Haryana Vet. (Dec., 2017) **56 (2),** 158-162 **Research Article**

CLINICAL INCIDENCES AND HAEMATO-BIOCHEMICAL CHANGES IN OBSTRUCTIVE UROLITHIASIS AFFECTED BUFFALO CALVES OF EASTERN HARYANA

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ABSTRACT

Obstructive urolithiasis is an important surgical condition of bovines. Data pertaining to obstructive urolithiasis in buffalo calves presented at Referral Veterinary Diagnostic and Extension Center, Karnal, Haryana from July 2015 to June 2016 was investigated to assess various risk factors contributing to this condition. During the study period, a total of 294 buffalo calves and 3 lactating buffaloes involving mainly obstructive urolithiasis, cystitis, urinary bladder rupture and nephritis were presented. The maximum occurrence of 75.06% was recorded in calves up to 6 months of age, followed by more than one year (14.47%) and 6 months to one year old (9.42%). Seasonal pattern showed increased incidence in winter months (82.15%). Haemato-biochemical profile of 25 cases of obstructive urolithiasis was studied in order to find out possible contributing factors and formulate preventive strategy. A non-significant variation for Hb, PCV, TEC, monocytes, eosinophils and basophils was observed in diseased compared to healthy calves. However, neutrophilic leucocytosis was observed in cases of urolithiasis compared to healthy calves. Among various biochemical parameters, levels of glucose, creatinine and blood urea nitrogen were significantly elevated as compared to healthy ones while hypoalbuminemia was found in diseased calves. Activities of aspartate aminotransferase and alanine aminotransferase were significantly higher in diseased calves. Significant hypocalcemia, hyperphosphatemia, hyponateremia, hypocholremia, hyperkalemia and low ratio of Ca:P (1.36:1) was recorded in diseased calves. Alkaline urine along with haematuria and proteinuria were observed in all cases of urolithiasis. From the present study it is concluded that the improper calcium-phosphorus ratio in feed, reduced water availability during winter, derangement of acid-base balance and electrolyte predisposes male buffalo calves to urolithiasis.

Key words: Buffalo calves, urolithiasis, haemato-biochemical, hyponateremia

Bovine obstructive urolithiasis is a common disease caused by formation of uroliths in the urinary tract with successive blockage to urine outflow subsequently leading to uremia and death (Loreeti *et al.,* 2003). Predisposing factors like age, types of feed and water, soil, mineral hormone, winter season, castration and urinary tract infections etc. have been identified as playing vital roles in pathogenesis of ailment (Singh *et al.,* 2008). Among bovines, buffalo calves had a significantly higher incidence of obstructive urolithiasis than cow calves (Kushwaha *et al.,* 2009). Thus, the present study was conducted to study the various risk factors contributing to this disease and haemato-biochemical profile of calf urolithiasis cases to find out possible contributing factors and formulate preventive strategy.

MATERIALS AND METHODS

Clinical Incidences and Haemato-biochemical Studies: Data pertaining to obstructive urolithiasis in buffalo calves presented at Referral Veterinary Diagnostic and Extension Center, Karnal, Haryana from July 2015 to June 2016 were collected. Affected buffalo calves were examined clinically and urine samples were collected in a sterile container for examination. During surgical intervention, uroliths were collected for future study. For haemato- biochemical studies, 10 ml of blood was collected from 25 buffalo calves by jugular venipuncture and 2 ml of blood was transferred into EDTA vials and the remaining blood was kept for serum separation. Haemoglobin (Hb), packed cell volume (PCV %), total erythrocyte count, total leukocyte count (TLC), differential leukocyte count (DLC) were estimated within 2-4 h of collection by using blood cell counter (MS4Se, HD consortium). Biochemical parameters viz. calcium, phosphorus, magnesium, glucose, serum creatinine, blood urea nitrogen, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), Gamma Glutamyl transpeptidase (GGT), total and direct bilirubin, lipid profile were estimated by using the commercial kits (Erba diagnostics Ltd, India) using fully automatic biochemistry analyzer (EM DESTINY 180, Erba) whereas sodium, potassium, chloride, iCa ions, pH were estimated by using an electrolyte analyzer (HDC-Lyte, HD Consortium). Statistical analysis was performed by t-test using SPSS ver 15.

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RESULTS AND DISCUSSION

Clinical Observations: The clinical signs exhibited by the diseased calves varied from anorexia, depression, and restlessness in early stages and in later stage showed bilateral abdominal distension, grinding of teeth, dribbling of urine, arching back, recumbency.

Occurrence of Urolithiasis: During the study period, a total of 294 buffalo calves and three lactating buffaloes were brought at Referral Veterinary Diagnostic and Extension Centre (RVDEC), Karnal, involving mainly obstructive urolithiasis, cystitis, urinary bladder rupture and nephritis. A maximum occurrence of 75.06% was recorded in calves of upto 6 months of age, followed by more than one year (14.47%) and6 months to one year of age (9.42%; Fig. 1). Sex-wise analysis revealed 99% occurrence in male animals. Monthly pattern showed an increased occurrence from October to March (Fig. 2). The seasonal pattern showed an increased incidence in winter months (82.15%; Fig. 2). Similar findings were observed previously (Makhdoomi and Gazi, 2013; Bayoumi and Attia, 2017).

Urine Examination: The pH of urine of clinically healthy calves ranged between 8.5 and 9.0 and in confirmed cases of urolithiasis, it ranged between 9.0 and 9.4 revealing significant difference among healthy and affected animals. In bovine obstructive urolithiasis, urine is usually alkaline. The release of ammonia due to the breakdown of urea in the retained urine renders it alkaline (Nagy, 2009). Urine pH plays an important role in the formation of uroliths (Parrah *et al.,* 2013). All the confirmed cases showed presence of protein in urine. But no protein was present in urine of clinically healthy animals. The presence of protein in urine might be due to renal damage, which may cause passage of large protein molecules into urine. Haematuria in all, glycosuria in 10 and ketonuria in three animals were also observed. These finding may be correlated with kidney damage due to urolithiasis. There is severe damage to the bladder and urethral mucosa by uroliths which leads to haematuria (Makhdoomi and Gazi, 2013). Glucosuria in combination with hyperglycaemia reflects a tubular resorption defect in which the renal tubules fail to reabsorb glucose from the glomerular filtrate (Appel *et al.,* 2008).

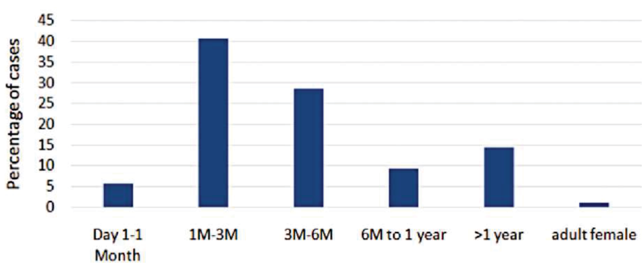

Fig. 1. Age wise analysis of incidence of calf urolithiasis

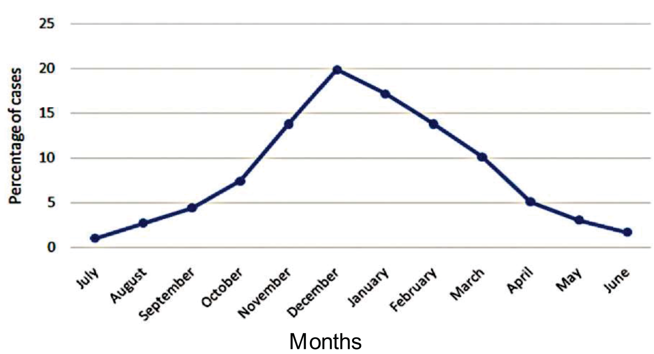

Fig. 2. Month wise analysis of calf urolithiasis cases

Haematological Observations: Blood picture exhibited non-significant variations for Hb, PCV, TEC and eosinophils in both the groups of animals and the values of all parameters were within the normal range (Sastry, 1985). A significantly higher neutrophil counts (56.64%) and lower lymphocyte counts in affected calves was noticed (Table 1). Neutrophilic leucocytosis may be due to stress, which causes release of adrenaline that reduces stickiness of neutrophils with erythrocytes causing increase in neutrophil pool. Neutrophilia was usually associated with uremia (Socket *et al*., 1986).

Biochemical Profile: Among various biochemical parameters studied, the levels of glucose, creatinine and serum urea nitrogen were found to be significantly elevated in confirmed cases of urolithiasis as compared to healthy group (Table 2) and results are in agreement with previous study (Mohamed and Wael, 2015). A creatinine test is used to measure the amount of creatinine in a patient's blood or urine. This helps determine how well the kidneys are able to filter small molecules. Elevated levels indicate kidney disease. As the disease progresses there is increase in the levels of creatinine (Makhdoomi and Marudwar, 1992).

The most prominent and descriptive measure of uraemia due to urolithiasis is blood urea nitrogen and creatinine that can be used as an index of uraemia because of their dependability and simplicity in assessment (Radostitis *et al.,* 2005). These findings are in agreement

Table 1
Haematological parameters of buffalo calves affected with urolithiasis

Parameters	Affected calves (n=25)		Healthy control (n=10)		P value
	Mean±SEM	Range	Mean±SEM	Range	
Hb (gm/dL)	11.3±0.38^{NS}	8-15.1	11.5±0.41	9.8-13.5	0.819
PCV(%)	34.8±1.10^{NS}	25-45	35.13 ±1.06	31.5-40	0.845
TEC($\times 10^6/\mu L$)	7.1±0.26^{NS}	4.95-9.26	6.79±0.27	5.3-8.1	0.528
TLC ($\times 10^3/\mu L$)	21.97±1.3**	8.9-32.5	8.562±0.672	6.12-11.12	<0.0001
Lymphocyte (%)	37.3±2.99*	20-65	49.3±0.93	45-55	0.0179
Monocyte (%)	1.5±0.14*	0-2	2.4±0.33	1-4	0.1069
Neutrophil(%)	58.6±2.87*	33-80	46.4±0.99	40-51	0.0126
Eosinophil (%)	2.6±0.23^{NS}	1-6	1.9±0.27	1-3	0.102

Hb=Haemoglobin; PCV=Packed cell volume; TEC=Total erythrocyte count, TLC=Total leukocyte count. Affected calves and healthy control differ significantly at *p<0.05, **p<0.01; NS=Non significant

with those of Gaji *et al.* (2015) and Bayoumi and Attia (2017). The confirmed cases showed significant hypoalbuminemia. Anorexia and chronic starvation may lead to depletion of TPP; however, dehydration could lead to increased concentration of TP in plasma (Benjamin, 1985). Similar results have been reported by Kumar *et al.* (1998). The ALT and AST enzyme activities were significantly higher in confirmed cases of urolithiasis while GGT activity was normal. Indirect bilirubin was significantly higher than healthy animals representing disturbance in bilirubin metabolism. Lipid profile viz. total cholesterol, triglycerides, LDL, and HDL was within the normal range for both the groups. Mohamed and Wael (2015) reported higher activity of AST and GGT in obstructive urolithiasis buffalo calves. They also estimated higher level of total cholesterol and normal level of total bilirubin in affected calves.AST is normally present in tissues like skeletal muscle, liver, RBC's, cardiac muscle and kidneys. Its high activity is non-specific indicator of the soft tissue damage (Carlson, 1990; Kerr, 2002).

Minerals and Electrolytes Profile: The ratio of Ca:P in blood of affected group was 1.36:1 as compared to healthy group (1.92:1) indicating disturbed ratio. Affected calves had significantly higher phosphorus and lowered calcium levels. Possible tissue hypoxia following retained

Table 2
Biochemical parameters of buffalo calves affected with urolithiasis

Parameters	Affected calves (n=25)		Healthy control (n=10)		P value
	Mean±SEM	Range	Mean±SEM	Range	
ALT (U/L)	55.1±3.95*	22-99	40±3.62	22-55	0.0252
AST(U/L)	145.5±6.47*	70-207	110.3±7.25	70-135	0.0036
GGT(U/L)	14.6±0.6^{NS}	10-19	15.6±0.7	12-17	0.065
Glucose (mg/)	106.7±4.50*	65-147	81.8±2.57	70.9-96	0.0018
TSP (g/dl)	6.5±0.10^{NS}	5.2-7.8	6.7±0.2	6.1-7.9	0.458
Albumin (g/dl)	2.6±0.08**	2.0-3.5	3.2±0.059	3-3.5	0.00064
BUN (mg/dl)	57.8±3.8**	35.3-105.7	16.9±0.84	11-20.3	<0.00001
Cr (mg/dl)	7.1±0.53**	2.4-11.7	0.9±0.045	0.7-1.1	<0.00001
TBIL (mg/dl)	1.3±0.1*	0.2-2.4	0.5±0.1	0.2-0.8	0.00353
DBIL (mg/dl)	0.2±0.04^{NS}	0.1-0.7	0.3±0.05	0.1-0.6	0.737
IBIL (mg/dl)	1.0±0.12**	0.1-2.3	0.2±0.04	0-0.4	0.000232
TG (mg/dl)	56.2±2.0^{NS}	39-80	48.6±3	40-65	0.0509
TC (mg/dl)	120.2±6.12^{NS}	54-170	132.6±3.7	112-144	0.240
HDL(mg/dl)	40±3.8^{NS}	11-72	46.5±2.4	39-56	0.302
LDL (mg/dl	69.4±4.5^{NS}	45-114.8	76.4±5.16	46.4-93.8	0.3813

Ca=Calcium; P=Phosphorus; Na=Sodium; K=Potassium; TSP=Total serum protein; ALT=Alanine aminotransferase; AST=Aspartate aminotransferase; GGT=Gamma-glutamyl transpeptidase; BUN= Blood urea nitrogen; Cr=Creatinine; TBIL=Total bilirubin; DBIL=Direct bilirubin; IBIL=Indirect bilirubin; Friedewald (1972) formula: LDL=TC-HDL-TG/5.0 (mg/dl)
Affected calves and healthy control differ significantly at *p<0.05, **p<0.01; NS=Non significant

Table 3
Haematological parameters of buffalo calves affected with urolithiasis

Parameters	Affected calves (n=25)		Healthy control(n=10)		P value
	Mean±SEM	Range	Mean±SEM	Range	
Ca(mg/dL)	8.7±0.24*	7-11	9.8±0.22	8.9-11	0.015
P(mg/dL)	6.4±0.21**	5-8.5	5.1±0.26	4-6	0.00007
Na^+(mmol/l)	131.3±2.14*	111.2-145.9	140±1.42	134.3-145.9	0.0187
K^+ (mmol/l)	6.7±0.2**	3.9-8.8	5.6±0.1	5.2-6.0	0.0054
iCa mg/dl	4.2±0.1*	3-5.4	4.8±0.1	4.4-5.4	0.0141
Cl^-(mmol/l)	88.2±2.12**	74.9-115.7	100.9±1.35	98.2-112.4	0.000881
Mg(mg/dL)	2.1±0.06**	1.54-2.62	2.5±0.06	2.25-2.89	0.001926

Ca=Calcium; P=Phosphorus; Na=Sodium; K=Potassium; iCa=ionized calcium; Cl^-=Chloride ion; Mg=Magnesium;
Affected calves and healthy control differ significantly at *p<0.05, **p<0.01; NS=Non significant

urine with breakdown of high energy phosphate compounds cause hyperphosphatemia which serve as a more reliable index under clinical situations (Makhdoomi and Gaji, 2013). There was significant hyponatremia, hypchloremia and hyperkalemia in urolithiasis cases. Ionized calcium was also significantly lower in affected calves (Table 3). Blood pH was toward alkaline side (8.1±0.1) and it was significantly (p<0.01) higher as compared to healthy group (7.5±0.1). Hyponatremia and hypchloremia could be attributed to the movement of sodium and chloride from interstitial compartment to the peritoneal cavity. Advanced obstructive nephropathy, poor renal perfusion, uroperitoneum and hemolysis result in hyperkalemia. Concurrent hyponatremia or hypocalcaemia may exacerbate the cardiotoxic effects of hyperkalemia and skeletal muscle weakness. A consequent digestive disorder results in hypochloremia due to sequestration of chloride ions in the digestive tract (Bayoumi and Attia, 2017). Retention of chloride in the gut occurs to compensate for large increase in potassium ions, decrease intake of chloride following anorexia, diffusion of chloride into the peritoneal cavity (Socket et al., 1986) and total body water expansion relative to total body chloride. Thus, urolithiasis leads to derangement of acid-base balance and electrolytes. Majority of the animals develop alkalosis but metabolic acidosis may also occur in advance stages (Makhdoomi and Gazi, 2013).

Osmotic pressure exerted due to hypertonic urine together with acute fibrinous peritonitis may promote rapid movement of large amounts of extra cellular water into the peritoneal cavity resulting in abdominal distension and dehydration. Various predisposing factors for urolithiasis include limited water intake, heavy concentrate-low roughage diets, alkaline water supplies, urine alkalinity, excess of sodium bicarbonate in the diet, vitamin imbalances e.g. hypervitaminosis D and hypovitaminosis A and high-protein rations (Drolet and Dee, 1999; Radostits et al., 2005). The feeding of livestock on heavy concentrate-low roughage or pelleted rations has been reported to result in increased production of mucoproteins which may act as a cementing agent favouring the formation of calculi (Radostits et al., 2000).

From the present study it was found that the improper calcium-phosphorus ratio in feed, reduced water availability during winter may predispose animals and calves to urolithiasis. Critical preventive measures such as providing a Ca:P ratio of 2:1 in ration, increasing the salt level to 4% of the diet in order to stimulate water consumption and to increase urine volume and the maintenance of adequate and abundant water supplies should be considered in calf rearing specially in winter season.

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