Pak. J. Agri. Sci., Vol. 51(1), 235-240; 2014
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

PATHOLOGICAL AND MOLECULAR BASED STUDY OF PNEUMONIC PASTEURELLOSIS IN CATTLE AND BUFFALO (*Bubalus bubalis*)

Riaz Hussain[1], Fazal Mahmood[2], Ahrar Khan[2,*], Muhammad Zargham Khan[2] and Abu Baker Siddique[3]

[1]College of Veterinary and Animal Science, The Islamia University of Bahawalpur, Bahawalpur-63000, Pakistan; [2]Department of Pathology, University of Agriculture, Faisalabad-38040, Pakistan; [3]Department of Microbiology, Faculty of Sciences and Technology GC University Faisalabad, Pakistan.
[*]Corresponding author's e-mail: ahrar1122@uaf.edu.pk

In present study the clinico-pathological findings were recorded in naturally infected cattle and buffaloes due to *Pasteurella multocida* during an outbreak at different livestock herds. There was no significant difference in mortality among various groups of buffaloes (P>0.78) and cattle (P>0.49). The infected animals showed clinical signs of moderate to acute anorexia, salivation, fever, depression, dysponea, submandibular edema, mucopurulent nasal discharge and respiratory grunts. Few of infected animals died due to septicemia. The necropsy of dead animals was performed and visceral organs lungs, kidneys, heart and lymph nodes were observed for gross and histopathological lesions. The tissue samples from these organs were fixed in formalin for pathological changes. Necropsy of dead animals revealed severe pneumonia, consolidation of lungs and intense pleural adhesions. Serosanguinous fluid was accumulated in pericardium and peritoneal cavities. Histopathologically affected lungs exhibited severe congestion, mononuclear cell infiltration, thick interlobular septae punctuated with macrophages, plasma cells and peri-vascular cuffing. Liver, kidneys and lymph nodes had degenerative changes in histological sections. The specificity of *P. multocida* was determined by colony characteristics on MacConkey's agar and morphological features with Gram's iodine. The PCR product size approximately 511bp from lung tissues confirmed a total of 82% (19/23) bacterial isolates from dead animals.

Keywords: *Hemorrhagic septicemia,* cattle, buffaloes, *Pasteurella multocida,* pathology, PCR

INTRODUCTION

Being an agricultural country, dairy farming plays crucial role in economy of Pakistan (Tariq *et al*., 2013). There are about 29.9 million heads of buffaloes and 33 million cattle, which are kept in small groups in the country (Ali *et al.,* 2012; Khaliq *et al*., 2013). Livestock population (33.0 and 29.9 million heads of cattle and buffaloes) in Pakistan accounts for approximately 55.1% of the agriculture value added and 11.5% to GDP by supplying 1.601 million ton beef and 43.562 million ton milk (Hussain *et al.*, 2012a; Hussain *et al.,* 2012b). Pakistan, being a sub-tropical region of South Asia, has favorable climatic conditions for the growth of different infectious agents and the dairy animals encounter various disease problems. Physiological stress of any kind decreases the capacity of the immune system of the animals and when compromised, it favors the occurrence of different diseases (Hussain *et al.,* 2012; Abubakar *et al.,* 2012; Jalees *et al*., 2013). *P. multocida* a Gram-negative bacterium is one of the major nasopharyngeal commensal pathogens responsible for respiratory diseases in animals. Pneumonic pasteurellosis due to *P. multocida* is a major endemic disease in Pakistan (Farooq *et al.,* 2007). The disease occurs mainly in cattle, buffaloes, camels, sheep and goats and has worldwide distribution (Sheehan *et al.,* 2007; Dabo *et al.,* 2007; George *et al.,* 2008; Dassanayake *et al.,* 2010). Acute and per-acute cases of bovine pasteurellosis have been reported in both young and older animals. Bovine pasteurellosis causes significant economic loss in dairy industry (Griffin, 1997; Mosier, 1997; Abubakar *et al.,* 2013). In spite of continuing investigations for several decades, the mechanisms by which the *P. multocida* provoke quick pathogenesis and acute respiratory diseases are poorly known. However, the disease develops under stressful conditions (Saharee *et al.,* 1993). The pathogenic components of organism include endotoxins and outer membrane proteins and previously have been reported for virulent factors responsible for immuno-pathological changes (Boyce and Adler, 2006; Singh *et al.,* 2011). Although the disease incidence is much greater during hot and humid weather; however, outbreaks may occur any time through out the year. Environmental temperature, humidity and stressful conditions enhance bacterial growth and shorten the incubation period of the pathogen approximately 30 hours resulting higher fatality among animals (De Alwis, 1995). Clinical signs and symptoms in infected animals are high fever, rapid breathing and nasal discharge (Khan *et al.,* 2011). Various tools such as the history of disease, clinical signs, necropsy and histopathological changes are valuable in establishing a presumptive disease diagnosis (Uzal and Onger, 2008) and serological assays and polymerase chain reaction

techniques can be used for confirmatory purpose (Biswas *et al.,* 2004; Ranjan *et al.,* 2011).

Pakistan, being a sub-tropical region in Indian subcontinent, has longer period of hot and humid environment, which favors for the rapid growth of several disease producing microbiota particularly *P. multocida*. The present paper describes the PCR based confirmation of pathogen, gross and microscopic lesions during an outbreak of pneumonic pasteurellosis in different livestock dairy herds kept at district Sahiwal Punjab province, Pakistan.

MATERIAL AND METHODS

***Farm locality and environmental temperature*:** The present study was carried out during an outbreak of bovine pasteurellosis at district Sahiwal, Punjab Province. This district is located at an altitude of about 173m and lies between longitudes 73°74′E and latitudes 30°30.15′N (Rahman *et al.,* 2012).

***Clinical observations*:** Clinical signs and history of disease were recorded from owner and attendants. The clinical signs in infected animals were mostly confined to respiratory system. Animals were suffering from high temperature, anorexia, brisket edema, perfuse salvation, protruded tongue, dysponea and with respiratory grunts. The animals were also under stressful condition due to shortage in green fodder, high temperature, humidity and lack of sufficient water supply. The sick animals were treated with antipyretic, steroids, and antibiotics drugs. However the treated animals did not show improvement in signs and death occurred in 2-4 h in per acute cases.

***Necropsies and histopathological investigations*:** Necropsies were conducted within 1-2 hours after death and gross changes were observed. Morbid tissues from lungs, liver, heart, kidney and spleen were collected and fixed in 10% neutral buffered formalin for paraffin method. Sections were stained with hematoxylin and eosin (Bancroft and Gamble, 2008).

***Culturing and colony morphological characteristics*:** The nasal swabs were inoculated on brain heart infusion broth and blood agar medium supplemented with 5% sheep blood. A bipolar characteristic of *P. multocida* was confirmed through staining smears with methylene blue stain and finally the pathogen was confirmed using polymerase chain reaction (Boyce *et al.,* 2000).

***PCR based confirmation*:** The bacterial DNA was isolated from the purified growth using Nucleic acid extraction kit (Vivantis, USA) following the manufacturer protocol and was stored at -20^{o}C for further use. Polymerase chain reaction was carried out to amplify region in polysaccharide capsule gene of *P. multocida* using specific primers (CAPF-FWD 5'-AAT-CGG-AGA-ACG-CAG-AAA-TCA-G-3' and CAPF-REV 5'-TTC-CGC-CGT-CAA-TTA-CTC-TG-3'). The PCR was carried out in the thermal cycler (PeqLab 25, Germany). A total of 27µl volume including PCR master mix (15µl) (Vivantis, USA), 5pm/ µl of each primer (forward and reverse) and the template DNA (2µl) was used. The thermal cycler conditions optimized for the reaction included initial denaturation at 94^{O}C for 3 min, followed by 25 cycles of denaturation at 94^{O}C for 30 sec, annealing at 54^{O}C for 1 min and extension at 72^{O}C for 1 min, while final extension was done at 72^{O}C for 3 min (Mahmood *et al.,* 2012). The amplified PCR product was electrophoresed using 100bp DNA ladder as molecular wt. marker and visualized in gel documentation system (Dolphin Doc, USA).

***Data analyses*:** The collected data was subjected to statistical analysis by applying Chi-square test. $P<0.05$ was considered as significance level. Where appropriate odds ratio and 95% confidence limits were determined by using SAS 9.1 statistical software (SAS, 2004).

RESULTS AND DISCUSSION

Pneumonic pasteurellosis in cattle and buffalo is reported to be a major disease in tropical and subtropical areas of Pakistan with high mortality (Asadullah *et al.,* 2006; Asma *et al.,* 2009; Khan *et al.,* 2011). The results of present study indicated no significant difference in mortality among various groups of buffaloes and cattle (Table 1). The clinical signs like anorexia, depression, dysponea and respiratory grunts were observed among all infected animals. Moderate to severe submandibular edema was present in animals of all age group. Salivation (86.04%), fever (81.39) and open mouth breathing (67.44%), were predominantly present in cattle and buffaloes (Table 2). Excessive amount of froth was accumulated in trachea (82.60%), pleural adhesions (91.30%), lungs were consolidated and covered with thick white pleura and costal were observed in animals showing severe respiratory signs. Kidneys (82.60) of affected animals showed hemorrhages. Serosanguinous fluid was accumulated in pericardium and petechial hemorrhages were also observed at the base of heart during postmortem examination (Table 3). Clinical signs and gross lesions observed in present study were similar to previous reports of pneumonic pasteurellosis in cattle and buffaloes (Hodgson *et al.,* 2005; Khan *et al.,* 2011). On necropsy, prominent bilateral cranio-ventral consolidation and congestion of lungs and large quantity of whitish froth in trachea were observed in both species (Fig.1). Fibrinous pleuritis and adhesion with the costal surfaces (Fig.2) and mediastinal lymph nodes were swollen, consolidated and were arranged in chain fashion. In some animals serosanguinous fluid was accumulated in pericardium and peritoneal cavities. In present study the non significant ($P>0.185$) difference was observed in frequency of gross and microscopic lesions (Table 3). Similarly a non significant difference in mortality percentage among all age groups of cattle ($P>0.370$) and in buffaloes ($P>0.972$) was recorded. Similar gross changes in naturally and experimentally infected cases of pasteurellosis

Table 1. Overall frequency of mortality in buffaloes and cattle died due to pneumonic pasteurellosis

Species/sex/age	Animal with clinical sings	Mortality		95% CI	Odd Ratio/ P value
		N	%		
Buffalo					
Male	7	3	42.85	12.27 to 78.40	OR= 0.75 [reciprocal = 1.33]
Female	16	8	50.0	26.59 to 73.41	
Age groups (years)					
2-3	5	2	40.0	7.35 to 81.76	Mantel-Haenszel P> 0.78
3.1-5	4	3	75.00	24.23 to 98.75	
5.1-9	9	4	44.44	16.05 to 75.96	
9.1-12	5	2	40.0	7.35 to 81.76	
Cattle					
Male	8	5	62.25	27.80 to 89.44	OR= 1.19 [reciprocal = 0.84]
Female	12	7	58.33	30.21 to 82.83	
Age groups (years)					
2-3	3	2	66.66	13.20 to 98.33	Mantel-Haenszel P> 0.49
3.1-5	4	3	75.0	24.23 to 98.75	
5.1-9	7	4	57.14	21.60 to 87.73	
9.1-12	6	3	50.0	14.66 to 85.34	

Table 2. Intensity and frequency of various clinical signs in cattle and buffaloes (n=43)

Clinical Signs	Intensity of Clinical Signs	Frequency		95% CI
		No	%	
Anorexia	Severe	27	62.79	47.72 to 76.19
Fever	Severe	35	81.39	67.71 to 90.97
Protruded tongue	Moderate	17	39.53	25.83 to 54.61
Sub-mandibular edema	Moderate	13	30.23	17.96 to 45.09
Open mouth breathing	Severe	29	67.44	52.48 to 80.12
Salivation	Severe	37	86.04	73.22 to 94.14
Throat swelling	Moderate	11	25.58	14.25 to 40.11
Respiratory grunts	Severe	11	25.58	14.25 to 40.11

Table 3. Overall frequency of necropsy lesions observed in animals (n=23) died of pneumonic pasteurellosis

Gross lesions	Intensity of lesions	Frequency		95% CI
		N	%	
Congested lungs	Severe	17	73.91	53.43 to 88.69
Edematous lungs	Sever	15	65.21	44.45 to 82.36
Forth in trachea	Severe	19	82.60	63.20 to 94.22
Pericardial hemorrhages	Moderate	13	56.52	36.10 to 75.39
Hemorrhages on kidney	Moderate	19	82.60	63.20 to 94.22
Serosanguinous fluid in pericardium	Moderate	13	56.52	36.10 to 75.39
Pleural adhesions	Severe	21	91.30	74.13 to 98.52
Clotted blood in heart	Severe	16	69.56	48.86 to 85.61
Serosanguinous fluid in peritoneum	Moderate	13	56.52	36.10 to 75.39

has been reported in goats (Zamri-Saad *et al.,* 1996; Goncalves *et al.,* 2010), sheep (Ayling and Nicholas, 2007), buffalo calves (Hodgson *et al.,* 2005), pigs (Kalorey *et al.,* 2008) and in mice (Praveena *et al.,* 2010).

Microscopically lungs sections exhibited severe pneumonic changes comprised of diffusely packed eosinophilic proteinious edema fluid and inflammatory exudates within alveoli, bronchioles and bronchi (Fig. 3). Congestion, extensive infiltration of leukocytes particularly mononuclear cells punctuated within interlobular septa in lung and thick interlobular septae were the characteristic features in present study (Fig. 4). Punctuation of mononuclear cells along the brim of lobular septae and sever perivascular cuffing were frequently observed. Previously such extensive gross and microscopic changes have never been reported in Pakistan in accessible literature however, it was observed in feedlot beef

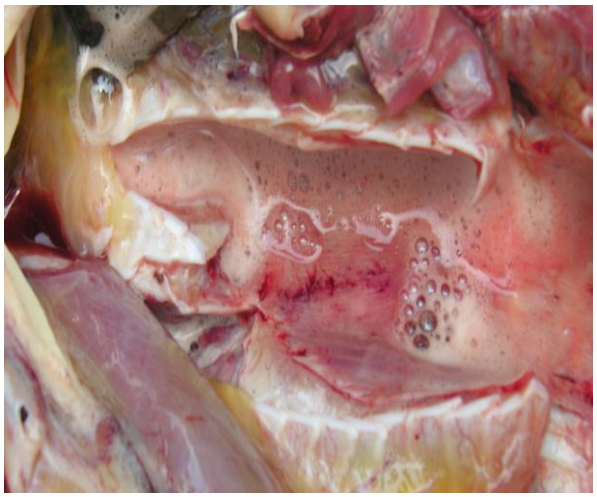

Figure 1. Photograph showing large quantity of frothy material in trachea of cattle

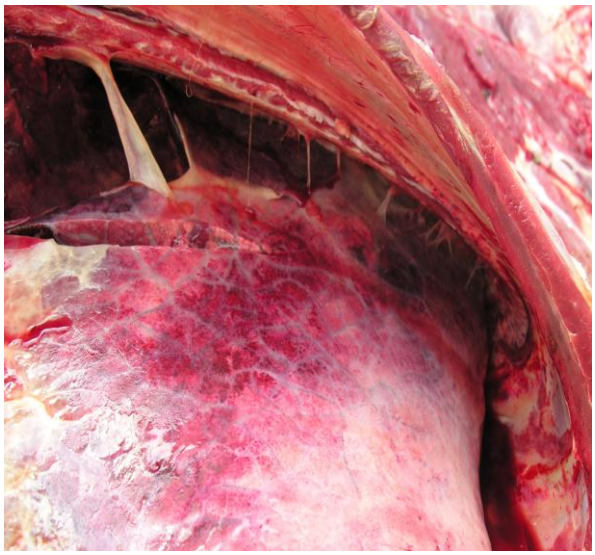

Figure 2. Photograph of thoracic cage exhibiting hepatization of lung in cattle, fibrinous pleuritis and pleural adhesion to costal surfaces.

calves (Gagea *et al.,* 2006), sheep (Ayling and Nicholas, 2007), atypical non-progressive pneumonia in goats (Goncalves *et al.,* 2010) and in experimentally induced cases of pneumonic pasteurellosis (Dowling *et al.,* 2002; Dagleish *et al.,* 2010). Histopathologically pleura and interlobular septae were markedly expanded by intense aggregates of neutrophils, fibrin strands and extravasated red blood cells.

Liver of animals were swollen and yellow in color. Histologically severe congestion and perivascular cytoplasmic vacuolation in hepatocytes, biliary hyperplasia, increased sinusoidal spaces and mild cellular infiltration was observed. Such observations in association with the pasteurellosis have never been reported in the cattle and buffaloes.

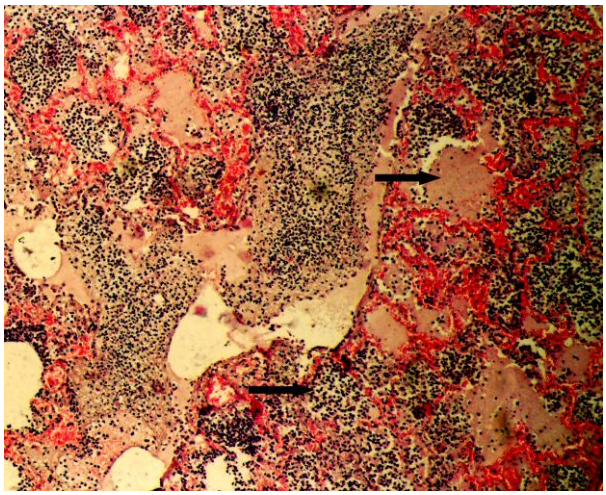

Figure 3. Photomicrograph of lungs showing extensive package of alveoli with inflammatory exudates (arrow) and eosinophilic proteinious edema fluid (arrow) H&E; X 200.

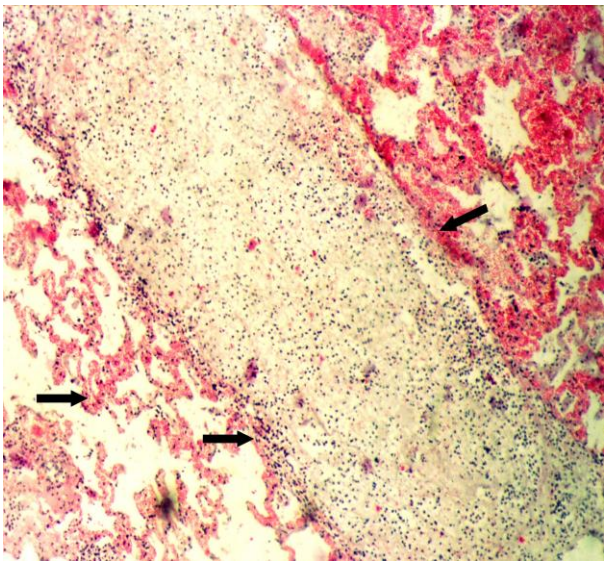

Figure 4. Severe congestion, extensive infiltration of leukocytes particularly mononuclear cells punctuated within interlobular septa (arrow) in cattle lung. H&E; X 200.

Degenerative changes were also observed in kidneys in the form of hyperemia and inflammation of glomeruli. Histologically lymph nodes indicated diffuse hemorrhages. Previously such pathological changes in liver, kidneys and lymph nodes have been reported in pigs (Kumar *et al.,* 2007), experimentally induced pasteurellosis in mice (Praveena *et al.,* 2010) and lambs (Rad *et al.,* 2009). However, previously such histological changes in visceral organs of calves died due to *Pasteurella multocida* have been reported (Dowling *et al.,* 2002).

The PCR confirmed 82.6% (19/23) bacterial isolates from lungs tissue samples obtained from dead animals during the present outbreak and yielded 511bp (Fig. 5) of PCR product using specific primers as previously reported (Boyce *et al.*, 2000; Townsend *et al.*, 2001; Ranjan *et al.*, 2011).

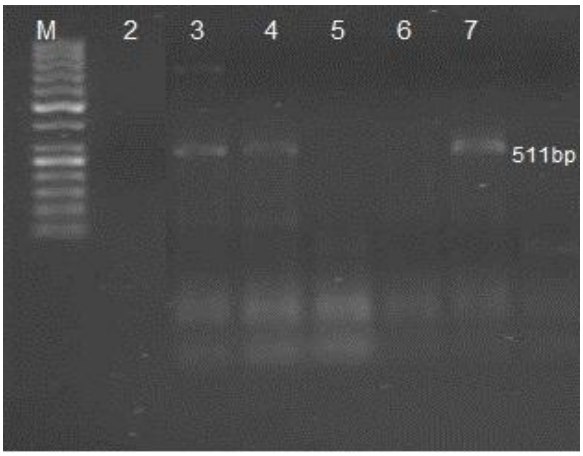

Figure 5. PCR based confirmation of *P. multocida*. Lane 1: 100bp ladder, lane 3, 4 & 7: PCR positive, lane 2, 5 & 6, negative samples.

***Conclusion*:** The Present study suggested that *Pasteurella* infection has strong correlation with the subtropical condition of the Punjab province of Pakistan. Aggravation in the disease was mainly due to closer confinement, hot and humid climate, abrupt change in feeding pattern and most importantly inclusion of unvaccinated animals in herd from different areas of the country.

***Acknowledgements*:** The authors are highly thankful to District livestock officer, Sahiwal Livestock and Dairy Development department Government of Punjab for extending help regarding specimen's collection and necropsy.

REFERENCES

Abubakar, M., M. Mansoor and M.J. Arshed. 2012. Bovine brucellosis: old and new concepts with Pakistan perspective. Pak. Vet. J. 32:147-155.

Abubakar, M.S., M. Zamri-Saad and S. Jasni. 2013. Ultrastructural changes and bacterial localization in buffalo calves following oral exposure to *Pasteurella multocida* B: 2. Pak.Vet. J. 33:101-106.

Ali, M.A., L.A. Lodhi and F. Hassan. 2012. Serum progesterone and estradiol-17β profiles in Nili Ravi buffaloes (*Bubalus bubalis*) with and without dystocia. Pak. Vet. J. 32:571-574.

Asadullah, K., S. Umar, A. Riaz, K. Hamayun, M. Younas and Z. Mohammad. 2006. Sero-surveillance of hemorrhagic septicemia in cattle and buffaloes in district Malakand, NWFP. J. Agri. Biol. Sci. 1:11-14.

Asma, A., S. Mahboob, M.S. Shah and S. Nadeem. 2009. Analysis of outer membrane proteins of *Pasteurella multocida* strains isolated from buffaloes affected with hemorrhagic septicemia. Aust. J. Basic Appl. Sci. 3:2360-2365.

Ayling, R.D. and R.A.J. Nicholas. 2007. Mycoplasma respiratory infections. In: I.D. Aitken (ed.), Diseases of Sheep, 4th Ed. Blackwell Publishing, Moredun, pp.231–235.

Biswas, A., S.B. Shivachandra, M.K. Saxena, A.A. Kumar, V.P. Singh and S.K. Srivastava. 2004. Molecular variability among strains of *P. multocida* isolated from an outbreak of haemorrhagic septicaemia in India. Vet. Res. Comm. 28:287–298.

Boyce, J.D. and B. Adler. 2006. How does *Pasteurella multocida* respond to the host environment. Current Opin. Microbiol. 9:117-122.

Boyce, J.D., J.Y. Chung and B. Adler. 2000. Genetic organization of the capsule biosynthetic locus of *Pasteurella multocida* M1404 (B: 2). Vet. Microbiol. 72:121–134.

Dagleish, M.P., J. Finlayson, C. Bayne, S. MacDonald, J. Sales and J.C. Hodgson. 2010. Characterization and time course of pulmonary lesions in calves after intratracheal infection with *Pasteurella multocida* A. J. Comp. Path. 142:157-169.

Dassanayake, R.P., S. Shanthalingam, C.N. Herndon, R. Subramaniam, P.K. Lawrence, J. Bavananthasivam, E.F. Cassirer, G.J. Haldorson, W.J. Foreyt, F.R. Rurangirwa, D.P. Knowles, T.E. Besser and S. Srikumaran. 2010. Mycoplasma ovipneumoniae can predispose bighorn sheep to fatal *Mannheimia haemolytica* pneumonia. Vet. Microbiol. 145:354-359.

De Alwis, M.C.L. 1995. Haemorrhagic septicaemia in cattle and buffaloes. In: W. Donachie et al. (ed.), Haemophilus, Actinobacillus and pasteurella. Plenum press, New York, pp.9-24.

Dowling, A., J.C. Hodgson, A. Schock, W. Donachie, P.D. Eckersall and I.J. Mckendrick. 2002. Experimental induction of pneumonic pasteurellosis in calves by intratracheal infection with *Pasteurella multocida* biotype A: 3. Res. Vet. Sci. 73:37-44.

Farooq, U., M. Hussain, H. Irshad, N. Badar, R. Muneer and Q. Ali. 2007. Status of haemorrhagic septicemia based on epidemiology in Pakistan. Pak. Vet. J. 27:67-72.

Gagea, M.I., K.G. Bateman, R.A. Shanahan, T. Van Dreumel, B.J. McEwen, S. Carman, M. Archambault and J.L. Caswell. 2006. Naturally occurring *Mycoplasma bovis*–associated pneumonia and polyarthritis in feedlot beef calves. J. Vet. Diag. Invest. 18:29-40.

George, J.L., D.J. Martin, P.M. Lukacs and M.W. Mille. 2008. Epidemic pasteurellosis in a bighorn sheep population

coinciding with the appearance of a domestic sheep. J. Wildlife Dis. 44:388-403.

Goncalves, R., I. Mariano, A. Nunez, S. Branco, G.E. Fairfoul and R. Nicholas. 2010. Atypical non-progressive pneumonia in goats. The Vet. J. 183:219-221.

Griffin, D. 1997. Economic impact associated with respiratory disease in beef cattle. Vet Clinics North America Food Anim. Pract. 13:367-377.

Hodgson, J.C., A. Finucane, P.D. Mark, A. Saeed, P. Roger and J.G Coote. 2005. Efficacy of vaccination of calves against hemorrhagic septicemia with a live aroA derivative of *Pasteurella multocida* B: 2 by two different routes of administration. Infec. Immun. 73:1475-1481.

Hussain, R, M.T. Javed and A. Khan. 2012a. Changes in some biochemical parameters and somatic cell counts in the milk of buffalo and cattle suffering from mastitis. Pak. Vet. J. 32:418-421

Hussain, R., A. Khan, M.T. Javed and F. Rizvi. 2012b. Possible risk factors associated with mastitis in indigenous cattle in Punjab, Pakistan. Pak. Vet. J. 32:605-608.

Kaloreya, D.R., S.Yuvaraja, S.S.Vanjaria, P.S. Gunjala, N.B. Dhanawadea, S.B. Barbuddheb and A.G. Bhandarkarc. 2008. PCR analysis of *Pasteurella multocida* isolates from an outbreak of pasteurellosis in Indian pigs. Comp. Immun. Microbiol. Infect. Dis. 31:459-465.

Khan, A., M.K. Saleemi, M.Z. Khan, S.T. Gul, M. Irfan and M.S. Qamar. 2011. Hemorrhagic septicemia in buffalo (*Bubalus bubalis*) calves under sub-tropical conditions in Pakistan. Pak. J. Zool. 43: 295-302.

Khaliq, T., Z.U. Rahman and I. Javed, 2013. High dose of recombinant bovine somatotropin do alter serum biochemical and hormonal profiles of Nili Ravi buffaloes. Pak. Vet. J. 33: 476-480.

Kumar, H., V. Mahajan and S. Sharma. 2007. Concurrent pasteurellosis and classical swine fever in Indian pigs. J. Swine Health Prod. 15: 279–283.

Mahmood, F., A. Khan, M.Z. Khan, R Hussain, S.T. Gul and A.B. Siddique. 2012. Pathological and molecular based study of naturally occurring lentivirus infection. Pak. Vet. J. 32:511-514.

Mosier, D.A. 1997. Bacterial pneumonia. Veterinary Clinics of North America. Food Anim. Pract. 13:483-493.

Praveena, P.E., S. Periasamy, A.A. Kumar and N. Singh. 2010. Cytokine profiles, apoptosis and pathology of experimental *Pasteurella multocida* serotype A1 infection in mice. Res. Vet. Sci. 89:332-339.

Rad, M., A.R. Movassaghi, K. Sharifi, Z. Naseri and H.A. Seifi. 2009. Two outbreaks of *Pasteurella multocida* septicemia in neonatal lambs. Comp. Clin. Path. 20:57-59.

Rahman, H.J.U., N. Ahmad, N.U. Rahman, S. Waheed, M. Ahmad, M. Younis and T. Ahmad. 2012. Effects of different levels of pigeon egg yolk in extenders on the post-thaw semen quality of Sahiwal bulls. Pak. Vet. J. 32:315-318.

SAS. 2004. SAS Statistical Software Version 9.1. SAS Institute Inc, Cary, NC, USA.

Sheehan, M., J.P. Cassidy, J.B. Brady, H.C. Ball, M.L.D. Doherty, P.J. Quinn, R.A.J. Nicholas and B.K. Markey. 2007. An aetiopathological study of chronic bronchopneumonia in lambs in Ireland. The Vet. J. 173:630-637.

Singh, K., J.W. Ritchey and A.W. Confer. 2011. *Mannheimia haemolytica*: bacterial-host interactions in bovine pneumonia. Vet. Path. 48:338-348.

Tariq, M., M. Younas, A.B. Khan and E. Schlecht, 2013. Body measurements and body condition scoring as basis for estimation of live weight in Nili-Ravi buffaloes. Pak. Vet. J. 33:325-329.

Townsend, K.M., J.D. Boyce, J.Y. Chung, A.J. Frost and B. Adler. 2001. Genetic organization of Pasteurella multocida cap loci ad development of multiplex capsular PCR typing system. J. Clin. Microbiol. 39:924-929.

Uzal, F.A. and J.G. Songer. 2008. Diagnosis of *Clostridium perfringens* intestinal infections in sheep and goats. J. Vet. Diag. Invest. 20:253-265.

Zamri-Saad, M. A., W.A. Effendy, M.A. Maswati, N. Salim and A.R. Sheikh-Omar. 1996. The goat as a model for studies of pneumonic pasteurellosis caused by *Pasteurella multocida*. Brit. Vet. J. 152:453-458.