B-4500
[1-8]

RESEARCH ARTICLE Indian Journal of Animal Research, Volume Issue : ()

Pathomicrobial and Molecular Investigations of Respiratory System Diseases Affecting Buffaloes

Charlie Sharma, Vikas Nehra, Deepika Lather,
Aman Kumar[1], Rajesh Chhabra[2], Gulshan Narang

10.18805/IJAR.B-4500

ABSTRACT

Background: Pathomicrobial and molecular investigations of respiratory system diseases was undertaken on twelve adult buffalo carcasses received for necropsy examination at post mortem facility of Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana during the period from August, 2018 to February, 2019.

Methods: After collection of the samples, laboratory work was undertaken in the laboratories of Department of Veterinary Pathology, Department of Animal Biotechnology and Central Laboratory of the College, LUVAS, Hisar, Haryana (India) in the year 2018-2019 regarding the examination of the clinical history, pathological, microbiological and molecular investigations.

Result: The gross pathological changes observed in lungs were variable degree of vascular changes, mild to severe consolidation, fibrin deposition along with adhesions to the thoracic wall. Histopathological findings revealed abnormalities of inflation such as pulmonary emphysema, atelectasis, pulmonary congestion and haemorrhages which was associated with different types of pneumonia *viz*. fibrinous bronchopneumonia, suppurative giant cell pneumonia, interstitial pneumonia and serous pneumonia. Lung, heart blood and tracheal swab samples collected from the buffalo carcasses revealed eighteen bacterial isolates which were identified by Vitek 2 system. These include *E. coli* (11 isolates), *Salmonella enteric enterica* (2 isolates), *Acinetobacter ursingii* (1 isolate), *Staphylococcus haemolyticus* (1 isolate), *Staphylococcus sciuri* (1 isolate), *Staphylococcus warneri* (1 isolate) and *Staphylococcus hominis* (1 isolate) mostly belonging to opportunistic pathogen category. *E. coli* serotypes confirmed from these cases were O83, O149 and O8. The results of *in-vitro* drug sensitivity testing revealed that most of bacterial strains were found sensitive to cefoperazone/sulbactum and co-trimoxazole. Molecular studies confirmed Bovine Herpes Virus-1 (BHV-1) infection in one case with Fibrinous bronchopneumonia through real-time quantitative PCR indicating the prevalence of the infection in the state.

Key words: Buffalo, Investigation, Molecular, Patho-microbial, Respiratory diseases, Sensitivity.

INTRODUCTION

The buffaloes play an integral role for improvement of rural economy in agriculture based developing countries. In the recent years, this productive, adoptive and multipurpose domestic animal has gained a significant attention nationally and internationally.

The world population of 200 million buffaloes has been distributed over 40 countries, but 97 percent population is confined to Asia and India with 109 million buffaloes hosting 57 percent of the total population (Hegde, 2019). Buffalo mortality due to respiratory affections is very common in Asian countries including India, regardless of the annual vaccination programmes being followed in the country.

Pneumonia causing respiratory distress in buffaloes is a major problem as it affects lungs confronting animal production and also resulting in economic loss (Villanueva *et al*., 2018). Deterioration of the hygienic conditions along with unusual rain, floods and improper managemental practices are some of the important factors that aggravate and promote pulmonary diseases in buffalo.

Respiratory problems have economic impacts in countries where livestock industry is an important segment of the agricultural sector, as well as these problems may cause significant economic losses for dairy farmers. A number of bacterial disease conditions like Pasteurellosis, Mycoplasmosis, *Mannheimia haemolytica* infection, *Histophilus somnus* infection, Tuberculosis; viral disease conditions like Bovine herpes virus-1 infection (BHV-1), Parainfluenza virus-3 infection (PI-3), bovine respiratory syncytial virus infection (BRSV), adenovirus; parasitic diseases primarily lungworm infestation and fungal infection like aspergillosis affect respiratory system of buffaloes

Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, Haryana, India.
[1]Department of Animal Biotechnology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, Haryana, India.
[2]College Central Laboratory, College of Veterinary Science, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, Haryana, India.

Corresponding Author: Vikas Nehra, Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, Haryana, India.
Email: drvikasnehra@gmail.com

How to cite this article: Sharma, C., Nehra, V., Lather, D., Kumar, A., Chhabra, R. and Narang, G. (2021). Pathomicrobial and Molecular Investigations of Respiratory System Diseases Affecting Buffaloes. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4500.

Submitted: 28-04-2021 **Accepted:** 19-07-2021 **Online:** 08-08-2021

resulting in the heavy mortality and decline in overall production (Fagiolo *et al*., 2005). Stress also plays an important role in causation of this respiratory disease complex and the various stressors include changes of feed, variation in ambient temperature, humidity and weather. The diagnosis of respiratory disease complex poses a significant challenge as numerous infectious aetiologies are operating either singly or concomitantly and the clinical signs of most of the infection usually mimic.

Correctly determining the cause of death permits one to apply effective measures to prevent further loss. That is why, it is desirable to know the clinico-pathological and microbiological aspect of the disease conditions causing mortality. So that, adequate therapeutic and preventive measures can be taken to prevent further losses. Antimicrobial resistance is another problem that veterinarians are facing now a days. Antimicrobial resistance is rising to dangerously high levels in all parts of the world. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases. *In-vitro* chemotherapeutic sensitivity testing provide knowledge about antimicrobial of choice, which further helps in specific treatment against isolated bacterial strain and thus increasing the overall health and productivity of animals and gains to the dairy industry. Keeping in view the above facts, the present study was envisaged to investigate the pathomicrobial and molecular investigations of respiratory system diseases affecting buffaloes.

MATERIALS AND METHODS

The present study was conducted on twelve adult buffalo carcasses suspected of respiratory disorders brought to the Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, Haryana from the month of August, 2018 to February, 2019.

Necropsy examination

All the carcasses were thoroughly examined externally for any injuries, markings, unusual secretions and subsequently any gross pathological lesions in the organs of the respiratory system (mainly lungs, trachea, mediastinal lymph nodes) and other associated systems (*viz.* heart, liver, spleen, kidney, intestine *etc*.) were carefully observed. Representative tissue samples primarily from respiratory system such as lung and trachea along with other secondarily affected organs like heart, mediastinal lymph node, intestine, liver, spleen and kidney were collected in 10% buffered formalin for histopathological examination through H and E staining (Luna, 1968) and the special staining procedures (Luna, 1968).

Microbiological studies

Isolation and identification of bacteria

To identify bacterial agents associated with respiratory diseases, samples from the heart blood, lung tissue and tracheal swabs were collected aseptically in sterile containers during post mortem examination. Aseptically collected samples were inoculated on Nutrient agar (NA), blood agar (BA) and/or Mac Conkey's Lactose agar (MLA) plates and were incubated at 37^{0}C for 24 hrs. The plates were examined for the presence and type of growth, hemolysis and were sub-cultured whenever required. Pure bacterial cultures were examined morphologically by Gram's staining and biochemical characterization using single colonies by Vitek-2 system (BioMerieux, Inc. Hazelwood, MO, USA).

In-vitro drug sensitivity assay

Bacterial isolates were subjected to antimicrobial sensitivity testing by using disc diffusion method as described by Bauer *et al*. (1966). Briefly, test culture was inoculated into tryptic soya broth using a sterile platinum loop and incubated at 35^{o}C for 2-5 hrs till development of turbidity. The broth culture was evenly spread by smearing over Mueller Hinton agar plates and the discs of standard concentrations were placed and pressed on the agar gently using a sterile forceps at a distance of 24 mm (centre to centre) to have a close contact with the medium. The plates were incubated at 37^{o}C for 24 h and the sensitivity was recorded as sensitive (S) and resistant (R) using zone size interpretation chart provided by the manufacturer.

Serotyping of bacterial isolates

Positive isolates of *E. coli* and *Salmonella* spp. were sent to the National Salmonella and Escherichia Centre (NSEC), Central Research Institute, Kasauli, Himachal Pradesh for serotyping.

Molecular studies

During post-mortem examination representative tissue sample of lung along with heart blood were collected in separate sterile vials and stored at -20^{o}C for molecular diagnostic studies by using polymerase chain reaction (PCR) technique. Collected samples were screened for BHV-1/ Infectious Bovine Rhinotracheitis (IBR) virus by using the real time quantitative PCR and for *Pasteurella multocida* (causing Haemorrhagic Septicaemia) by conventional PCR. Total DNA was extracted using the commercial kit (PureLink Genomic DNA mini kit, Invitrogen) as per manufacturer's protocol. DNA quantity was determined using A_{260} values in spectrophotometer and the purity was judged using $A_{260/280}$ ratio >1.5-1.8. The PCR amplification of DNA using primer specific for *Pasteurella multocida* and Bovine Herpes Virus-1 (Table 1) were standardized by varying the concentration of the reaction mixture and cycling conditions. Reaction mixture composition of PCR for *Pasteurella multocida* consisted of 2.75 µl PCR master mix (2X); 0.5 µl Forward primer (50 pmol/µl); 0.5 µl Reverse primer (50 pmol/µl); 3.0 µl Template DNA; 2.25 µl Nuclease free water. All the reactions were performed in vertical 96 well thermocycler. Thermal profile of the PCR for *Pasteurella multocida* is given in Table 2. PCR products were analyzed using conventional agarose

Table 1: Sequence of primers used for *Pasteurella multocida* and Bovine Herpes Virus-1.

Organism		Sequence (5′-3′)	Reference
Pasteurella multocida (Conventional PCR)	Forward primer	KMT1SP6 (5′ - GCTGTAAACGAACTCGCCAC - 3′)	Ullah *et al.* (2009)
	Reverse primer	KMT1T7 (5′- ATCCGCTATTTACCCAGTGG - 3′)	
BHV-1(Real-time PCR)	Forward primer	(5′- TGTGGACCTAAACCTCACGGT - 3′)	Kumar *et al.* (2014)
	Reverse primer	(5′- GTAGTCGAGCAGACCCGTGTC - 3′)	
	Probe	(5′- FAM-AGGACCGCGAGTTCTTGCCGC-TAMRA - 3′)	

Table 2: Thermal profile of the PCR for *Mycobacterium bovis*, *Mycobacterium tuberculosis*.

Organism	Temperature	Time	No of cycle	Remarks
Pasteurella multocida	95°C	1 min	1	Initial denaturation
	95°C	15 sec	35 cycles	Denaturation
	60°C	15 sec		Annealing
	72°C	30 sec		Extension
	72°C	7 min	1	Final extension

Table 3: Thermal profile of real-time quantitative PCR for BHV-1/IBR virus.

Stages	Steps	Temperature	Time	Cycles
Holding stage	Step 1	95°C	20 sec	1
Cycling stage	Step 1 (Template denaturation and enzyme activation)	95°C	3 sec	40
	Step 2 (Annealing/Extension)	60°C	30 sec	
Melt curve Stage	Step 1	95°C	15 sec	1
	Step 2	60°C	1 min	
	Step 3	95°C	15 sec	

gel electrophoresis in 1.0 % w/v agarose. The amplified products were run in agarose gel in 1x TBE buffer containing ethidium bromide at 0.1 µg/µl. Quantitative Gene ruler DNA ladder were used as molecular size ladder. The DNA bands were visualized and imaged using the Molecular imager ®Chemi DocTM XRS-imaging system (Bio-Rad).

For the analysis of the relative expression of target gB gene of BHV-1/IBR virus, real time PCR was carried out in the laboratory using real time PCR Applied Biosystems (Step one plus) for data acquisition and analysis. For the real time PCR reaction, TaqMan universal qPCR Master Mix (Applied Biosystem) was used and all the instructions were followed as per the instruction manual. Primer sequence used to target the gB gene of BHV-1 virus along with probe are given in table 1. The reaction mixture used to carry out the real time PCR (for 10.00 µl reaction volume) consisted of 5 µlTaq man qPCR Master mix (2x); 0.4 µl Forward Primer (10 µM); 0.4 µl Reverse Primer (10.0 µM); 0.4 µl Probe; 0.8 µl Nuclease free water; 3.0 µl Template. Thermal profile of real-time quantitative PCR for BHV-1/IBR virus is given in Table 3.

RESULTS AND DISCUSSION

The adult buffalo carcasses considered in the present study were suspected on the basis of history of respiratory signs such as dyspnoea, nasal discharge along with dullness and anorexia. The gross pathological examination of carcasses revealed vascular changes in most cases, fibrin deposition and adhesion of lungs to the thoracic wall (Fig 1 and 2) in three cases, hydrothorax (2 cases) and petechial haemorrhages in 3 cases. Tracheal examination revealed frothy exudate (2 cases), redness (3 cases) along with presence of regurgitated feed material (Fig 3). Apart from lungs, gross changes found in heart were fibrinous adhesions on pericardium (7 cases), petechial haemorrhages (4 cases, Fig 4) and hydropericardium (3 cases). Pericardial sac filled with fibrino-purulent fluid along with thick fibrin layer on heart was seen in one case (Fig 5) and this was found associated with traumatic pericarditis. Liver revealed presence of congestion (6 cases), pale coloured necrotic foci (2 cases) and hepatomegaly (4 cases). Spleen revealed petechial haemorrhages (3 cases) and splenomegaly (4 cases). Mediastinal lymph nodes were found enlarged in 5 cases. Secondarily, digestive system was involved in 5 cases showing presence of catarrhal enteritis (2 cases), congestion and haemorrhage (2 cases). The gross pathological changes observed in adult buffalo lungs were congestion, haemorrhage, mild to highly consolidated areas of lung parenchyma, fibrin deposition, bronchitis and broncheolitis. Akbor *et al*. (2007) also reported similar findings in bovine pathology studies. There are variable causes of these pulmonary lesions as viral or bacterial infections, parasitism, allergic disease or exposure to irritants or toxins, inhalation of toxic gases, toxins that

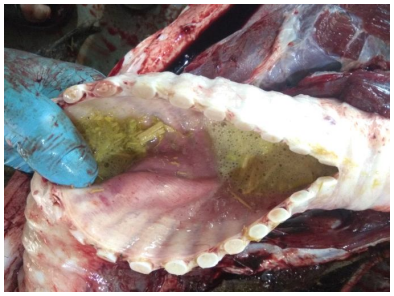

Fig 1: Thick sero-purulent layer on pleura along with purulent exudate in thoracic cavity (*Staphylococcus hominis*).

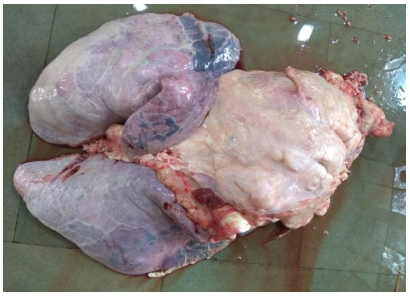

Fig 2: Focal consolidation of cardiac lobe of lungs along with fibrinous pericarditis and pleuritis (*Staphylococcus haemolyticus*).

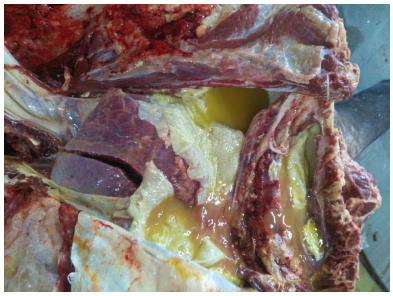

Fig 3: Trachea showing congested mucosa with regurgitated feed material (BHV-1/IBR virus).

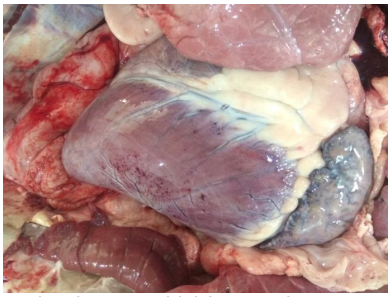

Fig 4: Heart showing petechial haemorrhages on myocardial surface (BHV-1/IBR virus).

Fig 5: Pericardial sac filled with fibrino-purulent fluid along with thick fibrin layer on heart (*Staphylococcus sciuri* in association with traumatic pericarditis).

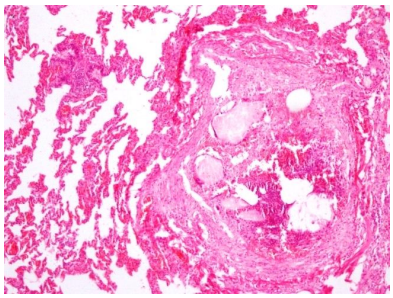

Fig 6: Presence of thrombosed mass in pulmonary blood vessel in a case of fibrinous bronchopneumonia (BHV-1/IBR virus). H&E 200X.

are metabolized by Cytochrome P450 in non-ciliated clara cells, hypersensitivity reactions or inflammatory reactions to inhaled irritants (Jubb *et al*., 2007). Apart from lungs, gross changes were also found in other associated organs such as heart, lymph nodes, liver, spleen and intestine. Belkhiri *et al*. (2009) and Devi (2011) found more or less similar lesions in their study.

Histopathological examination of lung tissues in adult buffaloes revealed abnormalities of inflation such as pulmonary emphysema, atelectasis, pulmonary congestion and haemorrhage which was associated with one or another type of pneumonia. Cases of pulmonary congestion and haemorrhages were mainly found associated with *E. coli* (O83) infection. Detailed histopathological changes observed in different types of pneumonia are described below in subsequent paragraphs.

Fibrinous bronchopneumonia was observed in one case which was characterized by congested alveolar capillaries, areas with emphysematous alveoli, accumulation of fibrinous exudate in alveolar lumen, thickened inter alveolar septa, infiltration of mononuclear cells mainly lymphocytes. There was presence of thrombosis in pulmonary blood vessels (Fig 6). Fibrin accumulation was also present in pleura with mild infiltration of mononuclear cells and presence of sero-fibrinous exudate in alveolar lumen indicating presence of fibrinous pleuritis (Fig 7). The pathogen detected in this case was BHV-1 that is responsible for causation of Infectious Bovine Rhinotracheitis (IBR). Verminous pneumonia was observed in one case characterized by the presence of cross sections of parasitic larvae in the lumen of alveoli (Fig 8) along with vascular changes as congestion and mild infiltration of mononuclear cells mainly lymphocytes. Suppurative giant cell pneumonia characterized by congestion, pink coloured liquefied pus material with infiltration of neutrophils, macrophages and giant cells (Fig 9), peribronchial lymphoid aggregates (Fig 10) was found in

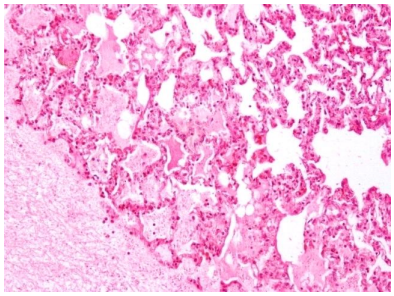

Fig 7: Fibrin accumulation in pleura along with presence of sero-fibrinous exudate in alveolar lumen (BHV-1/IBR virus). H&E 200X.

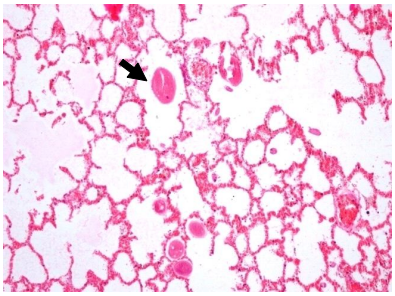

Fig 8: Verminous pneumonia characterized by the presence of cross sections of parasitic larvae (arrow) in the lumen of alveoli. H&E 200X.

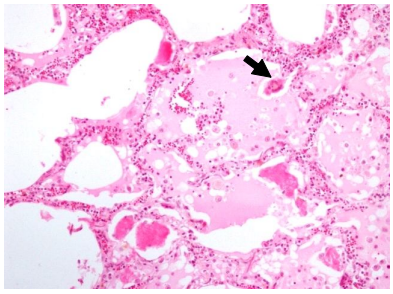

Fig 9: Suppurative giant cell pneumonia characterized by congestion, pink coloured liquefied pus material with infiltration of neutrophils, macrophages and giant cells (arrow). H&E 200X

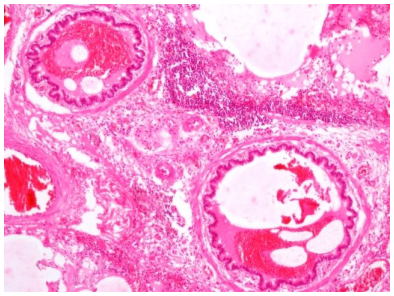

Fig 10: Presence of sero-fibrinous hemorrhagic mass in bronchial lumen and peribronchial lymphoid aggregates in a case of suppurative giant cell pneumonia (*E. coli* serotype O149, *Staphylococcus sciuri*). H&E 200X.

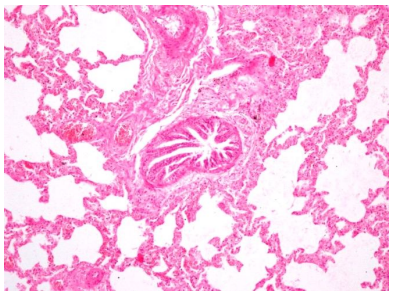

Fig 11: Interstitial pneumonia along with hyperplasia of bronchiolar epithelium (*Staphylococcus haemolyticus*, *Staphylococcus warneri*). H&E 200X.

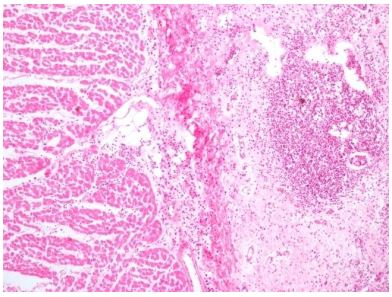

Fig 12: Pericarditis showing sero-fibrinous exudate, infiltration of leucocytes along with myocardial degeneration (*Staphylococcus sciuri*). H&E 200X.

one case. Organisms isolated were *E. coli* (O149) and *Staphylococcus sciuri*. Interstitial pneumonia was observed in one case and was characterized by the presence of congestion, haemorrhage, hyperplasia of bronchial and bronchiolar epithelium, thickening of alveolar septa, infiltration of mononuclear cells in the lung parenchyma and lymphoid follicular aggregates in the peri-bronchial regions (Fig 11). Organisms isolated in this case were *Staphylococcus haemolyticus* and *Staphylococcus warneri*. Serous pneumonia was present in two cases that were characterized by presence of congestion, haemorrhage, emphysema, serous fluid accumulation in the alveolar lumen, infiltration of lymphocytes and degenerated bronchiolar epithelium. Organisms isolated in this case were *E. coli* (serotypes detected were O83 and O8), *Staphylococcus hominis* and *Acinetobacter ursingii*. Tracheitis was a typical finding in many cases which was characterized by congestion along with infiltration of mononuclear cells in mucosal epithelium.

Apart from respiratory system histopathological changes were also observed in other associated organs as mediastinal and mesenteric lymph nodes which revealed presence of vascular changes, depletion of lymphocytes in cortical area and focal area of necrosis in medullary region. Pericarditis characterized by presence of sero-fibrinous exudate, infiltration of leucocytes in pericardium along with myocardial degeneration was seen in two cases (Fig 12). Liver showed telengiectasis, swollen hepatocytes with

cellular degeneration along with haemosiderosis. Spleen revealed severe congestion, haemorrhage and necrosis of lymphocytes in white pulp area. Kidney revealed presence of glomerulo-nephritis characterized by congested glomerular and tubular capillaries, degenerative tubular epithelium along with infiltration of mononuclear cells. There was also the presence of focal interstitial nephritis which showed congestion, tubular epithelium degeneration, infiltration of mononuclear cells in the interstitium.

Pulmonary emphysema and pulmonary congestion was reported previously by Joshi *et al*. (1994). In animals emphysema is always secondary to obstruction of out flow of air and occurs frequently in animals with bronchopneumonia as seen also in many cases in present study. Cases of buffaloes were found to be affected with different types of pneumonic conditions as Fibrinous bronchopneumonia, verminous pneumonia, Suppurative giant cell pneumonia and interstitial pneumonia. Similar observations were also reported by Ali *et al*. (2012). Earlier workers also observed similar results in adult buffalo carcasses as Fibrinous bronchopneumonia and pleuritis (Abdelbaset *et al.* 2014; Odugbol *et al*. 2005), verminous pneumonia (Mahmood *et al*. 2014), giant cell pneumonia (Devi 2011), serous pneumonia (Akbor *et al*. 2007), interstitial pneumonia (Sharma *et al*. 2011). Apart from respiratory system, histopathological changes observed in other associated organs as mediastinal and mesenteric lymph nodes, heart, liver, spleen and kidneys were also reported by Sushma *et al*. (2019) in their studies on ruminant carcasses.

Microbiological studies

A total of 36 different representative samples including that of lung, tracheal swab and heart blood were taken aseptically from all the carcasses. Out of these 36 samples, 20 samples showed growth on different agar plates. Vitek-2 system identified 18 different bacterial strains from these 20 cultures. The bacterial species isolated were *E. coli* (11 isolates), *Salmonella enteric enterica* (2 isolates), *Acinetobacter ursingii* (1 isolate), *Staphylococcus haemolyticus* (1 isolate), *Staphylococcus sciuri* (1 isolate), *Staphylococcus warneri* (1 isolate) and *Staphylococcus hominis* (1 isolate). Different serotypes of *Escherichia coli* detected were O83, O149 and O8.

From lung samples, *E. coli* (3 isolates), *Salmonella enteric enterica* (1 isolate), *Staphylococcus haemolyticus* (1 isolate), *Staphylococcus sciuri* (1 isolate) and *Staphylococcus hominis* (1 isolate) were detected. From heart blood, *E. coli* (1 isolate), *Salmonella enteric enterica* (1 isolate) and *Acinetobacter ursingii* (1 isolate) were detected. From tracheal swabs, *E. coli* (7 isolates) and *Staphylococcus warneri* (1 isolate) were detected.

Pathological association of isolated bacteria are already discussed along with pathological results.

***In-vitro* drug sensitivity testing**

The present investigation showed varying degree of sensitivity to the chemotherapeutic agents. *E. coli* strains were found to be most sensitive to chloramphenicol (94.29%), gentamicin (85.72 %), ceftriaxone/tazobactum (82.86%), cefoperazone/sulbactum (74.30%), streptomycin and co-trimoxazole (42.86%), ciprofloxacin (25.72%), tetracycline and enrofloxacin (22.86%), amoxyclav and ofloxacin (20.00%), moxifloxacin (17.20%), cloxacillin (14.30%), cefixime and erythromycin (8.60%). *E. coli* strains did not show resistance against any of the antibiotics.

Salmonella enterica enterica was found 100.00% sensitive to cefixime, gentamicin, streptomycin, chloramphenicol, co-trimoxazole and ceftriaxone/ tazobactum. *Salmonella entericaenterica* was 50% sensitive to cefoperazone/ sulbactum, cloxacillin, ciprofloxacin, enrofloxacin. On the other hand, *Salmonella enterica enterica* was 100.00% resistant to erythromycin, amoxyclav, tetracycline, ofloxacin and moxifloxacin.

Staphylococcus haemolyticus was found 100.00% sensitive to erythromycin, cefoperzone/sulbactum, tertracyclin, gentamicin, co-trimoxazole, chloramphenicol, cefixime, amoxyclav, streptomycin, moxifloxacin and ceftriaxone/tazobactum. On the other hand, *Staphylococcus haemolyticus* was 100.00% resistant to cloxacillin, ciprofloxacin, enrofloxacin and ofloxacin.

Staphylococcus sciuri was found 100.00% sensitive tocefoperzone/sulbactum, tertracyclin, gentamicin, moxifloxacin, co-trimoxazole, chloramphenicol, amoxyclav, cloxacillin, streptomycin and enrofloxacin. On the other hand, *Staphylococcus sciuri* was 100.00% resistant to erythromycin, cloxacillin, ceftriaxone/tazobactum, ciprofloxacin, cefixime and ofloxacin.

Staphylococcus warneri was found 100.00% sensitive to erythromycin, cefoperzone/sulbactum, tertracycline, gentamicin, moxifloxacin, co-trimoxazole, amoxyclav, streptomycin, enrofloxacin, ciprofloxacin and cloxacillin. On the other hand, *Staphylococcus warneri* was 100.00% resistant to ceftriaxone/tazobactum, chloramphenicol, cefixime and ofloxacin.

Staphyloccus hominis was found 100.00% sensitive to erythromycin, cefoperzone/sulbactum, tertracycline, gentamicin, moxifloxacin, co-trimoxazole, amoxyclav, streptomycin, enrofloxacin, ceftriaxone/tazobactum, chloramphenicol and ciprofloxacin. On the other hand, *Staphylococcus hominis* was 100.00% resistant to cloxacillin, cefixime and ofloxacin.

Acinetobacter ursingii was found 100.00% sensitive to ceftriaxone/ tazobactum, gentamicin, ciprofloxacin, co-trimoxazole, streptomycin and cefoperazone/sulbactum, amoxyclav, moxifloxacin, enrofloxacin and cloxacillin. On the other hand, *Acinetobacter ursingii* was 100.00% resistant to chloramphenicol, cefixime, tetracycline and ofloxacin.

Microbiological studies in adult buffaloes revealed the presence of *E. coli, Acinetobacter ursingii, Staphylococcus haemolyticus, Staphylococcus sciuri, Staphylococcus warneri, Staphylococcus hominis* and *Salmonella enteric enteric* from heart lood and lungs. Sayyari *et al*. (2011) also found almost similar pathogens in buffalo lungs. Though

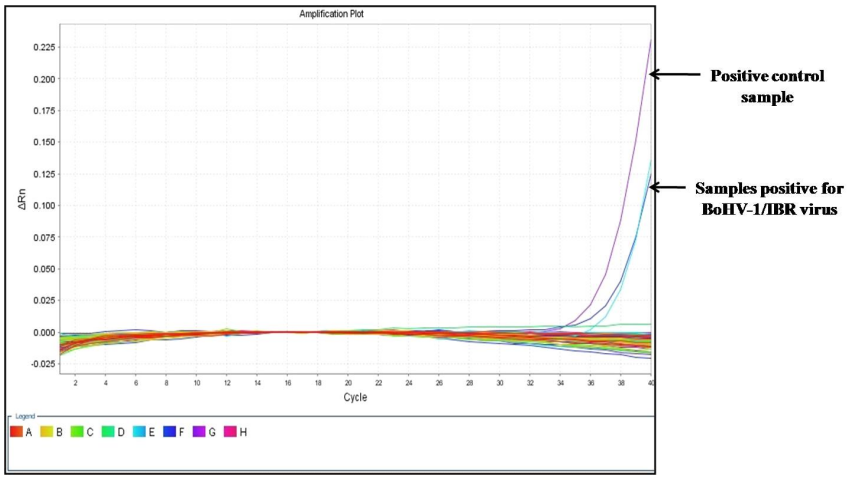

Fig 13: Linear amplification plot obtained after running the real-time quantitative PCR assay showing positive results insample of heart blood of adult buffalo for BHV-1/IBR virus

Staphylococcus haemolyticus has been isolated from adult buffaloes, there are very few reports available in the literature which shows isolation of *Staphylococcus sciuri, Staphylococcus warneri, Staphylococcus hominis* from respiratory system of adult buffaloes. *E. coli* strains isolated from adult buffaloes belonged to serotypes O83, O149, O8. Serotypes O8 and O83 of *E. coli* are normal inhabitant of buffaloes whereas O149 is an enterotoxigenic *E. coli*. Jamalludeen *et al*. (2009) also found similar types of serotypes in adult buffaloes in their study. *Salmonella enteric enterica* strain isolated from heart blood and lung of buffalo belonged to serotype *Salmonella welteverden*. Singh *et al.* (2010) has observed similar finding in their study. More or less similar results with respect to antimicrobial susceptibility resistance patterns have been reported previously by Singh *et al.* (2010) and Lehreena *et al*. (2012).

Molecular studies

Detection of *Pasteurella multocida* by conventional PCR assay

Conventional PCR assay was carried out from samples of lung and heart blood for detection of *Pasteurella multocida* in all the cases. All the twenty four samples were found negative as PCR products of expected size (560bp) did not appear in Agarose Gel Electrophoresis (AGE).

Detection BHV-1/Infectious Bovine Rhinotracheitis (IBR) virus by real-time quantitative PCR assay

Real time quantitative polymerase chain reaction assay was employed for the detection of BHV-1/IBR virus in the all the cases (lung and heart blood samples). Out of 24 samples screened only one sample of heart blood was found positive as shown in linear amplification plot obtained after running the Real-time quantitative PCR assay (Fig 13). The positive samples showed C_T value more than 33.83. Molecular studies confirmed the diagnosis of Interstitial pneumonia (BHV-1) in one case in present study through real time quantitative PCR indicating the prevalence of the viral infection in Haryana state. The BHV-1 positive buffalo was affected with fibrinous bronchopneumonia and other mixed type infection lesions. Thonur *et al*. (2012) also found similar results in their study on BHV-1. Bovine Herpes virus infection is reported to cause immunosuppression in buffaloes (Winkler *et al.* 1999). This immunosuppression may increase the susceptibility of host to opportunistic pathogens and thus aggravating the disease condition and multiple lesions.

CONCLUSION

In the present study, attempts were made to identify the number of pathogens associated with respiratory affections of adult buffaloes. Correlations of different pathogens with pathological findings were also attempted in cases having concurrent infections. Bovine Herpes Virus was reported in one adult buffalo having fibrinous bronchopneumonia along with mixed infections indicating the prevalence of this viral infection. Many opportunistic emerging pathogens were reported that can affect respiratory system of adult buffaloes under unfavourable stress conditions. Proper attention needs to be focussed on such pathogens and appropriate antimicrobial therapy should be directed to treat these conditions.

ACKNOWLEDGEMENT

Author(s) are highly thankful to the funding agency Rashtriya Krishi Vikas Yojna (RKVY) as the study was supported in part by the grant given to the Department of Veterinary Pathology, LUVAS, Hisar for RKVY-RAFTAAR project on “Establishment of immuno-histo-chemical laboratory and strengthening of immunotechnology laboratory for diagnosis of diseases of animals and poultry”.

REFERENCES

Abdelbaset, E., Abdelbaset, M.R., Ellah, A., ElGhaffar, S.K.A. and Sadiek, A.H. (2014). Acute-phase proteins in different pathological conditions at the lungs of buffaloes. Comparative Clinical Pathology. 23(4): 823-828.

Akbor, M., Haider, M.G., Ahmedullah, F., Khan, M.A.H.N.A., Hossain, M.I. and Hossain, M.M. (2007). Pathology of trachea and lungs of buffaloes. Bangladesh Journal of Veterinary Medicine. 5: 87-91.

Ali, S.A., Dawood, K.A. and Al-Oumashi, G.B. (2012). Hydatidosis of buffalo with secondary bacterial invaders. Kufa Journal for Veterinary Medical Sciences. 3: 104-10.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Truck, M. (1966). Antibiotic susceptibility testing by standardized single disc method. American Journal of Clinical Pathology. 45: 493.

Belkhiri, M., Tlidjane, M., Benhathat, Y. and Meziane, T. (2009). Histopathological study and pulmonary classification of bovine lesions. African Journal of Agricultural Research. 4(7): 584-591.

Devi, M.A. (2011). Pathomorphological studies on lung lesions in buffaloes. M.V.Sc. thesis submitted to SVVU, Tirupati.

Fagiolo, A., Roncoroni, C., Lai, O., Borghese, A. (2005). Buffalo pathologies. Buffalo production and research. Antonio Borghese (Ed.), FAO Regional Office for Europe, Rome, Chapter XIII. 249-296.

Hegde, N. G. (2019). Buffalo husbandry for sustainable development of small farmers in India and other developing countries. Asian Journal of Research in Animal and Veterinary Sciences. 3(1): 1-20.

Jamalludeen, N., Johnson, R.P., Shewen, P.E. and Gyles, C.L. (2009). Evaluation of bacteriophages for prevention and treatment of diarrhea due to experimental enterotoxigenic *Escherichia coli* O149 infection of pigs. Veterinary Microbiology.136: 135-141.

Joshi, M.D., Degloorkar, N.M., Moregaonkar, Kulkarni, G.B., Deshpande, B.B. and Digraskar, S.U. (1994). Age and sex wise incidence of pathological conditions of lung in buffalo and buffalo. Indian Veterinary Journal. 71: 768-770.

Jubb, K.V.F., Kennedy, P.C. and Palmer, N. (2007). Pathology of Domestic Animals. 5th Edn, Vol.2, pp: 523-653.

Kumar, A., Batra, K., Ghosh, A., Maan, N. S., Sunayna., Nanda, T. and Maan, S. (2014). PCR based screening of bulls for BoHV-1 infection in Haryana. Advances in Animal and Veterinary Sciences. 2: 35-38.

Lehreena, Gupta, R.P., Sindhu, N. and Nehra, V. (2012). Molecular characterization and antibiogram of *Escherichia coli* isolates implicated in calf mortality. Haryana Veterinarian 51: 41-44.

Luna, L. G. (1968). Manual of histologic staining method of Armed Forces Institute of Pathology, 3rd Edn., McGraw Hill Book Company, New York.

Mahmood, F., Khan, A., Hussain, R. and Anjum, M.S. (2014). Prevalence and pathology of *Dictyocaulus viviparous* infection in buffalo and buffaloes. Journal of Animal and Plant Science. 24(3): 743-748.

Odugbol, M.O., Turaki, U.A., Itodol, A.E., Okwori, A.E.J. and Yakubu, R.A. (2005). Experimental Haemorrhagic Septicemia of calves with Pasteurellamultocida serotype E:2: clinical, pathologic and microscopic studies. Journal of Tropical Livestock Science. 8(3): 133-137.

Sayyari, M. and Sharma, R. (2011). Pathological and bacteriological study of lobe distribution of lesions in buffalo (*Bubalus bubalis*). International Journal of Veterinary Research. 5(2): 125-128.

Sharma, R.K., Patil, R.D., Kishtwaria, R.S. and Asrani, R.K. (2011). An outbreak of pneumonic mannheimiosis in a livestock farm in sub-temperate region of India. Haryana Veterinarian. 50: 89-91.

Singh B., Agarwal M., Chandra M., Verma M., Sharma G., Verma J. and Singh V. (2010). Plasmid profile and drug resistance pattern of zoonotic Salmonella isolates from Indian buffaloes. The Journal of Infection in Developing Countries. 4: 477-483.

Sushma, Nehra, V., Deepika and Kundu, P. (2019). Histochemical staining procedures for differentiation of tissue/cell components in lambs and kids carcasses. The Pharma Innovation Journal. 8(2): 178-181.

Thonur, L., Maley, M., Gilray, J., Crook, T., Laming, E., Turnbull, D., Nath, M. and Willoughby, K. (2012). One-step multiplex real time RT-PCR for the detection of bovine respiratory syncytial virus, bovine herpesvirus 1 and bovine parainfluenza virus 3. BMC Veterinary Research. 8: 37.

Ullah, W., Abubakar, M., Arshed, M. J., Jamal, S. M., Ayub, N. and Ali, Q. (2009). Differentiation of closely related Vaccinal Strains of *Pasteurella multocida* using Polymerase Chain Reaction (PCR). Vet Scan. 4(1).

Villanueva, M.A., Mingala, C.N., Tubalinal, G.A.S., Gaban, P.B. V., Nakajima, C. and Suzuki, Y. (2018). Emerging infectious diseases in water Buffalo: An economic and public health concern. Intech Open. DOI: 10.5772/intechopen.73395.

Winkler, M.T.C., Doster, A. and Jones, C. (1999). Bovine herpesvirus 1 can infect CD4+ T lymphocytes and induce programmed cell death during acute infection of buffalo. Journal of Virology. 73(10): 8657-8668.