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Energy dispersive spectrophotometry based quantitative elemental estimation of equine enterolith

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ABSTRACT

A male horse of six years old showing episodic, mild to moderate abdominal pain with congested mucous membranes and mild dehydration. Rectal temperature, heart and respiratory rates were recorded 100.7 ^{0}F, 56 per min and 20 per min, respectively. Per-rectal examination revealed mucoid scanty faeces and hard mass of stone in the rectum and the case was diagnosed as of enterolithiasis. The enteroliths recovered were assessed morphologically and analysed using Energy Dispersive X-rays Spectrophotometer (EDS) for elemental analysis. The predominant elements present on the surface of the enterolith were O (72.43 %), P (9.10%), Mg (10.79%), Si (2.59%), Al (1.63%) and Na (1.49%). The mid layer was predominated by O (67.04%), P (8.31%), Mg (6.87%) and N (9.91%) and in core, O (73.13%), P (9.66%), Mg (8.24%) and N (7.89%).

Key words: Elemental analysis, Energy dispersive X-rays, Enterolith, Horse, Spectrophotometer (EDS).

INTRODUCTION

Equine enteroliths are mineral masses that can form in the colon of a horse. The horses with enteroliths are rarely under 4 years old (Lloyd *et al*., 1987), although an enterolith in an 11-month-old miniature horse has also been reported in past years (Hassel *et al*., 1999). Enteroliths are also known as intestinal stones or calculi. Usually these stones build up in thin layers around a bit of foreign matter (small piece of wood, wire, hair, nail or other material) that the horse has swallowed. A horse may have one or more enteroliths ranging in diameter from pea-sized to softball-sized or larger. The mineral contents are highly variable with 90% of a typical enterolith consists of struvite (a hydrous phosphate of magnesia and ammonia) and vivianite (a hydrous phosphate of iron). Combinations of sulfur, sodium, potassium, calcium, titanium, aluminum and nickel make up the remaining 10%. Magnesium vivianite also identified in enteroliths along with variable quantities of sodium, sulfur, potassium, and calcium (Stephen *et al*., 2004).

Enterolithiasis is characterized by episodic, mild to moderate, intermittent abdominal pain with development of progressive anorexia and depression (Lloyd *et al*., 1987, Stephen *et al*., 2004). The degree of pain depends on the degree of obstruction and amount of distention as a result of enterolith (Stephen *et al*., 2004). The heart rate also shows variation depending on the degree of pain. In partial luminal obstruction, there is passage of scant and pasty feces. In some cases, an enterolith may lodge into the small colon causing acute small colon obstruction (Stephen *et al*., 2004). The enteroliths may be diagnosed by abdominal radiography or at surgery and rarely by per-rectal examination, if present in the distal small colon (Anthony and Samuel, 2004).

HISTORY AND CLINICAL OBSERVATIONS

A male horse of six years old was brought to the clinic with the history of expulsion of one stone like object along with faeces one month back followed by discharge of mucus and scanty faeces. The animal was reported to have no defecation for last 10 days and developed progressive anorexia and depression. Water intake and urination was almost normal. Horse was also reported to show episodic, mild to moderate intermittent abdominal pain.

Clinical examination of the case revealed congested mucous membranes and mild dehydration. Rectal temperature, heart and respiratory rates were recorded 100.7 ^{0}F, 56 per min and 20 per min, respectively. Per-rectal examination revealed mucoid scanty faeces and hard mass of stone in the rectum. The caecum appeared to be distended and hard on the right flank. Faecal examination revealed absence of any parasitic ova. On the basis of history and clinical findings, the case was suspected to harbour enterolith and was managed accordingly.

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RESULTS AND DISCUSSION

The enteroliths recovered were assessed morphologically and analysed using Energy Dispersive X-rays Spectrophotometer (EDS) for elemental analysis. The dimensions of the recovered enteroliths were measured 3.4 x 4.9 and 3.0 x 4.6 cm^2 and weighing about 65.8 and 54 gm, respectively with irregular flattened surface (Fig. 1). The enteroliths were hard to cut as observed during elemental micro analysis.

The spectrum of elemental microanalysis of the enteroliths was done by Energy Dispersive X-rays Spectrophotometer (EDS or EDX) machine (BRUKER). The respective spectrum analysis of elemental microanalysis of the surface, mid-layer and core of the enterolith was done in Electron Microscopy Lab, Department of Anatomy, College of Veterinary Sciences and Animal Husbandry, GBPUA&T, Pantnagar, Uttarakhand, India. The results obtained are depicted in Table 1 and Fig (spectra) 1a, 1b and 1c. The data are expressed in both weight (Wt %) and atomic (At %) percent.

The predominant elements present on the surface of the enterolith were O (72.43 %), P (9.10%), Mg (10.79%), Si (2.59%), Al (1.63%) and Na (1.49%). The mid layer was predominated by O (67.04%), P (8.31%), Mg (6.87%) and N (9.91%) and in core, O (73.13%), P (9.66%), Mg (8.24%) and N (7.89%). It was also observed that N was absent only in the surface layer, whereas, Fe, Al, S and Cl were absent in both mid-layer and core. It was interesting to note that the Ca was absent only in the mid-layer and Na was absent only in core of the enterolith. The elements having smaller size of atoms like H, Li, C and B could not be quantified by EDS in the present study. Blue and Wittkopp (1981) had identified that enteroliths are mineralized masses typically composed of magnesium ammonium phosphate (struvite). However, magnesium vivianite also been identified in enteroliths, along with variable quantities of sodium, sulfur, potassium and calcium (Stephen *et al*., 2004).

In the present study, various minerals are identified from the enteroliths and the major minerals are oxygen, phosphorous, magnesium, nitrogen and silicon. Moreover, iron, potassium, calcium and aluminum are identified in minor quantities. These minerals are responsible for the formation of magnesium and calcium phosphate (apatite), iron phosphate (vivianite) and commonly struvite (magnesium ammonium phosphate). Relative abundance of calcium in colonic fluids is expected to favour the formation of calcium phosphates (apatite) rather than struvite (Hassel *et al*., 2001).

The formation of magnesium based minerals in the present case appears to be interesting and needs further studies. Elevated dietary intake of magnesium and protein may play a role in the formation of such enterolith in equids. Many horses that develop enteroliths are fed a diet consisting mainly of alfalfa hay, but in the present location probability of feeding such type of grass is nil due to its non-availability. The formation of such enteroliths could be due to the excessive feeding of phosphorus rich diet such as wheat bran in this locality and water with a high content of dissolved minerals or iron-laden feeds. The wheat bran contains very high content of protein and magnesium than grass hays, which is thought to contribute in the formation of crystals (Anne Rodiek, 2001). Dietary intake of feed material that increase the alkalinity of the colon have been suggested for the formation of enteroliths. Enteroliths most commonly reported to form around a nucleus of silicon dioxide (a flint like stone), ingested nails, rope and hair (Blue and Wittkopp, 1981). In the present study the presence of silicon and oxygen might have formed a silicon oxide core with formation of multiple enteroliths. Soiled grasses fed to the horses appears to be the main contributing factor of silica and formation of core

Table 1: Elemental quantitative analysis yield the weight and atomic percentages of the elements present in enterolith

Elements	Atomic Number	Series	Surface		Mid-layer		Core	
			Weight percent (Wt %)	Atomic percent (At %)	Weight percent (Wt %)	Atomic percent (At %)	Weight percent (Wt %)	Atomic percent (At %)
O (Oxygen)	8	K-series	59.85	72.43	67.04	74.32	64.34	73.13
P (Phosphorus)	15	K-series	14.56	9.10	14.51	8.31	16.45	9.66
Mg (Magnesium)	12	K-series	13.55	10.79	9.41	6.87	11.01	8.24
N (Nitrogen)	7	K-series	-	-	7.83	9.91	6.08	7.89
Si (Silicon)	14	K-series	3.76	2.59	0.19	0.12	0.56	0.36
Al (Aluminum)	13	K-series	2.27	1.63	-	-	-	-
Na (Sodium)	11	K-series	1.77	1.49	0.04	0.03	-	-
K (Potassium)	19	K-series	1.53	0.76	0.98	0.44	1.24	0.58
Ca (Calcium)	20	K-series	1.17	0.57	-	-	0.33	0.15
Fe (Iron)	26	K-series	1.06	0.37	-	-	-	-
S (Sulphur)	16	K-series	0.25	0.15	-	-	-	-
Cl (Chlorine)	17	K-series	0.22	0.12	-	-	-	-
Total			100.00	100.00	100.00	100.00	100.00	100.00

Fig 1: Photograph showing enteroliths recovered from faeces of the ailling horse

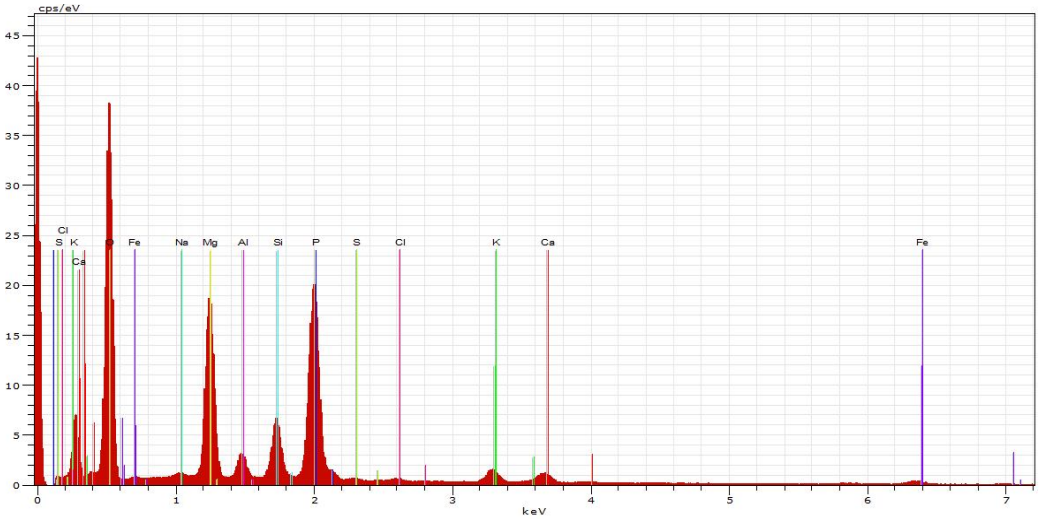

Fig 1a: Energy Dispersive X-rays Spectrophotometer elemental spectrum of enterolith surface

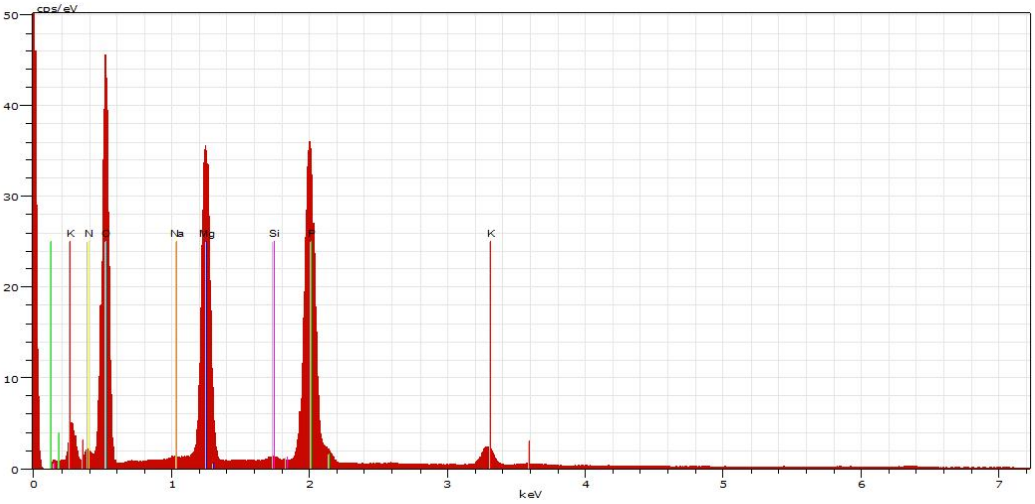

Fig 1b: Energy Dispersive X-rays Spectrophotometer elemental spectrum of enterolith mid-layer

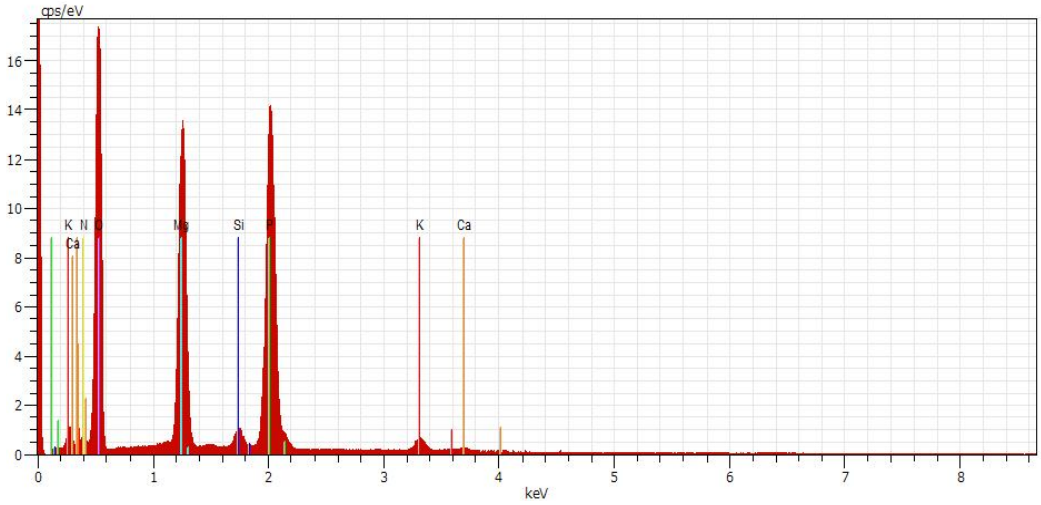

Fig 1c: Energy Dispersive X-rays Spectrophotometer elemental spectrum of enterolith core

of the calculi. Enteroliths found in the right dorsal and transverse colons has also been supported by Lloyd *et al*., (1987).

Episodic, mild to moderate intermittent abdominal pain, progressive anorexia and depression observed in the present case could be due to intestinal obstruction by the enteroliths. The presence of partial luminal obstruction might have allowed the passage of scanty, mucous containing pasty faeces for some time. In one study, 14% of horses presented for treatment with enterolithiasis had a history of passing an enterolith in the feces (Stephen *et al*., 2004) as seen in the present case. Solitary enteroliths are usually round, whereas multiple enteroliths have flat sides (Stephen *et al*., 2004) similar to our findings. Slightly elevated heart rate in this case could be due to mild degree of abdominal pain as well as mild dehydration. The prognosis is good after surgical removal of the enteroliths unless the colon ruptures during removal of an enterolith (Stephen *et al*., 2004). In the present case the horse was treated with intravenous fluid and electrolytes, systemic antimicrobial and multivitamins. Moreover, 3 liters of liquid paraffin was administered orally, using nasogastric tube, but no further improvement was observed over a period of 4-5 days and unfortunately the horse succumbed to death.

It can, therefore, be concluded that equine enterolithiasis in this locality may be due to excessive feeding of wheat bran as well as feeding of grasses containing silica. Medicinal treatment of enterolithiasis alone is not very effective in equine as observed in the present study. It is therefore, suggested that surgical removal of enterolith may be an alternate option under life-threatening conditions of enterolithiasis in equine.

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