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Research Article

AETIO-PATHOLOGICAL STUDIES OF DIGESTIVE AND RESPIRATORY AFFECTIONS IN BUFFALO CALVES

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ABSTRACT

Aetio-pathological studies of digestive and respiratory affections were undertaken in buffalo calves received for post mortem examination during a period of seven months (September, 2015 to March, 2016). Maximum mortality was in the age group upto 1 month and 1-3 months. Mortality was more in female calves. System-wise causes of death/mortality were highest due to combined involvement of both digestive and respiratory systems followed by involvement of respiratory system alone and digestive system alone. Main digestive and respiratory system affections were enteritis, hepatitis, gastro-enteritis and pneumonia. Pneumo-enteritis was the main affection noticed when combined involvement of both digestive and respiratory systems. Microbiological studies of samples collected from carcasses of buffalo calves revealed that, most prominent organism was *E. coli* followed by *Proteus* spp., *Staphylococcus* spp., *Klebsiella* spp. and *Salmonella* spp. Maximum number of bacterial species were isolated from intestine followed by lungs, tracheal swab and heart blood. 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 gentamycin and resistant to tetracycline. Examination of faecal samples of diarrhoeic as well as diseased and dead buffalo calves revealed that *Eimeria* spp. was the major infection followed by infestation of *Ascaris* ova, *Strongyle* spp. and *Strongyloid* spp.

Key words: Buffalo calves, *Eimeria* spp., *in-vitro* drug sensitivity, mortality, pneumo-enteritis

Management of young animals plays an important role in the development of the dairy and meat sector as calves are the future of the livestock industry. The care of buffalo calves is not only essential for sustenance of the dairy industry, but also essential in the context of preserving and maintaining good quality germplasm (Tiwari *et al.*, 2007). Therefore, to attain maximum gains from livestock industry, it is vitally important that young ones be reared into healthy and productive animals. Amongst the various causes, digestive and respiratory system disorders play an important role in the morbidity and mortality of buffalo calves. A number of infectious, parasitic, nutritional and metabolic conditions have been identified (Singh *et al*., 2013; Cho and Yoon, 2014; Shija *et al*., 2014). Calf diarrhoea occurs due to both infectious and non-infectious factors. Bacteria responsible for mortality in neonatal and young buffalo calves are *Escherichia coli, Salmonella* spp., *Pasteurella multocida, Clostridium perfringens and Staphylococcus aureus*. Several *E. coli* serotypes causing morbidity and mortality have been isolated from diarrhoeic calves (Wani *et al*., 2003).

Correctly determining the cause of death permits one to apply effective measures to prevent further loss. Keeping in view the above facts, aetio-pathological studies on digestive and respiratory affections in buffalo calves were undertaken.

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MATERIALS AND METHODS

Postmortem examination was carried out on ten buffalo-calf carcasses aged below six months that were presented to the Department of Veterinary Pathology, LUVAS, Hisar during September, 2015 to March, 2016. Following parameters were studied:

Postmortem Examination and Pathological Study: A detailed postmortem examination was conducted immediately on arrival of the carcass. Representative and appropriate tissue pieces from intestine, stomach part, liver, lung, trachea, heart and mesenteric lymph nodes were collected in 10% buffered formalin and processed for histopathological studies (Luna, 1968).

Microbiological Study: At necropsy, material for bacteriological studies was collected aseptically in sterile containers. Isolation of organisms was attempted from the heart blood, lung, trachea, and intestinal contents. The collected samples were put in nutrient broth or buffered peptone water and incubated at 37^0C for 24 h. The next day 1 ml of culture from buffered peptone water was transferred to selenite broth and incubated at 37^0C for 24 to 48 h, while those collected in nutrient broth were

inoculated on nutrient agar (NA) and Mac Conkey's Lactose agar (MLA) plates and incubated at 37^{0}C for 24 to 48 h. From MLA plate, lactose fermenting colonies were taken, inoculated on Eosin methylene blue (EMB) agar and incubated at 37^{0}C for 24 h and the isolates were stored in maintenance medium. From NA plates, golden orange colored, round, opaque and luxuriant colonies developed were selected and inoculated on Baired Parker agar and incubated at 37^{0}C for 24 to 48 h. From Baird Parker agar, the black colored colonies were selected and stored in maintenance medium. The culture from selenite broth was streaked on Brilliant Green agar (BGA) and incubated at 37^{0}C for 24 h. The plates were observed for colonies after incubation. From BGA plate, pinkish white colonies were taken and inoculated on Xylose-lysine deoxycholate agar (XLD) agar and incubated at 37^{0}C for 24 h and the isolates were stored in maintenance medium. Identification of all isolates was done following the procedure of Cruickshank and McCartney (1965).

All bacterial isolates were stained by Gram's staining and examined for their morphological characteristics. Biochemical tests IMViC (Indole, methyl red, Voges-Pro-skauer and citrate utilization test), sugar fermentation tests, nitrate reduction test, urease tests, H_2S production test on triple sugar iron medium, and catalase test were also performed.

***In-vitro* Drug Sensitivity Testing:** Different isolated bacterial strains were subjected to *in-vitro* drug sensitivity testing using antimicrobials by the disc diffusion method as suggested by Bauer *et al*. (1966). With the help of a platinum loop, small amount of growth from at least three isolated colonies of the organisms were transferred

Table 1
Mortality pattern according to age, sex, system-wise causes of mortality, bacterial species isolated from different samples and faecal examination of diarrhoeic/diseased and dead buffalo calves

Age groups	Age and sex-wise mortality in buffalo calves					
	Males (Number of cases)	Percentage of age-wise mortality in male	Females (Number of cases)	Percentage of age-wise mortality in females	Total	Percentage of age-wise mortality
Up to 1 month	2	50.00	2	33.34	4	40
1-3 months	1	25.00	3	50.00	4	40
3-6 months	1	25.00	1	16.66	2	20
Total sex-wise mortality	4 (40%)	-	6 (60%)	-	10	100

System-wise causes of death/mortality in buffalo calves						
System-wise causes of death/mortality	Digestive system alone	Respiratory system alone	Combination of both digestive and respiratory systems	Others systems/ causes	Putrefied carcass	Total number of cases
Total system-wise mortality	1 (10%)	2 (20%)	5 (50%)	1 (10%)	1 (10%)	10 (100%)

Bacterial species isolated from different samples collected from buffalo calves						
Bacterial species isolated	Intestine	Lungs	Tracheal swab	Heart blood	Total number of different bacterial species isolated	Percentage of different bacterial species isolated
E. coli	9	3	1	3	16	48.48
Klebsiella spp.	3	0	0	0	3	9.09
Salmonella spp.	0	2	0	0	2	6.07
Proteus spp.	3	1	3	0	7	21.21
Staphylococcus spp.	1	4	0	0	5	15.15
Total bacterial species isolated	16 (48.48%)	10 (30.30%)	4 (12.12%)	3 (9.10%)	33 (100.00%)	

Faecal examination in diarrhoeic/diseased buffalo calves						
Ascaris ova	*Strongyle* spp.	*Fasciola* eggs	*Trichuris* eggs	*Eimeria* spp.	Mixed infection	Negative sample
2	1	0	0	4	2 (*Ascaris* ova + *Eimeria* spp.)	1

Faecal examination in dead buffalo calves				
Strongyle spp.	*Eimeria* spp.	Mixed infection	Negative sample	Total number of cases
1	2	1 (*Strongyle* spp.+*Eimeria* spp.) 1 (*Strongyle* spp.+*Strongyloid* spp.)	4	09

Table 2
Gross pathological changes observed during postmortem examination of buffalo calves

Gross Changes	Intestine	Liver	Mesenteric lymph nodes	Abomasum	Lungs	Heart	Kidneys	Spleen	Trachea	Urinary bladder	Gall bladder
Congestion	2	2	-	3	6	-	4		-	-	-
Haemorrhages	1	-	-	-	-	-	2	1	-	-	-
Necrotic foci	-	2 (One having diffuse pale necrotic change)	-	-	-	-	1	-	-	-	-
Fibrinous deposition	-	-	-	-	1	-	-	-	-	-	-
Firmness and induration	-	2	-	-	-	-	-	-	-	-	-
Consolidation	-	-	-	-	3	-	-	-	-	-	-
Emphysema	-	-	-	-	1	-	-	-	-	-	-
Abscess	-	-	-	-	1	-	-	-	-	-	-
Exudate	4 (Catarrhal)	-	-	-	1 (Whitish cheesy)	-	-	-	2 (Whitish frothy)	-	-
Enlargement	-	1	3	-	-	-	-	-	-	-	-
Thickened mucosa	1	-	-	2	-	-	-	-	-	-	-
Distension	-	-	-	-	-	-	-	-	-	2	1

into a tube of trypticase soya broth and incubated for two to five hours at 37^0C so as to obtain a turbidity, equivalent to that obtained by adding 0.5 ml of 0.048 M $BaCl_2$ (1.175 % $BaCl_2$, $2H_2O$) to 99.5 ml of 0.36M NH_2SO_4 (1 % v/v). The broth culture was then evenly spread by smearing over the surface of Mueller Hinton agar plates. Different antibiotic discs of standard concentrations were then used. The plates were then incubated at 37^0C for 18 to 24 h and observed for sensitivity by measuring the zones of inhibition. Results were noted as sensitive (S) and resistant (R) on the basis of the table provided by the manufacturer (Himedia) for zone size interpretation.

Parasitological Studies from Faecal Samples: A total of 19 faecal samples were collected from both dead (9) and diarrheic (10) buffalo calves at University farm, Teaching Veterinary Clinical Complex, and postmortem hall of the Department of Veterinary Pathology, LUVAS, Hisar. Examination of these faecal samples was performed as per method of Soulsby (1982) by floatation and sedimentation methods.

RESULTS AND DISCUSSION

As per the information provided, most of the animals had died suddenly with no clinical signs and symptoms, while others were being treated for diarrhoea, dehydration and weakness. Table 1 depicts the mortality pattern according to age, sex, system-wise causes of death/mortality, bacterial species isolated from different samples and faecal examination of diarrhoeic/diseased and dead buffalo calves. The proposed study starting from September, 2015 to March, 2016 of buffalo calves revealed that maximum age-wise mortality was in age group of upto 1 month and 1 to 3 months. Sumit *et al.* (2011) reported that mortality was maximum in age group of below 1 month of age and Verma *et al.* (1980) reported maximum mortality below 3 month of age in buffalo calves.

Sex-wise mortality was higher in female calves as compared to male calves. Seema (2004) also reported maximum mortality in female buffalo calves. In contrast, Kinjavdekar *et al*. (1994) reported a significantly higher mortality in male as compared to female calves. No effect of sex on mortality rate in buffalo calves was reported by Khan and Khan (1996). System-wise causes of death/mortality were highest due to combined involvement of both digestive and respiratory systems followed by involvement of respiratory and digestive system alone. Gastritis, enteritis, pneumo-enteritis, hepatitis and pneumonia were the main conditions encountered in buffalo calves. These results were supported by the findings of Sumit *et al.* (2011); Islam *et al*. (2015) and Ali *et al*. (2015).

Microbiological study of different samples collected from carcasses of buffalo calves revealed presence of *E. coli* followed by *Proteus* spp., *Staphylococcus* spp., *Klebsiella* spp. and *Salmonella* spp. Maximum number of bacterial species were isolated from intestine followed by lungs, tracheal swab and heart blood. Furthermore, in some cases these bacterial species were also isolated from heart blood indicating that they might have caused bacteraemia or septicaemia. It is worthwhile to mention here that in these cases, lesions were seen in number of organs indicating systemic infection. Hussain and Saikia (2000), Carlson *et al.* (2002), Singh (2005), Lehreena *et al*. (2010), Ahmed *et al*. (2015) reported isolation of the similar bacterial species from carcasses of buffalo calves. The results of *in-vitro* drug sensitivity to different bacterial species isolated from different samples collected from carcasses of buffalo calves revealed that *E. coli* was found sensitive to gentamycin, ciprofloxacin; *Salmonella* spp. to amoxyclav, streptomycin, gentamycin, amoxicillin; *Klebsiella* spp. to ceftriaxone; *Proteus* spp. to amikacin, ceftriaxone, cefotaxime, ciprofloxacin,

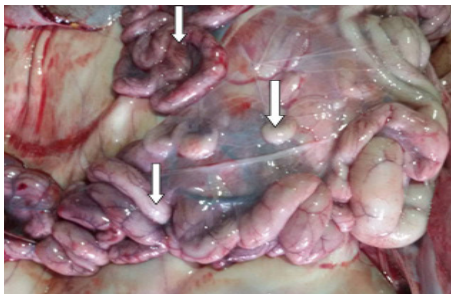

Fig 1. Haemorrhage in intestine along with swollen mesenteric lymph nodes (Buffalo calf, *E. coli* infection)

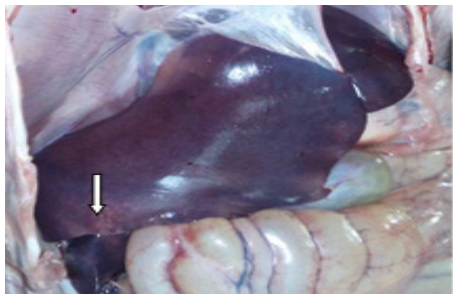

Fig 2. Dark and severely congested liver with focal necrotic foci (Buffalo calf, *Salmonella* spp. infection)

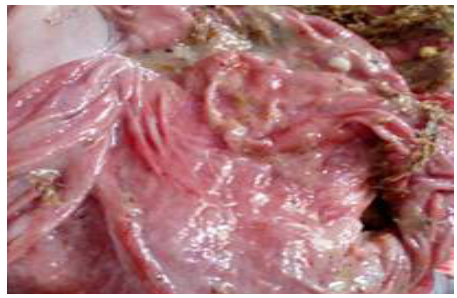

Fig 3. Congestion and thickened mucosal folds of abomasum (Buffalo calf, *E. coli* infection and *Strongyle* spp. infestation)

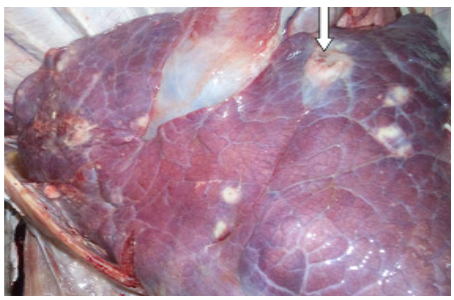

Fig 4. Focal abscesses in lung (Buffalo calf, *Klebsiella* spp. infection)

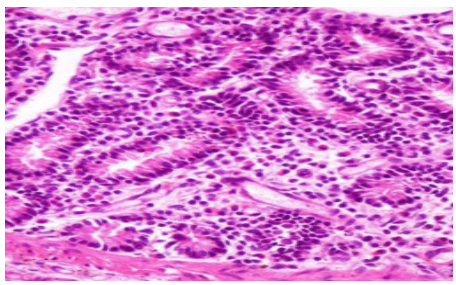

Fig 5. Intestine: Necrotic enteritis characterized by infiltration of leucocytes in mucosa *Klebsiella* spp. infection) H & E x 400

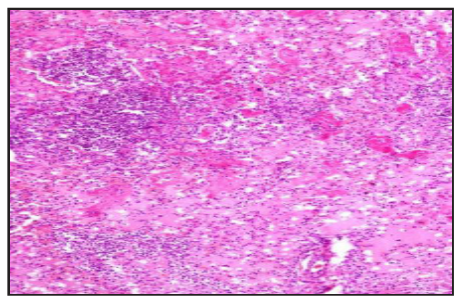

Fig 6. Lungs: Suppurative pneumonia characterized by sero-purulent exudate in alveoli, infiltration of leucocytes and necrosis, (Buffalo calf, *Salmonella* spp. infection) H & E x 100

Staphylococcus spp. to gentamycin, amikacin, amoxyclav, ceftriaxone whereas *E. coli* was found resistant to tetracycline; *Salmonella* spp. resistant to polymyxin B, tetracycline and ampicillin; *Klebsiella* spp. to chloramphenicol; *Proteus* spp. resistant to tetracycline and amoxicillin and *Staphylococcus* spp. to amoxycillin and ampicillin. Almost, similar results with respect to antimicrobial susceptibility resistance patterns have been reported previously by Lehreena *et al*. (2012).

Examination of faecal samples of diarrhoeic/ diseased buffalo calves revealed *Eimeria* spp. as the major infection followed by infection of *Ascaris* ova, *Strongyle* spp. and mixed infection of *Ascaris* ova along with *Eimeria* spp. Similarly, examination of faecal samples of dead buffalo calves revealed *Eimeria* spp. as the major infection, followed by *Strongyle* spp., mixed infection of *Strongyle* spp. along with *Eimeria* spp. and *Strongyle* spp. along with *Strongyloid* spp. were also noticed. The detection of ascaris infection at early age could apparently suggest it to be vertical infection as reported by Selim and Tewfic (1966). However, it is difficult to account for the presence of coccidial oocysts in faecal samples at early age of life. It is presumed that buffalo calves while licking the udder and other parts of body, contaminated with coccidial oocysts, might have taken the oocysts and passed as such in the faeces.

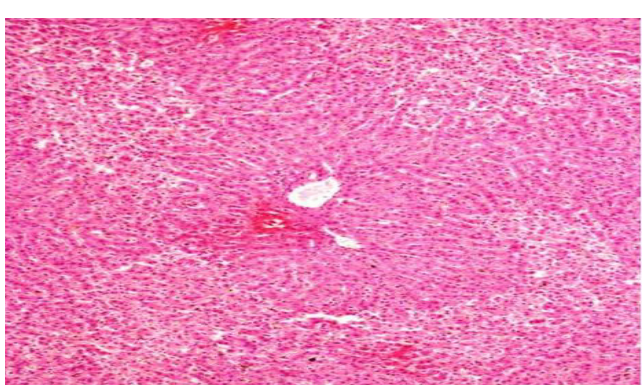

Fig 7. Liver: Necrotic hepatitis characterized by congestion in blood vessels and focal areas of necrosis (Buffalo calf, *Salmonella* spp. infection) H & E x 100

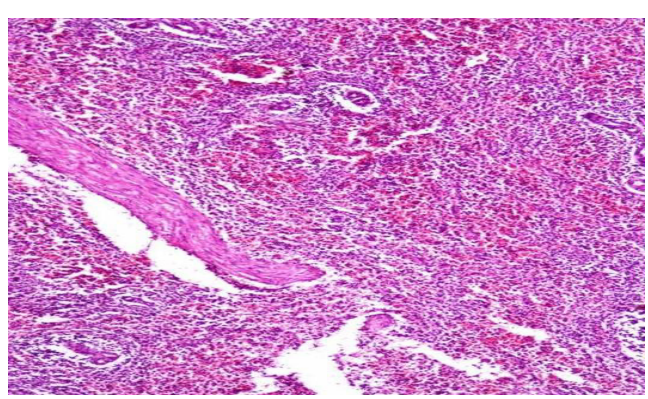

Fig 8. Spleen: Haemorrhages and depletion of lymphocytes in white pulp along with focal area of necrosis (Buffalo calf, *E. coli* and *Klebsiella* spp. infection) H & E x 100

Ribeira *et al*. (2000) and Singh *et al*. (2008) also reported such similar type of infections from diarrhoeic buffalo calves. *Strongyle* spp. infection in buffalo calves was also previously reported by Pal *et al.* (2001).

Gross pathological changes observed during postmortem examination of buffalo calves shown in Table 2. In the present study, gross lesion in intestine was catarrhal exudate indicating catarrhal enteritis followed by haemorrhages (Fig. 1), congestion and thickened mucosa. In mesenteric lymph node most prominent change was swelling (Fig. 1). In liver, most prominent changes were congestion and focal necrotic foci (Fig. 2) and ribs imprints along with pale diffuse necrotic areas, firmness and induration and hepatomegaly. Apart from this distension of gall bladder was also noticed. In abomasum, most prominent changes were congestion of mucosa along with thickening of mucosa (Fig. 3). In lungs, most prominent changes were congestion followed by consolidation, emphysema, focal abscesses (Fig. 4) and presence of whitish cheesy exudate and fibrinous layer. In kidney, major changes observed were congestion followed by haemorrhages and necrotic foci. Spleen revealed presence of petechial haemorrhages. There was distension in urinary bladder due to accumulation of urine. In trachea, whitish frothy exudate was observed. Apart from above, hydrothorax with sero-sanguinous fluid and adhesion of umbilicus with peritoneum along with inflammation of the umbilicus were also seen. More or less gross changes have also been reported by Charan and Pawaiya (1997); Khan and Khan (1997) and Carlson *et al*. (2002) in buffalo calves.

Histopathologically, intestine exhibited enteritis characterized by infiltration of leucocytes in mucosa (Fig. 5) and formation of diphtheritic membrane and necrosed mucosal glands. Other changes noticed were hyperplasia of goblet cells, necrosis of villi and congestion in mucosa and serosa. In abomasum, major microscopic lesions observed were necrosis of glands in mucosa along with mild leucocytic infiltration and congestion in mucosa. Similar findings have been reported by Singh *et al*. (1996). Mesenteric lymph nodes revealed presence of depletion of lymphocytes in cortical area and hyperplasia of cells, infiltration of leucocytes and focal area of necrosis in medullary region. The observations of Maity *et al*. (2000) support these findings. In liver, major microscopic lesions observed were hepatitis characterized by perivascular infiltration and dilatation of sinusoids. Other changes were presence of necrotic hepatitis characterized by presence of focal areas of necrosis of hepatocytes, congestion along with haemorrhages in parenchyma (Fig. 6). Cloudy swelling of hepatocytes and infiltration of leucocytes in portal triad area were also seen in few of the cases. Similar findings were reported by Motto *et al*. (1989) and Singh *et al*. (2000).

In lungs, there was suppurative pneumonia characterized by presence of sero-purulent exudates in alveoli, infiltration of leucocytes primarily neutrophils in parenchyma (Fig. 7). Bronchiolar lumen was found to be filled with leucocytes. Fibrinous pneumonia was also observed characterized by the presence of grey hepatization, thickening of interlobular septa due to presence of thick fibrinous layer and infiltration of leucocytes. Serous pneumonia characterized by serous fluid in alveoli, congestion and thrombosis in blood vessels, hemosiderosis and infiltration of leucocytes in parenchyma, necrosis of bronchiolar epithelium. Apart from this emphysema was also noticed. Trachea revealed mild tracheitis characterized by congestion and mild infiltration of leucocytes. Similar changes have been reported by Jubb *et al*. (1993) and Singh *et al*. (1996).

Kidneys revealed infiltration of leucocytes in interstitium, congestion and haemorrhages in parenchyma and also in capillaries of glomeruli along with tubular degeneration. In spleen, haemorrhages, depletion of lymphocytes in white pulp along and focal area of necrosis (Fig. 8) were observed. In heart, fragmentation and degenerative changes of muscle fibers was noticed along with presence of sarcocysts and vacoulative changes. Other changes noticed were congestion, infiltration of leucocytes and fatty changes also observed. These changes were also observed by Khan and Khan (1997) and Singh *et al*. (1996)

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