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## Study on the frequency and chemical composition of urinary stones of Moghani sheep in Northwest of Iran

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**ABSTRACT:** Nephrolithiasis is one of the most important urinary tract diseases of ruminants and a costly problem for many sheep and goat owners. Therefore, it is important to analyse the composition of renal stone to prevent the formation of these kind of stones. The present study aimed to determine the frequency and the chemical constituents of urinary stones of Moghani sheep in Northwest of Iran. The kidneys, ureters, and urinary bladders of 384 Moghani sheep were examined carefully. The prevalence of urinary stones was 3.7%. All the stones were in the pelvis of the kidney and no stones were found in the ureter or urinary bladder. Macroscopically, 85.7% of the urinary stones showed a rough (jagged) appearance and 12.3% of them a smooth appearance. The largest and smallest urinary stones were 7 mm and 2 mm in diameter and 0.600 g and 0.087 g in weight, respectively. The composition of calculi was pure or mixed and included calcium oxalate (44.44%), magnesium ammonium phosphate (33.33%) and calcium carbonate (22.22%). In conclusion, the predominant constituent of urinary stones was Calcium oxalate, followed by magnesium ammonium phosphate and calcium carbonate. More detailed studies should be designed to clarify the role of environmental and nutritional factors in the development of urinary stones.

**Keywords:** Small ruminants; Nephrolith; Calcium oxalate; Calcium carbonate; Struvite.

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## INTRODUCTION

Nephrolithiasis is one of the most important urinary tract diseases of ruminants and a costly problem for many sheep and goat owners (Makhdoomi and Gazi, 2013; Jones et al., 2017; Alimi et al., 2018). The urinary calculi etiology is complex and multifactorial that usually results from a combination of several nutritional, physiological and management factors (Constable et al., 2017; Smith et al., 2020). Uroliths may occur due to dehydration, limited intake or deprivation of water, excessive or imbalanced intake of minerals, vitamin imbalances (such as hypervitaminosis and hypovitaminosis), mineralized artesian water, alkaline water supplies, excess of sodium bicarbonate in diet, urine alkalinity, heavy concentrate-low roughage diets, and high protein rations (Makhdoomi and Gazi, 2013; Constable et al., 2017; Smith et al., 2020). The highest prevalence of urolithiasis in ruminants has been reported to occur in late fall and winter which is theoretically due to decreased water intake and increased urine concentration (Bailey, 1981).

Uroliths have various chemical compositions that appear to depend largely on the dietary intake of individual elements (Constable et al., 2017; Smith et al., 2020). Urinary stones are classified according to their chemical composition including magnesium ammonium phosphate (struvite), silica, calcium oxalate, and calcium carbonate (Constable et al., 2017; Smith et al., 2020). Calculi containing calcium carbonate are more common in animals on clover-rich pasture or when oxalate-containing plants abound (Ewoldt et al., 2008; Smith et al., 2020). Sheep usually have calculi composed of struvite, magnesium ammonium phosphate. High concentration of magnesium in feedlot rations also cause a high prevalence of magnesium ammonium phosphate calculi in lambs (Constable et al., 2017; Smith et al., 2020). Ammonium, calcium, and magnesium carbonate are also common constituents of calculi in cattle and sheep on pasture.

Renal and ureteral calculi can produce partial or complete obstruction of one or both sides of the upper urinary tract. Nephroliths usually develop within or adjacent to the renal pelvis and obstruction can lead to hydronephrosis (Constable et al., 2017; Smith et al., 2020). The major reported clinical signs include anorexia, suspended rumination, and decreased water intake. Animals suffering from partial obstruction shows dribbling of blood-tinged urine, painful attempts of urination, grinding of teeth, colic, tenesmus,

urethral pulsation, weight shifting and the tendency of rectal prolapsed (Constable et al., 2017; Smith et al., 2020). Knowledge of urinary stone compounds can be very helpful in diagnosis, treatment and prevention. Therefore, the present study aimed to determine the frequency of urinary stones in sheep and its chemical components.

## MATERIAL AND METHODS

In this study, 384 Moghani sheep slaughtered in Northwest of Iran were examined for urinary stones during the six months in fall and winter, 2020. The kidneys, ureters and bladder of sheep were examined carefully for urinary stones.

In the first stage, the stones were washed with distilled water to remove fibrin from their surface. The weight of the stones was measured using digital scales and their size was measured with a calliper.

Information on diet and age of sheep with stones was recorded in a questionnaire. The water of the region was analyzed by standard methods. Heavy metals were measured using atomic absorption spectrophotometer and other parameters were analyzed based on the Standard Methods (Panahifard et al., 2017).

### Urinary stone analysis

To determine the chemical composition of stones by manual colorimetric method, a urinary stone analysis kit (Darman Faraz Kave, Iran) was used. Homogeneous powder of each urinary stone was prepared and dissolved in sulfuric acid. The sublimation of carbon dioxide indicated the presence of carbonate. Sodium hydroxide, calconcarboxylic acid and titriplex III reagent were used to detect calcium content of the urinary stones. Oxalate content of urinary stones were detected by adding borate buffer, iron chloride and sulfosalicylic acid to the solution. Ammonium content of urinary stones was determined by adding Nessler A and hydroxide sodium to the solution. Ammonium molybdate and 4-methylamino phenol sulfate were used to detect phosphate content of urinary stones. Magnesium content of urinary stones was detected by adding borate buffer and color reagent to the solution. Uric acid of urinary stones was determined by adding molybdato phosphoric acid and borate buffer to the solution. Ammonia 10%, sodium sulfite powder and sodium Nitroprussiate reagent were used to detect cystine content of urinary stone (Maurer and Gots, 1976; McIntosh and Salter, 1942)

## RESULTS

14 out of 384 (3.7%) slaughtered sheep in North West of Iran had urinary stone (Figure 1). All the stones were in the pelvis of the kidneys. Urinary stones were unilateral (left or right kidney), and each affected sheep had only one urinary stone. 71.4% of urinary stones were in the left kidney and 28.6% of urinary stones were in the right kidney. Macroscopically, 85.7% of the urinary stones had a rough (jagged) appearance and 12.3% of them had a smooth appearance. The largest and smallest urinary stones were 7 mm and 2 mm in diameter and 0.600 g and 0.087 g in weight, respectively (average of 3.9 mm and 0.295 g). They were mostly grey white or yellowish brown (Figure 1). Among all urinary stones, one stone was found in sheep under one year old, five stones in sheep between 2 and 4 years old and 8 stones in sheep over 4 years old.

Six (42.9%) urinary stones were composed of pure calcium oxalate and three (21.4%) urinary stones were consisted of calcium carbonate and magnesium ammonium phosphate. Two (14.3%) urinary stones were composed of pure magnesium ammonium phosphate and two (14.3%) urinary stones were consisted

of calcium oxalate and magnesium ammonium phosphate. The last one (7.1%) was composed of pure calcium carbonate.

The results of water analysis are given in Table 1. The values of all parameters were lower than the standard (WHO, 2008).

## DISCUSSION

The frequency of urinary stones and their composition in Moghani sheep were evaluated in the present study. All the sheep under study were slaughtered as healthy animal and urinary stones were an accidental finding in these animals.

Urinary stones can be formed in the renal pelvis, the ureter and most frequently in the bladder (Makhdoomi and Gazi, 2013; Constable et al., 2017). In this study, all the stones were found in the renal pelvis. In a study by Riet-Correa et al. (2008) on 28 rams and 16 goats with urolithiasis the main stones were in the urethra and urinary bladder and most of the affected animals were died from hemorrhagic necrotizing urethritis and rupture of the urethra (Riet-Correa et al., 2008). In another study on sheep and goats most



Figure 1.

**Table 1.** Mean chemical parameters of drinking water in Northwest of Iran

Parameter	Measured values(mg/L)	Permissible values* (mg/L)
pH	7.7	6.5-8.5
Total Hardness	265	500
TDS	646	1000-1500
Cl <sup>-</sup>	110	250-400
HCO <sub>3</sub> <sup>-</sup>	190	300
SO <sub>4</sub> <sup>-2</sup>	167	250-400
NO <sub>2</sub> <sup>-</sup>	7.6	3
NO <sub>3</sub> <sup>-</sup>	0.03	50
PO <sub>4</sub> <sup>-3</sup>	0.04	50
CO <sub>3</sub> <sup>-2</sup>	0	0-3
F <sup>-</sup>	0.4	1.5
Ca <sup>+2</sup>	66	75-300
Mg <sup>+2</sup>	27	30
Na <sup>+</sup>	98	100-200
Fe <sup>+2</sup>	0.13	0.3
Pb	0.0077	0.05
Cd	0.0007	0.005
Cu	0.046	1
As	0.0075	0.05

\* WHO, 2008

(**Abbreviations:** TDS: Total dissolved solids, Cl<sup>-</sup>: Chloride ion, HCO<sub>3</sub><sup>-</sup>: Bicarbonate ion, SO<sub>4</sub><sup>-2</sup>: Sulfate ion, NO<sub>2</sub><sup>-</sup>: Nitrite ion, NO<sub>3</sub><sup>-</sup>: Nitrate ion, PO<sub>4</sub><sup>-3</sup>: Phosphate ion, CO<sub>3</sub><sup>-2</sup>: Carbonate ion, F<sup>-</sup>: Fluoride ion, Ca<sup>+2</sup>: Calcium ion, Mg<sup>+2</sup>: Magnesium ion, Na<sup>+</sup>: Sodium ion, Fe<sup>+2</sup>: Iron ion, Pb: Lead, Cd: Cadmium, Cu: Copper, As: Arsenic)

of the stones were also seen in the urethra and urinary bladder (Jones et al., 2017). It seems that urinary stones in the renal pelvis of sheep are not as serious as the urinary stones in the ureter or the bladder.

Urinary stones were detected in the kidneys of 14 out of 384 (3.7%) slaughtered sheep. Amarपाल et al. (2004) reported the prevalence of 1.04% nephrolithiasis in sheep in India. Rakesh et al. (2014) reported the prevalence of 0.6% nephrolithiasis in goats in India. Guven et al. 2003 reported different prevalence of nephrolithiasis of sheep in different regions of Turkey as 5.70% in Kars, 7.02% in Ardahan and 3.92% in Igdir. Sheikh et al. (2018) reported the prevalence of 4.66% urolithiasis in goats in Kashmir. The frequency of urinary stones varies in different parts of the world, which may be due to differences in the amount of water compounds in each region. Eating habits can also play a role in the incidence of urinary stones. Concentrate feeding and alkaline urine has been shown to result in greater prevalence of urolithiasis than the free grazing system and acidic urine (Sen et al., 2018).

In the present study, higher prevalence of urinary stones (57.14%) was observed in sheep over 4 years old, followed by 35.71% in age group 2-4 years and

7.14% in sheep below 2 years. Our results were inconsistent with other studies on affected animals referred to the clinic such as Jones, et al. 2017 which reported higher prevalence of urolithiasis in small ruminants below 3 years (Jones et al., 2017). Sheikh, et al. 2018 also reported a higher prevalence of urolithiasis in goats below 2 years (Sheikh et al., 2018). In the study of Sheikh et al. (2018) on goats it was found that the animals within the age limit 0-6 months showed a higher prevalence of urolithiasis than the later stages. Feeding of high proteinaceous diet during early age and changes brought about by weaning could be the reasons (Sen et al., 2018; Skeikh et al., 2018).

The preventive approach of urinary stones is to determine the risk factors related to diet, management, environment and mineral analysis of urinary stones to accurately identify the causative agents. Calcium oxalate, silica, magnesium ammonium phosphate and calcium carbonate are the most common types of crystals found in ruminants (Constable et al. 2017). In the present study, the composition of calculi were pure or mixed and included calcium oxalate (44.44%), magnesium ammonium phosphate (33.33%) and calcium carbonate (22.22%). Calcium oxalate was the major constituent in the urinary stones, in the

present study. Oryan, et al. 2015 also reported calcium oxalate as a major urinary stone in cattle, in Iran. Grazing on the pasture plants containing large quantities of oxalate is the main factor in the development of the urinary calcium oxalate (Constable et al., 2017; Jones et al., 2017). In Western Australia, the majority of urinary stone in sheep have silica as the predominant mineral element. Feeding on oatmeal or oat flakes and acidic urine has been reported as the reason (Gardiner, 1965). The composition of urinary stones can vary depending on the geographical location of the area (Makhdoomi and Gazi, 2013). Magnesium ammonium phosphate urinary stones occur due to high grain feeding and low dietary calcium to phosphorous ratio (Jones et al., 2012; Jones et al., 2017). They also form when the urine become supersaturated with magnesium, ammonium or phosphorous and when urine pH is  $> 6.5$  (Jones et al., 2012). Calcium carbonate urinary stones are more likely found in animals consuming large amounts of legumes (Jubb and Kennedy, 1970; Jones et al., 2012; Jones et al., 2017). Alkaline urine (pH  $> 7.0$ ) also supports the formation

of calcium carbonate stones (Constable et al., 2017). Therefore, feeding of ammonium chloride (at 0.5%-2.0% of dry matter intake) may prevent urinary stones caused by magnesium ammonium phosphate or calcium carbonate (Constable et al., 2017).

## CONCLUSIONS

The frequency of urinary stones and their composition in Moghani sheep slaughtered in northwest of Iran were evaluated in the present study. The prevalence of urinary stones was 3.7%. The predominant constituent of urinary stones was Calcium oxalate, followed by magnesium ammonium phosphate and calcium carbonate. More detailed studies should be designed to clarify the role of environmental and nutritional factors in development of urinary stones.

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