Figure 5. *Mycoplasma mycoides* subspecie *mycoides* colonies with nipple like appearance.

Table 1. Prevalence of *Mycoplasma species* infection in Cattle based on cultural characteristics in Nasarawa State.

LGA	LOCATION	TISSUE	No. Examined	No. Positive	Prevalence	Odd Ratio	P-value	95% CI	Chi-square
AKWANGA	ABATTIOR	P/F	50	1	2	1	0.6523 - 8.825	0.5	0.171
	CATTLE MKT	N/S	50	2	4				
KARU	ABATTIOR	P/F	50	0	0				
	CATTLE MKT	N/S	50	3	6				
LAFIA	ABATTIOR	P/F	50	0	0				
	CATTLE MKT	N/S	50	6	12				
TOTAL			300	12	4				

KEY: P/F: Pleural fluid; N/S: Nasal Swab; MKT: Market; CI: Confidence Interval.

Table 2. Biochemical characteristics of *mycoplasmas* isolated from cattle in Nasarawa State.

No. of	BIOCHEMICAL TEST							
Isolates recovered (%)	GC	AH	TR	PP	UH	CH	FS	**Isolates**
7 (58.3%)	+	−	+	−	−	+	−	*MmmSc/Mmc*
3 (25%)	−	+	−	+	−	−	−	*M. Alkalescens*
2 (16.6%)	−	−	−	+	+	−	−	*M. bovis*

Key: GC: glucose fermentation; **AH:** arginine hydrolysis; **TR:** tetrazolium reduction; **PP:** phosphatase production; **UH:** urea hydrolysis; **CH:** casein hydrolysis.

Table 3. Detection of *MmmSC* infection by direct PCR in Nasarawa State.

LGA	Sample type	No. Examined	No Positive	prevalence	Odd Ratio	95% CI	P-value	Chi-square
Akwanga	N/S	50	29	58	0.3056	0.5614 - 0.8419	0.000083	13.13
	P/F	50	26	52				
Karu	N/S	50	30	60				
	P/F	50	38	76				
Lafia	N/S	50	39	78				
	P/F	50	41	82				
Total		300	203	67.7				

Reaction confirmed local isolates were processed for sequencing and the sequence of the PCR products obtained through specie specific primers shared maximum sequence homology of 96% to 99% of 16 S rRNA gene of *Mycoplasma mycoides* subspecie *mycoides* with other strains in the gen bank. The phylogenetic tree was constructed by using software version 7.0.5.2 and compared with 14 available sequences in NCBI gen data bank. The constructed tree indicated that local isolates from the three agro ecological zones of Nasarawa state, Nigeria, were similar to the sequences in gen bank by 99%, but differ from the T1/44 vaccine with 42% similarity (Figure 4).

4. Discussion

The isolation, biochemical and molecular characterization of *mycoplasma* isolates in three agro ecological zones of Nasarawa state were established. The homology of the 16S rRNA gene of *Mycoplasma mycoides* subspecie *mycoides*

with local strains was determined in this study. The Polymerase Chain Reaction technique was very specific and rapid, with short duration of reaction time. This is similar to previous reports [4] [16] [23] [25] [26]. The presence of *Mycoplasma mycoides* subspecie *mycoides* and other members of the cluster were investigated successfully with isolation from the nasal swabs and pleural fluid. However, there was low isolation rate (4%) probably due to use of antimicrobials by herders for prophylactics or contaminants by other bacterial pathogens as reported previously [25] [27] [28].

Optimal growth of *Mycoplasma* micro colonies were observed on the pleuropneumonia like agar media after 72 hours of incubation at 37˚C from the nasal swabs (91.6%), compared to pleural fluid (8.3%) using cultural technique in this investigation. This is in agreement with previous reports, in which there was numerous isolation of *Mycoplasma* species from nasal swabs [29] [30] [31] [32]. Culturing and enrichment technique was demonstrated in this study. This was also compared with direct PCR. The direct PCR was highly sensitive and specific producing higher positive results. Biochemical characterization of the 12 *Mycoplasma* isolates indicated that 7 (58.3%) fermented glucose, reduced tetrazolium chloride and hydrolyzed casein (**Table 2**). They did not hydrolyse arginine nor urea and neither did they produce phosphatase and film and spots, and were presumptively identified as members of the *Mycoplasma mycoides* sub-cluster which consists of *Mycoplasma mycoides* subspecies *mycoides* (*Mmm*) and *Mycoplasma mycoides* subspecies *capri* (*Mmc*). The two *Mycoplasma* subspecies share these biochemical properties even though *Mycoplasma mycoides* subspecies *capri* is a pathogen mostly of small ruminants [22]. Within the other 5 *Mycoplasma* isolates, three (25%) isolates reacted to arginine hydrolysis and show phosphatase activity but did not reduce tetrazolium chloride, did not hydrolyze casein and urea nor fermented glucose and did not produce film and spots and were presumptively identified as *M. alkalescens*. While two (16.6%) isolates only reduced tetrazolium chloride and produced phosphatase and film and spots and was presumptively identified as *M. bovis*. *Ureaplasma* and other urea-utilizing *Mycoplasma* species were screened out, as none of the *Mycoplasma* isolates hydrolyzed urea.

The *Mycoplasma* isolates found in this study were isolated from the nasal swab and pleural fluid. This could be explained due to the fact that the main predilection site for *Mycoplasma mycoides* subspecie *mycoides* is the respiratory tract where it causes pathological lesions in the lungs with production of yellowish straw colored pleural fluid in chronic cases. This environment of the respiratory tract is rich in the *Mycoplasma mycoides* subspecie *mycoides* organism which might spread throughout the lower respiratory tract to the upper respiratory tract where it can be also isolated from the nostrils through nasal swabs. This is in agreement with previous reports [5] [11] [31].

The Polymerase Chain Reaction amplification specific for *Mycoplasma mycoides* subspecie *mycoides* was found to be positive for seven isolates and the positive control T1/44 vaccine strain with a molecular size of 1.1 k bp and 548 bp specific

for *Mmm* and *Mmc*. The identification of *Mycoplasma mycoides* subspecie *mycoides* and *Mycoplasma mycoides* cluster at molecular size of 1.1 k bp and 548 bp respectively is in agreement with other findings [4] [5] [25] [33] [34].

Also in this study, the 16S rRNA gene of the *Mycoplasma mycoides* subspecie *mycoides* gene of local isolates in Nasarawa state was 96% to 99% similar to sequences obtained worldwide in NCBI Gen Bank and 42% similar to CBPP vaccine strain T1/44.

It is concluded that *Mycoplasma mycoides* subspecie *mycoides* is endemic in three zones of Nasarawa state. The isolated specie of *Mmm* has close homology with strains of *Mmm* in the Gen Bank, but with evolutionary distance with the vaccine strain T1/44. Therefore the successful isolation and characterization of local isolates of *Mmm* will provide an opportunity for the development of an indigenous multivalent vaccine for the control of CBPP in Nasarawa state, Nigeria.

Acknowledgements

I truly appreciate the Director/Chief Executive, Dr D. Shamaki, National Veterinary Research Institute Vom, Nigeria for the support. Also my supervisor Prof. M. I. Adah Department of veterinary Medicine, University of Agriculture, Makurdi Benue state Nigeria. Inquaba biotech Ltd., for the reagents and finally to all staff of Mycoplasma laboratory and Biotechnology Laboratory National Veterinary Research Institute Vom, Nigeria.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Thiaucourt, F., Dedieu, I., Maillard, I.C., Bonnet, P., Lesnoff, M., Laval, G. and Provost, A. (2003) Contagious Bovine Pleuropneumonia Vaccine, Historic Highlights, Present Situation and Hopes. *Development in Biologicals*, **114**, 147-160.

[2] Office International des Epizooties (OIE) (2014) Contagious Bovine Pleuropneumonia. OIE Terrestrial Manual, OIE, 1-16.

[3] Nicholas, R.A.J., Ayling, R.D. and McAuliffe, L. (2008) Contagious Bovine Pleuropneumonia. In: Nicholas, R., Ayling, R. and McAuliffe, L., Eds., *Mycoplasma Diseases of Ruminants*, CABI Publishing Wallingford, UK, 69-97. https://doi.org/10.1079/9780851990125.0069

[4] Alhaji and Babalobi, O.O. (2015) Molecular Epidemiology of Contagious Bovine Pleuropneumonia by Detection, Identification and Differentiation of Mycoplasma Mycoides Subspecie Mycoides in Niger State, Nigeria. *Sokoto Journal of Veterinary Science*, **13**, 1-8. https://doi.org/10.4314/sokjvs.v13i3.1

[5] Ankeli, P.I., Raji, M.A., Kazeem, H.M., Tambuwal, F.M., Francis, M.I., Ikpa, L.T., Fagbamila, M.I., Luka, P.D. and Nwankpa, N. (2017) Seroprevalence of Contagious Bovine Pleuropneumonia in Plateau State, North-Central Nigeria. *Bulletin of Animal Health and Production in Africa*, **65**, No. 2.

[6] Aliyu, M.M. and Egwu, G.O. (2000) Prevalence of Contagious Bovine Pleuropneu-

monia (CBPP) in Northern Nigeria. *Preventive Veterinary Medicine*, **47**, 263-269. https://doi.org/10.1016/S0167-5877(00)00170-7

[7] Chima, J.C., Mohammed, A., Lombin, L.H. and Majiyagbe, K.A. (1999) Field Validation of Monoclonal Antibody Based Competitive ELISA for the Detection of Antibodies to Contagious Bovine Pleuropneumonia. *Proceedings 2nd RCM of IAEA/FAO CRP on monitoring of CBPP in Africa Using ELISA*, Lusaka Zambia, 27 September 1999, 11-12.

[8] Danbirni, S., Okaiyeto, S.O., Pewan, S.B. and Kudi, A.C. (2010) Concurrent Infection of Contagious Bovine Pleuropneumonia and Bovine Tuberculosis in Bunaji Nomadic Cows. *Research Journal of Animal Science*, **4**, 23-25. https://doi.org/10.3923/rjnasci.2010.23.25

[9] Egwu, G.O., Adamu, M., Mshelia, G.D. and Bukar-Kolo, Y.M. (2012) A Sustainable Laboratory Approach for Contagious Bovine Pleuropneumonia (CBPP) Monitoring in Nigeria: Comparison between Two Serological Test in an Endemic Area Complemented with Post Morten Lesions. *African Journal of Microbiology Research*, **6**, 5890-5895. https://doi.org/10.5897/AJMR11.1612

[10] Francis, M.I., Ankeli, P.I., Ikpa, L.T., Maichibi, M.S. and Fagbamila, I.O. (2017) First Report of Contagious Bovine Pleuropneumonia in a Herd of Friesian Cattle at Birnin-Kudu, Jigawa State. *Vom Journal of Veterinary Science*, **12**, 41-45.

[11] Nwankpa, N.D., Muraina, I., Ogunjumo, S.O., Okewole, P.A., Chukwu, O.C., Luther, N.J., Jambalang, A.R. and Abiayi, E. (2004) Incidence of Contagious Bovine Pleuropneumonia (CBPP) A Comparison of Local Zebu and Imported Cattle. *The Proceedings of the 41st Annual Congress of the Nigerian Veterinary Medical Association*, Jos, 27-29.

[12] Tambuwal, F.M., Egwu, G.O., Shittu, A., Sharubutu, G.H., Umaru, M.A., Umar, H.U., Mshelia P.C. and Garba, S. (2011) Vaccination Coverage and Prevalence of Contagious Bovine Pleuropneumonia (1999-2008) in Two Trans Boundary States of North Western Nigeria. *Nigerian Veterinary Journal*, **32**, 169-173.

[13] Foluso, E.F. (2003) Status of Contagious Bovine Pleuropneumonia (CBPP) in Nigeria with Emphasis on Control Strategies. In: *Proceedings FAO/OIE/IBAR/IAEA Consultative Group on Contagious Bovine Pleuropneumonia Third Meeting*, Rome, 161.

[14] Nicholas, R., Bashiruddin, J., Ayling, R.D. and Miles, R. (2000) Contagious Bovine Pleuropneumonia: A Review of Recent Developments. *Veterinary Bulletin*, **70**, 827-838.

[15] Woubit, S., Lorenzon, S., Peyraud, A., Manso-Silvan, L. and Thiaucourt, F. (2004) A Specific PCR for the Identification of Mycoplasma Capricolum subsp. Capripneumoniae, the Causative Agent of Contagious Caprine Pleuropneumonia (CCPP). *Veterinary Microbiology*, **104**, 125-132. https://doi.org/10.1016/j.vetmic.2004.08.006

[16] Muhammad, K.S., Umer, S., Shakoor, Hamayun, K., Hanif, U.R., Said Sajjad, A.S. and Muhammad, I. (2016) Molecular Characterization of Local Isolates of Mycoplasma Capricolum Subspecie Capripneumoniae in Goats (*Capra hircus*) of Khyber Pakthunkhwa, Pakistan. *Pakistan Veterinary Journal*, **37**, 90-94.

[17] Hotzel, H., Sachse, K. and Pfutner, H. (1996) A PCR Scheme for the Differentiation of Organisms Belonging to the Mycoplasma Mycoides Cluster. *Veterinary Microbiology*, **49**, 31-43. https://doi.org/10.1016/0378-1135(95)00176-X

[18] Manso-Silvan, L., Perrier, X. and Thiaucourt, F. (2007) Phylogeny of the Mycoplasma Mycoides Cluster Based on Analysis of Five Conserved Protein-Coding Se-

quences and Possible Implications for The Taxonomy of the Group. *International Journal of systematic and Evolutionary Microbiology*, **57**, 2257-2258. https://doi.org/10.1099/ijs.0.64918-0

[19] Kumar, P.R., Bhanderi, B. and Pal, B.C. (2011) Isolation, Identification and Molecular Characterization of Mycoplasma Isolates from Goats of Gujurat, State India. *Veterinarski Arhiv*, **81**, 443-458.

[20] Nendir, U. (2015) Isolation and Molecular Characterization of *Mycoplasma capricolum* Subspecie Capripneumoniae in Three Local Governments of Plateau State. Ahmadu Bello University, Zaria.

[21] Office International des Epizooties (OIE) (2012) World Animal Health Information Database, Version 2. World Animal Health Information Database, Paris, France.

[22] Nicholas, R.A.J., Ayling, R.D. and McAuliffe, L. (2008) Contagious Bovine Pleuropneumonia. In: Ayling, R., McAuliffe, L. and Nicholas, R., Eds., *Mycoplasma Diseases of Ruminants*, CABI Publishing Wallingford, UK, 69-97. https://doi.org/10.1079/9780851990125.0069

[23] Miles, K., Churchward, C.P., McAucliffe, L., Ayling, R.D. and Nicholas, R.A. (2006) Identification and Differentiation of European and African/Australian Strains of Mycoplasma Mycoides Subspecie Mycoides Small Colony Type Using Polymerase Chain Reaction Analysis. *Journal of Veterinary Diagnostic Investigation*, **18**, 168-171. https://doi.org/10.1177/104063870601800205

[24] Hall, T.A. (1999) Bio Edit: A User-Friendly Biological Sequence Alignment Editor and Analysis Programme for Windows 95/98 NT. *Nucleic Acids Symposium Series*, **41**, 95-98.

[25] Bashiruddin, J.B., Nicholas, R.A.J., Santini, F.G., Ready, R.A., Woodward, M.J. and Taylor, T.K. (1994) Use of Polymerase Chain Reaction to Detect Mycoplasma DNA in Cattle Contagious Bovine Pleuropneumonia. *Veterinary Records*, **134**, 240-241. https://doi.org/10.1136/vr.134.10.240

[26] Enyaru, J.C.K., Biryomumaisho, S., Balyeidhusa, A.S.P., Ebong,C., Musoni, A., Manzi, M., Rutagwenda, T., Zimurinda, J., Ansiimwe, T. and Gahakwa, D. (2012) Comparison of Competitive ELISA, PCR and Loop Mediated Isothermal Amplification of Mycoplasmal DNA in Confirmatory Diagnosis of an Outbreak of Contagious Bovine Pleuropneumonia in Eastern Rwanda. *International Journal of Animal and Veterinary Advances*, **4**, 22-28.

[27] McAuliffe, L., Ellis, R.J., Ayling, R.D. and Nicholas, R.A.J. (2003) Differentiation of *Mycoplasma* Species by 16S Ribosomal DNA PCR and Denaturing Gradient Gel Electrophoresis Fingerprinting. *Journal of Clinical Microbiology*, **41**, 4844-4847. https://doi.org/10.1128/JCM.41.10.4844-4847.2003

[28] Pourbakhsh, S.A., Abtin, A.R., Ashtari, A., Bayatzadeh, M.A., Barani, S.M. and Asli, E. (2014) Detection of *Mycoplasma capricolum* subsp. *capricolum* from Goats of Qom Province, Iran. *Razi Vaccine and Serum Research Institute*, **70**, 45-50.

[29] Hirose, K., Kobayashi, N., Kawasaki, Y., Zako, M., Kotani, K., Ogawa, H. and Sato, H. (2003) Isolation of Mycoplasma from Nasal Swabs of Calves Affected with Respiratory Diseases and Antimicrobial Susceptibility of Their Isolates. *Journal of Veterinary Medicine and infectious Diseases*, *Veterinary Public Health*, **50**, 347-351. https://doi.org/10.1046/j.1439-0450.2003.00681.x

[30] Nathues, H., Woeste, H., Doehring, S., Fahrion, A., Doherr, M. and Grosse Beilage, E. (2012) Detection of *Mycoplasma* Hyopneumoniae in Nasal Swabs Sampled from Pig Farmers. *Veterinary Records*, **170**, 623. https://doi.org/10.1136/vr.100817

[31] Musa, J.A., Bale, J.O.O., Kaseem, H.M., Nwankpa, N.D., Di Provvido, A., Sacchini,

F., Zilli,K., Abass, A., Scacchia, M. and Pini, A. (2016) Molecular Detection of Nigerian Field Isolates of *Mycoplasma mycoides* Subspecie Mycoides as Causative Agents of Contagious Bovine Pleuropneumonia. *International Journal of Veterinay Science and Medicine*, **4**, 46-53. https://doi.org/10.1016/j.ijvsm.2016.10.007

[32] Ankeli, P.I, Raji, M.A., Kazeem, H.M., Odugbo, M.O., Umaru, N.J., Fagbamila, I.O., Ikpa, L.T., Nwankiti, O.O., Muraina, I.A. and Nwankpa, N.D. (2016) Isolation and Identification of *Mycoplasma mycoides* from the Ear Canal of Cattle in Plateau State, North-Central Nigeria. *Veterinary Sciences. Research and Review*, **2**, 52-59. https://doi.org/10.17582/journal.vsrr/2016.2.2.52.59

[33] Muuka, G.M., Banda, F., Bournavoglia, D., Pini, A. and Seacchia, M. (2013) Clinical Cases of Contagious Bovine Pleuropneumonia (CBPP) among Calves in Vaccinated Cattle Herds in Zambia: A Case Study. *Journal of Veterinary Science Medical Diagnosis*, **2**, 4. https://doi.org/10.4172/2325-9590.1000124

[34] Thiacourt, F., Lorenzon, S., David, A. and Breard, A. (2000) Phylogeny of the Mycoplasma Mycoides Cluster as Shown by Sequencing of a Putative Membrane Protein Gene. *Veterinay Microbiology*, **72**, 251-268. https://doi.org/10.1016/S0378-1135(99)00204-7