Haryana Vet. (June, 2020) **59(1),** 42-46

Research Article

AETIO-PATHOLOGICAL STUDY OF RESPIRATORY AFFECTIONS IN BUFFALO CALVES

CHARLIE SHARMA*, VIKAS NEHRA, DEEPIKA LATHER, RAJESH CHHABRA[1], AMAN KUMAR[2], GULSHAN NARANG and R.P. GUPTA

Department of Veterinary Pathology, [1]College Central Laboratory, [2]Department of Animal Biotechnology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar-125004, India

Received: 12.06.2019; Accepted: 01.08.2019

ABSTRACT

Aetio-pathological study was undertaken for the respiratory affections of twelve buffalo calves received for necropsy at post mortem facility of LUVAS, Hisar during the period of seven months from August, 2018 to February, 2019. Maximum mortality was noticed in the age group of 0-3 months followed by 3-6 months. The mortality was higher in females as compare to males. Grossly, the lesions observed were congestion, consolidation, fibrinous deposits on the pleura and hydrothrax. Histopathologically, sero-fibrinous and interstitial pneumonia were observed. 18 bacterial cultures from lung, heart blood and tracheal swab revealed *E. coli* (14 isolate), *Staphylococcus vitulinus* (1 isolate), *Aerococcus viridans* (1 isolate), *Providencia struatii* (1 isolate) and *Acinetobacter baumanni* (1 isolate). Serotypes of *E. coli* detected from these isolates were O83, O134, O149, O120, O118, O2. Maximum numbers of bacterial species were isolated from tracheal swab followed by lung. The results of *in vitro* drug sensitivity to different bacterial species isolated from carcasses of buffalo calves revealed that most of bacterial strains were found sensitive to cefoperazone/sulbactum and resistant to cloxacillin.

Keywords: Buffalo calves, Interstitial pneumonia, *In Vitro* Drug Sensitivity, Sero-fibrinous pneumonia, Serous pneumonia

Livestock plays an important role in Indian economy through the animal produce like milk, meat, wool, draught power etc. thereby contributing significantly to total agricultural economy. Calves are the livestock industry of the future. The care of buffalo calves is not only essential for sustenance of the dairy industry but is also essential in the wake of preserving and maintaining good quality germplasm (Tiwari *et al.*, 2007). According to USDA (USDA Part 1, 2002) respiratory diseases are responsible for 21.30 % of mortality in pre-weaned calves and 30.4% of deaths in weaned heifers. Diseases of buffalo calves results in heavy financial losses, which may occur, not only in the form of mortality but also because of decreased productivity from recovered buffalo calves of both the sexes, limited scope for selection of breeding animals, delay in rebuilding of herds and loss of lactation etc.

Therefore, to attain maximum gains from livestock industry, it is vitally an important aspect that young ones should be reared into healthy and productive animals. Respiratory system disorders/diseases play an important role in the morbidity and mortality of buffalo calves (Singh *et al.*, 2013). BRD (Bovine respiratory disease complex) in bovines is a multifactorial disease complex including infection with a wide range of conditionally or obligatory pathogenic viruses, bacteria and exposure to stressors (Duff and Galyean, 2007). Although, there are innumerable number of pathogens that contribute to BRD, the clinical signs of most of the infections usually mimic. Typical signs include rapid respiration, anorexia, nasal and/or ocular discharge, depression, fever, bronchitis, rhinitis, pneumonia and reproductive failure. A number of bacterial diseases conditions like colibacillosis, pasteurellosis, mycoplasmosis, *Mannheimia haemolytica* infection, *Histophilus somnus* infection, salmonellosis. (Griffin *et al.*, 2010); viral disease conditions like bovine herpes virus-1 infection (BHV-1), parainfluenza virus-3 infection (PI-3), bovine respiratory syncytial virus infection (BRSV), adenovirus, infectious bovine rhinotracheitis (IBR) and bovine viral diarrhoea virus infection (BVDV) infection (Fulton *et al.*, 2017); parasitic disease primarily lung worm infestation and fungal infection like aspergillosis affects respiratory system of buffalo calves. This early morbidity and mortality represents an important source of economic loss to the farmer due to loss of the present value of the calf and loss of genetic potential for herd improvement.

MATERIALS AND METHODS

Clinical History: Clinical history, treatment given and other details were recorded from the post mortem requisition form and the mortality pattern was analyzed.

Pathological examination: The carcasses were examined for any injuries, markings etc. and observed for any gross pathological lesions in the respiratory system (lungs, trachea, mediastinal lymph nodes) and other associated systems (heart, liver, spleen, kidney, intestine etc.) carefully. Representative tissue samples primarily from respiratory system such as lung and trachea along with other affected organs like heart, mediastinal lymph node, intestine, liver, spleen, kidney were collected in 10% buffered formalin for histopathological examination.

*Corresponding author: charliesharma4@gmail.com

Microbiological studies: Lung, heart blood and tracheal swab samples were collected aseptically in sterile petri plates/swab for microbiological studies. Aseptically collected heart blood, tracheal swab and lung tissues were inoculated on Nutrient agar (NA), blood agar (BA) and/or Mac Conkey's Lactose agar (MLA) plates and were incubated at 37 ºC for 24 h. 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 the College Central Laboratory facility of the College of Veterinary Science, LUVAS, Hisar. Pure cultures of bacteria were stored on solid agar for further studies.

Drug sensitivity assay: All the isolates of bacteria were subjected to antimicrobial sensitivity test by disc diffusion method. Briefly, test culture was inoculated into tryptic soya broth using a sterile platinum loop and incubated at 35 ºC for 2-5 h till development of turbidity. The broth culture was evenly spread by smearing over Mueller Hinton agar plates and the antibiotic 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 for 24 h at 37 ºC 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* were sent to the National Salmonella and Escherichia Centre (NSEC), Central Research Institute, Kasauli, Himachal Pradesh for serotyping.

RESULTS AND DISCUSSION

As per the information recorded from the post-mortem requisition form, most of the buffalo calves had sudden death with no clinical signs and symptoms. Besides, in some of the cases, treatment given prior to death was also written. The buffalo calves which were having signs of respiratory problem, dyspnoea and weakness were treated with dextrose, normal saline solution and antibiotics. The animals which were having signs of pneumonia were found treated with antibiotics like amoxyclav and ceftriaxone. Maximum age wise mortality in buffalo calves was in age group of up to 3 month (9 cases, 75.00 %) followed by 3-6 months (3 cases, 25.00 %). Shakya *et al.* (2017) also reported the similar observations in their study.

Sex-wise mortality was more in female buffalo calves (5 cases, 41.66 %) of up to 3 months as compared to male buffalo calves (4 cases, 33.33 %). Similar finding was also reported by Seema (2004) in buffalo calves. In case of buffalo calves of 3-6 month age, mortality was highest in male buffalo calves (2 cases, 16.66 %) as compare to female buffalo calves (1 case, 8.33 %).

Grossly in buffalo calves, different lesions such as consolidated purple red or grey color areas, congestion, patchy haemorrhages, pale lungs with white coloured fibrin deposition on pleural surface (Fig. 1) and focal areas of consolidation were noticed. Trachea revealed the presence of congestion in the lumen. Apart from lungs, gross change were also found in other associated organs such as heart, lymph nodes, liver, umbilical cord and spleen. Gross lesions observed in heart were congestion, presence of ecchymotic haemorrhages, hydropericardium showed presence of serosanguinous fluid in the pericardial sac (Fig. 2). Liver showed congestion, pale coloured necrotic foci along with distended gall bladder and hepatomegaly. In one case, distension of gall bladder alone was also noticed. Spleen revealed mild splenomegaly. Mediastinal lymph nodes were found enlarged. Adhesion of umbilicus with peritoneum along with omphalitis characterized by presence of purulent material in navel region was also observed. Khin *et al.* (2010) also observed similar pathological findings in his study.

Histopathological findings in buffalo calves revealed abnormalities of inflation viz. pulmonary emphysema, atelectasis, pulmonary congestion and haemorrhage which was associated with one or another type of pneumonia. Three types of pneumonia were seen in buffalo calves including interstitial pneumonia, sero-fibrinous pneumonia and serous pneumonia. Interstitial pneumonia (03 cases) was characterized by thickening of inter alveolar septa, congestion, emphysema, infiltration of mononuclear cells mainly lymphocytes, atelectasis, thickening of blood vessel walls, damage to the bronchiolar epithelium, edema and haemorrhages (Fig. 3) These findings related to interstitial pneumonia are in accordance with the earlier findings of Mahmood *et al.* (2000) in buffalo calves. Sero-fibrinous pneumonia (05 cases) was characterized by presence of sero-fibrinous fluid in alveolar lumen; infiltration of mononuclear cells mainly lymphocytes, congestion, haemorrhage along with emphysema (Fig. 4). Fibrinous pleuritis was evident in sero-fibrinous pneumonia cases that were characterized by the thickening of pleura along with congestion and fibrin accumulation (Fig. 5). Trachea revealed the presence of tracheitis characterized by the desquamation of tracheal epithelium along with infiltration of mononuclear cells

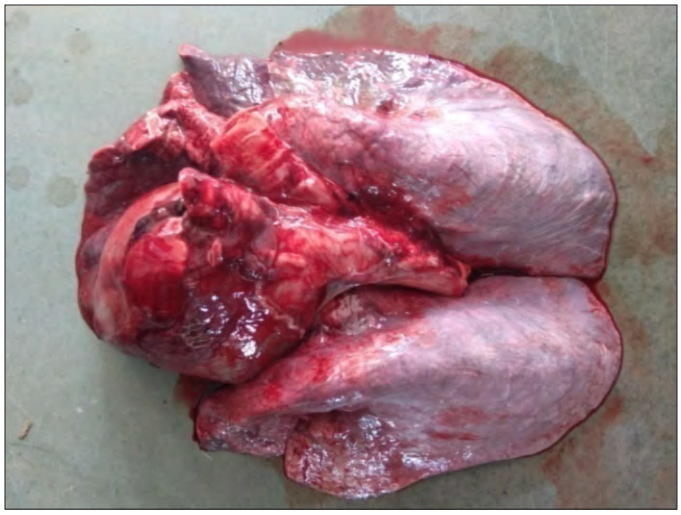

Fig. 1. Pale lung with white colored fibrin deposition on pleural surface (Buffalo calf)

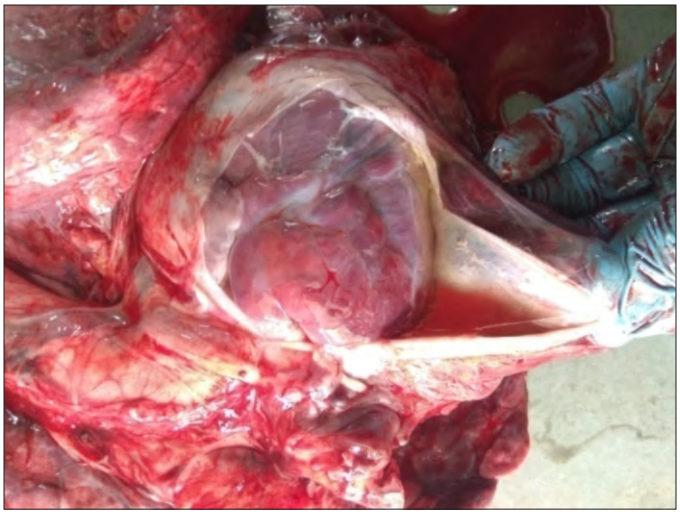

Fig. 2. Hydropericardium showing blood tinged fluid in pericardial sac (Buffalo calf)

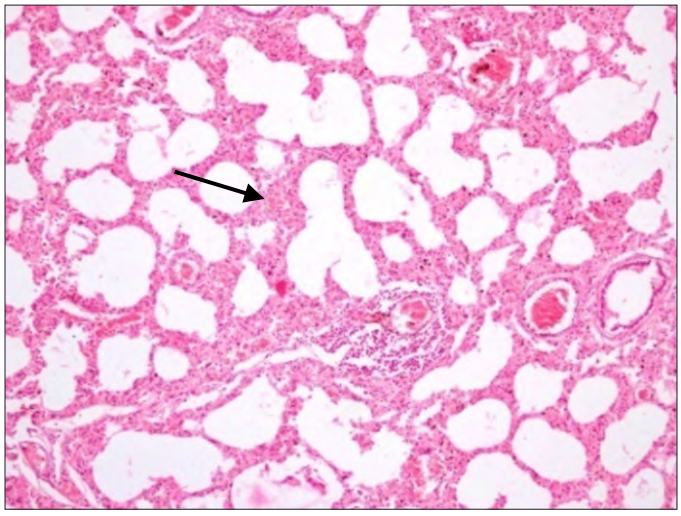

Fig. 3. Interstitial pneumonia showing thickened inter-alveolar septa (arrow), congestion and mononuclear cell infiltration (Buffalo calf, *Providencia stuartii*, *E. coli* serotype O2). H&E 200X

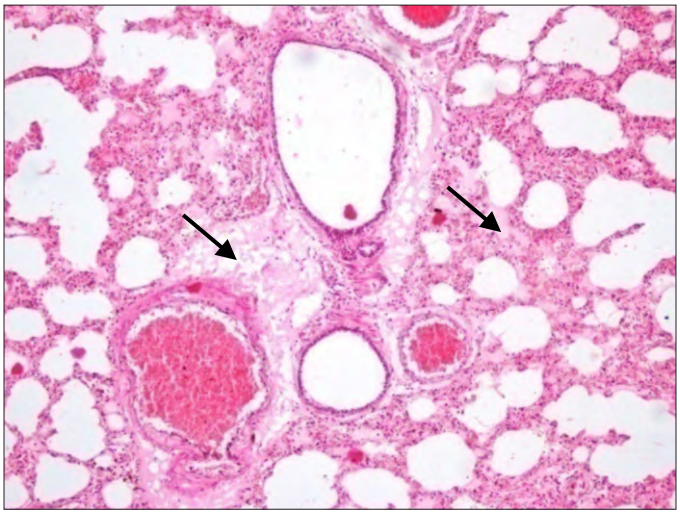

Fig. 4. Sero-fibrinous bronchopneumonia showing congestion, presence of sero-fibrinous fluid in inter-alveolar septa (arrow) along with lymphocytic cells infiltration (Buffalo calf, *E. coli* serotypes O120, 118, 134; *Aerococcus viridans*). H&E 200X

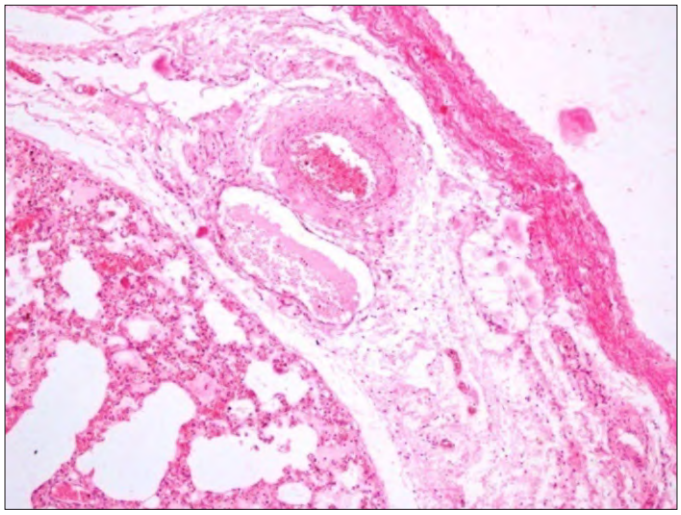

Fig. 5. Fibrinous pleuritis showing thickening of pleura, congestion, leucocyticinfiltration and presence of fibrin (Buffalo calf, *E. coli* serotypes O120, 118, 134; *Aerococcus viridans*). H&E 200X

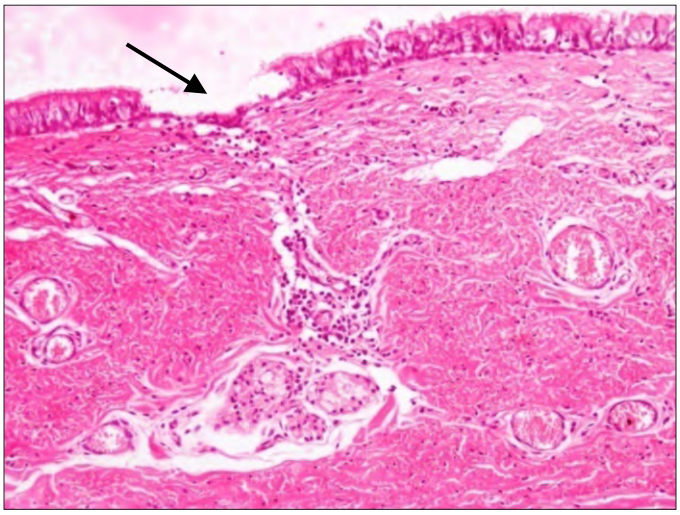

Fig. 6. Tracheitis showing desquamation of tracheal epithelium (arrow) in the mucosal layer along with infiltration of mononuclear cells (Buffalo calf, *E. coli* serotypes O120, 118, 134; *Aerococcus viridans*). H&E 200X

(Fig. 6). Praveena *et al.* (2014) also described the similar changes in their study. Serous pneumonia (01 case) was characterized by presence of serous fluid in alveoli, congestion; infiltration of leucocyte and degeneration of bronchial epithelium.

Histopathological changes were also observed in other associated organs of buffalo calves. In heart, fragmentation and degenerative changes of cardiac muscle fibres was noticed along with presence of sarcocysts. Other changes noticed were congestion, haemorrhage and infiltration of mono nuclear cells predominantly lymphocytes. In liver, necrotic hepatitis characterized by presence of focal areas of necrosis of hepatocytes, leucocytic cells infiltration in portal triad area and telangectiasis was observed. Cloudy swelling of hepatocytes was observed. In spleen, haemorrhages and depletion of lymphocytes in white pulp along with focal area of necrosis were observed. Intestine revealed presence of severe parasitic enteritis due to intestinal coccidiosis characterized by the presence of different stages of coccidial oocysts, congestion and lymphocytic cells infiltration.

Microbiological study of different samples collected from carcasses of buffalo calves revealed presence of *E. coli*, *Acinetobacter baumanni*, *Providencia stuartii* and *Staphylococcus vitulinus*. *E. coli* was isolated from 14 samples which included 9 of tracheal swabs, 4 of lungs, 1 of heart blood. *Acinetobacter baumanni* and *Staphylococcus vitulinus* was isolated from one lung sample each and *Providencia stuartii* was isolated from one tracheal swab sample. Though, *Acinetobacter baumanni* has been isolated from pneumonic cases of cattle but reports are not available in the literature which shows its isolation from buffalo calf lungs or tracheal swab. Ribeiro *et al.* (2000) isolated *Providencia struatii* from buffalo calves. Maximum numbers of bacterial species were isolated from tracheal swab followed by lungs and heart blood. *E. coli* strains isolated from buffalo calves belonged to serotypes O2, O8, O120, O118, O83, O149 and O134. O83. Here, it is necessary to mention that serotypes O2 and O8 of *E. coli* are normal inhabitant of buffalo calves and buffalo, whereas O149 is an Enterotoxigenic *E. coli*. Jamalludeen *et al.* (2009) first time reported serotypes O134 and O120 from buffalo calves lung. O118 is pathogenic but it mainly causes enteritis and as per literature it has not yet been isolated from respiratory tract. O118 is of zoonotic importance as it causes enteritis in humans. Beutin *et al.* (2000) also revealed the presence of serotype O118. Furthermore, here it is pertinent to mention that organism isolated from cases of pulmonary congestion and haemorrhages was *E. coli* (serotypes O83, O134, O149); from the interstitial

Table 1

Overall in vitro drug sensitivity (%) testing against different bacterial species isolated from different samples collected from buffalo calves carcasses

Group of antibiotics	Antibiotics/ drugs used	*E. coli*	*Providencia stuartii*	*Staphylococcus vitulinus*	*Acinetobacter baumanni*	*Aerococcus viridans*
Macrolides	Erythromycin	8.60	0.00	100.00	100.00	66.60
Cephalosporin/Beta lactamase inhibitors	Cefoperazone/ sulbactum	74.30	100.00	100.00	100.00	66.60
	Ceftriaxone/ tazobactam	82.86	100.00	0.00	100.00	66.60
Penicillin+ Beta lactamase inhibitors	Amoxyclav	20.00	100.00	100.00	100.00	66.60
Penicillin	Cloxacillin	14.30	0.00	0.00	0.00	66.60
Chloramphenicol	Chloramphenicol	94.20	100.00	100.00	100.00	100.00
Cephalosporins	Cefixime	8.60	100.00	0.00	0.00	0.00
Tetracyclin	Tetracycline	22.86	100.00	100.00	100.00	100.00
Sulphonamide + DHFR inhibitor	Co-Trimoxazole	42.86	100.00	100.00	100.00	33.40
Aminoglycoside	Streptomycin	42.86	100.00	100.00	100.00	66.60
	Gentamycin	85.72	100.00	100.00	100.00	100.00
Fluoroquinolones	Ofloxacin	20.00	100.00	0.00	100.00	66.60
	Moxifloxacin	17.20	100.00	100.00	100.00	100.00
	Ciprofloxacin	25.72	100.00	100.00	100.00	66.60
	Enrofloxacin	22.86	100.00	0.00	0.00	0.00

pneumonia cases were *E. coli* (serotype O2) and *Providencia stuartii*; from the sero-fibrinous pneumonia cases were *E. coli* (serotypes O120, 118, 134) and *Aerococcus viridians*; from serous pneumonia case were *E. coli* (serotype O134) and *Acinetobacter baumanni*.

In-vitro drug sensitivity against different bacterial species revealed that *E. coli* strains were found to be most sensitive to chloramphenicol, gentamycin, ceftriaxone/ tazobactum, cefoperazone/sulbactum, streptomycin and co-trimoxazole, ciprofloxacin, tetracycline and enrofloxacin, amoxyclav and ofloxacin, moxifloxacin, cloxacillin, cefixime and erythromycin. *Providencia stuartii* was found to be highly sensitive to cefoperazone/sulbactum, gentamycin, ceftriaxone/tazobactum, streptomycin, ciprofloxacin, cefixime, chloramphenicol, amoxyclav, co-trimoxazole, tetracycline, enrofloxacin, ofloxacin and moxifloxacin. *Acinetobacter baumanni* was found highly sensitive to ceftriaxone/tazobactum, gentamycin, ciprofloxacin, co-trimoxazole, streptomycin, tetracycline, chloramphenicol, cefoperazone/sulbactum, ofloxacin, amoxyclav, and moxifloxacin. *Staphylococcus vitulinus* was found highly sensitive to erythromycin, cefoperzone/ sulbactum, tertracyclin, gentamycin, co-trimoxazole, chloramphenicol, amoxyclav and streptomycin (Table 1).

Whereas, *Providencia stuartii* was found highly resistant to cloxacillin and erythromycin. *Acinetobacter baumanni* was found to be highly resistant to enrofloacin, cefixime and cloxacillin. *Staphylococcus vitulinus* was highly resistant to ceftriaxone/tazobactum, cloxacillin, cefixime, enrofloxacin and ofloxacin. More or less similar results with respect to antimicrobial susceptibility resistance patterns have been reported previously by Sarvan (2017).

On the basis of the present study it is reasonable to conclude that respiratory disorders in buffalo calves were mainly caused by bacterial pathogens viz. *E. coli*, *Providencia stuartii*, *Aerococcus viridians*, *Acinetobacter baumanni* etc. and these species were mainly responsible for the causation of three different types of pneumonia particularly interstitial pneumonia, sero-fibrinous pneumonia including fibrinous pleuritis and serous pneumonia. *E. coli* belonged to serotypes O83, O134, O149, O2, O118 and O120. *In vitro* drug sensitivity testing revealed that in buffalo calves, most of the bacterial species were highly sensitive to amoxyclav and ceftriaxone/ tazobactum. The *in vitro* antibiotic sensitivity tests may be useful in effective drug administration to reduce treatment cost and to prevent the diseases in better way.

REFERENCES

Beutin, L., Bulte, M., Weber, A. and Zimmermann, S. (2000). Investigation of human infections with verocytotoxin-producing strains of *Escherichia coli* (VTEC) belonging to serogroup O118 with evidence for zoonotic transmission. *Epid. Infect.* **125(1)**: 47-54.

Duff, G.C. and Galyean, M.L. (2007). Board-invited review: Recent advances in management of highly stressed, newly received feedlot cattle. *J. Anim. Sci.* **85**: 823-840

Fulton, R.W., Neill, J.D., Salili, J.T., Landis, C., Burge, L.J. and Payton, M.E. (2017). Genomic and antigenic characterization of bovine parainfluenza-3 viruses in the United States including modified live virus vaccine (MLV) strains and field strains from cattle. *Virus Res.* **235**: 77-81.

Griffin, D., Chengappa, M.M., Kuszak, J. and McVey, D.S. (2010). Bacterial Pathogens of the Bovine Respiratory Disease Complex. *Vet. Clin. North Am. Food Anim. Pract.* **26**: 381-394.

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. *Vet. Microbial.* **136**: 135-141.

Khin, M.N., Zamri-Saad, M. and Noordin, M.M. (2010). Pathological Changes in the lungs of calves following intratracheal Exposure to *Pasteurella multocida* B:2. *Pertanika J. Trop. Agric. Sci.* **33(1)**: 113 - 117.

Mahmood, F., Khan, A., Khan, I.A., Ali, H.M. and Younus, M. (2000). Patho-bacteriological investigation of an outbreak of *Mycoplasma bovis* infection in calves. *Microb. Pathogen.* **107**: 404-408.

Praveena, P.E., Periasamy, S., Kumar, A.A. and Singh, N. (2014). Pathology of experimental infection by *Pasteurella multocida* serotype A:1 in buffalo calves. *Vet. Pathol.* **51**: 1109-1112.

Ribeiro, M.G., Langoni, H., Jerez, J.A., Leite, D.D.S., Ferreira, F. and Gennari, S.M. (2000). Identification of enteropathogens from buffalo calves with and without diarrhoea in the Ribeira Valley, State of São Paulo, Brazil. *Braz. J. Vet. Res. Anim. Sci.* **37(2)**: 00-00.

Sarvan (2017). Clinico-pathological studies of gastrointestinal tract disorders in bovines with special reference to calf diarrhoea. Ph. D. thesis submitted to LUVAS, Hisar, India.

Seema (2004). Etio-pathomorphological studies of buffalo calves mortality. M.V.Sc. thesis submitted to CCS HAU, Hisar.

Shakya, A., Roy, B. and Baghel, R.P.S. (2017). Studies on buffalo calf mortality in Jabalpur district of Madhya Pradesh. *Buffalo Bull.* **36(3)**: 521-524.

Singh, K., Mishra, S.K., Jakhar, K.K. and Lather, D. (2013). Pathological investigation on buffalo calves suffering from gastrointestinal tract disorders. *Haryana Vet.* **52**: 38-42.

Tiwari, R., Sharma, M.C. and Singh, B.P. (2007). Buffalo calf health care in commercial dairy farms: a field study in Uttar Pradesh (India). *Livest. Res. Rural Dev.* **19(3)**: 62-64.

USDA. Part 1 (2002). Reference of dairy health and management in the United States. Fort Collins (Co.): USDA: Aphis: VS, Ceah. National Animal Health Monitoring System N377.1202.