Some pathological and biochemical studies on fattening calves with urolethiasis in some areas at Sharkia governorate

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Abstract
urolithiasis was recognized and investigated in fattening calves were raised in some areas of Sharkia governorate due to feeding on unbalanced ration with an excess of underground water so the clinical cases showed restlessness, colicy pain, straining and olige urea. Calculus material composed mainly of magnesium, ammonium and phosphate. Urine analysis revealed increase in urinary sediment, PH and specific gravity. Phosphorus and magnesium concentration greatly increased while calcium was decreased. Biochemical studies showed increase in total protein, uric acid, blood urea nitrogen, serum creatinine, phosphorus and magnesium concentrations, while calcium levels were decreased. RBcs, Hb, Pcv and total WBcs were increased with neutrophilia. Pathological examination of the kidneys showed thickening of renal blood vessels, congestion accompanied with cellular and hyaline casts within renal tubules, while microscopical examination of urinary bladder revealed hemorrhage and congestion.
The present study was planning to study the formed urolithiasis in fattening calves in some areas of Sharkia governorate to determine its causes and so trying control or decrease these causes leading to increase production of meat.

Key words:
Urolethiasis, fattening calves, pathological, biochemical, kidneys, urinary bladder.

Introduction

Obstructive urolithiasis is the retention of urine subsequent to lodgment of calculi anywhere in the urinary conduct from up to urethral orifice. The disease results in heavy economic losses to the livestock industry as it is considered the fifth most prevalent cause of death in feedlot. Nephroliths are uroliths located in renal pelvis and / or collecting diverticula of kidney **(Gasthuys *et al.,* 1993)**.

Nephroliths may be clinically silent, obstruct the renal pelvis or ureter, predispose to pyelonephritis or result in compressive injury to renal parenchyma leading to renal failure **(Hoppe *et al.,* 1998)**.

Uroliths in cattle, sheep, and goats are common **(Macbeth 2008, Osborne *et al.,* 2009, Eccles 2010 and pan *et al.,* 2010)**.

It occurs in both sexes, although obstruction occurs most frequently in males and steers **(Nagy, 2011)**.

Urolith formation usually results from a combination of physiological, nutritional, genetic disarrangement and management factors. Decreased water intake, urinary stasis, altered urine pH, relative lack of inhibitors of crystallization; urinary tract infection, vitamin A deficiency, and high estrogen intake have all been

implicated as risk factors. Urolithisasis is mainly attributed to excessive or imbalanced intake of minerals **(Divers, 1990; Hossain *et al.,* 2003; pan *et al.,* 2010; VinodhKumar *et al.,* 2010 and Nagy, 2011)**.

The role of water hardness in the development of urolithiasis is controversial where water hardness is defined as the amount of calcium and magnesium found in water and is expressed as the concentration of calcium carbonate **(Schwartz *et al.,* 2002)**.

Early investigators reported an inverse relationship between drinking water hardness and calcium urolithiasis **(Churchill *et al.,* 1978 and Sierakowski *et al.,* 1979).**

Animals that consume additional water will be less likely to form highly concentrated urine and, as a result, urine will contain lower concentrations of calculogenic minerals. This will in turn minimize formation of crystals and uroliths. The simplest way of reducing the super saturation of urine is to increase the urinary volume **(Borgi *et al.,* 1999; Eccles, 2010 and pan *et al.,* 2010).**

Materials and Methods

This study was performed from July 2013 to January 2014. 25 male fattening calves (3-9) months old were used in this study, 15 suffering from severe colic and recumbancy which diagnostic may be urine retention and 10 used as control.

Urine analysis. urine analysis was carried out on the urine samples when available.

Ration examination. chemical analysis to the diet of affected animals to examine its contents from protein, ash, inorganic phosphorus, magnesium and calcium by the methods of association of official agricultural chest **(AOAC, 1980)**.

Water examination. five water samples were collected from underground and tap water, each sample were half liter for chemical analysis in central Lab., Faculty of Veterinary Medicine Zagazig University.

Blood examination. two blood samples from every animal were collected from jugular vein puncture in dry centrifuge tubes. The first blood sample was collected on heparin for haemogram **(Schalm, 2000)**, the second sample was collected without anticoagulant and the sera were separated and analyzed for determination of total protein, serum uric acid according to **(Henry, 1974)**, urea was determined according to **(Beale and Croft, 1961)** and creatinine by **(Husdan and Rapaport, 1968)**. Serum, calcium and inorganic potassium determined by **(Fernandez and Kohn, 1971)**, serum inorganic phosphorus detected by **(Goldenberg, 1966)** otherwise magnesium was applied by **(Glinders, 1971)** .

Examination of calculi. calculi were collected from urinary organs and analyzed chemically as described by calculi were collected from urinary organs and analyzed chemically as described by **(Gowenlock, 1988).**

Pathological examination. the macroscopic findings as well as the microscopic findings were recorded. The collected specimens from the kidneys and urinary bladder were fixed in 10% formalin solution .Paraffin sections of 5-micron thick were prepared and stained with hematoxalyin and eosin **(Bankcroft and Steven, 1996)** and then examined microscopically.

Statistical analysis of data was carried out according to **(Tamahane and Dunlop, 2000).**

Results

The collected calculi showed brown coloration with rough or smooth surface and it varied in diameter from 0.4-0.8 cm and ranged from 1.5-4.5 gm in weight, chemical analysis of calculus are shown in table (1).

Urine analysis as well as inorganic phosphorus (I.p), magnesium (Mg), Calcium (Ca) level in urine of fattening calves affected with urolithiasis are shown in tables (2,3).

Haemogram and biochemical changes in affected cases as compared with control are illustrated in tables (4, 5).

Chemical composition of consumed ration and analysis of underground and tap water are discussed in tables (6, 7).

Pathological findings.

The macroscopical examination of affected kidneys in 15 cases revealed variable size of cysts on the surface **Fig. (1)**. on cross section the parenchyma was grayish in color with impeded microcalculi in the medulla and pelvis. The urinary bladder was filled with urine; congested and edematous. The Histopathological examination of the kidneys showed mononuclear cell infiltrating renal medulla **Fig. (2)**, cellular cast inside renal tubules **Fig. (3)**, shrinkage of glomerular tuft, hyaline casts in renal tubules **Fig. (4)**, cystic dilatation of some renal tubules and or hyaline casts inside the lumina **Fig. (5)**, thickening in renal blood vessel's wall together with mild congestion and perivascular edema were seen **Fig. (6)**. Coagulative necrosis and degeneration were found in convoluted and collecting tubules of some cases.

Microscopical findings of the urinary bladder showed severe hemorrhage and congestion in outer tunica muscularis and subserosa**.**

Discussion

Urolithiasis syndrome is considered a big economic problem in fattening calves occurred in certain areas where the pasture and diet have a high content of calcuogenic contents.

Upon obtained results in tables (1, 3, 5, 6, 7), it appears that the chemical composition of urinary calculi depended mainly on type of diet. Phosphorus and magnesium salts were the main ingredients in the chemical composition of the urinary calculi as well as calcium and ammonia were relatively less table (1). These results are in agreement with **(Mohga Abd El Razik *et al.*, 2000).**

According to physico chemical findings of urine, table (2) showed that the urine was strongly alkaline because the nature of ration and water table (6&7). These results were reported by **(Borgi *et al.*, 1999; Eccles, 2010 and pan *et al.*, 2010)** whom mentioned that animals consumed additional water will be less likely to form highly concentrated urine and as a result, urine will contain lower concentrations of calculogenic minerals. This will in turn minimize formation of crystals and uroliths. Meanwhile, as mentioned by **(Radostitis *et al.*, 1995)** high level of minerals in diets increase the urinary salts which prompting the process of crystallization and precipitation but also in ruminant feeding high concentrate , low roughage diet increases the levels of urinary muco proteins. This notes explained the phosphate nature of formed calculi. Magnesium phosphate solubility decreased by increasing urine pH creates suitable media for uroliths formation minerals **(Divers, 1990, Hossain *et al.*, 2003, pan *et al.*, 2010; VinodhKumar *et al.*, 2010 and Nagy, 2011)**. Urinary sediments appeared microscopically in all affected cases which consisted mainly of triple and amorphous phosphate in the form of elongated coffin-lid crystals, calcium oxalate and calcium carbonate with increased RBcs amounts, pus cells and desquamated epithelial cells. These findings were in agreement with **(Mohga Abd El Razik *et al.*, 2000).**

Proteinuria and haemoglobinuria were reported in most affected cases indicated renal diseases. Regarding to chemical constituents of urine table (3) phosphorus, magnesium and potassium values were elevated than healthy animals used as control.

Obtained hematological results table (4) revealed a marked elevation in erythrocytic count, hemoglobin concentration and packed cell volume which may be caused by a decreased in plasma volume as mentioned by **(Boyed *et al.*, 1974)**. These results were similar to these obtained by **(Mohga Abd El Razik *et al.*, (2000)**. Such elevation may be attributed to dehydration as the owners of the animals usually preferred deprivation the animals of water to avoid urinary bladder rupture. Lecuocytosis with marked neutrophilia, lymphopenia, eosinopenia and monocytopenia were prominent in affected calves as shown in (table 4) as mentioned by **(Coles, 1986)** who corrugated the efficacy of stress (pain) and release of cortisone.

Marked elevation of uric acid, blood urea nitrogen and creatinine was recorded as in (table 5) may be attributed to pathogenesis occurred

in kidneys. This expectation was confirmed by **(Coles, 1986 and Radostitis *et al.*, 1995).**

Affected calves showed a significant increase in serum total protein level (table 5) regarding to the control ones. These results may be attributed to severe dehydration due to water deprivation among calves. These results were in accordance with the results obtained by **(El-Sayed, 1996)**.

Serum phosphorus and magnesium (table 5) were significantly increased in affected calves and may be attributed to high phosphorus intake tables (6&7) in addition to decreased calcium level (hypocalcemia) which attributed to hyperphosphatemia as the pathophysiologic relationship changed. Imbalance of the normal calcium: phosphorus ratio, abnormal quantities excretions of phosphorus into the intestine led to formation of non absorbable calcium salts and poor utilization of vit. D. All these factors were considered the causative role of hypocalcemia following nephrolithiasis. These results were in agreement with the results obtained by **(Agag *et al.*, 1988)** whom study the phosphatic urolithiasis etiology in fattening lamb.

Hyperkalemia may occur with renal failure especially in acute phase with anurea or oligurea as supported by **(Coles, 1986)**.

Histopathological examination of the kidneys showed destruction of some renal tubules, mononuclear cell infiltrating renal medulla, cellular and hyaline casts inside renal tubule, shrinkage of glomerular tuft, perivascular edema, coagulative necrosis and degeneration were found in convoluted and collecting tubules (Figs 2-7). While microscopical findings of the urinary bladder showed severe hemorrhage and congestion in outer tunica muscularis and subserosa (Fig.8). These results were confirmed by **(Mohga Abd El Razik *et al.*, 2000)**. Such results may be attributed to the migration of urinary calculi to the urethra and appearance of clinical signs of urine retention in affected calves.

Finally it could be concluded that urolithiasis was a problem with many causes with majority to ration type and minerals in water (hardness of water aid in urolithiasis formation.

Hence our attention must be given to fattening calves feeding on adequate balanced mineral content in addition to good mangemental factors may decrease the risk of calculi formations.

Table 1. Chemical analysis of urinary calculi.

Organic matter	Inorganic matter	phosphorus	Ammonium	Calcium	Magnesium	Carbonate
-	+	+++	+	+	+++	-

Table 2. Physical changes of urine of fattening calves affected with urolithiasis.

Physical		Control (10)	Affected (15)
	Specific gravity	1.010-1.020	1.030-1.040
	PH	7.2-7.6	7.6-8.3
	Turbidity	Transparent	Turbid (+)
	Deposit	traces	++
Microscopical	Desquamated EP. cells	Few	+
	R.B.Cs	Few	+
	Pus cells	Nil	+
	Albumin urea	Nil	++
	Hemoglobin urea	Nil	+

Table 3. Chemical changes of (Ph, Mg, Ca), level in urine of fattening calves affected with urolithiasis.

Animals		Ph mg/dl	Mg mg/dl	Ca mg/dl
Control (10)	Mean ± S.E	26.90 ± 1.9	23.29 ± 2.18	5.45 ± 0.47
Affected (15)	Mean ± S.E	48.76 *** ± 1.3	53.40 *** ± 3.16	3.7 * ± 0.40

Significant at p<0.01 *
Very highly significant at p<0.001***

Table 4. Haemogram (mean values+ S.E) of fattening calves affected with urolithiasis compared with Normal one.

Animals		Hb gm/100	Pcv %	RBcs X10^6	WBcs X10^3	Differential leucocytic count				
						Neut	Lymph	Eos	Bas	Mon
Control (10)	Mean ± S.E	9.86 ± 0.42	28.79 ± 1.3	6.07 ± 0.35	10.27 ± 0.37	3.50 ± 0.26	5.1 ± 0.41	0.80 ± 0.1	0.50 ± 0.07	0.35 ± 0.05
Affected (15)	Mean ± S.E	12.35 * ± 0.68	36.5* ± 1.97	9.6*** ± 0.56	13.74 ** ± 0.62	7.93 *** ± 0.34	4.75 ± 0.62	0.77 ± 0.13	0.18 ** ± 0.02	0.11 ± 0.02

Significant at p<0.05 *
Highly significant at p<0.01**
Very highly significant<0.001***

Table 5. (mean + stander error) of some biochemical blood values and serum minerals of fattening calves affected with urolithiasis

Animals		T.p gm%	Uric acid mg/100 ml	Urea mg/dl	Creatinine mg/dl	Ca mg/dl	Ph mg/dl	Mg mg/dl	K mEq/l
Control (10)	Mean ± S.E	5.6 ± 0.29	2.91 ± 0.2	22.6 ± 0.84	1.12 ± 0.26	10.28 ± 1.06	5.82 ± 0.89	2.70 ± 0.49	5.00 ± 0.51
Affected (15)	Mean ± S.E	7.60** ± 0.37	4.9** ± 0.38	73.60 *** ± 2.6	3.51* ± 0.66	8.55 ± 0.44 N.S	8.8 * ± 0.72	4.2* + 0.30	7.1 ± 0.98 N.S

Significant at p<0.05 *
Highly significant at p<0.01**
Very highly significant<0.001***
N.S: Non significant

Table 6. Chemical composition of consumed ration, Compared with permissible limit of dietary ration.

Chemical composition	ration % Dietary	Hay %	Permissible limit of dietary ration
Crude protein	16.3 %	12 %	16.2%
Ash	6.4 %	4.5 %	5.4%
Calcium	0.45 %	1.2 %	0.5%
Phosphorus	0.75%	0.85%	0.3%
Magnesium	0.32%	0.3%	0.23%

N.B: Unbalanced ration containing lower calcium: phosphorous ratio.

Table 7. Analysis of underground and tap water.

composition Chemical		Under ground water	Tape water
ppm	Total soluble salts	2200.0 **	492.0
mg/ L	Bicarb	3.10	2.20
mg/ L	Chloride	19.20	4.9
mg/ L	Sulphate	11.51	0.92
mg/ L	Calcium	9.3	3.00
mg/ L	Magnesium	5.5	1.68
mg/ L	Phosphorus	* 0.89	0.72

N.B: Underground water has high soluble salts and phosphorus.

Fig. (1). Kidneys showing cysts filled with serous fluids in its surface. (H& E x 300).

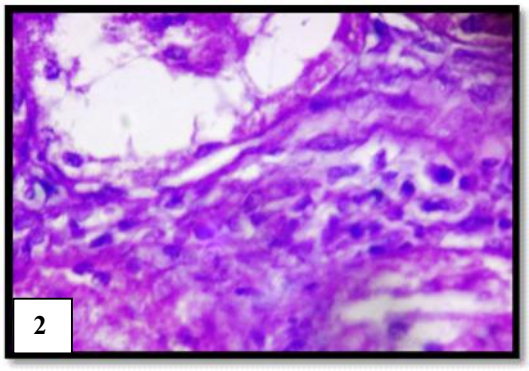

Fig. (2). Kidneys showing mononuclear cell infiltrating renal medulla (arrow). (H& E x 520).

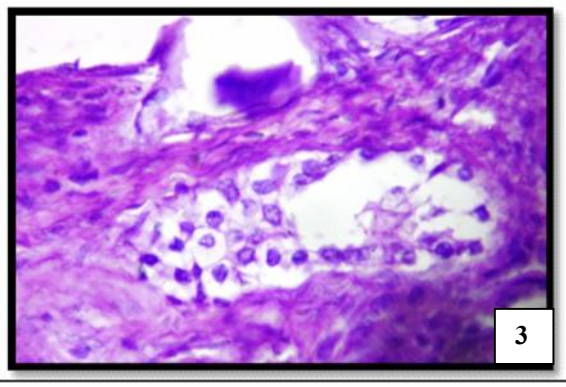

Fig. (3). Kidneys showing cellular cast inside renal tubules. (H& E x 300).

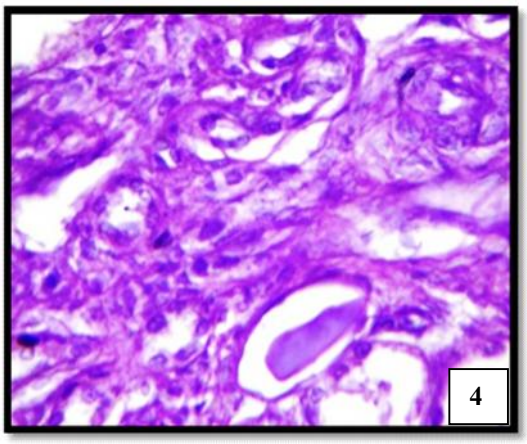

Fig. (4). Kidneys showing hyaline casts in renal tubules. (H& E x 520).

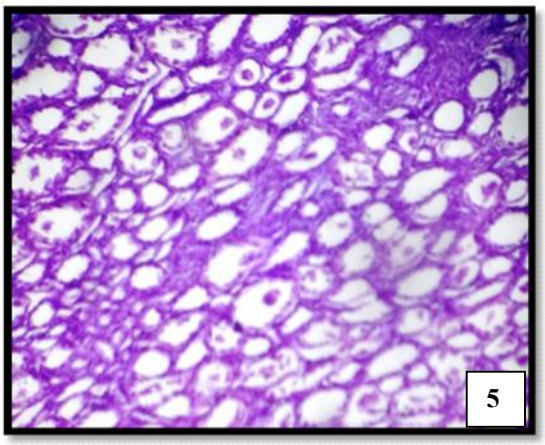

Fig. (5). Kidneys showing cystic dilatation of some renal tubules and or hyaline casts inside the lumina. (H& E x 300).

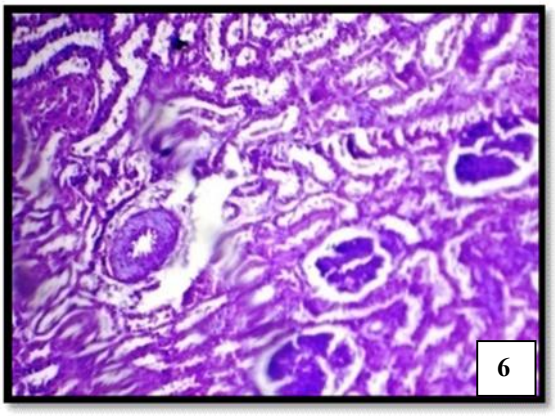

Fig. (6). Kidneys showing thickening in renal blood vessels associated with perivascular edema. (H& E x 300).

References

Agag, B.I., Hafez, M.A.M., Safwat, E.E.A. and Nadia, M. Aly. (1988). Phosphatic urolithiasis as a field problem in goat farm. J. Egypt Vet. Med. Associated., 48, (1): 25.

AOAC (1980). Association of Official Chemists official methods of analysis 13 th Ed. Washigton S.D.C.

Bankcroft, G.D. and Stevens, A. (1996). Theory and Practice of Histopathological Technique. 4th edition, Churchill living stone Edinburgh London, Melbourne and New York.

Beale, R.N. and Croft, D.J. (1961). Determination of BUN. j. Clin. Path.14 (4): 418-424.

Borgi, L., Mischi, T., Schianchi, T., Briganti, A., Guerra, A., Allegri, F. (1999). Urine volume: stone risk factors and preventive measure. Nephrone 1999; 81 (1): 66-70.

Boyed, J.W., Baker, J.R. and Leyland, H. (1974). Neonatal diarrhea in calves. Vet. Rec. 95:310.

Churchill, D., Bryant, D., Fodor, G., Gault, M.H. (1978). Drinking water hardness and urolithiasis. Ann Intern Med; 88 (4): 513-514.

Coles, E.H. (1986). Veterinary clinical pathology.4th Ed. W.B. Saunders Company.

Divers, T.J. (1990). Diseases of the renal system. In: Smith BP (Ed). Large Animal Internal Medi-cine. CV Mosby Co., St Louise.

Eccles, J. (2010). Obstructive Urolithiasis (Bladder Stones) in Cattle. Northern Terri-

tory Government, K57, 1-3. Accessed December: http:// nt.gov.au/d.

El-Sayed Zabady, M.K. El-sayed (1996). Studies on urolithiasis in ruminants. M.V.Sc thesis. Faculty of veterinary Medicine Cairo University.

Fernandez, F.J. and Kohn, H.L. (1971). Clinical methods of atomic absorption spectrophotometer. Clin. Chem. News 13: 24-28.

Gasthuys, F., Steenhaut, M., De Moor, A., Sercu, K. (1993). Surgical treatment of urethral obstruction due to urolithiasis in male cattle: a review of 85 cases. Vet. Res. 133 (21): 522-526.

Glinders (1971). Colorimetric method for the determination of magnesium. Clin. Chem. 17: 662.

Goldenberg (1966). Determination of inorganic phosphorus. Clin. Chem (12): 871-882.

Gowenlock, A.H., McMurray, J.R. and Mclauchan, D.M. (1988). Practical clinical biochemistry, 6th ed. Londen: Heinemann Medical book, 756-761.

Henry, R.Z. (1974). Clinical chemistry, principle and techniques, 2nd edition, Harper and raw .p.52.

Hoppe, B., Hesse, A., Bromme, S., Rietschel, E., Michalk, D. (1998). Urinary excretion substances in patients with cystic fibrosis: risk of urolithiasis? Pediatr Nephrol 12(4): 275-279.

Hossain, R .Z., Ogawa, Y., Hokama, S., Morozumi, M., Hatano, T. (2003). Urolithiasis in Okinawa, Japan: A relatively high prevalence of uric acid Stones. Int J Urol; 10: 411-415.

Husdan, H. and Rapaport, A. (1968). Estimation of creatinine by Jaffe reaction a comparison of three methods. Clin. Chem. 14,(3): 222-238.

Macbeth, B.J. (2008). Obstructive urolithiasis, unilateral hydronephrosis, and probable nephrolithiasis in a 12-year old Clydesdale gelding. Can Vet J; 49: 287-290.

Mohga, S.Z. Abd El Razik, Ibtisam, M.G. El Din and El Kabbani, A.W. (2000). Urolithiasis syndrome as a problem among fattening buffalo calves in eastern delta. Faculty of Veterinary Medicine Mansoura University. Volume II, No 2 ISSN 1110-7219.

Nagy, D.W. (2011). Urolithiasis in small ruminants (Proceedings). Accessed 1 Agust: http://veterinarycalendar.dvm360. Com/ ArticleStandard/Article/detail/650466.

Osborne, C.A., Albasan, H., Lulich, J.P., Nwaokorie, E., Koehler, L.A., Ulrich, L.K. (2009). Quantitative analysis of 4468 uroliths retrieved from farm animals, exotic species, and wildlifes to the Minnesota Urolith Center: 1981 to 2007. Vet Clin North Am Small Anim Pract; 39: 65-78.

Pan, X.L., Wen, Z.S., Zou, X.T., Zhou, E.K., Kou, H.Q., Xu, Z.R., Zhang, W.J. (2010). Chemical composition and microstructure of uroliths associated with the feeding of high-level cottonseed meal diet to sheep. Res. Vet. Sci; 91(3): 405-411.

Radostitis, O.M., Blood, D.C. and Gay, C.C. (1995). Vet. Medicine 8th Ed. Tindall, London Philadelphia, 14-69.

Schalm, O.W. (2000). Veterinary Hematology, 5th Ed. Wolter Kluwer Company, Philadelphia, Baltimore, New York and London.

Schwartz, B.F., Schenkman, N.S., Bruce, E.J., Leslie, S.W., Stoller, M.L. (2002). Calcium nephrolithiasis: effect of water hardness on urinary electrolytes. Urology; 60: 23-7.

Sierakowski, R., Finlayson, B., Landes, R. (1979). Stone incidence as related to water hardness in different geographical regions of the United States. Urol Res; 7:157-160.

Tamhane, A.C. and Dunlop, D.D. (2000). Statistics and data analysis from elementary to intermediate upper saddle River, USA.

VinodhKumar, O.R., Swarnkar, C.P., Shinde, A.K., Singh, D. (2010). Clinical, mineral and haemato-biochemical studies of urolithiasis in weaner lambs. AJAR; 5(15): 2045-2050.

بعض الدراسات الباثولوجية والبيوكيميائية فى عجول التسمين التى تعانى من وجود حصوات بالمجارى البولية ببعض المناطق فى محافظة الشرقية

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قسم الباثولوجيا والباثولوجيا الاكلينيكيه

معهد بحوث صحة الحيوان - المعمل الفرعي بالزقازيق

الملخص العربى

تم إجراء هذه الدراسة على عدد 25 عجل من عجول التسمين . 15 عجل يعانوا من احتباس بالبول ويتناولواعلائق غير متوازنه ويستخدموا مياه الاباركمصدر لمياه الشرب بالاضافه الى 10 عجول سليمه اكلينيكيه وتتناول علائق متوازنه, استخدمت كمجموعه ضابطه وقد فحصت الحيوانات بعد اخذ عينات من البول والدم وايضا العليقه ومياه الشرب المقدمه للحيوانات.وظهرت الاعراض الاكلينكيه فى وجود مغص والام مستمره بالبطن واحتباس فى البول والامتناع عن تناول العليقه ومياه الشرب.كما اوضحت نتائج تحليل البول للحالات المصابه بارتفاع فى قلوية البول والكثافه النوعيه, كمااوضحت النتائج ارتفاع نسبة الفوسفور والماغنيسيوم وايضا خلايا طلائيه متهتكه بالبول.

وبتحليل الحصوات التى وجدت فى الجهاز البولى وجد انها تتكون من ترسيبات لاملاح الماغنسيوم والفسفور والامونيوم.

وبتحليل مصل دم العجول المصابه باحتباس البول وبمقارنتها بالسليمه وجد زياده معنويه فى البروتين الكلي وحمض البوليك واليوريا نيتروجين والكرياتينين والفوسفور والماغنيسيوم وايضا وجد نقص معنوي فى عنصر الكالسيوم.

كما اوضحت صورة الدم ارتفاع فى نسبة كرات الدم الحمراء والهيموجلوبين وكرات الدم البيضاء,خاصة الخلايا المتعادله .

كما اوضحت الدراسه الباثولوجيه وجود تغيرات فى نسيج الكلى والمثانة.

من النتائج السابقه ننصح بالاهتمام بالتغذ يه السليمه والمتوازنه فى مكوناتها من علائق واملاح معدنيه وتحليل دوري لمياه الشرب ومعالجتها للتقليل من حدوث الحصوات ومدي خطورتها على الكلى.