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Article

Electron microscopy and X-ray diffraction analysis of equine enteroliths from the Aburrá Valley in Antioquia, Colombia

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Abstract:

The objective of this study was to determine the mineralogical composition of equine enteroliths from the Aburrá Valley in Antioquia, Colombia. Samples of eight enteroliths from eight horses were subjected to semi-quantitative X-ray diffraction (XRD) and transmission and scanning electron microscopy (TEM/SEM) analysis. The TEM/SEM of the analyzed enteroliths reported the presence of carbon, oxygen, phosphorus, magnesium, calcium, silicon, potassium, bromine, iron, sulfur, and aluminum. The XRD identified struvite, newberyte, kyanite, low quartz, actinolite, nitratine, cordierite, and vivianite. Both techniques used in the analysis of the enteroliths were correlated by matching the mineral compounds with the detected chemical elements. The main mineral components of the enteroliths were magnesium phosphates, struvite and newberyte being the most common.

Keywords: Colic, Enterolithiasis, Equine, Struvite.

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Introduction

Enteroliths are concretions derived from mineral precipitations around a nucleus or nidus of organic or inorganic material, located in the gastrointestinal tract[1,2]. These foreign bodies have different shapes, among which the most common are those of spherical or tetrahedral and irregular conformation, with different sizes and weights[3]. Geographic regions with a high predisposition to enterolith formation due to specific mineral components of the soil, water, and plant species have been identified[2-8].

Risk factors such as water sources and high consumption of alfalfa hay with high levels of magnesium, nitrogen, and phosphorus in the diet may contribute to the formation of enteroliths, as the struvite formed by these minerals predisposes to their formation[9]. Alfalfa facilitates the formation of magnesium oxide by promoting an alkaline pH, which favors conditions for the deposition and formation of enteroliths; hence, this legume in the diet of horses is described as a potential risk factor. Among other factors involved, the environment, intestinal pH, hypomotility, and the presence of nuclei are reported to make the formation of these foreign bodies possible[1,8,10].

In addition to exogenous predisposing factors, endogenous factors such as breed, sex, age, and physiological particularities of horses are described for the presentation of enteroliths and phytobezoars[4,9]. For example, 15-yr-old horses have been found to have enterolithiasis in the major colon, and 13-yr-old horses, in the minor colon[3]; however, this condition is also reported in animals of all ages[2,4], beings less common in young animals because of the time required for its development[7].

The speed of enterolith formation in the intestinal tract is variable, as it is related to particularities of the luminal microenvironment of the colon, type of feed —mainly concentrate—, and management in confinement[8,9,10], growth form from the nucleus, and presence of minerals and trace elements[11]. Alterations in intestinal pH can contribute to both the formation and dissolution of enteroliths, thus affecting the time of formation[1]. In these situations, studies are needed to identify and determine the involvement of predisposing factors in order to establish appropriate preventive measures and avoid surgical solution as a last resort[12]. Therefore, the objective of this study was to evaluate the mineralogical composition by electron microscopy (chemical elements) and X-ray diffraction (chemical compounds) of enteroliths obtained from horses in Colombia.

Material and methods

It was used enteroliths collected (by surgical extraction and spontaneous excretion) from horses (Colombian Criollo and Argentine Silla) aged 12 to 16 yr, fed with commercial concentrate, Angleton hay (*Dichantium aristatum*), salt, and water at will, in the Aburrá Valley, in Antioquia, Colombia. Once photographically registered, the enteroliths were weighed and classified by appearance, shape, and size, and later fragmented with an electric saw, allowing the identification of their nidus or central nucleus. The slices facilitated the evaluation of color and internal architectural features such as texture and porosity. Eight samples of enteroliths from an equal number of horses were analyzed in laboratories specialized in mineralogy, crystallography, or characterization of materials; by X-ray diffraction and transmission and scanning electron microscopy (TEM/SEM).

Fragments of enteroliths were pulverized and subsequently placed on the quartz crystal for mineralogical composition analysis, through the semi quantitative X-ray diffraction (XRD) technique (Empyrean® Series II - Alpha 1, Model 2012, Madrid, Spain). The analysis of crystalline phases and quantification was performed with the HighScore Plus software and the ICDD/PDF-4-2012 database for phase identification, with standard reflection configuration, angle 2^{θ} - 5-80°, step: 0.0263°, time: 46.359 sec. Mineral identification was obtained by comparing the diffraction patterns of the enterolith samples with the standard patterns.

On the other hand, samples of the enteroliths were cut into slices that were polished on both sides, subsequently dehydrated on a hot plate, and prepared according to the routine procedure for examination with TEM/SEM (FEI Tecnai® G2 F20), along with a dispersive X-ray spectroscopy for the scanning of the study material.

The data were tabulated and systematized in MS Excel spreadsheets, analyzed with descriptive statistics, and presented in frequency tables with reports in percentages of the elements and mineral compounds in the composition of each of the enterolith samples. This study was approved by the Ethics Committee for Animal Experimentation (CEEA, Spanish acronym) of the University of Antioquia, Medellin - Colombia (protocol No. 1062016).

Results

Figure 1 shows the size, shape and texture of the collected enteroliths. Spherical, polyhedral and irregular shapes with smooth, rough, and porous surfaces were predominant. The figure also shows the macroscopic and electron microscopic texture of some of the enteroliths. The enteroliths ranged in size from 5 to 15 cm, and weighed 664.14 ± 385.01 g (maximum 1,157 g; minimum 127 g). On the other hand, material of plant origin (fiber and seeds) was identified in all the cores of the enteroliths studied, when they were fragmented with the saw.

Figure 1: Enteroliths obtained from equines

a) Shape, size and texture of the enteroliths. b) Electron microscopy image of enteroliths by texture and conformation of struvite crystals.

Table 1 shows the chemical elements in compositional percentages reported during the analysis of each enterolith by TEM/SEM. The elements with the highest percentage were carbon (C), 46.06 %; oxygen (O), 26.85 %; phosphorus (P), 11.55 %; magnesium (Mg), 5.97 %, and calcium (Ca), 3.71 %, with minerals such as silicon (Si), 2.74 %; potassium (K), 1.24 %; bromine (Br), 0.35 %; iron (Fe), 0.71 %; sulfur (S), 0.61 %, and aluminum (Al), 0.17 %. The presence of these elements —especially those considered as trace elements— varied among the enteroliths.

Table 1: Compositional percentages of mineral elements in enteroliths from eight horses from Valle de Aburrá in Antioquia, Colombia, analyzed by transmission and scanning electron microscopy (TEM/SEM)

Enterolith	**Element (%)**										
	C	**O**	**P**	**Mg**	**Ca**	**Si**	**K**	**Br**	**Fe**	**S**	**Al**
1	31.86	21.07	32.46	12.32	0	0	2.29	0	0	0	0
2	38.07	30.61	11.32	2.56	13.96	2.88	0.60	0	0	0	0
3	57.46	24.00	3.27	1.27	4.12	4.37	1.35	0	1.98	1.48	0.71
4	27.16	27.22	30.18	13.29	0	0	2.15	0	0	0	0
5	61.81	23.79	1.18	0	1.18	9.37	0	2.66	0	0	0
6	32.56	30,45	7.13	11.23	5.21	3,56	2.34	0	3.45	3.40	0.67
7	63.45	27.04	2.34	4.23	1.56	1.23	0	0.15	0	0	0
8	56.12	30.67	4.56	2.89	3.67	0.56	1.23	0	0.30	0	0

Table 2 shows the chemical compounds detected in each enterolith by XRD analysis. The mineral compounds with the highest concentration were struvite (magnesium ammonium phosphate hexahydrate [MgNH4PO4-6H20]), 78.68 %; newberyte (magnesium acid phosphate), 11.23 %; kyanite (aluminum silicate), 3. 18 %; low quartz (silicon oxide), 2.36 %; actinolite (inosilicate), 2.15 %; nitratine (sodium nitrate), 1.45 %; cordierite, (magnesium cyclocyclicate), 0.46 %, and vivianite, (hydrated iron phosphate) 0.45 %. None of the samples contained more than five of these compounds.

Table 2: Concentration percentages of mineral compounds in horse enteroliths from the Aburrá Valley in Antioquia, Colombia, analyzed by X-ray diffraction (XRD)

Enterolith	Compound (%)							
	Struvite	Nitratin	Newberyte	Low quartz	Cordierite	Actinolite	Vivianite	Kyanite
1	83.8	7.8	1.1	6.9	0.3	0	0	0
2	99.6	0	0.4	0	0	0	0	0
3	81.9	3.3	3.1	4.0	0	7.8	0	0
4	81.5	0	18.1	0.1	0	0.3	0	0
5	96.1	0	0.5	0	3.4	0	0	0
6	96.4	0	0	0	0	0	3.6	0
7	55.7	0	33.3	7.9	0	3.1	0	0
8	34.5	0.5	33.4	0	0	6.0	0	25.5

Discussion

The literature reports that equine enteroliths are formed mainly by the precipitation of struvite, with increased presence of Mg, nitrates, phosphates, and high concentrations of cations within an alkaline environment in the colon(5,7,13). In addition, high Mg concentrations in the equine colon have been associated with alfalfa-based diets (> 50 %) and are considered to predispose the formation of enteroliths. However, not all horses fed alfalfa develop enterolithiasis; this indicates the existence of other factors that may induce the formation of these concretions, such as individual issues, hypomotility, bacterial flora, diets, buffering capacity, and water quality, which may influence the intestinal pH and the colonic mineral content(6,9,10).

This study did not analyze the predisposing factors of enterolith formation or the evolution of the clinical pictures of horses diagnosed with enterolithiasis. This is a recognized limitation of this work, as it does not allow to infer the participation of these factors in the formation of enteroliths; only the composition of these factors is described. However, the enteroliths come from a geographical area of the department of Antioquia, Colombia, where it is unusual to feed horses with alfalfa and there is no desert context, in contrast with previous reports where regions of the world with a high alfalfa supply and sandy soils have been reported to have the highest frequencies of occurrence of enterolithiasis(1-4,6,7), indicating that the genesis of enteroliths may be multifactorial.

The variety of shape, size and texture, and configuration of the nidi were similar to those of other reports(13). However, unlike in other studies, all nidi were identified, being the predominant plant material, in contrast with other studies that have described materials other than plant material(1,9). It was not possible to verify the single or multiple presence of enteroliths; spherical enteroliths are interpreted as single presence of foreign bodies, and polyhedral enteroliths, as multiple presence(14,15), as complete information on the medical history of the equines was not available.

Struvite is identified as the predominant mineral compound in enteroliths as in other studies(6,7,13). Likewise, the presence of vivianite, although in a lower proportion in the composition, was similar to that reported by Hassel *et al*(13). Conversely, the presence of newberyte, kyanite, low quartz, actinolite, nitratine, and cordierite —mineral elements and trace minerals determined by TEM/SEM (Table 1)— has not been reported; however, it cannot be inferred that this is a characteristic of enteroliths obtained from animals from this geographic region, given the low number of samples. Particularly noteworthy is the finding of more than three compounds in most of the enteroliths, with the exception of the one constituted by struvite and vivianite.

Although the presence of eight compounds was determined in the group of selected enteroliths, the presence of apatite (Ca phosphate) was not found, in consonance with the study by Hassel *et al*(13), although a larger number of samples are required to confirm this finding. However, in canines and felines, struvite urinary stones may be accompanied by apatite stones(16,17), indicating special conditions and interaction or substitution of ions that can influence the crystallization of apatite, as is the case of K and Mg(18).

As for the major elements, the concentrations of P, Mg, K, Ca and organic C and trace elements such as Fe, were similar to those reported in the petrographic and mineralogical studies carried out by Rouff et al. (11) in samples of enteroliths from different geographic regions. On the other hand, the present study reported concentrations of S, Si, Br, and Al, but did not detect the presence of Zn or Mn. In addition, copper (Cu) was not detected in any of the studies, despite being found in the nutritional analysis of equine feed carried out by the same authors(11). Therefore, it is possible that the precipitation and crystallization of mineral compounds depends not only on saturation but also on the interaction of ions and pH conditions in the colonic fluid(18). Based on the above, it is possible to hypothesize that the difference in contexts and feeding systems of the equines may affect the ionic saturation in the colonic fluid, which could partly explain the amount of major and trace elements determined in this work.

Despite the type of food supplied to the horses and the presence of certain minerals in their colonic fluid, these do not form compounds, or such compounds are not detected in the composition of the enteroliths, a fact that reinforces the hypothesis of the existence of other predisposing factors involved in their formation and growth(11,13,18). However, it is interesting that struvite is the major component of the enteroliths analyzed in several parts of the world, which might suggest the existence of a potential analogy with the formation of struvite urinary calculi, in which there is evidence of microbial metabolism rather than mineral saturation(19,20). However, this process is complex, and there is still no evidence that it occurs in the equine colon(9,21).

The recognition of trace elements and organic impurities in the composition of struvite is important, as the higher the concentration of these elements, the greater the susceptibility to decomposition(11). In addition, canine and feline apatite stones are more resistant than struvite stones(16,17); however, they are absent in equine enterolithiasis. Therefore, it is possible to consider medical treatments to dissolve the enteroliths and diet manipulation strategies to prevent their formation, given that the mineralogical analyses showed high impurities of organic material and trace elements that make them susceptible to disintegration and, depending on the composition, solvents like carbonated beverages such as Coca-Cola® may be utilized for this purpose (12).

Conclusions and implications

Both techniques (TEM/SEM and XRD) used in the analysis of the enteroliths were correlated by matching the mineral compounds with the detected chemical elements. In sum, the main mineral components of the analyzed enteroliths were Mg phosphates, the most common of which are struvite and newberyte, unlike vivianite which was also detected, but in a lower proportion than previously reported[13]. Other compounds were also reported to be distributed in all the analyzed samples; however, studies with a larger number of samples and with relevant information on the management, feeding, and clinical condition associated with enterolithiasis of the animals are required to determine the association with the mineralogical composition of the enteroliths.

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