Tropical Biomedicine 37(2): 273–281 (2020)

Prevalence and molecular detection of contagious bovine pleuropneumonia in large ruminants in Punjab, Pakistan

Anjum, A.[1*], Usman, S.[1], Aslam, A.[1], Faiz, M.[2], Usman, S.[3], Imran, M.S.[1], Hussain, I.[1], Usman, M.[4], Badar, S.[5], Iqbal, M.Z.[4], Dar, A.[6] and Haq, H.M.A.[7]
[1]Department of Pathology, University of Veterinary & Animal Sciences, Lahore, Pakistan
[2]King Edward Medical University, Lahore, Pakistan
[3]Nishtar Medical University, Multan, Pakistan
[4]Department of Clinical Medicine and Surgery, University of Veterinary & Animal Sciences, Lahore, Pakistan
[5]Livestock & Dairy Development Department Punjab, Pakistan
[6]Foot and Mouth Disease Research Center, Veterinary Research Institute, Lahore, Pakistan
[7]Poultry Research Institute, Rawalpindi, Pakistan
[*]Corresponding author e-mail: ahsan.anjum@uvas.edu.pk
Received 22 April 2019; received in revised form 17 February 2020; accepted 18 February 2020

Abstract. Contagious bovine pleuropneumonia (CBPP) is a highly contagious disease of cattle caused by *Mycoplasma mycoides* subsp. *mycoides*. It is characterized by anorexia, fever, dyspnea, polypnea, cough, and nasal discharges. Gross lesions in the lung such as marbling, sequestra, thickening of interlobular septa, and consolidation are evident. Serological tests including complement fixation test and competitive enzyme-linked immunosorbent assay and molecular tests such as polymerase chain reactions are used for diagnostic purposes. In this study, lung samples of suspected large ruminants (cattle n=560, buffalo n=293) were collected from abattoirs of three districts of Punjab namely Lahore, Kasur and Jhang. PCR was performed with specific primers, targeting the 16S ribosomal RNA gene to detect the positive cases. The results indicated that 49 samples (8.75%) of cattle were positive, with maximum prevalence was observed in Jhang with 16 positive samples (10.06%), but CBPP was not detected in any buffalo sample. High prevalence of disease was seen in cattle of more than seven years of age, in female cattle, and in cross-bred cattle. Age and gender were found significantly associated ($P<0.05$) with the prevalence of the disease. Gene sequencing of identified 5 isolates of *Mycoplasma mycoides* subsp. *mycoides* had more than 99% similarities with the strains isolated from China, Italy, Australia and Tanzania and were categorized into a monophyletic group but strain isolated from Portugal had more than 55% variable regions, hence clustered separately. This study confirms the presence of contagious bovine pleuropneumonia in the country which can be a threat to the livestock export market and warrants the implementation of control measures to mitigate the economic losses associated with the disease.

INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is a contagious respiratory disease of cattle that is caused by *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*). CBPP is prevalent in sub-Saharan African countries with a major impact on livestock production (Abdela & Yune, 2017). The disease has a long incubation period of about 6 weeks. In adults, the disease is characterized by fever, anorexia respiratory distress including dyspnea, polypnea, cough, and nasal discharge, while in young animal swelling of joints is mainly associated with the disease (Francis *et al.*, 2018). On necropsy, gross lesions in lungs including thickening of interlobular septa, consolidation, red and grey hepatization, marbling, severe fibrinous exudative pleuropneumonia, and sequestra formation are evident (Massimo, 2018). CBPP was included in former List "A" diseases of OIE

and is considered as a transboundary animal disease (TAD). CBPP is an important disease with significant economic impact as the mortality rate of 10-70% in African countries has been reported (Yansambou *et al.*, 2018). Other economic impact includes high production cost, medication expenditures, weight loss, decreased working ability, decreased fertility rate, diminution in cattle trade, a decline in investment in livestock sector resulted in the imposition of rigorous limitation to international trade on CBPP-affected countries in accordance with World Organization of Animal Health (OIE) regulation. In Africa, an annual loss of approximately 2 billion US$ has been documented (Onono *et al.*, 2017).

The disease is transmitted through the inhalation of pathogen however, airborne transmission cannot be neglected. Chronically infected animals act as a carrier of *Mmm*. Even a single carrier in a herd can cause a potential threat to the whole population of animals (Le Get, 2018). In case of outbreak of the disease, control measures including the culling of suspected and diseased animals are essential to prevent the transmission of disease. All these measures depend upon the proper identification of disease and etiologic agents (Malicha *et al.*, 2017).

World Organization for Animal Health has approved two serological tests for diagnosis of CBPP; complement fixation test (CFT) and a competitive enzyme-linked immunosorbent assay (cELISA), but these tests are recommended at herd level only (Muuka *et al.*, 2011). Conventionally, isolation of *Mmm* by culture and identification by biochemical and antigenic techniques have drawbacks such as low sensitivity due to contamination, low specificity due to cross-reactivity with other *Mycoplasma* species furthermore, these techniques are time-consuming, require labor and laboratory procedures. For the serological assessment of CBPP in a specific region, the ELISA technique gave good results in a short period of time along with minimal chances of contaminations as compared to conventional culture or biochemical techniques (Bashiruddin *et al.*, 2005; Anjum *et al.*, 2019). The only drawback of the ELISA technique is it's slow sensitivity against the acute cases when compared to the results of ELISA with the PCR and loop-mediated isothermal amplification (LAMP) techniques (Muuka *et al.*, 2011; Enyaru *et al.*, 2012).

Since the development of PCR in 1994, considerable developments have been introduced to improve the sensitivity and specificity to diagnose CBPP (Hotzel *et al.*, 1996; Schnee *et al.*, 2011). In this study, the conventional PCR technique was used for prevalence and molecular detection of CBPP in slaughtered cattle and buffalo lung samples, collected from abattoirs of three districts of Punjab, Pakistan.

MATERIALS AND METHODS

Study area and sample collection

In this study, lung samples of suspected cattle (n=560) and buffalo (n=293) were collected from different abattoirs of three districts (Lahore, Kasur, Jhang) of Punjab, Pakistan (Figure 1). Samples were kept in transfer media containing heart infusion broth, and shifted to the molecular pathology lab, Department of Pathology, University of Veterinary & Animal Sciences, Lahore, Pakistan for further processing.

Genome extraction and PCR

Lung samples were used for DNA extraction using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Forward and reverse primers of oligonucleotide sequences (16S rRNA–F 'AAAATGAGAGTTTGATCCTGG' and 16S rRNA–R 'AGAAAGGAGGTGAT CCATCCG') were derived from the 16S ribosomal RNA gene with an amplicon size of 1525bp, using Oligo primer analysis software version 6.71. All the amplifications were conducted in Mastercycler® nexus (Eppendorf, Hamburg, Germany). For 20 µL reaction mixture, 10 µL PCR Master Mix (2X) (Thermo Scientific™, Massachusetts, United States), 1 µL DNA template, 1 µL forward primer, 1 µL reverse primer, and 7 µL nuclease-free water was used. For amplification, the reaction mixtures were pre-heated to 95°C for 5 min followed by 30

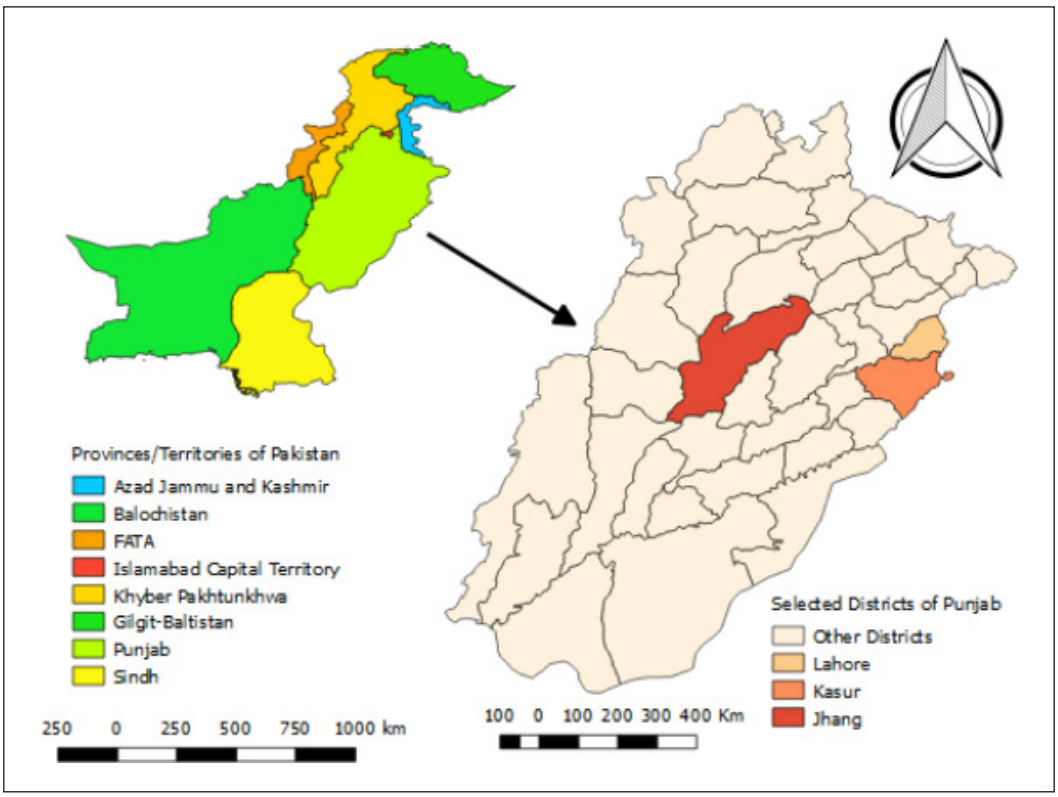

Figure 1. Areas for sample collections from abattoirs of three districts of Punjab, Pakistan.

cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 50 sec and a final extension at 72°C for 7 min. The same primers were used for the negative control reaction mixture under the same amplification conditions. The PCR products were run in 1% tris-acetic acid-EDTA buffer by using 1% gel electrophoresis and analyzed the amplicon size in gel documentation (AlphaImager™, USA) by their band size. The PCR product was further processed for nucleotide sequence analysis targeting the 16S rRNA gene for the detection of CBPP.

Phylogenetic analysis and homology percentage

Nucleotide sequences were compared to check the homology identity with the already reported strains available in NCBI-GenBank databases. Nucleotide sequences were aligned by using Clustal W pairwise alignment tool and MEGA-X software was used for phylogenetic analysis. Neighbor-joining algorithm was applied to construct the tree, with bootstrap percentage values estimated from 1000 replications and values over 50% were displayed. The percentage homology among the *Mmm* isolates was calculated through pairwise sequence distance matrix tool using DNASTAR Lasergene MegAlign version 7.1.0 (44) software.

Statistical analysis

All data were expressed in means ± standard deviation form. All values were documented in Microsoft excel files. Pearson chi-square test was applied to determine the prevalence of CBPP along with the associated risk factors. The level of significance was accepted at 95% confidence interval. Statistical analysis was performed at statistical package for the social sciences (IBM SPSS version 23.0) software.

RESULTS

PCR results indicated that *Mycoplasma mycoides* subsp. *mycoides (Mmm)* was found in 49 (8.75%) cattle lung samples, but *Mmm* was not detected in any buffalo lung sample. The number of positive samples in Lahore, Kasur, and Jhang were 8.26%, 8.20%, and 10.06% respectively, no significant difference (P>0.05) was observed regarding the prevalence of CBPP with respect to cities as shown in Table 1.

The analysis of data indicated that the number of positive samples in cattle of less than 4 years, 4-7 years and more than 7 years of age were 0.79%, 8.16%, and 32.08% respectively. A significant difference (P<0.05) was found with the prevalence of CBPP in terms of age factor as shown in Table 2.

The number of positive cases observed in male and female cattle were 5.86% and 11.50% respectively. Pearson chi-square value was 0.01, which indicated the prevalence of CBPP was statistically associated (P<0.05) with the gender of cattle as shown in Table 2.

The analysis of data depicted that positive cases of CBPP in local and cross-bred cattle were 4.05% and 9.47% respectively. Pearson chi-square value was 0.06, which showed that the prevalence of CBPP was not statistically associated (P>0.05) in terms of the breed of cattle as shown in Table 2.

The analysis of the results of our study revealed that PCR had amplified a 1525 base pair amplicon specific for the 16S rRNA gene of *Mmm*. The nucleotide sequences of identified isolates namely *Mmm* isolate PAK/C85, *Mmm* isolate PAK/C187, *Mmm* isolate PAK/C291, *Mmm* isolate PAK/C354 and *Mmm* isolate PAK/C499 were submitted to NCBI-GenBank databases under the accession numbers MK692950, MK692951, MK692952, MK692953, and MK692954 respectively.

Phylogenetic analysis of the sequences indicated that field isolated *Mycoplasma* isolates were closely related to the *Mmm* group as compared to other *Mycoplasma* species. Field isolated *Mmm* isolate Pak/C87 being closely related to *Mmm* strain Ben326 isolated from China, was clustered together. Vaccinal strain *Mmm* strain T1/44 (Tanzania), *Mmm* strain Gladysdale (Australia) and *Mmm* Ben strains (1, 50, 181 and 426) isolated from China, having high percentage identity, formed a monophyletic group with field identified isolates, while *Mmm* strain

Table 1. Prevalence of contagious bovine pleuropneumonia in selected districts of Punjab

City	N	Positive	Negative	Prevalence (%)	*P*-value*
Lahore	218	18	200	8.26%	0.79
Kasur	183	15	168	8.20%	
Jhang	159	16	143	10.06%	

*Pearson chi-square.

Table 2. Prevalence of CBPP in cattle with respect to different possible risk factors

Factors	Classification	N	Positive	Negative	Prevalence (%)	*P*-value*
Age	<4 years	127	01	126	0.79%	<0.0001
	4-7 years	380	31	349	8.16%	
	>7 years	53	17	36	32.08%	
Gender	Female	287	33	254	11.50%	0.01
	Male	273	16	257	5.86%	
Breed	Local breed	74	3	71	4.05%	0.06
	Cross-bred	486	46	440	9.47%	

*Pearson chi-square.

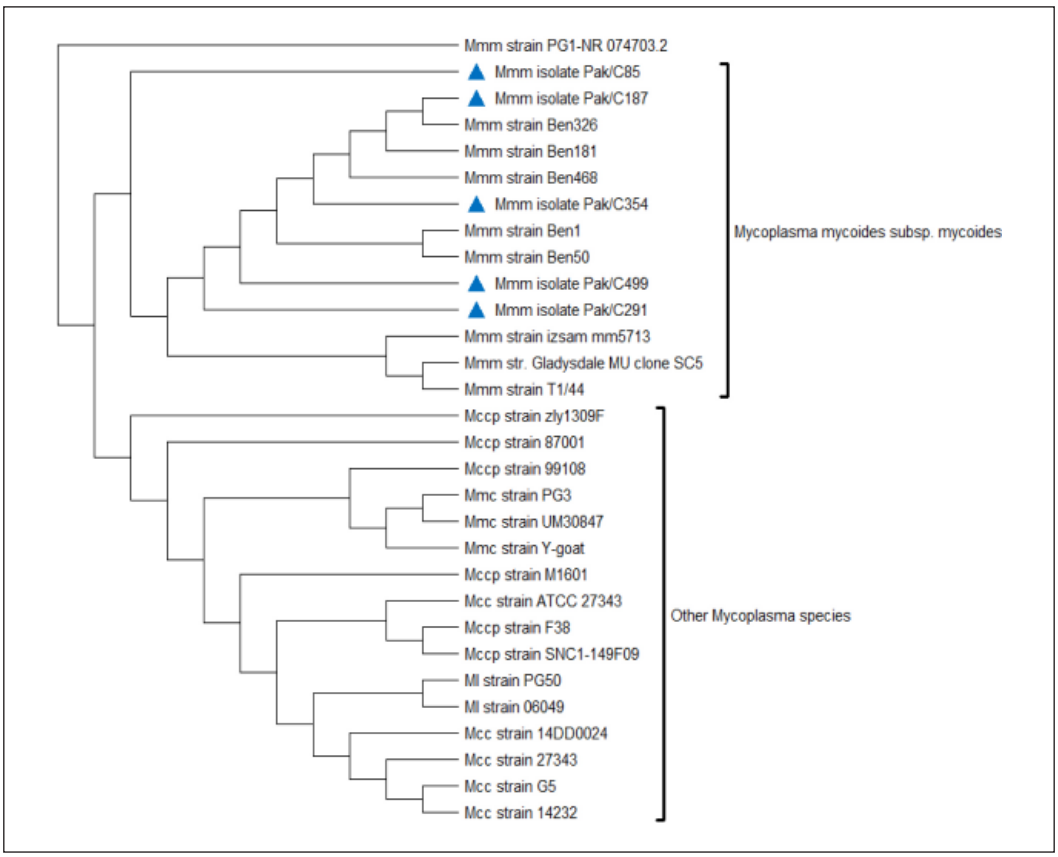

Figure 2. Phylogenetic tree based on the 16S rRNA gene sequences of 5 field isolates (marked) and 25 NCBI-GenBank databases reference strains of *Mycoplasma mycoides* group.

Table 3. Pairwise distance analysis of nucleotide sequences of 16S rRNA gene (field identified isolates with other reference strains reported in NCBI-GenBank databases)

Strains	Nucleotide Identity (%)				
	PAK/C85	PAK/C187	PAK/C291	PAK/C354	PAK/C499
Ben 1	99.8	99.9	99.9	99.9	99.7
Ben 50	99.8	99.9	99.9	99.9	99.7
Ben 181	99.7	99.9	99.9	99.7	99.7
Ben 326	99.7	99.9	99.8	99.8	99.8
Ben 468	99.7	99.7	99.6	99.7	99.7
Izsam_mm5713	99.9	99.8	99.9	99.8	99.8
T1/44	99.9	99.8	99.9	99.8	99.8
Gladysdale	99.9	99.8	99.9	99.8	99.8
PG1	43.2	43.0	43.2	43.1	43.2

PG1(Portugal) with highly variable nucleotide sequence, was branched separately than that of other member of *Mycoplasma* groups as shown in Figure 2.

Pairwise sequence distance matrix analysis revealed that identified isolates had 99.6–99.9% nucleotide identity (%) with the strains reported in China, Australia, Tanzania, and Italy, while *Mmm* strain PG1(Portugal) had greater variations with 43.0-43.2% nucleotide identity (%) as shown in Table 3.

DISCUSSION

Contagious bovine pleuropneumonia is an important disease, having economic consequences listed by the World Organization of Animal Health. It is a major militating factor affecting cattle production in terms of animal protein supply and economic value. Diagnostic techniques for CBPP have been reviewed by many authors in recent years. DNA amplification by PCR provides a successful and rapid detection tool for *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*). In this study, 8.75% (49/560) lung samples of cattle were found positive thorough PCR targeting the 16S rRNA gene, the results are similar to our previous document in which using a sandwich enzyme-linked immunosorbent assay, we confirmed the seroprevalence of CBPP in Lahore, Kasur and Jhang districts, with 8.26, 8.20 and 10.06% seropositivity respectively with no observable significance difference (P>0.05) (Anjum *et al.*, 2019). The results are in agreement with the work of Musa *et al.* (2016), who found that CBPP can be confirmed from consolidated lung samples through PCR. In previous studies, *Mycoplasma mycoides* subsp. *mycoides* has been confirmed in lung samples thorough PCR targeting the 16S rRNA gene (Persson *et al.*, 1999; Bashiruddin *et al.*, 2005). Miserez *et al.* (1997) developed a nested PCR, targeting the *lipoprotein P72* gene of *Mmm* and found it to be sensitive enough to detect the antigen at a very low concentration even of 10-10^3 viable cells per mL. Hence detection of CBPP through PCR can be considered as a robust and rapid tool.

In our study, a significant difference (P<0.05) was observed regarding the prevalence of CBPP in cattle when categorized according to their age. Similarly, age was associated significantly (P<0.05) when compared to the results with seroprevalence of CBPP in Pakistan. The seropositivity of CBPP was greater in older animals as compared to young animals (Anjum *et al.*, 2019). Our results are in agreement with previous studies of Olabode *et al.* (2013), who reported the highest occurrence of disease in older animals as compared to young animals. Swai *et al.* (2013) conducted an abattoir-based survey and investigated that animals aged 6-18 months had a significantly high rate of disease as compared to younger ones. Hence, chances of disease incidence increase with age. Our findings tend to agree with previous studies conducted in Ethiopia by (Kassaye & Molla, 2013), who investigated the seroprevalence of CBPP in animals of less than 4 years, 4-7 years and more than 7 years of age were 3%, 5.2% and 9.5% respectively. Daniel *et al.* (2016) reported that the prevalence of CBPP in young and adult animals was 25.5% and 30.3% respectively, with no age-related statistical significance (P>0.05). Similarly, our results were in contradiction with the findings of Mamo *et al.* (2018), who reported that age was not significantly associated with the seropositivity of CBPP. It can be attributed to similar exposure to all animals in a herd as the disease is contagious and one animal might become continuous source of infection to other animals.

In our study, 11.50% (33/287) and 5.86% (16/273) lung samples were found positive in female and male cattle respectively, which were similar to findings of (Anjum *et al.*, 2019), who reported that seroprevalence of CBPP was significantly associated (P<0.05) with gender. Our findings were in agreement with the results of Malicha *et al.* (2017), who reported that the prevalence of the disease in male and female cattle was 12.9% and 28.1% respectively, hence the distribution of disease in female cattle was significantly (P<0.05) high. Our results were also in line with the findings of Séry *et al.* (2015), who reported that the prevalence of CBPP in male and female cattle was 15.6% and 19.72% respectively. Similarly, Olabode *et al.* (2013) also reported 33.3% and 66.7% distribution of CBPP in male and female cattle respectively. Mamo *et al.* (2018), also documented that percentage seroprevalence of CBPP in male and female were 7.1% and 8.8% respectively. Our results were also similar to Schnier *et al.* (2006), who reported a significantly higher prevalence in female cattle as compared to male cattle in Kenya. Higher prevalence of the disease in cows can be attributed to their

lower immunity level following reproductive stress as buttressed by the age frequency of the disease accompanied by the fact that male cattle are sold at younger age while cows are kept at herd for breeding purposes. In contrast to our findings, Daniel *et al.* (2016) reported that seroprevalence of disease in male and female cattle was 30.9% and 27.04% respectively, though statistically insignificant association (P>0.05) was found in terms of gender.

Table 2 revealed that 4.05% (3/74) and 9.47% (46/486) lung samples were found positive in the local breed and cross-bred cattle respectively, the results are similar to our previous study, where seroprevalence of CBPP was detected non-significantly (P>0.05) higher in cross-cred cattle as compared to local breed (Anjum *et al.*, 2019). The analysis of this study showed that the breed was not significantly associated (P>0.05) with the prevalence of the disease. Our results tend to be in line with the findings of Daniel *et al.* (2016), who reported that the percentage of CBPP in cross-bred animals was high in Ethiopia, with percentage prevalence in local and cross-bred animals was 27.4% and 37.2% respectively.

CONCLUSION

In conclusion, this study is valuable for reporting the current status of CBPP in Pakistan. For the first time, we have detected the *Mycoplasma mycoides* subsp *mycoides* (*Mmm*) in the lung samples of cattle through PCR. Hence, surveillance of CBPP throughout the country and implementation of the stamping-out strategies including culling of diseased animal/herd or vaccination campaigns, to control the disease, is strongly recommended.

Acknowledgments. This study was financially supported by the Higher Education Commission (Pakistan), Research Grant No. 20-4049/NRPU/R&D/HEC/14/689.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

Abdela, N. & Yune, N. (2017). Seroprevalence and Distribution of Contagious Bovine Pleuropneumonia in Ethiopia: Update and Critical Analysis of 20 Years (1996-2016) Reports. *Frontiers in Veterinary Science* **4**(1): 100-108.

Anjum, A., Aslam, A., Akhtar, R., Yaqub, T., Naseer, J., Mushtaq, A., Munir, M.A. & Khan, A.U. (2019). Seroprevalence of contagious bovine pleuropneumonia in cattle of Punjab, Pakistan and assessment of risk factors. *Indian Journal of Animal Research* 1-4.

Bashiruddin, J.B., de Santis, P., Persson, A., Ball, H. & Regalla, J. (2005). Detection of Mycoplasma mycoides subspecies mycoides SC in bovine lung and lymph node tissues by culture, sandwich ELISA and polymerase chain reaction systems. *Research in Veterinary Science* **78**(3): 199-205.

Daniel, G., Abdurahaman, M., Tuli, G. & Deresa, B. (2016). Contagious bovine pleuropneumonia: Seroprevalence and risk factors in Western Oromia, Ethiopia. *Onderstepoort Journal of Veterinary Research* **83**(1): 958-965.

Enyaru, J., Biryomumaisho, S., Balyeidhusa, A., Ebong, C., Musoni, A., Manzi, M., Rutagwenda, T., Zimurinda, J., Asiimwe, T. & 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**(1): 22-28.

Francis, M., Oragwa, A., Ankeli, P., Liba, J., Ejeh, E., Raji, M., Ameh, J. & Egwu, G. (2018). Prevalence of contagious bovine pleuropneumonia based on gross lesions in cattle at slaughter in Adamawa State, Nigeria. *Sokoto Journal of Veterinary Science* **16**(1): 31-37.

Hotzel, H., Sachse, K. & Pfützner, H. (1996). A PCR scheme for differentiation of organisms belonging to the Mycoplasma mycoides cluster. *Veterinary Microbiology* **49**(1-2): 31-43.

Kassaye, D. & Molla, W. (2013). Seroprevalence of contagious bovine pleuropneumonia at export quarantine centers in and around Adama, Ethiopia. *Tropical Animal Health and Production* **45**(1): 275-279.

Le Get, R. (2018). Contagious bovine pleuropneumonia within the 19th century miasmatic landscape. *Australian Veterinary Journal* **96**(8): 285-290.

Malicha, G., Alemu, S., Aklilu, F. & Abraha, A. (2017). Study of Seroprevalence and Associated Risk Factors of Contagious Bovine Pleuropneumonia in Sidama Zone, Southern Ethiopia. *Journal of Veterinary Science and Technology* **8**(5): 1-5.

Mamo, Y., Bitew, M., Teklemariam, T., Soma, M., Gebre, D., Abera, T., Benti, T. & Deneke, Y. (2018). Contagious Bovine Pleuropneumonia: Seroprevalence and Risk Factors in Gimbo District, Southwest Ethiopia. *Veterinary Medicine International* **2018**(1): 1-7.

Massimo, M. (2018). Lung lesion score system in cattle: proposal for contagious bovine pleuropneumonia. *Tropical Animal Health and Production* **50**(1): 223-228.

Miserez, R., Pilloud, T., Cheng, X., Nicolet, J., Griot, C. & Frey, J. (1997). Development of a sensitive nested PCR method for the specific detection of Mycoplasma mycoides subsp. mycoides SC. *Molecular and Cellular Probes* **11**(2): 103-111.

Musa, J.A., Bale, J.O., Kazeem, H.M., Nwankpa, N.D., Di Provvido, A., Sacchini, F., Zilli, K., Abass, A., Scacchia, M. & Pini, A. (2016). Molecular detection of Nigerian field isolates of Mycoplasma mycoides subsp. mycoides as causative agents of contagious bovine pleuropneumonia. *International Journal of Veterinary Science and Medicine* **4**(2): 46-53.

Muuka, G., Hang'ombe, B.M., Nalubamba, K.S., Kabilika, S., Mwambazi, L. & Muma, J.B. (2011). Comparison of complement fixation test, competitive ELISA and LppQ ELISA with post-mortem findings in the diagnosis of contagious bovine pleuropneumonia (CBPP). *Tropical Animal Health and Production* **43**(5): 1057-1062.

Olabode, H., Mailafia, S., Adah, B., Nafarnda, W., Ikpa, L., Jambalang, A. & Bello, R. (2013). Serological Evidence of Contagious Bovine Pleuro-Pneumonia antibodies in trade cattle (BosIndicus) sold in Kwara state-Nigeria. *Online International Journal of Microbiology Research* **1**(1): 14-19.

Onono, J., Wieland, B., Suleiman, A. & Rushton, J. (2017). Policy analysis for delivery of contagious bovine pleuropneumonia control strategies in sub-Saharan Africa. *Revue Scientifique et Technique-Office International des Epizooties* **36**(1): 195-205.

Persson, A., Pettersson, B., Bölske, G. & Johansson, K.-E. (1999). Diagnosis of Contagious Bovine Pleuropneumonia by PCR–Laser-Induced Fluorescence and PCR-Restriction Endonuclease Analysis Based on the 16S rRNA Genes of Mycoplasma mycoides subsp. mycoidesSC. *Journal of Clinical Microbiology* **37**(12): 3815-3821.

Schnee, C., Heller, M., Jores, J., Tomaso, H. & Neubauer, H. (2011). Assessment of a novel multiplex real-time PCR assay for the detection of the CBPP agent Mycoplasma mycoides subsp. mycoides SC through experimental infection in cattle. *BMC Veterinary Research* **7**(1): 47-54.

Schnier, C., Mtui-Malamsha, N., Cleaveland, S., Kiara, H., Grace, D., McKeever, D. & Zadoks, R. CBPP seroprevalence and associated risk factors in the Maasai-ecosystem of south-western Kenya. In: Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics: Cairns Australia; 2006: 150-165.

Sery, A., Sidibe, C.A., Cisse, O., Diallo, M., Kone, M., Waret-Szkuta, A., Roger, F., Thiaucourt, F. & Niang, M. (2015). Seroprevalence of contagious bovine pleuropneumonia (CBPP) in Mali. *Tropical Animal Health and Production* **47**(2): 395-402.

Swai, E., Mwezimpya, I., Ulicky, E., Mbise, A. & Moshy, W. (2013). An abattoir survey of contagious bovine pleuropneumonia lesions in slaughtered cattle in selected districts in Northern Tanzania. *Asian Pacific Journal of Tropical Biomedicine* **3**(4): 303-306.

Yansambou, M.S., Ferreira, A.C., Bada-Alambedji, R. & Botelho, A. (2018). Seroprevalence of Contagious Bovine Pleuropneumonia in Niamey Region, Niger. *Journal of Veterinary Medicine and Research* **5**(8): 1151.